CHEMICAL REVIEWS

Review

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Can Controversial Nanotechnology Promise Drug Delivery?

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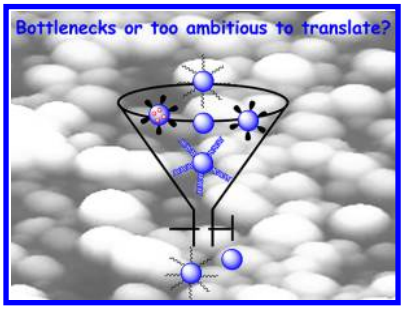

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1. INTRODUCTION

Observing and visualizing objects, with the help of tools, which cannot be seen with the naked eye, had always raised curiosity among the scientific community and given scientific breakthroughs and technological advancements. This pursuit resulted in the development of the optical (light) microscope during the 16−17th centuries and was popularized during the 18th century. As this quest was continued, the phase contrast microscope was invented by Fritz Zernike that fetched him the Nobel Prize in 1953. Phase contrast microscope has promoted cell culture studies and made it possible to study the cell cycle. Later, further developments in optical microscope, such as the confocal microscopy and fluorescence microscopy, have helped the science of the small world. However, the magnification provided by the light microscope is limited to 1000 times. To improve the resolution more than 1000 times, modes other than light have been employed. The electron microscope (EM) that uses electron beam instead of light was developed to suffice the purpose. Nowadays, with the use of high-resolution transmission electron microscope (HRTEM), it is possible to achieve a magnification of 50 million times to a resolution below 0.5 Å.[1] The other mode of imaging technique utilizes an indirect method, where the force between the sample and the cantilever in the microscope are measured to generate a false image that resembles the sample. The prototype that uses cantilever is known as the scanning tunneling microscope (STM). The atomic force microscope (AFM) is the improved version of STM, using which resolutions up to molecular or atomic scale can be obtained.[2] The ability to observe the miniscule objects is helping in the rapid growth of modern science as it is evidence based on the support of a proposed hypothesis. Observation of the material at molecular scale and the ability to manipulate them at that scale is thus creating a lot of curiosity in all fields of science. The most important criterion

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for the enormous amount of interest at nanoscale lies in the fact that many of the materials at this scale show different properties such as optical (e.g., gold), electrical (e.g., carbon nanotubes), physical (e.g., gold), and chemical (e.g., gold) in comparison to their bulk counterparts.[3] The arena where the quantum mechanics start to play an important role in understanding the behavior of materials starts from the nanoscale. The science of observing, manipulating, and utilizing the material below the scale where the quantum behavior and classical behavior start to overlap can be considered as the nanoscience.

The roots for the birth of nanotechnology can be found way back in the twentieth century. The famous prophetic lecture by Richard Feynman, at Caltech in the year 1959, titled "there's plenty of room at the bottom" is credited for the initiation of the nanotechnological revolution. Feynman first explored the possibility of manipulating material at the scale of individual atoms or molecules.[4] In 1974, Norio Taniguchi, a researcher at the University of Tokyo, first used the term nanotechnology concerning the ability of manipulating materials at the nanometer level. The wave of interest in nanotechnology over the last couple of decades has spread across almost all branches of science.[5,6] Nanoscale compounds of carbon black, fumed silica (SiO_2), titanium dioxide (TiO_2), and zinc oxide (ZnO) were widely used before the term nanotechnology was introduced. There are intrinsic nanoscale properties that result from the confinement of atoms and electrons within the boundaries of a few nanometers.[7] These effects are most dominant at sizes below a few tens of nanometers (less than about 30 nm).

Presently, products developed as drugs, therapeutic agents, nutraceuticals, or cosmetics under the nanotechnology label might not always have the quantum behavior. However, they interact with the biological components in a unique way when compared to the same components with larger dimensions. Pharmaceutical properties like stability of the drugs, release of the drugs from formulations, dissolution rate, absorption, distribution, metabolism, excretion, and pharmacological response are reported to be affected by the formulations containing nanoscale drugs or carriers. Further, it is widely believed by the research community that the nanosystems have a distinct advantage over other carriers in that the former can be designed to navigate to sites of interest in the body. On the other hand, in other types of delivery systems, leaving locally acting agents and surgical implants, release of the drugs takes place far away from the target site. It is interesting to note how the area of targeted drug delivery exploiting nanosystems will progress, where once discovered drugs based on a particular target are being used for other diseases.

Nanotoxicology is the newest subdiscipline of nanotechnology that is believed to have played a major role in prompting the regulators to frame strict guidelines to regulate the products containing nanomaterials. While the efforts of the regulators are appreciated, it is unclear at this stage how would one come up with a universal guide to regulate products that are expected to contain versatile materials with different physicochemical properties.

There is a lot of misconception in the area of drug delivery where researchers tend to make comparisons that delivery is a better option when compared to discovering new drugs with respect to time, money, as well as therapeutic efficacy, while most of the drug delivery research to date is on product life cycle extension, and the academic advancements on nanosystems are making the situation even more difficult. This Review is an attempt to highlight the gaps in the area of drug delivery using nanosystems and possible remedies to fill the gaps.

1.1. Definition of Nanotechnology

Why do we need the definition or the tag?

The pivotal criterion to evaluate the definition is to divide the world of technology into nanotechnology and non-nanotechnology.[8] Precise definitions of the terms related to nanotechnology are essential to outline the regulations, formulate the legal terminology for patents, and for commercialization of the products developed using nanotechnology. Widespread acceptance of the terms and their meanings will provide harmonization of the terminology, common understanding, and consistent usage of the terms.[3,8−12] It is also necessary to define nanotechnology and related terms for regulatory purposes, because certain properties of the materials are varied from their bulk counterparts at this scale. The properties attained through nanotechnology might be toxic in some cases and raised environmental health and safety (EHS) concerns.[12]

The "1−1000 nm rule" is based on the prefix "nano" that denotes one billionth of a meter and is most easily comprehensible for users across various disciplines. This definition, however, has been improved to reflect the fact that the component nano brings some novel functionality to the technology. Chip design can illustrate the implications of nanosize in a better way. The advances in chip design were reflected by Moore's law that stated that roughly every 2 years the overall number of transistors, which can be fitted in a commercial integrated connection, is doubled. Further observations evolved, like roughly every 18 months the capacity of microchips doubles, and that every 18 months the relevant structure sizes are divided in half. However, these developments soon reached a limit. The thickness of the gate oxide layer of around 2 nm corresponds to no more than eight atoms of silicon. The development of 90 nm node devices already faces the problems of quantum mechanical effects (like tunneling) that lead to leak currents, because the kinetic energy of electrons can exceed the potential energy of the barrier layer. For the 65 nm node, the gate-oxide layer requires new material with a high dielectricity, which has to be adapted to the conditions of the wafer production. Hence, it is becoming evident that reduction of size beyond a limit is challenging the classical application of the material because the quantum effects start playing a greater role.

The transition to nanotechnology can therefore be described as "step crossing this limit" and entering an area, in which new rules like the quantum size rules prevail. Because these effects appear in the nanoscale range of the metric system, nanotechnology can be described as a research area in which this limit is reached or strategies are developed to overcome it.[8] Sometimes the effect that is seen is due to entities that are engineered to that range and at other times due to discovery or invention of entities that exist in the nanometer range. Specific size-dependent properties are magnetic, mechanic, electronic, optical, thermodynamic, and thermal features as well as the abilities for self-assembly and recognition.

All definitions refer explicitly to the length scale. This is done in a general way (in the area of nanometer), by mentioning a concrete limit (below 100 nm) or by describing it in detail. The description of the relevant order of magnitude is maybe the most pragmatic way to define nanotechnology. The described

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new effects and phenomena or new functions take place around and within this order of magnitude. However, it remains arbitrary, because there is no direct causality from size to these effects or functions.

The "1−100 nm rule" says that an engineered nanoparticle may be defined as any intentionally produced particle that has a characteristic dimension from 1 to 100 nm and has properties that are not shared by non-nanoscale particles with the same chemical composition.[12]

According to the National Nanotechnology Initiative, nanotechnology is the research and technology development at the atomic, molecular, or macromolecular levels, in the length scale of 1−100 nm range, to provide a fundamental understanding of phenomena and materials at the nanoscale and to create and use structures, devices, and systems that have novel properties and functions because of their small and/or intermediate size. The novel and differentiating properties and functions are developed at a critical length scale of matter typically under 100 nm. Nanotechnology research and development includes controlled manipulation of the nanoscale structures and their integration into larger material components, systems, and architectures.

In some particular cases, the critical length scale for novel properties and phenomena may be under 1 nm (e.g., manipulation of atoms at ∼0.1 nm) or be larger than 100 nm (e.g., nanoparticles reinforced polymers have the unique feature at ∼200−300 nm as a function of the local bridges or bonds between the nanoparticles and the polymer).[8]

Nanomedicine, although a branch of nanotechnology still covers a wide range of strategies in the health care management, including nanodiagnostics, nanotherapeutics, and nanomaterials for pharmaceutical applications. Some of the major contributions of the nanomedicine are analytical or diagnostic applications. The discussion about applications of nanomedicine in diagnostics, imaging techniques, and regenerative medicine is out of the scope of this Review, which covers drug delivery aspects.

Nanomedicine according to the National Institutes of Health (NIH) roadmap initiative refers to a highly specific molecular intervention for diagnosis or therapy.[13] Drug delivery aided by nanotechnology can be realized by the development of nanoscale delivery systems or nanoscale manipulations on the materials used for drug delivery.

Nanoparticles for pharmaceutical purposes are defined by the Encyclopedia of Pharmaceutical Technology as solid colloidal particles ranging in size from 1 to 1000 nm. They consist of macromolecular materials and can be used therapeutically as drug carriers, in which the active principle (drug or biologically active material) is dissolved, entrapped, or encapsulated, or to which the active principle is adsorbed or attached.[14] Some properties of material are due to the atoms, and some are due to the bulk level properties that we are used to see routinely. Unlike the properties of the bulk material, which follow classical laws, the nanoscale materials show varying (novel) properties due to the influence of quantum effects, increased role of surface, and collective properties of the object.[15] Despite this scientific reasoning, there are other factors that play a role in formalizing a definition for nanotechnology related terms. Three purposes can be clearly marked as scientific, public, and regulatory.[11]

1.2. Research Fields with Most Active Research in Nanotechnology

An overwhelming number of publications are based on the research in nanotechnology. Although the number of journals dedicated to nanoscience and nanotechnology are steadily increasing, the reports on nanoscience and nanotechnology appear in all kinds of scientific journals. There have been many reports mining the data of these publications, and few disciplines emerge as clear front runners in the field. Table 1 shows the results from four such studies[16−19] showing the average percentage of publications associated with different disciplines.[20] Biochemistry and molecular biology, biotechnology and applied microbiology, and biochemical research methods are the other most studied research topics.

Table 1. Most Actively Pursued and Reported Disciplines of Research in Nanotechnology

research discipline	percentage of publications
materials science, multidisciplinary	13.3
physics, applied	12.4
chemistry, physical	9.2
physics, condensed matter	8.0
chemistry, multidisciplinary	5.5
nanoscience and nanotechnology	4.9
polymer science	3.8
metallurgy and metallurgical engineering	2.7
materials science, coatings and films	2.5
chemistry, analytical	2.4
engineering, electrical and electronic	2.3
physics, multidisciplinary	2.2
electrochemistry	2.1

The number of publications as indexed from Web of Science, Science Citation Index, are the highest from Europe and USA, although Asian countries especially China[21] and South Korea are making fast progress.[22] While the Asian countries are now making a significantly high number of publications, the number of patents coming out from these regions is still very small as compared to those from Europe and USA.[23] Kostoff et al.[24,25] have researched detailed geographical metrices on the nanotechnology publication landscape and sieved the key applications.[26] Table 2 presents the list of compounds most widely used at nanoscale.[3]

Nanotechnology aided drug delivery is an exciting avenue that can provide insights into fundamentals behind how we control matter to the point of dictating the effect of material on body and how the body deals with this material. Being studied at the (a) molecular level for interaction with biological systems, (b) particle level for product design, and (c) bulk level for processing (manufacturing), drugs, and their delivery vehicles are at the forefront of the payload of nanorockets.

The topics of research most relevant to drug delivery have focused on the following:[23]

(a) polymers, especially on the molecular chain structures, and the structures and molecular weights of polymer aggregates in solution, especially water-based;
(b) addition of block copolymers, or polymeric micelles, to promote self-assembly and improve material properties and structures;
(c) artificial and biological membranes, including their structure determination, and formation of the artificial

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Table 2. Nonexhaustive List of Nanomaterials Either Currently Used Commercially or Being Produced in Significant Quantities for Research or Development Purposes[3]

aluminum	nickel
antimony oxide	platinum
bismuth oxide	praseodymium oxide
carbon black	silanamine
cluster diamonds	carbon nanotubes
colloidal gold	titanium dioxide
dimethyl siloxide	zinc oxide
germanium oxide	aluminum hydroxide
iron oxides	barium carbonate
manganese oxide	calcium oxide
neodymium oxide	chromium oxide
palladium	cobalt oxide
polystyrene	dendrimers
samarium oxide	fullerenes
silver	iron
terbium oxide	lithium titanate
yttrium oxide	nanoclays
aluminum oxide	niobium
antimony pentoxide	polyethylene
boron oxide	rhodium
cerium oxide	silicon dioxide
cobalt	tantalum
copper(II) oxide	tungsten
dysprosium oxide	zirconium oxide
indium oxide	molybdenum oxide
lanthanum oxide	

membranes; some emphasis was placed on nanoscopic structures using hydrated single lipids and lipid mixtures, where the nanostructures formed by these extruded vesicles/liposomes ranged from isolated unilamellar vesicles to flat sheet membranes;

(d) particles in fluids, especially colloids, typically a particle core with surfactant shell, and use of emulsions and microemulsions polymerization to generate these particles;

(e) nanoparticles, their size distribution, and properties of particle aggregates;

(f) nanoparticles, with primary emphasis divided between gold/noble metal nanoparticle mixtures and magnetic nanoparticles in magnetic fluids, and secondary emphasis on ZnO nanoparticles; also addresses production of nanoparticles or nanobubbles by core−shell separation;

(g) silver, especially nanoparticles (core−shell nanostructures), colloids, particles, and determination of their structural, chemical, and electrical properties;

(h) detection of proteins and inhibitors, emphasizing their active binding sites

(i) DNA, emphasizing oligonucleotides used in hybridization studies to detect and study specific nucleic acid fragments, such as single- or double-stranded DNA.

1.3. Natural Carrier Molecules and Particles of Nanosize

Understanding the natural carrier molecules and their ability to deliver a wide range of nutrients to different parts of the body helps in producing more efficient drug delivery systems. This will allow us to learn the natural functions of the carrier molecules and help us to mimic or modulate the delivery of therapeutic agents. Nanoscience is important in understanding the biological phenomenon as many of the biomolecules and cellular components lounge in the nanoscale (Table 3). Cells contain biomacromolecules and nanoscale machines, which are complex molecular aggregates. There are various kinds of natural nanostructures present in the cells serving mechanical, protective, and physiological functions. Understanding the molecular nanostructures leads us to the better understanding of nanomedicine. The majority of the cellular components are in the nanoscale as the cell size itself ranges from 1 to 100 μm. The phospholipid bilayer surrounding the cells is around 5 nm in size.[27] Actin filaments and microtubules have the diameters of around 8 and 25 nm, respectively.[28] Mitochondria are around 500 nm in width and 1000 nm in length.[29] Protein synthesis in the cells takes place at the ribosomes, which are spherical and are around 25 nm in diameter.[30]

Specifically in relation to the drug delivery, natural carrier molecules can be studied to understand the effect of nanosize in the uptake, transport, and delivery of nutrients and other essential molecules. With the introduction of nanotechnology, it has been understood that most of the cellular mechanisms take place at the nanoscale. Similarly, many of the natural carrier molecules exist in humans at the nanoscale.

Two important carriers that help in the absorption of the nutrients are casein micelles and bile salt aggregates. Casein micelles are part of the milk that supplies essential nutrients to the infants from the mother. Casein micelles are also present in the milk obtained from the cows and other domestic animals, which forms the major part of the human diet. Normal bovine milk contains around 3.5% of proteins. The function of milk proteins is to supply young mammals with essential amino acids for the development of muscular tissue. The major part of the milk occurs as colloidal particles containing casein micelles.[31,32]

Table 3. Carriers in Biological System

carrier/transporter	size (nm)	composition	role
bile micelle[33]	4	bile salts	facilitates absorption of fats from the intestine
bile vesicle[33]	60	bile salts	facilitates absorption of fats from the intestine
casein micelle[38]	50−500	casein protein	supply of essential amino acids
chylomicrons	75−1200	lipoproteins	carrier of dietary cholesterol and fats
LDL-particle	22	lipoproteins	major transporter of cholesterol and triglycerides
HDL-particle	8−12	lipoproteins	transport of cholesterol from body parts to liver
VLDL-particle	30−80	lipoproteins	transport of triglycerides and cholesterol from liver to body parts
cellular vesicles	30−1000	lipoproteins	internalization, storage, and secretion of wide variety of compounds
human serum albumin[39]	8	soluble monomeric protein	transports thyroid hormones, fat soluble hormones, unconjugated bilirubin, and many drugs
virus	20−400	capsule and genetic material	pathogenic and can penetrate mucosal barriers

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Bile salt aggregates are either bile salt micelles or bile salt vesicles, which help in the absorption of the fats from the gut. Bile salts are amphiphilic molecules with a concave hydrophilic surface and a convex hydrophobic surface. These salts form aggregates in the aqueous solutions and help in the absorption of fats from the gastro intestinal tract (GIT). Hydrodynamic diameters of bile salt micelles are around 4 nm, and those of the bile salt vesicles are about 60 nm.[33]

Lipoprotein particles are the major transporters of the cholesterol and triglycerides in the human body. There are different classes of lipoprotein particles in the humans. The external surfaces of the lipoprotein particles are hydrophilic and the internal surface is lipophilic where the cholesterol and triglycerides can reside and be transported across the bloodstream. Lipoproteins also contain signaling moieties on their surfaces, which help in delivering the cholesterol and triglycerides to specific cell types. Lipoprotein particles are categorized on the basis of their densities related to the amounts of cholesterol, triglycerides, and apolipoproteins. The densest and smallest are called high density lipoproteins (HDL), the least dense and the largest of the lipoproteins are the chylomicrons. After chylomicrons, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL), and HDL can be arranged in the descending order of size and ascending order of density. LDL particles transport the majority of cholesterol and are responsible for the transport of cholesterol to the peripheral tissues. HDL plays a crucial role in reverse transport of cholesterol from peripheral tissues to the liver.

Chylomicrons are generated in the intestinal walls and transport fats from the intestine to muscle and other tissues. These are the exogenous cholesterol and triglyceride transporters. After transporting the cholesterol and triglycerides to tissues, chylomicrons circulate back to liver, which are now rich in cholesterol and are called chylomicron remnants. The endogenous transporters of cholesterol to peripheral tissues are VLDL particles. These are produced by the liver and contain triacylglycerol and cholesterol. During the transport in the bloodstream, VLDL particles lose triacylglycerol to leave IDL particles, which contain a higher percentage of cholesterol. The IDL molecules are either taken up by the liver for metabolism into other biomolecules or continue to lose triacylglycerols in the bloodstream until they form LDL particles. All of the lipoprotein carriers fall under the nano region, suggesting the importance of size in the transport of nutrients.

Albumin, the major protein found in the human body, also serves as a transporter of a wide range of nutrients. It transiently binds to thyroid hormones, fat soluble hormones, and many drug molecules to help in the transport to different parts of the body. Each cell has vacuoles of nanometric size. These intracellular vesicles can help in uptake, storage, and secretion of a variety of functional molecules.

Virus is a small nanometric particle that infects living cells and replicates only inside the live cells. These particles help in understanding the penetration of nanoparticles across different routes of delivery and also give insights in the design of nanoparticles that can avoid recognition from the mononuclear phagocytic system (MPS). Viral components are also used to develop a novel class of vaccines and delivery systems known as virosomes.[34,35]

Most of the biomolecules and therapeutic molecules of natural origin are synthesized using biotechnological methods.[36] Similarly, literature reports also suggest the utility of molecular biology techniques to produce carrier molecules.[37]

1.4. Applications of Nano in Drug Delivery: History and Advancements

Drug delivery has evolved from conventional pills to modified dosage forms offering a variety of release profiles fit for the need such as immediate, sustained, pulsatile, and delayed release.[40−42] During the last 50 years, drug delivery has witnessed rapid growth in the research and development. With lots of studies and technological advancements taking place at a rapid pace for obtaining modified release through different routes, this science has made advancements in biodegradable implants, intravenous pumps, transmucosal delivery systems, and inhalers. New advancements in drug delivery technology are making the old drugs work better and delivering novel classes of drugs, which are otherwise difficult to deliver.[41] Targeted delivery approach is the long sought theory of drug delivery. The idea of targeted drug delivery was first developed by the "magic bullet" concept of Paul Ehrlich.[43] The role of immunological techniques was the central theme in the magic bullet concept, and it still plays a key role in targeting, with other physical and chemical advancements in delivery systems. Targeted drug delivery is the most advanced technological development in the history of drug delivery. Some of the recently marketed formulations make use of the pathological defects in the tumor to deliver the drugs to the target, which is known as passive targeting. Active targeting is under development, which uses a targeting moiety on the carrier system. Introduction of nanotechnology has revolutionized the science and technology of drug delivery. Nanotechnology has provided the foundations for targeting strategies along with more developmental promises in other kinds of delivery approaches.

Pharmaceutical companies and academic researchers worldwide are exploring new strategies to (i) deliver potent molecules with poor biopharmaceutical properties, (ii) increase the efficacy of existing drugs, (iii) reduce the adverse effects, (iv) facilitate alternate routes of administration, and (v) achieve site specificity. Efficient delivery is achieved by physicochemical approaches (salt forms, polymorphs, chemical modifications), design (osmotic pumps, matrix or depots, gels), or use of excipients (formulation design). The aim of effective drug delivery is to transport the drug to the target site while minimizing the distribution in nontarget areas.

Many new molecules are identified to be pharmacologically active through the combinatorial chemistry and high throughput screening adapted in the drug discovery process.[44] However, a very small fraction of these compounds reach the clinical stage. As this fraction is so small, applying a formulation tool in the pipeline drastically improves the percentage reaching the final stage[45] and makes the drug discovery investment more worthy. Many of the compounds with proven pharmacological activities are dropped from further development in the pipeline to reach the clinical stage due to poor physicochemical properties, instability, or adverse toxic effects. Even overcoming these hurdles and showing preclinical success does not warrant the clinical use of a huge number of compounds due to the factors affecting absorption, distribution, metabolism, and excretion. The application of a drug delivery tool in this process addresses one or more of the following questions related to the drug:[46] (1) physicochemical or metabolic instability, (2) poor aqueous solubility or permeability, (3) insufficient drug concentration at the target tissue, (4) high

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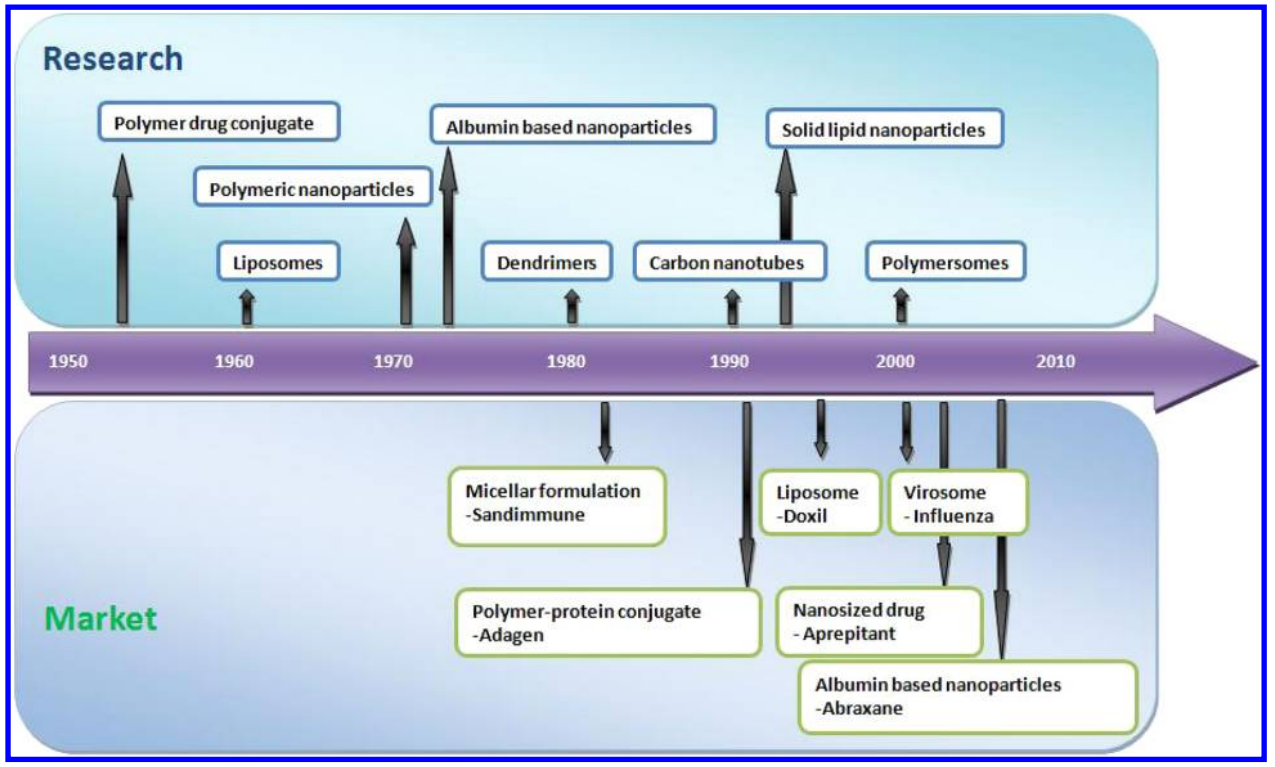

Figure 1. Nano drug delivery systems research and market timeline.

volume of distribution, (5) nontarget organ toxicity, and (6) rapid clearance from the body.

The net clinical efficacy of a compound is not only dictated by the desired action, but also the relative lack of those that are undesirable. For any drug, increase in bioavailability can also increase the adverse effects because they too are pharmacodynamic manifestations of the drug. This distinction is even more demanding for anticancer drugs because the same fundamental activity is warranted in the target cells and to be avoided in the normal cells. The dose of anticancer drugs is titrated so as to administer the maximum tolerable dose. Any increase or decrease in systemic bioavailability of such a molecule will thus disturb the delicate equilibrium against the health of the patient.

The focal point of product development studies is converging on means that impart such ability to the delivery system that can attain, even for otherwise poorly bioavailable compounds, sufficient concentrations across the intestinal barrier. The main challenges for drug delivery can be summarized as solubility, permeability, and targeting. Among the various novel drug delivery systems, nanosized vehicles have attracted significant interest in this direction. The following section reviews the basics and applications of nanotechnology in drug delivery.

Achieving drug delivery by the aid of nanotechnology is not new,[47,48] but the past few years have seen an overwhelming research thrust in this area.[49−52] Drugs and novel drug delivery systems developed with the aid of nanotechnology are being investigated to improve the delivery of drugs through all possible routes of drug administration.[41,53−63] The size of the drugs and drug delivery systems developed utilizing nanotechnology is helping them to be applied in all routes of administration. In a way, nanotherapeutics are route-independent delivery systems, although the pharmacokinetics of the same nanotherapeutic might vary depending upon the route of administration.

During the 1950s and 1960s, miniaturized drug delivery systems were introduced. The Wurster process was developed to microencapsulate drug molecules. Development of polymer−drug conjugates was also introduced during this period. Liposomes were explored as the biomimetic membranes.[64] In the 1970s, polymeric nanoparticles were introduced and found to encapsulate drug molecules and deliver them to the cells through endocytosis.[48,65] Albumin-based nanoparticles were also reported during that time. It took approximately 20−30 years for these novel types of nanotherapeutics to reach the market for clinical use (Figure 1). Polymeric nanoparticles are still in the pipeline to reach the market.

The use of colloidal gold as rejuvenating medicine has been long known in ancient civilizations.[66] The ayurvedic system of medicine, one of the oldest, uses a preparation of colloidal gold known as "swarn bhasm" (gold ash) for a variety of therapeutic effects. The quest for exploring miniature vehicles for modern drug delivery was pioneered by Professor Peter Paul Speiser at the Federal Institute of Technology, Zurich. His group first experimented with beads[67,68] and microparticles[69] and then reported a nanoparticulate product of polymerized micellar system for vaccine delivery.[70] Interestingly, Benacerraf et al.[71] were studying interactions of the MPS with carbon nanoparticles of about 25 nm and radiolabeled albumin microaggregated particles since the 1950s, and their procedure was later refined by another group in the Department of Radiological Science at Johns Hopkins Medical Institutions in Baltimore,[72] a modification of which was used in one of the earliest attempts for drug delivery.[73] Nanoparticles are now a major focus area in the field of drug delivery for both new and already marketed compounds.

The timeline in drug delivery with respect to nanotechnology can be viewed as drug delivery before and after the nanotechnology boom with the current transition period.[74] Many of the presently known nanoparticulate drug delivery

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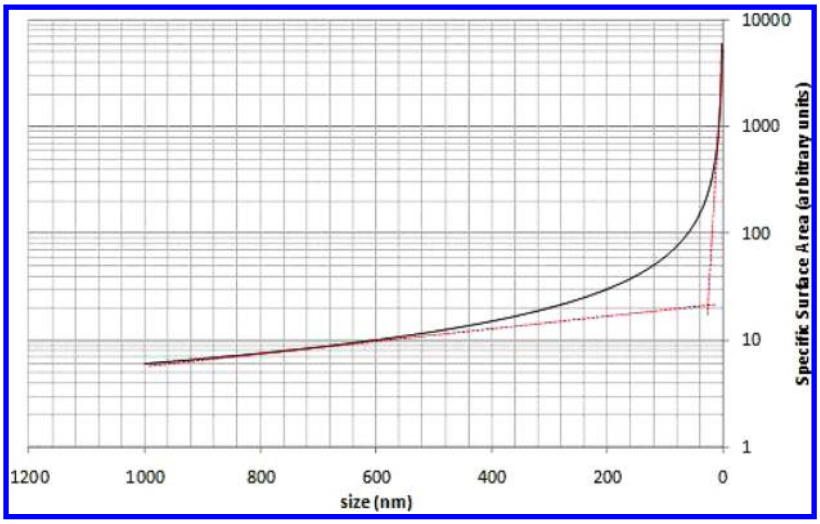

Figure 2. Plot of specific surface area versus size hints toward higher role of surface properties below 100 nm.

systems are produced with the previously existing technologies, for example, milling or emulsification techniques. Polymer−drug conjugates and the vesicular systems were developed long before the definition of nanotechnology was introduced.

The proof of concept of nanoenabled drug delivery came through previously approved drugs, but many new drug candidates are under development using nanotechnology enabled strategies,[75,76] mostly anticancer drugs.[77] The majority of these products constitute a nontargeted delivery system,[75] and are therefore classified as first generation nanotherapeutics.[78] Liposomes and polymer−drug conjugates constitute the major category of first generation products approved for clinical use.[54,55] The first generation nano drug delivery systems are passive-targeted systems that localize into the target site due to some underlying physiology. The most classical case is presented by the enhanced permeation and retention (EPR) effect, which results in accumulation of macromolecules in the tumor through altered vasculature and lymphatic drainage.[79] The biological profile of these systems can be improved by enhancing circulation using surface hydrophilicity imparting agents like poly(ethylene glycol) (PEG).[80,81]

2. THE CORE OF THIS REVIEW

While we debate whether it is the size, composition, or the technology that is driving it, the landscape of nanotechnology research fields is changing faster than we blink.[82] The following sections review the basic fundamentals that need to be considered to understand the behavior of material and interaction with biological matrices. We review how traditional understanding is being rapidly refined by the use of nanotechnology in innovative technologies, targeted delivery, and designer materials.

2.1. Is It the Size?

The prefix "nano" in the nanotechnology is solely related to the dimensions of the material. The technology completely revolves around the theme that understanding and manipulating the material at nanoscale often results in the improved properties or additional new properties of the material. Especially in drug delivery, the nano size allows the access of biological matrixes to the carrier systems of that size. This section reviews the advances in nanotechnology as a function of size alone vis a vis other factors in drug delivery. Size of the nanoparticles often represents the diameter for spherical particles, which are nanometric in all three orthogonal directions. For anisotropic nanoparticles, generally the diameters along the short axis and the long axis were reported, and often the aspect ratio is calculated for such nanoparticles. Anisotropic nanomaterials such as actin filaments and carbon nanotubes are sometimes nanometric in only two orthogonal directions. Planar structures such as lipid bilayer and nanofilms are nanometric in only one orthogonal direction.

2.1.1. Review of Size Considerations for Drug Delivery. Among the nanopharmaceuticals, size plays a crucial role in every case. The nanocrystals (nanosized drug particles) are developed for improved dissolution rate, and the encapsulated nanoparticles are developed for favorable pharmacokinetics and other benefits. The following sections discuss the importance of size and the properties affected by size among various types of nanopharmaceuticals. In case of nanocrystals, biological barriers are the same as other formulations as these were intended for the improvement of the dissolution properties of the drugs. However, in case of nanosized carriers in which drugs are encapsulated or bound either physically or chemically, biological barriers other than routine barriers that are encountered by conventional formulations come into picture.

2.1.1.1. Properties Associated with Size. Some intrinsic properties of the particles are additive, and some are nonadditive. Additive properties can reflect the summation of contributions from individual atoms or molecules within a particle. Nonadditive properties of the material depend on the cooperative effects. Many properties of the materials are nonadditive including their strength, melting temperatures, chemical activity, and opto-electronic properties that are often determined by the surface layer. These properties attain their maximum values only when a critical proximity length from the core to the surface has been reached.[7] Below critical proximity length, nanostructured materials can become harder or softer. If the material is polycrystalline, it becomes harder due to the absence of dislocations as the size decreases, and other materials become softer because of the reduced number of bonds holding the atoms together in a smaller particle. There are two kinds of forces that exist in a particle, body forces and surface forces. Body forces involve all of the molecules in the particles, whereas surface forces are exerted only by the

molecules present at the surface. As the size decreases, the role of surface forces increases and can significantly affect the material properties. van der Waals forces can be treated as body forces, which are responsible for attraction between particles that are decreased with decreasing particle size.

Similarly, forces acting between particles can be long and short ranged, which are also important while dealing with the nanomaterials. The topics about the forces acting upon nanoparticles and physicochemical effects of nanosize have been recently reviewed.[7,83] The size effect is not uniform across the materials; crystalline substances exhibit this tendency more than the amorphous materials. The decreasing size results in the increase of surface energy, which is then compensated by crystallographic changes such as lattice contraction, lattice deformation, defects, rearrangements, and changes in morphology.[12] Some of these changes reduce the degree of crystallinity and thus can increase solubility, which is one of the important criteria for drug delivery.

2.1.1.2. Specific Surface Area. Specific surface area (SSA) is defined as the surface area per unit of mass or volume. SSA is also considered to be a determining parameter to consider a material as a nanomaterial. If a material's SSA by volume exceeds 60 m^2 cm^{-3}, then that material can be classified as a nanomaterial.[84] As the size decreases, the SSA increases, and the critical size for nanoproperties to start appearing is in the zone of exponential increase in SSA. Those properties that arise due to the surface effects have this critical size range much smaller than 100 nm, as depicted in Figure 2. Higher surface area results in enhanced adsorption capabilities of the material. The larger specific surface area is also responsible for increased reactivity of the material with the biological environments as the number of surface molecules increases with decreasing size.

The increases in specific surface area with size reduction might be beneficial or toxic especially for drug crystals. If the material is antioxidant in nature, size reduction to nanoscale might prove to be more effective due to the increased reactive molecules on the surface. Similarly, the adsorption (loading) of the drugs can be increased with higher specific surfaces of the material. Increased toxic effects might include oxidation and protein adsorption of the nanoparticles, which might be due to the higher specific surfaces.[85]

2.1.1.3. Dissolution Rate. Increases in dissolution rate and the saturation solubility are two distinct properties utilized by the nanosized drugs. Many of the nano formulations that are either marketed or in the research pipeline contain particle sizes of around 100 nm. The dissolution rate of the nanosized drugs is very rapid, in some cases, and these drugs can act as liquid dosage forms such as drug solutions.

The surface area dictates the dissolution rate of the solid active pharmaceutical ingredient (API) in a solvent. The dissolution rate is proportional to the surface area available, which can be described by the Nernst–Brunner/Noyes–Whitney equation:

$$\frac{dX}{dt} = \frac{AD}{h}\left(C_S - \frac{Xd}{V}\right)$$

where dX/dt is the dissolution rate, Xd is the amount dissolved, A is the particle surface area, D is the diffusion coefficient, V is the volume of fluid available for dissolution, C_S is the saturation solubility, and h is the effective boundary layer thickness.

2.1.1.4. Saturation Solubility. Increase in saturation solubility with reduction in size has also been reported for nanoparticles. The Ostwald–Freundlich equation is used to explain the phenomenon of increased solubility of nanoparticles:

$$\ln \frac{C}{C_\infty} = \frac{2\gamma V_m}{rRT}$$

where C is the solubility of the particle, C_∞ is the solubility of the large particle, V_m is the molar volume of the compound, γ is the interfacial tension between the solid surface and the surrounding medium, R is the universal gas constant, and T is the absolute temperature.

Nanosizing of the drug crystals results in high energy surfaces, which help in the increased saturation solubility.[86−88] Saturation solubility of the nanosized particle increases to several folds according to the above equation. Rise in the saturation solubility is sometimes greater than the increase predicted by the Ostwald–Freundlich equation. Increased surface area of the drug particles increases dissolution rate and influences bioavailability.[89] Some contrary reports suggest that the increase is not more than that predicted by the Ostwald–Freundlich equation.[87] The reason for this contradiction might be due to the methods employed for the determination of solubility of the nanoparticles. Light scattering, turbidity measurements were more suited methods than the separation methods to understand this phenomenon. Even though these results conflict in the extent of increase in solubility, it can be implied that with particle size reduction solubility is increased.

2.1.1.5. Barriers to Particle Uptake and Transport. The nanoparticles have to cross various types of barriers to get absorbed from different routes of administration. Nanoparticles that are intended for rapid dissolution, like nanocrystals, need to pass through barriers similar to those of the conventional formulations. Nanoparticles encapsulating drugs, which are intended to enter the bloodstream, have to overcome the barriers that are discussed in this section. The nanoparticle delivery systems also need to survive the clearance mechanisms in vivo for sufficient duration to show the required efficacy.[90−92] The important clearance mechanism that exists for nanoparticles is the mono nuclear phagocytic system (MPS). The clearance mechanism by the MPS competes with the efficacy of the nanoparticles and results in suboptimal therapy. Wholly, three types of barriers exist for the nanoparticles in delivering the drugs to the target site via various routes, barriers affecting the absorption from the site of administration, the MPS during the blood circulation, and the vascular barriers at the target site affecting the extravasation of nanoparticles (Figure 3).

The solubilized compounds can cross the cellular layers majorly by passive diffusion, although some molecules can only be taken up via active transport mechanisms. In contrast, passive diffusion is limited or not possible for the particulate matter. The cell membrane acts as the barrier for nanocarriers larger than 1 kDa, preventing their entry into the cells. Other mechanisms of uptake are important in the internalization of the nanoparticles by the cells. Nanoparticles are internalized by the phagocytosis, macropinocytosis, or endocytosis in mammalian cells. Large particles are taken up by the phagocytosis or macropinocytosis. Small particles such as nanoparticles are generally taken up by the endocytosis, which can be clathrin mediated, caveolar mediated, or clathrin and caveolin independent endocytosis. Particles larger than a few micrometers are generally taken up by the phagocytosis, and the

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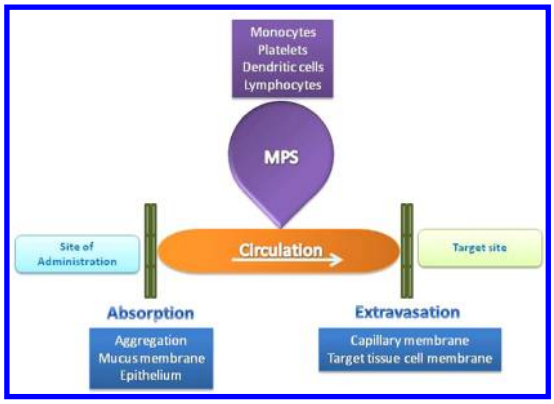

Figure 3. Barriers in nanoparticle uptake. Nanoparticles have to cross various barriers during the transit to the target tissue. Particles need to pass through different barriers during absorption, circulation, and extravasation phases. During absorption, particle aggregation might result in inefficient particle uptake. Particles need to cross the mucus membrane at a rate that is higher than its turnover rate. Particles have to cross the epithelial membrane through endocytosis, predominantly. After reaching the circulation via different routes, particles have to avoid the uptake through the MPS. Finally, particles need to cross the capillary membrane and pass through the target tissue cell membrane to elicit the desired action.

smaller ones are taken up by the macropinocytosis.[54] In a recent study, the cellular uptake of gold nanoparticles with sizes 50 nm or smaller showed that the nanoparticle uptake is size dependent, where 50 nm nanoparticles are taken up in larger quantities than the smaller particles. It has been reported that the gold nanoparticles in these size ranges are taken up by the receptor-mediated endocytosis.[93] Although studies suggest specific mechanisms of endocytosis for specific type of nanoparticles, it was also reported that the nanoparticles might get access to the cells by various random mechanisms of endocytosis.[94]

Nanoparticles are able to internalize in all of the cell types investigated.[54,94,95] The rate and the extent to which nanoparticles were taken up were different with the type of cells employed. In a study, three different kinds of epithelial cells were used to study the uptake of fluorescent dye rhodamine entrapped poly(lactide-*co*-glycolic) acid (PLGA) nanoparticles.[95] The three cell types are opossum kidney (OK) cells, model of renal proximal tubule; Caco-2 cells, model of the gut epithelium; and human bronchial epithelial (HBE) cells, model of the respiratory airway. OK cells took up PLGA nanoparticles quickly, and it appeared that a saturation limit was reached at about 4 h. In both the Caco-2 and the HBE cells, the rate of uptake was considerably slower than in the OK cells. Saturation limit was not observed in HBE cells within the 24 h period. To determine whether the uptake mechanism of nanoparticle entry into epithelial cells was mediated by endocytosis, cells were coincubated with nanoparticles and maintained at 4 °C. Endocytosis, an energy dependent process, is blocked at low temperatures. It was observed that nanoparticle uptake was significantly decreased at 4 °C, suggesting that indeed uptake is mediated by endocytosis. Analysis of immunofluorescently labeled compartments in OK and HBE cells showed that internalized nanoparticles colocalized with markers of early endosomes and the Golgi complex. Nanoparticles strongly colocalize with early endosomes at 2 h after incubation, followed by a decrease in early endosome association and an increased association/colocalization with the endoplasmic reticulum and Golgi over time. It was inferred that the nanoparticles may largely escape endolysosomal degradation.[95] It is also important to note that the rate and extent of endocytosis are dependent on the cell density. Cell-specific variations in the sorting and handling of internalized nanoparticles can be expected.[96] The major route of nanoparticle uptake for particle sizes less than 200 nm is through clathrin mediated endocytosis, and particles with 50 nm diameter are most rapidly taken up by different cell types.[97] Thus, it is essential to understand the mechanisms of nanoparticles uptake in various cells that are most likely encountered by the nanoparticles during their transit across the biological tissues.

Spherical particles up to 3 μm are readily taken up by the nonphagocytic human cervical carcinoma epithelial (HeLa) cells.[94] Uptake in HeLa cells revoked the previous criteria that particles should be less than 150 nm to be internalized by the nonphagocytic cells. Cationic particles have shown more rapid internalization in comparison to their anionic counter parts having the same size and shape. The shape has a definite role in the internalization of the nanoparticles. The rod-shaped particles with high aspect ratio have been shown to be internalized more rapidly than the spherical particles having the same volume. It was found that the internalization of nano and microparticles follows multiple pathways irrespective of their size. Similar observations with phagocytes were also reported reiterating the role of shape in the internalization of nanoparticles.[98,99]

It is well understood that the nanoparticles gain access to the internal compartments of the cells through endocytosis majorly.[96,100] Endocytosis has recently been shown to be dependent on the hypoxic environment.[101] Thus, it is also necessary to understand the pathology where hypoxia might be involved, which can influence the absorption or extravasation of nanoparticles affecting the target tissue concentrations.

Apart from membranous barriers reducing the entry into a tissue or cell, MPS, which was previously known as reticuloendothelial system (RES), can act rapidly on the particulate matter to clear them from the circulation. Thus, this mechanism also acts as a barrier to the particulate matter preventing the blood residence time. Mononuclear phagocytic cells include monocytes, dendritic cells, and macrophages.[102] Monocytes are circulated in the blood, whereas dendritic cells and macrophages are present in tissues like spleen, liver, lungs, and bone marrow.

The uptake of nanoparticles can take place in the blood by the monocytes, platelets, dendritic cells, and lymphocyte.[103] In tissues, resident dendritic cells and the macrophages might do the job of nanoparticle uptake. Blood clearance of nanoparticles is thought to involve three steps. First is the opsonization of the foreign body, followed by recognition and ingestion of nanoparticles by the phagocytes. Opsonization generally takes place in the blood and can take seconds to days to complete. Opsonins come into contact with the nanoparticles through Brownian motion, and the adhesion takes place with the help of van der Waals, electrostatic, hydrophilic, hydrophobic, or ionic forces when at sufficient distance.[104] Opsonization can occur mostly by the nonspecific binding of the opsonin proteins to the nanoparticles.[104,105] Opsonin proteins include immunoglobulins, apolipoproteins, clotting factors, and complement proteins.[91,92] Opsonization makes the nanoparticles recognizable by the phagocytes to engulf them. The internalized nanoparticles are broken down by the enzymes and oxidative reactants, which are released by the macrophages. If the

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particulate matter is nonbiodegradable, it is sequestered and stored in the mononuclear phagocytic organs like liver (Kuppfer cells), spleen (marginal zone and red pulp macrophages), and bone marrow. Thus, opsonization acts as an effective barrier for nanotherapeutics in preventing the access of nanoparticles to the target site.

Because of the smaller size, nanoparticulate delivery systems can be used through all possible invasive and noninvasive routes of administration to reach the circulation, in a way imparting these delivery systems a state of route-independent delivery systems. However, there may be drastic differences in the bioavailability and tissue distribution of the nanotherapeutics through different routes, which might have a strong influence on the therapeutic outcome. The barrier types for nanoparticles vary depending upon the route of administration.

The majority of the research is dedicated to the invasive administration of the nanotherapeutics via intravenous route, which is also reflected in the marketed nanotherapeutics. Two major concerns in the systemic delivery of nanoparticles are limited vascular permeability and uptake by the MPS.[92] This route offers less number of membranous barriers for the systemic delivery of therapeutics as there exists no absorption phase after administration of the nanotherapeutics intravenously. The role of blood vessel walls is to control the extravasation of the blood components into the surrounding tissues. Effective transvascular penetration is essential for the nanotherapeutics to be effective to show therapeutic benefits in the tissues. Blood vessels are composed of an internal epithelial layer, which can be continuous, fenestrated, or discontinuous. The continuous epithelium appears in lungs, fenestrated epithelium in glands, mucosa of the gastrointestinal tract and kidney, and the discontinuous epithelium is seen in the liver and the bone marrow.[90,106] The fenestrae have interconnections with pores of nanometric size. For intravenously administered nanoparticles and particles intended to reach the site of action through blood circulation via other routes, a particle size cut off of 200 nm is necessary to escape the engulfing mechanisms of the spleen.[107] If the particles larger than 200 nm are to be used, other ways of avoiding spleen uptake are necessary. This can be achieved by the decoy particle administration, which saturates the cells of the MPS or hydrophilic surface modification of the particles to stay in the blood circulation for prolonged periods of time.

In some diseases, defects in the vasculature appear, and these defects can be utilized by the nanotherapeutics in enhancing the drug usage. Angiogenesis during tumor growth results in defective vasculature and a deficient lymphatic drainage, which forms the basis of EPR effect for the nanotherapeutics to localize in tumors.[108] This accumulation occurs passively by diffusion of the nanoparticles across the vascular endothelium from the intercellular spaces created during the pathology rather than transport across the vascular endothelium. Other than cancers, abnormal blood vessels with enhanced permeation are observed in a number of ocular diseases. These pathological changes in the vasculature make the nanotherapeutics suitable drug delivery systems for the drugs used in these conditions. Nanotherapeutic clearance from blood is necessary, but the accumulation in target tissues is needed instead of localization in the liver or spleen. However, in cases where the EPR effect plays a role in passive accumulation of the nanotherapeutics, slow localization is warranted. For localization in tissues where no EPR effects are seen, active targeting can be useful.

Invasive routes other than intravenous route deploy the nanoparticles in the interstitium. From the interstitium, nanoparticles enter the lymph and through lymph they can enter the blood circulation. The absorption phase is evident in these conditions where the barrier is the lymph vessel wall. Lymph vessel walls are more permeable than the blood vessel walls, thus promoting the absorption of nanoparticles from the interstitium.[109,110] This route of administration is mostly used to target lymph nodes or for local effect, although systemic delivery is also possible with surface engineering.[111]

The major barrier other than cell membranes or MPS is the mucus layer when the nanotherapeutics are delivered via transmucosal routes. Mucus coating in the eyes, gastrointestinal tract, airways, and female reproductive tract acts rapidly on the foreign particulate matter to remove them. This protective nature of the mucus poses an important hurdle in the delivery of particulate matter to the mucosal linings. Mucus is a very complex network, composed of branched glycoproteins, lipids, macromolecules, electrolytes, and cells. The presence of hydrogen bonding, electrostatic, and hydrophobic interactions along with the physical entanglement in the mucus pose an effective barrier for particles.[112] Orally administered nanotherapeutics, if they are intended to be taken up intact, have to penetrate the dynamic and sticky mucus layer whose properties are mainly dictated by gel forming mucin MUC2 molecules.[113] Mucus is split into two distinct layers in the GIT: the inner layer is adherent firmly and adjacent to the endothelium, on top of which a loosely adherent outer layer rests. Mucus blanket is 10−200 μm thick depending up on the location throughout the gastrointestinal tract, being in its thickest form in the stomach and thinnest in the ileum, from the epithelial surface of the intestine.[114−116] Mucus thickness present in other tissues might be in the range 1 μm to several hundred micrometers. Mucus is continuously secreted with a mean turnover time of 4−6 h.[116] To penetrate the mucus blanket, the nanotherapeutics should be nonadherent to the mucus and small enough to inhibit steric inhibition from the mucus fiber mesh. Apart from these properties, the viscous nature also acts against the particle movement to the epithelial surface.[117]

The interest in oral route lies in its compliancy because of the possible self-administration by the patient and availability of larger surface area for absorption. Intestinal tract containing Peyer's patches whose primary role is to prevent the bacterial infestation received a lot of attention in the absorption of nanoparticles through oral route. Initially it was thought that the nanoparticles are taken up only by the microfold (M) cells of Peyer's patches, but now it is understood that the enterocytes can also participate in the internalization of the nanoparticles.[118,119] Peyer's patches in the small intestine are claimed to be the primary gateways of particle uptake,[120] delivering the particles to the circulatory system through the lymphatics, with the absorption more pronounced and rapid for smaller particles. Other parts of the small intestine are also known to be participating in the uptake of the nanoparticles, and some studies find little distinction between lymphoid and nonlymphoid areas of the small intestine. This phenomenon seems to be dependent on the particle characteristics and needs further understanding. The advancements in the nanotechnology for drug delivery and the understanding of the role of gut associated lymphoid tissue (GALT) are challenging the traditional rule of complete solubilization before the absorption

of any drug through the gut, considering the fact that passive diffusion is not necessary for the transport of nanoparticles.[119] After approaching the epithelial cells, particles can be taken up by endocytosis, as this is thought to be the major uptake mechanism, while the passive diffusion of nanoparticles is also considered by some researchers.[121] From there particles are thought to be introduced to the lymph before reaching the blood circulation. Larger particles of around 1 μm are retained by the M cells and do not enter the circulation.[122] The mucus lining the internal surface of GIT acts as a physical diffusion-barrier to drug absorption[123] and at the same time provides a method to increase the retention time of these delivery systems in the GIT by mucoadhesion.[124] The localization can be promoted with the use of covalently (or otherwise) conjugated cell markers with affinity for transporters and overexpressed receptors.[125]

Apart from these considerations in the oral route, the stability of the nanoparticles is crucial for efficient delivery of the therapeutic. Gastro intestinal lumen, which has varying pH along its length and the presence of enzymes, also acts like a barrier in the particle uptake from the GIT.[126] Thus, it is necessary for the nanoparticles to be stable enough to retain their initial size and the encapsulated drug (Table 4).

Table 4. Factors Affecting Absorption of Orally Administered Nanoparticles[a]

character	effect[b]
size	the smaller the size, the greater is the absorption up to 1 μm; larger particles tend to retain in the M cells or are not absorbed
shape	particles with high aspect ratio are believed to be taken up in larger quantities and faster rate than the spherical particles
surface charge	nonionic hydrophobic particle show higher absorption than charged particles
surface ligands	particles tagged for epithelial cell receptors might increase the absorption
flexibility/ elasticity	flexibility and elasticity might help in passage through narrow capillaries (a phenomenon red blood cells represent)
physical and chemical stability	agglomeration and interaction with the mucus might diminish the advantages offered by nanosize; biodegradability will affect the release of the load

[a]Modified from ref 118. [b]These effects are mostly empirical; firm observations and experimental evidence are needed to support these assumptions.

The pulmonary route is characterized by a surface area greater than 100 m^2 and a very thin barrier 0.1 μm thick of alveolar epithelium for the uptake of drugs.[127,128] The particle deposition is an important criterion in the absorption of drugs from the pulmonary epithelium. Once deposited, the particle has to pass through the mucus barrier, catabolic enzymes in the tracheobronchial region, and macrophages in the alveolar region.[129] At nanoscales the particle deposition in the lungs is diffusion governed rather than the impaction or gravitational sedimentation governed. 100 nm particles deposit throughout the respiratory tract where about 20% are deposited in the alveolar and 5% are deposited in the tracheobronchial region.[130] Mucociliary clearance mechanism operates to clear the mucus toward larynx/pharynx. Insoluble particles larger than 6 μm are cleared within 24 h by the mucociliary clearance mechanism from the pulmonary epithelial surface. However, it was found that the smaller particles such as nanoparticles are retained for longer durations, suggesting an inverse relationship between the particle size and the clearance.[131] Nanoparticles with enhanced mobility can partition through the mucus into the periciliary spaces from where they can be taken up by the lung macrophages or bronchial epithelial cells, causing a reduction in the clearance. Macrophage uptake is another barrier the particles have to overcome to show the therapeutic efficiency. Alveolar macrophages take up particles and are transferred to lymphatic circulation and ultimately to the lymphatic nodes. However, the uptake of particles by macrophages with sizes less than 100 nm or below is reported to be low. Thus, the role of macrophages is less important when compared to the mucociliary clearance through the pulmonary route.[56]

Skin is mostly explored for the local delivery of nanoparticles. The structure of the skin is divided into three distinct layers. Stratum corneum is the topmost outer layer, epidermis lies in the middle, and the deeper layer is the dermis. Nanoparticles can penetrate the skin via the subcutaneous surface, furrows, or through the openings of the hair follicles. Nanoparticle delivery to the dermis or epidermis without barrier modifications has been achieved with little success. A recent study suggests that the nanoparticles can be injected into the skin using needle-free liquid jet injectors for skin delivery of the loaded therapeutic agent.[132]

Although nanoparticles are believed to be route independent and proven to some extent in a variety of pathological conditions or bioavailability enhancement in general, we do not fully understand the exact mechanisms of uptake and transport through those routes yet, but the negative hype of toxicology is hampering the progress to a certain extent. On the other hand, efforts are being put on making an impossible task possible anticipating better mileage over the competitors be it a product, a grant, or a publication, which is also not the way out in healthcare setup, and one has to look at the implications of breaching the biological barriers on a time scale given the pathological condition being treated. Although safety and efficacy will be the two ultimate drivers, it is always interesting to understand the underlying mechanisms on how the particulate matter performs; we feel such attempts should be made using disease models and therapeutic doses.

2.1.1.6. Effect of Size on Circulation and Disposition in Body. Biodistribution of nanotherapeutics is determined by a number of factors including size, surface properties and composition of the nanoparticles, and the route of administration. Nanoparticle size plays an important role in the circulation time and disposition at the tissues from the circulation. The effect of the particulate carrier system size has been extensively studied with spherical particles, and some size-dependent properties pertaining to the circulation and disposition have been reported. MPS recognizes the foreign particles entering the circulation, and generally particles around 15 μm are cleared very rapidly and are accumulated in the liver and spleen. Smaller particles around 5 nm are cleared through the renal filtration rapidly. The circulation times for the particles between 5 nm and 15 μm vary depending upon the surface properties, shape, and nature of the material.

Dendrimers less than 5 nm in size can permeate through the blood vessel walls rapidly to reach the surrounding tissues with ease.[133] Dendrimers with 3−6 nm are excreted rapidly from the kidneys.[134] In the 6−8 nm range, dendrimers are observed to localize in the tumor tissues. As the size increased to 15 nm, the tendency of dendrimers to be taken up by the macrophages also increased.[135]

The morphology of the epithelium surrounding the blood vessels varies with the type of tissue. Capillaries of the lungs and the muscles contain the endothelium, which is continuous and allows only small molecules to traverse across (<3 nm).[106] Other types of endothelia include the fenestrated and continuous types. Both of these types possess large intercellular spaces, which might allow the translocation of nanoparticles slightly larger in size.

Radiolabeled polystyrene nanoparticles were not found in the lung or heart tissue.[122] This suggests that the vascular endothelium has a major role in the accumulation of nanoparticles in different tissues. The lung and muscle capillaries have a continuous epithelium, thus preventing the extravasation of these nanoparticles in these tissues.

To avoid the recognition by the MPS and to increase the circulation times, hydrophilic coating of the particles is performed, which is also known as the stealth property, generally by surface modification with PEG. PEG is generally coated onto the surface of the nanoparticles to impart stealth properties resulting in prolonged circulation times. Steric stabilization is created by the PEG on the surface, which imparts hydrophilicity to the particles that prevents or delays the adhesion of the opsonins onto the particles. Other polymers used for imparting stealth properties to the nanoparticles include poly(oxazoline), polyvinyl alcohol, poly(glycerol), poly-*N*-vinylpyrrolidone, poly[*N*-(2-hydroxypropyl)-methacrylamide], and poly(amino acid)s. Although the long circulation times and stealth property are desired initially, this property might hinder the drug release at the site of action. To increase the chances of drug release at the site of action, the polymer coating has to shed away after arrival at the destination to produce the desired effects.

The size of the nanoparticles has been shown to have a substantial role in the protein absorption and recognition by the macrophages. Smaller particles absorbed lower amounts of proteins in comparison to the particles with larger sizes made up of similar material.[136] Particles with larger size are cleared more quickly than the smaller particles, and this phenomenon is also observed even with stealth nanoparticles.[137] The primary filtering mechanisms, spleenic sinusoids, and Kuppfer cell fenestrations of the liver are in the range of 150−200 nm. These structures trap the particles larger than this size. Liposomes with size ranges from 40 to 450 nm were studied in mice, and after 4 h it was found that the liposomes larger than 100 nm showed increased accumulation in the spleen. It has also been found that the concentration of the liposomes circulating in the blood decreased exponentially with increasing size of the liposomes.[138] Similarly, small (18 nm) shell cross-linked nanoparticles also showed increased circulation time in comparison to larger (37 nm) nanoparticles of same composition.[139] The blood clearance times of nanoparticles are inversely proportional to the particle size.[90] Blood residence times of the nanoparticles that are dependent on the particle size were shown to correlate with the tumor accumulation.[140]

Lymphatic presence of nanoparticles is observed through various routes. Transport from the lymph to blood circulation is also important when the nanoparticles are delivered via non intravenous invasive routes and oral route. Particle size less than 100 nm is preferred for the nanoparticle transport via lymph to the blood from interstitium.[57,111]

In a study, liver accumulation of particles smaller than 100 nm was found to be higher due to the escape of liposomes from the blood to the liver. The same study also suggested that liposomal size less than 200 nm is necessary for long blood residence time. The conclusion drawn from the study suggests that particle size around 100 nm is necessary for prolonged circulation times of liposomes, which was correlated with the tumor localization of the nanoparticles.[138]

The renal excretion of the quantum dots is reported to be size dependent with dot size less than 5.5 nm showing efficient and rapid excretion, while those >15 nm size survived renal excretion.[141] The size of the gold nanoparticles has been shown to influence the extent of organ distribution. Smallest nanoparticles of size 10 nm have shown widespread organ distribution with presence in blood, liver, spleen, kidney, testis, thymus, heart, lung, and brain, whereas the larger particles (250 nm) were detected only in the blood, liver, and spleen.[142]

Having said and understood that the particle kinetics is heavily influenced by the size and matter, how much of this understanding with polystyrene, gold, quantum dots, carbon nanotubes, etc., will be implicated in the area of drug delivery, while not mixing it with occupational health hazards where understanding of these materials could be very important. It is also very important to note that the route of administration/exposure, the duration of exposure, and the dose of these particles are the key in particle kinetics followed by their favorable or unfavorable outcomes.

2.1.1.7. EPR Effect in Tumors. The EPR effect in tumors was first reported by Matsumura and Maeda in 1986,[79] and validated in further reports.[143−145] This remarkable discovery was made by Maeda and colleagues during their studies on the poly(styrene-*co*-maleic) acid conjugated to neocarzinostatin (SMANCS), a polymer−drug conjugate intended for the treatment of cancer. The discovery gave rise to a new targeting phenomenon called passive targeting, in which the size of the therapeutic molecules is responsible for concentrating the drug in the tumors. Long-standing magic bullet concept took a step forward by the introduction of passive targeting, which had shown clinical improvements by reducing the toxicities and increasing the drug response in cancer treatment.[146] EPR is a cumulative outcome of tumor hypervasculature due to neovascularization, leakiness of the vasculature, poor lymphatic drainage, and structural and functional incompleteness of the tumor vasculature.[147] Most solid tumors show hypervasculature to ensure rapid growth in comparison to the normal tissue with exceptions of pancreatic and prostate cancers and large metastatic cancers of the liver. Tumor angiogenesis starts when the tumor becomes larger than 0.8−1 mm in size;[148,149] however, recent findings suggest that the neovascularization starts even at the tumor sizes of around 0.2 mm.[150,151] The new blood vessels in the tumors show deformations with wide fenestrations, discontinuous endothelial lining, lack of smooth muscle cells and pericytes, and irregular basement membrane.[152,153] In normal tissues, lymphatic vessels are more permeable to macromolecules than the blood capillaries, helping in the removal of these molecules from interstitial spaces. In solid tumors, this lymphatic drainage is impaired. Deformed neovasculature coupled with the defective lymphatic drainage leads to the EPR effect for macromolecules. Drug transport across the vascular wall takes place predominantly by diffusion from the discontinuous endothelium. This EPR effect is being used by the drug delivery systems to passively target the tumors to accumulate there. The larger size of pores in the tumor vasculature and reduced lymphatic drainage allows polymers, large compounds, and particulate carriers to accumulate, thereby providing an opportunity to concentrate

the drug in the target locales. EPR effect is demonstrated using the dye Evans blue, which binds to albumin and acts like a true macromolecule. After 24 h of intravenous administration of the dye, Evans blue/albumin complex accumulated in the tumor indicated by blue coloration, but not in normal tissues. Larger tumors are colored blue only at the boundaries as the EPR effect diminishes in the core of larger tumors because of the necrosis and hypovasculature.[154] The factors controlling the growth of the tumor vasculature are similar to normal tissue, but the growth is poorly regulated in solid tumors, resulting in intratumoral differences in the vasculature. Because of this reason, the periphery of the tumor is highly vascularized and the inner tissue is poorly vascularized.[155]

By the well-documented EPR effect,[79,156] nanoparticles can localize in the tumor. This would require the particles to be bigger than a particular cutoff. The minimum particle size required is above 10 nm to escape the renal filtration. The upper limit varies with the type of tumor being targeted.[157] For macromolecules or polymer conjugates, the molecular weight >40 kDa is desired to benefit from the EPR effect.[108] Recent reviews summarized the use of nanotechnology in cancer chemotherapy and indicated that the capillary escape cut off size can be as large as 200 nm to 1.2 μm in some animal tumor models.[158,159] The EPR effect might be the key to the efficacy of the nanoparticle formulation because of their preferential accumulation in the tumors.[156,160] On the basis of the EPR effect, many of the macromolecules and nanoparticles that can target the cancers passively were being developed. While developing the drug delivery systems for tumor targeting, utilizing the EPR effect, some other factors apart from size need to be taken into consideration. The size of the fenestrations varies with the type and stage of the tumor, and other approaches to increase the EPR effect need to be considered. For the augmentation of EPR effect and for homogeneity in larger tumors, the induction of systemic hypertension by using angiotensin II or use of nitric oxide releasing compounds is being investigated. As the tumor vasculature lacks smooth muscle cells, infusion of angiotensin II would have a minimal effect on these vessels, while the normal vessels constrict leading to systemic hypertension. Under these conditions, normal vessels constrict, but the tumor vessels remain relaxed leading to vascular leakage to the tumor, resulting in accumulation of macromolecules and particles of optimum size into the tumors.[161] Similarly, nitroglycerine and isosorbide dinitrite, which act through the release of nitric oxide, are also reported to increase the EPR effect and accumulate macromolecules in tumors.[162]

Longer circulation times are also necessary for the passive targeting of nanotherapeutics in the tumors by the EPR effect.[163] Strategies to improve the circulation times of the nanoparticles should be considered for effective anticancer therapy. PEGylated or stealth liposomal formulations with long circulation times are currently under clinical usage to treat various cancers.

Anticancer drugs are the most benefited drugs from nanotherapeutics due to the EPR effect.[164] The EPR effect can also be utilized for the localized delivery of nanocarriers in other pathological conditions such as arthritis or infection;[165,166] however, the promise has not yet been realized.

2.1.2. Performance of Drug Delivery as a Function of Size. The name of the nanoparticles is self-explanatory of the importance of size in performance of these drug delivery systems. The majority of the nanotechnology research is focused below 100 nm size; however, for biomedical applications this size range is often between 100 and 200 nm. This is because of the particle entrapment in the spleen and liver, which generally trap particles above 200−250 nm. For particles that do not enter the systemic circulation, size can be slightly higher but below 1 μm to show the enhancement in dissolution rate or saturation solubility in the GIT.

The notable achievement of the nanoscale of the drug delivery systems is the ability to target the site of interest, although active targeting is still in the research stages and needs extensive understanding of the phenomenon. The engineered nanoparticles can passively or actively target the diseased sites, avoiding unwanted adverse effects of the drugs at nontarget site. The combination of magnetism and immunomodulation of the nanoparticles enhances the delivery of the drugs to the sites of interest. Magnetism can bring the nanoparticles to the site of interest, and the targeting moieties on the surface of the nanoparticles cause the nano drug delivery systems to penetrate the cells and deliver the drug to the intracellular targets.

Extent of absorption through oral route for nanoparticles is proven to be size dependent. Radiolabeled polystyrene latex particles with different sizes were studied for the extent of uptake through the oral route. 50 nm particles showed 34% uptake, and 100 nm particles showed 26% uptake. The majority of the absorbed dose is found in the blood, liver, spleen, and bone marrow. Particles larger than 100 nm were not found in the bone marrow, and particles larger than 300 nm were absent from the blood.[122] It is very difficult to extrapolate these findings to the use of nanoparticles in drug delivery as such, which involves many more deciding factors than just the particle size; in that sense, will polystyrene absorption reflect other polymers currently in use for drug delivery, and, if yes, then how much of the drug will be delivered in the 34% or 26% particles, and will that dose be therapeutically viable?

Not only size reduction has been shown to give beneficial clinical outcomes, but increasing the size of the protein therapeutics has also proven to improve the efficacy. Most of the protein−PEG conjugates marketed today are based on the advantages in circulation times and nonimmunogenicity they offer. Renal clearance of the protein molecules is decreased by conjugating with different molecular weights of PEG to increase the circulation times of the proteins. Although PEGylation gives rise to other advantages such as steric stability and resistance to clearance by the mononuclear phagocytic system, increase in the hydrodynamic size of the therapeutic is also of considerable importance. Conjugation of anticancer drugs like neocarnizostatin with styrene maleic acid (SMA) has increased the size of the drug molecule and helped in increased localization of the drug in the tumors by the EPR effect. The EPR effect illustrated the importance of size in the tumor therapy.

Small particles have been shown to be taken up through and across the biomembranes by unique mechanisms, which can address the bioavailability problems of the poorly bioavailable compounds.[167,168] These particles can produce local effect at the site of administration and also systemic effect by entering the circulatory system. The performance of nanotechnology-based drug delivery systems is influenced mostly by size[169,170] and surface properties (charge and hydrophilicity), shape, and flexibility.[171]

It is now widely accepted that the performance of nanoparticulate formulation heavily depends on the particle size. Recently, we have demonstrated that the drug bioavailability and the duration for which it stays in the blood

are dictated by the particle size, which is further governed by the copolymer composition and molecular weight.[172,173] Nanoparticles with sizes around 200 nm can be internalized by the nonphagocytic cells. Nanoparticles with sizes of 35 nm can reach the cell nucleus.[174] It was recently shown that the large cationic PAMAM dendrimers induce platelet aggregation but not the small dendrimers.[175] Dendrimers penetrate the skin in a charge-dependent manner. Cationic dendrimers are reported to have greater penetration than neutral or anionic dendrimers.[176] Research in nanotherapeutics so far yielded some size dependencies in the delivery that are listed in Table 5.

Table 5. Size Dependency in Drug Delivery

phenomenon	size limits
intravenous delivery	particles larger than 5 μm cannot be administered due to the danger of capillary occlusion and embolism
saturation solubility	increase in the saturation solubility of drugs is observed below the particle size of 1 μm[177]
oral absorption	nanotherapeutics absorption from the gut presents an inverse relationship with increasing size up to 1 μm; particles larger than this size are retained by the M cells[118]
mucus penetration	larger particles (500 nm) were found to more rapidly penetrate the mucus than smaller particles[178]
skin penetration	largely impermeable to nanoparticles
lymphatic targeting	30−100 nm particles can be taken up rapidly from the interstitium and can be retained in the lymph; smaller particles might rapidly leak into the blood circulation[111]
tumor passive targeting	nanotherapeutics are expected to be in the size range of 5−1200 nm to localize extenstively in tumors
kidney targeting	around 80 nm particles of PEGylated gold nanoparticles tend to accumulate more than smaller or larger particles[179]
intracellular uptake	uptake is size dependent although varies with the type of cells employed in the study
uptake by macrophages	particles with 2−3 μm size are taken up at higher extent than are smaller and larger particles[99]
extravasation from endothelium	depends on the type of tissue; particles larger than 100 nm are trapped in spleen; leaky vasculature in tumors helps in passive targeting of particles with size ranges of 5−1200 nm
renal clearance	nanotherapeutics smaller than 5 nm are cleared by the kidneys rapidly

2.2. Does Matter Matter?

Starting from the atoms, it is known that the ensemble of matter, although exhibiting same fundamental chemical properties, can have substantially different physical properties with implications on interaction with the (especially biological) surroundings.[180,181] Here, we review the effect of material properties of the material in combination with the size function.[182]

2.2.1. Effect of Material Chemistry. Material properties of the nanotherapeutics are majorly responsible for the delivery of the active ingredient. In polymer drug conjugates, the chemistry of the linker and the polymer with the drug imparts new properties to the drug altogether.

The chemistry of the polymer drug conjugates has to be taken at different stages including the polymer drug conjugate as a whole, polymer, and linker after releasing the drug and the degradation products of the polymer. Polymer conjugation must be sufficiently strong during the storage, transit after administration, and should release the drug at the target site at an appropriate rate. Especially for polymers variations in molecular weight, architecture and polydispersity should result in alterations of the pharmacokinetics of the polymer. Thus, a good control over the polymer molecular weight and polydispersity is necessary for drug delivery.[183]

The protein polymer conjugates marketed show different chemistry with respect to the native protein itself. The polymer conjugation is necessary to provide stability by imparting steric hindrance to the labile groups on the proteins and delaying the uptake of the protein by the MPS. PEG, the most widely used polymer in drug delivery, has advantages such as superior biocompatibility and hydrophilicity. Only PEG has been used in different molecular weights to conjugate the proteins, oligonucleotides, and antibodies. All of these developments can be attributed to the properties of PEG, which are not yet matched by any polymer in the clinical setting. With the success of protein conjugates, PEGylation has also been used in the liposomal formulations that are marketed with improved safety and prolonged circulation times.

Protein-based nanoparticles, especially human serum albumin, help in transvascular penetration of the nanoparticles. Immunotoxicity needs to be assessed if proteins other than human origin are to be used in the drug delivery.

During milling to produce the nanosized drug particles, salt forms are generally avoided as they tend to form free base forms during the preparation process, which might affect the therapy.

The toxicity of the material can be attributed to its chemistry. Biodegradability and biocompatibility are the two important issues dependent on the material chemistry in nanotherapeutics. Biodegradability is predominantly affected by the chemical structure of the material. Most of the polymers undergoing hydrolytic degradation are carboxylic acid derivatives such as polyesters, polyamides, poly(anhydrides), poly(ortho esters), poly(phosphoesters), poly(cyanoacrylates), and poly(phosphazenes). Hydrolysis is affected by the type of degradable bond in the order of ester, carbonate, urethane, or amide. High crystallinity and hydrophobicity hinder the hydrolysis of the polymer. Hydrolysis is a rapid process and can be controlled by appropriate selection of the polymer molecular weight, type of linkage, or copolymer composition. Some polymers like polyurethanes and polycarbamates can be degraded by oxidation in the macrophages. Hydrolytic degradation can be observed as bulk or surface erosion; in surface erosion the outer layers of the polymer are first eroded and followed by degradation, whereas in the bulk erosion polymer degradation takes place uniformly in the polymer. During surface erosion, the polymer molecular weight in the noneroding area is unaffected, but in the bulk erosion, the molecular weight of the polymer reduces with time.

The flexibility in polymer synthesis/polymer structures can facilitate development of versatile materials that can respond to diverse stimuli either biological or chemical in nature, which can be beneficial in drug delivery. The pH sensitivity of the nanoparticle core plays an important role in the delivery of the payload; for example, pH-sensitive poly(β-amino ester) can rapidly release the drug at the tumor site as compared to those nanoparticles without pH core.[184] Similarly, certain materials respond to temperature, for example, nanoparticles made from hydrochlorinated poly(isoprene).[185] Spherical shell cross-linked nanoparticles containing poly(isoprene) core with glass transition temperature (T_g) −63 °C are shown to deform at room temperature upon deposition onto mica substrate, while the particles with T_g 33 °C shown no deformation. The rigidity of these nanoparticles can be reduced by heating the nanoparticles over the glass transition temperature.

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Polymer chemistry is also known to affect the complement system. Polymers with nucleophilic groups such as amino and hydroxyl groups are known to activate the complement system.[186,187] Complement activation by the polymers is related to the chain length, architecture, chemical makeup, and solution properties. Free amino groups were responsible for the activation of alternative complement pathway and form amide bonds with the regulatory proteins. Dextrans and sulfated dextrans with molecular weights more than 60 and 10 kDa, respectively, are known activators of the complement system.

Polymer molecular weight is shown to influence the particle size, entrapment efficiency, and pharmacokinetics of the nanoparticles prepared by the emulsion−diffusion−evaporation method. In a study, influences of five different molecular weights of the PLGA (15, 45, 85, 137, and 213 kDa) and three different monomer compositions (MW ≈ 90 kDa) were studied on the nanoparticles preparation, entrapment efficiency, and pharmacokinetics of the estradiol encapsulated nanoparticles in rats.[173] The increase in polymer molecular weight led to an increase in particle size, and this is due to the increase in polymer viscosity as the molecular weight increased, causing resistance for the breakdown of the emulsion droplets. The 50:50, 65:35, and 85:15 lactide to glycolide monomer ratios of PLGA with similar molecular weight of around 90 kDa formed similar sized nanoparticles, suggesting no influence of monomer ratio on the particle size. Higher molecular weight polymers improved the drug loading efficiency due to the increase in hydrophobic interactions between the drug and the polymer; monomer ratios influenced the drug entrapment efficiency with higher lactide content showing higher entrapment due to the increased hydrophobic interactions of the polymer and estradiol. In vitro release and in vivo pharmacokinetics suggested that the increase in molecular weight and lactide content of the PLGA resulted in slower release of estradiol from the nanoparticles. Maximum plasma concentration (C_{max}) was higher, and the time (T_{max}) to attain that concentration was lower for estradiol nanoparticles prepared with low molecular weight or low lactide ratio PLGA; on the other hand, the increase in molecular weight or the lactide content of PLGA resulted in lower C_{max} and an increase in the T_{max}.

Material properties such as flexibility are important in the nano delivery systems such as liposomes. Membrane flexibility of the phospholipid vesicles plays an important role in the surface interactions. Particles with higher degree of flexibility are shown to possess greater binding ability.[188]

Apart from the core or matrix material of the nanoparticles, stabilizers' chemical properties also play an important role in the delivery of nanotherapeutics. The selection of stabilizers for the milling is rather empirical at the moment. However, studies conducted so far suggest polymeric or small molecular weight surfactant molecules can be used as stabilizers. Stabilizer interactions with the polymer core are important in some instances. Pluronic stabilizers are triblock copolymers with center core polypropylene oxide and side polyethylene oxide blocks. In emulsion methods, the stabilization of the nanoparticles prepared using pluronic stabilizers is dependent on the hydrophobic interactions of the nanoparticle core and the center block polypropylene oxide of the pluronic.

The matrix materials in some instances might also have an important role in a specific route of administration. Nanoparticles when delivered through the pulmonary route first encounter the surfactant layer, which increases the wettability of the particles. This will help in the penetration of nanoparticles through the gel phase initially followed by sol phase. Human serum albumin nanoparticles were more slowly cleared when compared to the sulfur nanoparticles in the bronchi of dogs. The slow clearance is attributed to the possibility of positioning in the mucus. The sulfur particles were thought to be located in the gel phase, and the albumin particles might have penetrated deep into the sol phase making the clearance slower. This observation can be extrapolated to other types of materials considering the solubility where more soluble forms will be cleared slowly from the pulmonary surface.[189]

2.2.2. Effect of Charge. The inherent stability as well as instability of the colloidal particles are affected by the Brownian motion. Interfacial forces play a key role in the thermodynamic stability of emulsions and suspensions. Colloidal stability is a balance between the basic physicochemical phenomena of van der Waals attractive forces and electrostatic repulsion as explained by the DLVO theory. Surface charge is essential for the dispersed particles to be separated and stable for longer storage periods, and almost all of the particles in dispersed system acquire surface charges of different magnitudes. According to the DLVO theory, electrical repulsive forces are the major forces keeping the particles apart in a dispersed system. Without the repulsive forces, particles (colloids) tend to aggregate, resulting in sedimentation and crystal growth. Suspensions can be flocculated or deflocculated. In deflocculated suspensions, the particles tend to settle as individual entities, and the sedimentation can result in caking, which is very difficult to redisperse. In flocculated system, particle tends to form loose aggregates, which tend to settle rapidly in comparison to the deflocculated system, but the cake formed is loose and can be redispersed with moderate agitation. DLVO theory, which is widely used to predict the stability of the suspensions, assumes that the particles interact with each other by van der Waals attractive forces and electrical repulsive forces. The total potential energy of the particle−particle interaction is the sum of the repulsion potential and the attractive potential. The attractive potential is dependent on the particle size and the interparticulate distance, while the repulsion potential is dependent on the particle size, interparticulate distance, ion concentration, pH, zeta potential, and dielectric constant of the medium. When the ionic concentration increases in the medium, the thickness of the electrical double layer reduces, resulting in aggregation. Zeta potential is the electrical potential at the shear plane, which is widely used to predict the stability of the suspension, and higher values indicate higher stability. To impart electrical stabilization to the nanoparticles, surfactants are used in the manufacture of nanoparticles.

Apart from the electrical stabilization, steric stabilization also plays an important role in the nanoparticles stabilization. Steric stabilization is achieved by the long tail-like structures of surfactants and other stabilizers that get physically adsorbed on the particle surface. These protrusions prevent the particles from coming too close and attain the primary energy minima. Here, a distinction is required to be made between stabilizers that have one point of attachment to those that have many. The latter group is mostly composed of widely used polymers like polyvinyl alcohol (PVA). The reason for this observation lies in the fact that adsorption is relatively a low energy phenomenon and the stabilizer is only weakly associated with the surface. This process is reversible, and the molecules keep on attaching and detaching to maintain the equilibrium. Thus, single point

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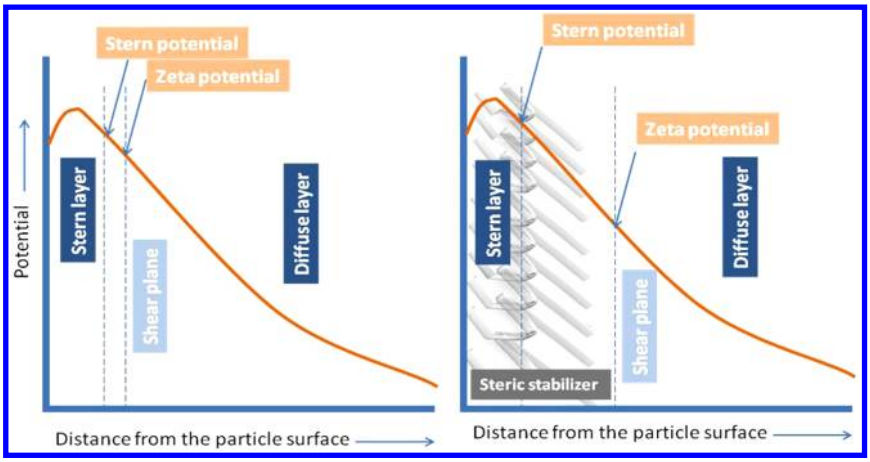

Figure 4. Effect of steric stabilizer on the zeta potential.[190]

attachment can transiently leave the surface naked, the surface being exposed for either a replacement stabilizer molecule or another particle. However, the polymeric multipoint attachments ensure that even if some of the attachments are broken, there are others that will still provide a zone of inhibition for physical approach. Thus, the adsorption process of steric stabilizers is a nonequilibrium process. It should be noted that the Brownian motion can contribute to both the stability (by acting against gravity sedimentation) and the instability (by inducing collisions or reducing the interparticle distance). Because of the presence of steric stabilizers, the Stern layer thickness is increased around the nanoparticles and gives enhanced stability.[190] Because of the increase in the thickness of the Stern layer, the zeta potential value shows a reduction that does not correlate with the stability, which means in case of steric stabilization lower zeta potential values might show the stability that can be comparable to the system with high zeta potential where no steric stabilization exists. Generally, it is believed that a zeta potential value of 60 mV gives high stability to the suspension, around 30 mV gives good stability, and less than 5 mV results in rapid aggregation. However, this is only valid when pure electrostatic stabilization is utilized with small molecular weight surfactants, and its validity diminishes in case of higher molecular weight surfactants and the presence of steric stabilization (Figure 4).

Sedimentation is rarely observed in case of nanosuspensions because of the presence of stabilizers and the relatively smaller sizes. In addition to this, many of the nanosuspensions are freeze-dried to increase the stability. However, in some cases like metered dose inhalers, drying is not intended; thus alternative approaches like altering the morphology, for example, hollow porous particles, give enhanced stability from sedimentation or creaming, which is the deposition of particles on the surface of the medium.

Apart from the effects on the stability, surface charge also plays a crucial role in the interaction with the biological system. Many of the cellular components are negatively charged; thus it can be understood that the cationic particles will interact more with the biological system. However, this interaction is not needed always. For a formulation to be present in the circulation for longer durations, it should not interact intensively with the blood components or blood vessels, but at the same time it should interact with the target tissue. So, the type of charge and charge density must be optimized for that specific need. Polymers with charge are more rapidly cleared from the circulation than nonionic polymers. In ionic polymers, the cationic polymers have lower circulation times in comparison to the anionic polymers. Neutral and anionic polymers are safer than the cationic polymers. Cationic polymers are responsible for cell and nuclear membrane damage and disrupting blood brain barrier. However, cationic polymers are rapidly internalized by the cells and provide efficient intracellular delivery.

In a study, four different types of fullerene compounds (C_{60}, C_{60}−OH, C_{60}−COOH, C_{60}−NH_2) were used in *Escherichia coli* W3110 and *Shewanella oneidensis* MR-1. Positively charged C_{60}−NH_2 has been shown to inhibit the growth and uptake of the microorganisms studied, whereas the neutrally charged C_{60} and C_{60}−OH had shown moderate inhibition, and negatively charged C_{60}−COOH has no effects on the microorganisms. The importance of charge can be understood from the observation that the fullerenes caused the membrane damage but did little harm to the energy metabolism in the microorganisms.[191] It was also demonstrated that the positive charge on the bis-fulleropyrrolidines is necessary to elicit anti viral activity.[192] Similar is the case with silver nanoparticles, which show the antimicrobial activity because of the positive charge.[193] Positively charged silver nanoparticles show the antimicrobial activity at much lower concentrations than silver cation, probably due to additional activity on the membranous enzymes.[194]

Charge also plays an important role in the drug loading in the development of nanotherapeutics. Albumin contains high amounts of charged amino acids in its primary structure. Because of this reason, albumin-based nanoparticles can electrostatically adsorb the drugs, either positively or negatively charged, without the requirement of other compounds.[195,196] The desorption of negatively charged oligonucleotide from the bovine serum albumin is affected by the pH and ionic strength medium, which indicated that the attachment of therapeutic moiety to albumin is due to the electrostatic interaction.[197]

Biodegradability of gadalonium chelate copolymers is reported to be dependent on the charge. Gadalonium diethylene triamine pentaacetate cystine copolymers (GDCP), which are negatively charged, and gadalonium diethylene triamine pentaacetate cystine diethyl ester copolymers (GDCEP), which are neutral, were prepared, and the degradation rates were studied in rat plasma. The results indicate that the negatively charged GDCP was degraded slowly in comparison to the GDCEP. The degradation of the

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Table 6. Role of Surface Characteristics of Nanoparticles in Drug Delivery

property	surface modification	observations
stabilization	electrostatic stabilizer or steric stabilizer	decreased agglomeration, increased shelf life
stealth property	PEGylation	decreased opsonization and phagocytosis by MPS
mucoadhesion	thiolation	increased adsorption of particles to the mucus
mucus penetration	dense covalent coating with low molecular weight PEG	increased penetration rate through the mucus
lymphatic distribution	surface coating with poloxamer 407	uncoated or less dense coat increases lymphatic residence time, and higher density coating results in increased blood levels[111]
safety	charge reduction	cationic charges are thought to be responsible for toxicity; surface coating with nonionic stabilizers decreases the toxicity
specificity	antibody tag	can be used to enhance the permeability or to achieve active targeting
blood brain barrier penetration	coating with polysorbate 80 transferrin; transferrin receptor monoclonal antibodies	increased localization of drug in the brain

gadalonium chelate copolymers occurs through the breakage of disulfide bonds by the free thiols in the disulfide−thiol exchange reaction. The free thiols in plasma under physiological conditions possess negative charges and thus are not able to approach the negatively charged polymer but are able to break the disulfide bonds on the neutral polymer via the disulfide−thiol exchange reaction. Thus, the surface charge of the polymer affects the degradation rates in case of gadalonium chelate copolymers.[198]

Charge can also be used to prepare stimuli responsive drug delivery systems. Liposomes with variable surface charges can be prepared by altering the lipid composition. pH-sensitive liposomes are prepared with phosphatidyl choline and dimethylammonium propane with a pK_a of 6.7. Poly(ethylene glycol)-*b*-polycation polymer can be electrostatically attached to these liposomes at pH 7.4. At lower pH, the liposomal surface becomes cationic due to which the cationic polymer detaches from the liposome. This phenomenon can be used to deliver the liposomes to the lysosomal compartment of the cells while protecting the liposomes in circulation.[199] In another study, gold-capped mesoporous silica nanoparticles were developed for photoinduced intracellular release. Five nanometer gold nanoparticles were linked with a cationic photoreactive linker to 100 nm silica nanoparticles. When these nanoparticles were photoirradiated with ultraviolet light, the photolabile linker is cleaved, and the gold nanoparticles were separated from the silica due to repulsive forces.[200]

Surface charge influences the opsonization of the nanoparticles. In vitro studies demonstrate that the neutral particles are less prone to opsonization than the charged particles.[201] Nanoparticles with high positive or negative surface charge are taken up nonspecifically by the macrophages and deposited in the liver after systemic administration.[202] Particles sizes around 150 nm with the zeta potential less than 15 mV resulted in the survival of nanoparticles from the uptake by the macrophages.[203] Small variations (10 mV) in the zeta potential of the nanoparticles can significantly affect the uptake by nonphagocytic cells.[203]

Particle movement across the tumor tissue was studied using differently charged nanoparticles, and it was found that highly charged nanoparticles are less likely to move along the tumor because of the electrostatic attraction with the extra cellular matrix in the tumor.[204] It was also found that the hydrophobic interaction with the extracellular network is also responsible for the limited movement of the highly charged nanoparticles.[205]

It was also observed that the surface charge plays an essential role in the endosomal escape of nanoparticles after internalization. Mesoporous silica nanoparticles were prepared with different functionalities representing different charges and charge densities. The negatively charged nanoparticles were able to escape the endosomes within 6 h probably due to the proton sponge effect.[206]

Cytotoxic effects of some nanomaterials are also dependent on the surface charges. Cytotoxic effects of amorphous silica were shown to be dependent on surface charge and the morphological features but not on the crystalline component of silica.[207] Synthetic amorphous silica displayed a higher rate of dissolution than the reverse precipitation in biological media.[208] Thus, when the nanomaterial is dissolvable at higher rate than the precipitation rate, the material properties play a minimal role in the toxicity, but the surface charges are responsible for the toxicity.[209]

2.2.3. Role of Surface Characteristics. The surface properties of the nanoparticles can have a significant influence on their storage stability, pharmacokinetics/biological interaction, possibility to adsorb bioactive/drugs on the surface, and also offer flexibility to attach a variety of ligands facilitating tissue or cell specific targetability (Table 6).

Surface energy determinations will help to find out an appropriate stabilizer for the nanoparticle preparation by milling.[210] From the contact angle measurements, it is possible to get the estimate of the surface free energy. If the surface free energy of the stabilizer is similar to the drug's surface free energy, nanoparticles of the drug can be prepared with small variations in the size using that stabilizer.[211] Stabilizers in nanoparticles impart them electrostatic and steric stabilization. Small surfactant molecules will only render electrostatic stabilization, whereas larger molecular weight surfactants or polymers serve both purposes. Electrostatic stabilization increases the charge on the surface, which can be estimated by determining the zeta potential; however, steric stabilization can only be observed. For a system with stabilizer(s) imparting electrostatic and steric stabilization, a lower zeta potential can also result in the increased stability as zeta potential gives the measure of only electrostatic stability but does not include the steric stabilization values.[212] There are also instances where the nanosuspension has the ability to self-stabilize without the help of stabilizers. For example, 2-devinyl-2-(1-hexyloxyethyl)-pyropheophorbide (HPPH) nanosuspensions were stable for 3 months without any stabilizer because of the deprotonation of the carboxylic end groups of the HPPH molecules.[213]

Surface characteristics play an important role in the physical stability and interaction with the biological membranes. Nanoparticle surface has been engineered to see the effects of surface coating on the rate of mucus penetration of nanoparticles. Hanes and colleagues[116,214] assumed that engineering the particle surface with near neutral charge is needed for efficient penetration through the mucus. PEG coating was

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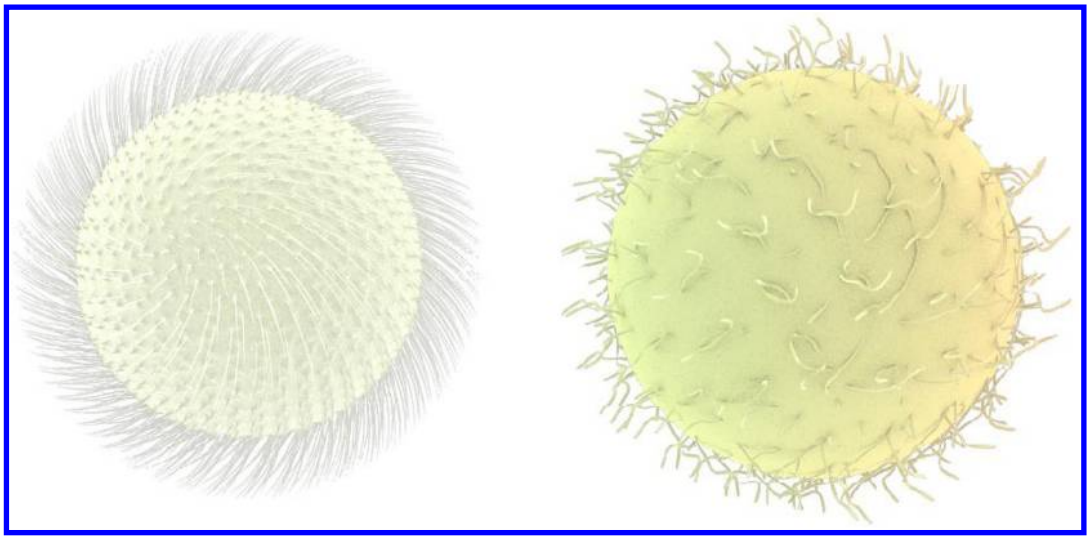

Figure 5. Surface modification with hydrophilic polymer. Brush border conformation (left) and mushroom conformation (right) can be achieved on the surface by polymers.

known to increase the mucoadhesion.[215,216] To delineate the reasons for different properties assigned to the nanoparticles by PEG coating, different molecular weight PEGs and different surface coating densities were employed in a study determining the mucus penetration rates of the nanoparticles. It was observed that the high density coating of low molecular weight PEG on the surface increases the mucus penetration rate in comparison to the low density low molecular weight PEG.[217] This might be similar to other observations where the conformation of the surface polymeric chains was shown to be altered with the surface density from mushroom-like conformation to brush-like conformation. A new PEG-based stabilizer, Vitamin E conjugated 5 kDa PEG, was developed to serve the purpose of surface coating of biodegradable polymeric nanoparticles.[218] Several materials like poly ethylene oxide, phospholipids, and polysaccharide coatings have been investigated as coatings resistant to bioadhesion in diverse applications.[219]

The toxic effects of doxorubicin have been reduced with surface modification of human serum albumin nanoparticles with polysorbate 80 coating.[220] The polysorbate 80 coating resulted in the altered pharmacokinetics of the nanoparticles with lower hematotoxicity and cardiotoxicity.[221] Coating with polysorbate 80 increased the area under the plasma concentration time curve (AUC) and decreased the volume of distribution of the doxorubicin.[222] These coated particles are also known to reduce the testicular toxicity of uncoated nanoparticles. Sertoli cells serve the purpose of phagocytosis in testicular tissue, and coated particles reduce the toxicity by reduced phagocytosis by these cells.[223]

The aspect ratio of the nanoparticles is being investigated for in vitro stability and in vivo functions. Recently, attempts were made to modify the flocs, as low density open flocs resulted in higher stability of the nanosuspensions with high aspect ratio particles.[224]

Surface curvature or geometry is also known to play an important role in the phagocytosis by macrophages. It was shown that the curvature or geometry at the initial contact with the cells plays an important role in the macrophage uptake in vitro.[98,225,226] Although the studies by Mitragotri and colleagues were carried out using micrometer-sized particles, their studies with varying particle geometries suggest the role of particle geometry in the uptake by the macrophages. How much of this basic understanding in vitro will translate to clinic will remain to be answered.

Modifying the surface properties to improve the circulation times by PEGylation has been proved highly successful in case of PEGylated proteins and PEGylated liposomes, which have been in wide clinical use for more than a decade. This surface modification not only resulted in longer circulation times but also led to the required dose reductions, thus reducing the toxicity of the formulation. Blood clearance of naked nanoparticles or liposomes is significantly higher in comparison to the PEGylated surfaces. Methoxy PEG chains on the surface of the liposomes induced the complement activation and opsonization, but the phagocytosis is restricted by the steric constraint offered by the methoxy PEG chain.[227] PEG chains on the nanoparticle surface depict mushroom or brush configuration with low and high surface coverage, respectively (Figure 5).[104] In mushroom configuration, the chains are closer to the surface, and in the brush configuration, the chains are extended away from the surface. Several theories have been proposed for the understanding of increased circulation half-lives of the PEGylated nanoparticles. PEG surface modification creates a water bound surface on the particles, which prevents the adsorption of opsonins. Additionally, some theories assumed that the excess quantities of PEGylated nanoparticles overload the opsonization system of the body and give the false impression of stealth properties.[105] The most widely accepted theory is based on the steric interactions between the proteins and PEG chains, which resists the adhesion of proteins on the surface of the PEGylated nanoparticles, thus preventing the opsonization.[228] PEG chains can exist in extended conformation when in solution. The proteins are attracted to the particles by van der Waals attractive forces during which the proteins encounter the protruded PEG chains and try to compress them. This compression leads to more condensed and high energy conformation of the PEG chains. This results in the generation of repulsive forces and prevents the protein adhesion to the particle surface. Optimum thickness of the PEG chains is necessary for effective stealth properties of the nanoparticles.[104]

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Poloxamer 407 coating can be discussed as another better example of surface engineering of nanoparticles.[229] Poloxamer 407 has ethylene oxide chains, and when coated at lower concentrations on to the nanoparticle surface they represent a flat or mushroom-like conformation. When the concentration of poloxamer is increased, ethylene oxide chains represent a brush-like conformation. These particles were introduced to the interstitium in the foot pad of the rats along with uncoated nanoparticles. Uncoated nanoparticles retained in the footpad for longer durations than the coated nanoparticles. Coated nanoparticles were taken up rapidly from the interstitium. The uptake was rapid for the particles depicting brush conformation of the ethylene oxide on the surface when compared to the particles with mushroom-like conformation of ethylene oxide on the surface. However, the particles with brush-like conformation were retained in the lymph nodes due to the uptake by macrophages.[111] The brush border conformation reduced the uptake of nanoparticles by the macrophages, and the particles appeared in blood, which was not seen with the other two kinds of nanoparticles. This specific experiment ascertains the importance of surface engineering in modulation of lymphatic uptake of nanoparticles.

Transferrin (Tf) or transferrin receptor monoclonal antibodies (TfR-mAb) can be used to modify the surface of the human serum albumin nanoparticles to achieve brain targeting.[230,231] Polysorbate 80 coated nanoparticles were reported to cross the blood brain barrier to deliver the encapsulated drugs.[232] The coating of nanoparticles with polysorbate 80 is thought to help in the adsorption of Apolipoprotein E onto their surface while in the circulation, and mimics low density lipoprotein (LDL) particles that are then taken up by the LDL receptors on the vascular endothelial cells and transported to the brain.[232] Coating of estradiol loaded polymeric nanoparticles with polysorbate 80 has also resulted in increased brain levels of estradiol after oral administration; however, no direct evidence of particle transport was reported.[233] Modifying the surface of the liposomes with virus-enveloped glycoprotein gives a novel vaccine delivery systems called virosomes.[234]

Nanoparticles can be assembled as composites with modifications on the surface. A study utilizing the Fischer's lock and key principle developed nanoparticles that assemble into composite structures. The spherical particles were used as keys, and the monodisperse particles with a spherical cavity were used as locks. Keys are bound to locks irreversibly via the depletion interactions.[235] Increasing the surface roughness of the protein nanomatrix particles reduced the cohesion force and decreased the inhaler retention, resulting in enhanced aerosol performance.[236]

2.2.4. Effect of Agglomeration. The huge surface area of the nanoparticles makes them thermodynamically unstable, and they tend to agglomerate to result in a stable system that has lesser surface free energy. Agglomeration in the nanosuspensions can result in settling, creaming, crystal growth, and dose variations.[237] During the preparation of nanosuspensions, addition of stabilizers prevents the agglomeration of the nanoparticles by imparting steric and electrostatic stabilization and wetting ability to the surface. Quantitative studies on the uptake of nanoparticles by the biological systems should also consider the aggregation, agglomeration, and sedimentation, which might occur simultaneously in the physiologically relevant media.[238]

Agglomeration is the collection of particles that are loosely bound with either of the following interactions, van der Waals forces, electrostatic forces, physical entanglement, or surface tension.[9,239] The surface area of the agglomerate equals the total of the individual surface areas of the participating particles. Aggregation is different from agglomeration where the chemical or metallic bonds bring the particles to close contact, often resulting in the surface area slightly smaller than the sum of the individual surface areas of the participating particles. Agglomerates can be reversible, whereas aggregates are generally not reversible. Agglomeration or aggregation can alone significantly alter the behavior of the nanotherapeutics. These effects are mostly related to the toxic effects of nanotherapeutics during usage. Agglomeration of nanotherapeutics is very important to study because some are known to agglomerate at a very rapid rate in culture media and biologically relevant media.[240] The rate of agglomeration of nanoparticles is dependent on the concentration and the size of the nanoparticles. Agglomeration can be prevented by electrostatic or steric stabilizers or by using open flocs in the dispersion.[241,242] Sometimes, excess surfactants could also cause the agglomeration in nanodispersions.[243]

Ceria nanoparticles with 20−50 nm size tend to agglomerate at a more rapid rate than the 250−500 nm particles. The uptake of smaller nanoparticles only takes place as agglomerates, as the smaller particles transporting across the cell culture medium is diffusion controlled, whereas larger particles can settle on the cells and can be taken up at a more rapid rate than the 20−50 nm ceria nanoparticles. Thus, 20−50 nm ceria nanoparticles can only reach the cells as agglomerates to internalize, but for 250−500 nm particles, although the agglomeration time is slower, individual particles can settle on the cells and can be taken up, resulting in higher uptake of larger particles than smaller particles (Figure 6).[238]

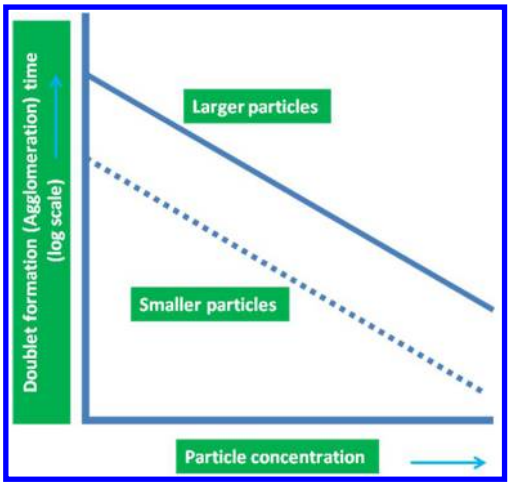

Figure 6. Rate of agglomeration is dependent on the particle size and the concentration of particles.[238]

The rate of agglomeration is an important criterion in the toxicological studies. Silver nanoparticles are reported to be agglomerated in the RPMI 1640 medium, which is generally used to dilute silver nanoparticles. Agglomeration can be prevented by addition of fetal calf serum to the RPMI 1640 medium in cell studies.[244]

It was observed that GIT mucus covers the particles and forms slugs irrespective of size, density, and composition of the

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nanoparticles in dogs and rats.[245,246] This type of aggregation can prevent the absorption of nanoparticles from the gastrointestinal tract. Through the pulmonary route, the agglomeration of nanoparticles can influence the deposition of the nanoparticles on the epithelial surface and the clearance through the mucociliary clearance and macrophage uptake. Agglomeration increases the size of the nanoparticles, and the deposition turns to be impaction and sedimentation dependent, which is diffusion dependent for nanoparticles, and this increase in size leads to faster clearance. Similarly, the recognition by the macrophages also increases for the agglomerates.[56]

During the formulation of nanoparticles, freeze-drying is often the last step that increases the particle stability and storage life. This process induces freezing and drying stress on the particles affecting the physical stability of the nanoparticles.[247] Thus, the freeze-drying process has also been implicated in the stability of the nanoparticles. The selection of cryoprotectants and process variables is crucial to retain the initial particle sizes and to prevent agglomeration of the nanoparticles during the freezing and drying process. Some studies suggest that rapid freezing rates can be suitable for nanoparticles in custom-made apparatus, which can be used for higher freezing rates.[248] Routinely used freeze-dryers do not allow such high rates, and the prenucleation freezing rate of the product often differs from the shelf inlet temperature that is reported in most of the cases.[249] Quench cooling induces a high freezing stress on the nanoparticles and is not a preferred method for freezing the nanoparticles.[250] Quench cooling method is not preferred also because of the inhomogeneity in the batches. Apart from the freezing rate, selection of the primary packing material, cryoprotectant, and the fill volume are also responsible for retaining the initial particle size distribution after the freeze-drying. The formulation process generally employs steric and/or charge inducing stabilizers to prevent the agglomeration of the particles. Additionally, cryoprotectants are added to prevent the agglomeration of nanoparticles during the freeze-drying process. It was found that the rate of freezing has a minimal effect in comparison to other factors like steric stabilizer and cryoprotectant concentrations during the freeze-drying process.[250,251]

2.2.5. Can We Deduce Rules for Material Selection? It is necessary to understand the drug and the disease, before a delivery system is designed but not the other way around. With the increasing interest in translational research with product development as a theoretical end point, many novel materials are finding drug delivery as an obvious choice. Most of these materials are tested using some model dyes/drugs that are easy to monitor, which could be one of the major drawbacks for poor/no translation from basic research to clinical, leaving aside the lack of appropriate models to test them. This can be well understood by the fact that the most successful liposomal product(Doxil) goes well with doxorubicin but not with other anticancer drugs that are also seeking delivery systems. Therefore, rule number 1 should be to understand the drug and the disease.

Many materials that were not thought of as drug delivery systems before by the pharmaceutical scientists are being explored for the purpose. The materials now include metals, carbon nanostructures, lipids, proteins, magnetic materials, and polymers for the development of drug delivery apart from the materials like surfactants and other excipients, which are already in use. Although this multidisciplinary nature increased the quantity of research in the drug delivery field in academia, it also surmounted the toxicity concerns of the materials. This is one of the main drawbacks of the nanotechnology aided drug delivery in translation of the medicine from preclinical level to the clinical setup. Many of the heavy metals are not easily eliminated from the body and pose a major risk. The development of these types of drug delivery systems can be promoted with the risk versus benefits offered by the delivery systems.

The nanotechnology also placed the soft material research on a rapid pace. Micellar systems, vesicles, polymeric nanoparticles, and lipidic systems were extensively investigated for the drug delivery of many of the challenging molecules. The success of the soft material-based drug delivery systems includes the approval of cremophor-based drug delivery systems, liposomal amphoterecin. The polymeric nanoparticles have not yet seen the success in the market maybe because the polymers used for the drug delivery are not on the generally recognized as safe (GRAS) list.

Polymer selection for drug delivery is generally based on its origin, chemical nature, backbone stability, and water solubility.[252] Natural polymers are often biodegradable and cheap, but the problem with their use is due to batch to batch variation and high immunogenicity. Synthetic polymers are readily available with controlled composition and properties. The major drawback of the synthetic polymers is their nonbiocompatibility. PLGA and PEG are exempt from this limitation due to their use in humans over a period of time.

The polymer conjugates involve the attachment of novel polymers to the therapeutic molecules. Polymer biodegradability is very crucial for the success and safety of the therapy. A nondegradable backbone poses a major risk of toxicity. Even when the polymer chain is small and can be excreted by renal filtration, there are chances of lysosomal accumulation of polymer, which might lead to the "lysosomal storage disease" syndrome. PEG and hydroxypropyl methacrylate (HPMA) are extensively used in marketed products and well tolerated clinically, but the main backbone is nonbiodegradable for both of these polymers with cleavable linkers to control the release of the therapeutic moiety. Although transient, the presence of intracellular vacuolation in the animal studies with PEG protein conjugates is raising awareness about the use of biodegradable polymers. It is important that the development of new polymer should be based on their biodegradability, safety, and industrial feasibility. Biodegradable polymer polyglutamic acid (PGA) being used to generate new anticancer agents is degraded by the lysosomal thiol-dependent proteases.[253] Other polymers that undergo enzymatic or hydrolytic degradation are also under development such as dextrin,[254] hydroxyethyl starch,[255] polysialic acid,[256] etc. These are either branched or linear polymers; other classes of biodegradable dendritic polymers under development are polyglycerols.[257] Apart from the biodegradability, while developing the conjugates, the safety of covalent linking chemistry and possible development of toxic degradation products while storage must be taken into consideration.[183] The total control of the parameters such as molecular weight, polydispersity, localization of charge, or hydrophilicity−hydrophobicity balance is necessary to adjust the biodistribution, circulation half-life, activity, and toxicity of the conjugates.

One of the novel classes of carrier materials in drug delivery are carbon nanotubes. Carbon nanotubes can be single walled or multiwalled. The diameters are in the nanometer range, and the lengths can reach several micrometers. Pristine nanotubes

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are the prototype nanotubes. Surfaces have been modified noncovalently or covalently, which is known as functionalization. Various modifications have been carried out on the carbon nanotubes to suit the delivery of various types of drugs.[258] However, the main concern about the carbon nanotubes is their nonbiodegradability and poor biocompatibility. The important question in the usage of carbon nanotubes is their safety. Safety is the primary concern in drug delivery; however, sometimes if benefits offered by the delivery system outweigh the risk associated with the system, they are accepted. Apart from the safety in the body, biopersistance and environmental impacts also need to be taken into consideration while using the carbon nanotubes. Although efforts have been made to make the carbon nanotubes biocompatible, nonbiodegradability remains a major concern. A recent report hints that the functionalized nanotubes can be degraded by oxidizing enzymes, which is providing new perspectives in the development of carbon nanotubes for drug delivery and other medical applications.[259,260] Incomplete phagocytosis due to very long lengths is sometimes reported to be the cause of carbon nanotube toxicity and resemblance to asbestos.[261,262] Reducing carbon nanotube length and making them biodegradable might be compulsory for these agents to be developed as drug delivery systems. The majority of the drugs that need nonconventional delivery strategies mostly exhibit some degree of toxicity, and it would be interesting to see how the carbon nanotubes will cope with the latter being toxic themselves. The toxicity of the vehicle has always been a concern, and this can be better explained by the amount of research being conducted on developing alternative delivery systems that avoid the use of Cremophor EL, a vehicle that is toxic and used in Neoral (Cyclosporine) and Taxol, due to the drug's poor solubility. To complicate this further, drug delivery systems are investigated in managing the chronic diseases that need long-term administration and nondegradable materials, and those that are not easily cleared will have even more problems. Thus far, the research on carbon nanotubes for drug delivery has been given extra mileage academically; however, not much progress has been made toward product development involving the pharmaceutical industry.

2.3. Digging Deep

The fundamental tenets on which nanoscale drug delivery is based, like Peyer's patches, particle uptake mechanisms, barrier transport, enhanced permeation, and retention, shall be discussed[263] covering new developments like the effect of particle shape.[94] In the background of basic physical principles of Brownian motion and free energy, we can define the unique interactions of material with biological systems.[12]

2.3.1. Bottom Up and Top Down Approaches. As the nanoscale lies between the molecular scale and macroscale, nano regime can be approached by using the "bottom up" technique from molecular scale or the "top down" technique from a macroscopic scale.

The "top down" approach is more predominant than the "bottom up" approach in obtaining nanoparticles of the API. In the "top down" approach, the large particles are mechanically reduced to nanoscale using wet milling or high pressure homogenization. This approach is presently used to produce drug nanoparticles in large industrial scales.

Bottom up approaches are the easiest way to produce drug nanoparticles given that the drug has sufficient solubility in the pharmaceutically acceptable solvents. The simplest method is the hydrosol process where the drug is solubilized in a water miscible solvent and then added to water.[264] Addition of organic drug solution to the water alters the solvent power of the system, and, as a result, precipitation takes place. To prevent the particle size growth, often stabilizers are added. Evaporative precipitation into aqueous solution, spray freezing into liquid, and ultra rapid freezing are the other known methods of the bottom up approach for the preparation of drug nanoparticles.[265−268]

A problem with the top down approach is the imperfect surface. At nanoscales, the surface to volume ratio is very high, and any imperfections in the surface might drastically affect the performance of the nanoparticles. In the bottom up approach, nanomaterials are manufactured with the efficient and effective control of the arrangement of atoms, molecules, macromolecules, or supramolecules.[269] The nanomaterials produced by the bottom up approach will have reduced Gibb's free energy, representing thermodynamic equilibrium state. The nanostructures created using the bottom up approach typically show fewer defects on the surfaces and are more homogeneous with better short- and long-range ordering.

Industrial technologies are available for the production of drug nanoparticles. Elan's NanoCrystal technology and SkyePharma's Dissocubes technologies utilize the top down approach. DowPharma and BASF Pharma Solutions utilize the "bottom up" approach, which involves the controlled precipitation or crystallization of the solubilized API. A combination of both approaches is adapted in Baxter's NANOEDGE technology.[270] A newer top down approach known as particle replication in nonwetting templates (PRINT) has been developed for the preparation of drug loaded nanoparticles using polymers, which results in monodisperse particles with high precision on the shape of the nanoparticles.[271]

The preparation of dendrimers is a bottom up approach, where the dendrimers are prepared from the building blocks that ultimately form the core, branches, and shell of the dendrimers. Most of the preparation methods for nanoparticles from preformed polymers can be accommodated under the bottom up approach as the process involves solubilization and precipitation with high shear processes.

2.3.2. Specificity and Affinity. The unique advantage of nanotherapeutics is their ability to reach the target site either by passive or by active means, while minimizing the toxicity and improving efficacy. In antifungal treatment, liposomal formulation of amphoterecin B has shown improved efficacy and reduced toxicity as compared to that of plain amphotericin B toward fungal infections majorly through its slow release. Although initial invasion and replication of many fungi takes place in the lungs, there is also considerable dissemination of fungal burden in the brain, kidneys, liver, and spleen with time. It is necessary for an antifungal agent to be available at all of these sites to effectively reduce the fungal burden, because the diagnosis is often late and slow release formulations delivered at minimum effective doses will be beneficial in such conditions. In vitro studies have revealed that the liposomal formulations are attached to the yeast cell walls, from where the amphoterecin B was released and disrupted the cell walls.[272] Liposomal amphoterecin B has an added advantage over the plain amphoterecin B due to its ability to cross the blood brain barrier.[273]

PEGylated doxorubicin is another liposomal formulation with reported low toxicities in comparison to the other

doxorubicin formulations. Doxorubicin is a hydrophilic molecule associated with cardiotoxicity and neutropenia in clinical usage. In liposomal formulation of doxorubicin, the drug is placed in the inner aqueous core, and the outer surface is PEGylated. Surface coating with PEG prolonged the circulation times of the formulation, which was found to be essential for improved tumor accumulation of these formulations.[146] Liposomal formulations due to their tiny size are benefited by the EPR effect to selectively accumulate in the tumors. Because of this selective accumulation and control over the drug release while in the circulation, reduced toxicity of the liposomal formulation resulted. The accumulation of nano sized drug delivery systems due to the EPR effect is known as passive targeting. Polymer therapeutics (polymer−drug conjugates) of nanosize can also be passively targeted to the tumor tissues. Nanoformulations are also useful for brain targeting of many drugs, which are otherwise impermeable to the blood brain barrier.[273] Surface modifications have been suggested to improve the brain localization of drugs. Lymphatic targeting is another approach considered with the nanotherapeutics.[111] Unlike blood, lymph circulation is unidirectional, and it is possible to make the nanoformulations retained in the lymph for prolonged durations. Active targeting is realized when the surface of the nanotherapeutics is modified with a specific antibody or a ligand. Active targeting is used to improve the absorption or extravasation of nanotherapeutics or to extensively localize the formulation in the target tissue.

Drugs response in vivo hardly reaches the saturation response. The saturation response is the response that can be achieved with the usage of a drug. This is limited due to the toxicity at other tissues, which is often dose dependent. Thus, the drugs for clinical usage possess two plasma concentration limits based on their in vivo response. First is the minimum effective dose, which is the dose required to achieve a measurable clinical response. Second is the maximum tolerable dose, which is the dose after which the drug shows unwanted adverse or toxic effects. However, if the drug exposure to the nontarget tissue is avoided, there is a chance of achieving saturation response utilizing the full potential of the drug. It is also possible to reduce the minimum effective dose required for a drug by avoiding nontarget tissue distribution and increasing the specificity to the target tissue. The dose reduction becomes an important consideration for high dose drugs, especially for those drugs that require constant administration through intravenous administration. Therapeutic window that generally signifies the range of doses used can be expanded sufficiently to reduce adverse effects of the drug treatment (Figure 7).

For a nanosized drug formulation, the substance, size, shape, and surface properties majorly determine the specificity toward a target site (Figure 8). Substances can be pH responsive, temperature responsive, or can have magnetic properties or photothermal properties, all which can increase the specificity of a delivery system. Size is crucial for long circulation and passive targeting. Shape is also known to improve the properties of the nano delivery system especially by improving the uptake by cells to reach intracellular targets. Surface properties can render stealth properties toward MPS, can minimize adverse effects like complement activation of the nano delivery system, and surface functionalization with antibodies will help in the active targeting. The four properties, substance properties, size, shape, and surface properties, collectively can improve the specificity of a nanotherapeutic toward certain targets in vivo. Apart from the inherent

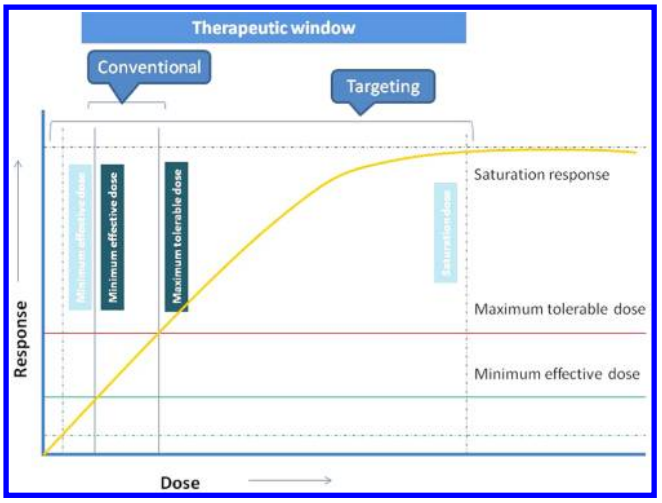

Figure 7. Improvement in the therapeutic window by targeting. The region between minimum and maximum effective doses can be increased by avoiding the nonspecific actions of drugs. Saturation response is the maximum achievable response by a drug candidate in ideal conditions; however, under in vivo circumstances due to the effect of the drug on other tissues, drug response is limited to a response achieved by the maximum tolerable response. However, with ideal targeted nanosystems, the drug is expected to act only on the receptor of interest resulting in increased therapeutic window.

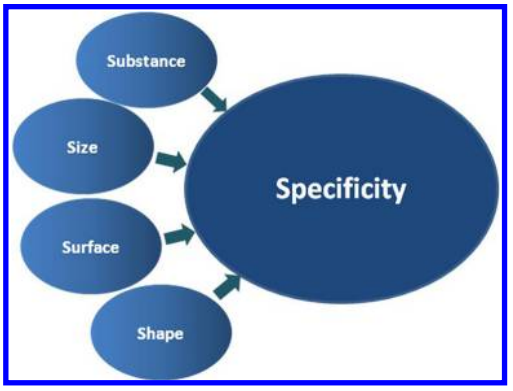

Figure 8. Targeting with nanotherapeutics. Size plays an important role in the absorption of the nanotherapeutics from transmucosal routes, circulation in the bloodstream, and clearance. Size is also known to play an important role in avoiding the phagocytosis by the mononuclear phagocytic system and improving the circulation times in blood for surface-modified nanotherapeutics. EPR effect in passive targeting by tumors is also determined by the size of the nanotherapeutics. Nanotherapeutics smaller than 5 nm are excreted by the renal clearance. Larger particles tend to accumulate in the mononuclear phagocytic organs. Higher surface charges are known to hinder the long circulation of the nanotherapeutics and also affect the penetration into the tissues of interest. Surface modifications by specific antibodies will help in the active targeting of the nanoparticles. Shape of the nanocarriers is also known to play a key role in circulation times. Optimized size, shape, and surface can lead to target specificity of the nanotherapeutics, which is the ultimate goal of an ideal drug delivery system.

properties of the nanoparticles, targeting can also be achieved with external influences like magnetic fields and electromagnetic radiation.

Use of magnetism for curing diseases is very historic. Nowadays, magnetism is being explored to target the nanotherapeutics to the precise location where it is intended.

The requirements of a magnetic drug delivery system include the magnetic core, surface molecules, and the therapeutic molecules. These magnetic nanoparticles because of their smaller size can easily circulate in the capillaries and can be easily localized at the target sites close to the body surface using magnetic field gradient. The drug or therapeutic molecule should be attached such that it possesses sufficient strength not to release the drug or therapeutic molecule immediately but should release in a controlled manner at the site of interest. The design can be engineered to present the drug on the surface or can be encapsulated inside the magnetic nanoparticles.

In the "good old days", pharmaceutical scientists believed developing a delivery system will be less investment with low risk and high return, when compared to new drug discovery. However, in recent days that does not fit very well anymore, with scientists taking more tedious approaches to deliver drugs just like the new drug discovery programs struggling to find a right target and then to synthesize the molecule to act on that target.

2.3.3. Delivery of Biologics. In the coming years, the biologics are going to take a major share in the drug market. Insulin, thyroid hormone, and factor VIII are among the first protein drugs approved for human usage during the 1920s. With the advent of molecular biology techniques during the 1960s, the development of biologics has seen a rapid growth. The production of biologics has shifted from extraction form tissues to synthesis using recombinant biotechnology. Because of the large size and labile nature of the biologics, suitable delivery methods are needed for efficient clinical usage. There are already some biologics in the market of which some have benefited from the nanotherapeutic approaches. The protein−polymer conjugates are the major class of nanotherapeutics delivering the biologics. Various kinds of proteins have been successfully delivered by these protein−polymer conjugates. Protein−polymer conjugates have been discussed elaborately in section 3.1.2.1 on polymer therapeutics.

Many of the recent biologics have been approved in the last 10 years. Delivery strategies coupled with the developments in the biologics are resulting in successful clinical applications of the biologics. 25% of the drugs in the drug development pipeline are biologics. Patents of the first generation biologics are nearing the expiry date, and the search for biosimilars by the pharmaceutical industry has picked up the pace.[274]

It is also important to maintain the functional structure of the proteins during the formulation development. For small peptides, the functions are dependent on the functional groups, but the functions of proteins are dependent additionally on the noncovalently bound 3D structure. This might be the reason for the poor efficacy of protein encapsulation in microspheres.[275,276] Multiple daily administration of insulin through invasive routes has created a requirement for the noninvasive insulin delivery. Oral delivery of insulin is a challenge for the delivery scientist even today.[277]

Short interfering RNAs have demonstrated therapeutic effects in viral infections, cancers, and hereditary disorders. siRNAs can have a broader range of applications than the small molecules, but due to the rapid clearance, high molecular weight, and high negative charge, these RNAs will require a drug delivery system that can specifically deliver the RNA to the target cell, avoiding toxicity to other cells.

2.3.4. Pharmacokinetics and IVIVC. Nanotherapeutics display diverse pharmacokinetic profiles with variations in size, shape, composition, and surface; however, one cannot ignore the role of bioactive encapsulated in the particle. Significant variations are observed with the nanoformulations from all routes of administration in various studies. This can be attributed to the sustained release, altered tissue distribution, and target specificity observed with the nanotherapeutics. Conventional delivery systems release the drug in the body at a specific site for a determined time, from where it has to be absorbed except for the intravenous route. Nanotherapeutics can release the drug until its lifetime expires in the blood circulation and sometimes can specifically release the drug only at the target site.

In pharmacokinetics, the four major components are absorption, distribution, metabolism, and excretion (ADME). Important parameters in the pharmacokinetics include clearance, volume of distribution, and plasma half-life. Compartmental models are developed to determine various pharmacokinetic parameters of the drug molecules.[278−280] Physiologically based pharmacokinetic model (PBPK) is often suitable for most of the molecules. The suitability of these models for the nanoparticulates is very limited. The reasons include prolonged absorption phases, extended releases in the compartments, selective accumulation in different tissues, and varying excretion mechanisms. For a small drug molecule, biopharmaceutical properties are often sufficient to determine the pharmacokinetics, but for nanoparticles additionally delivery system characteristics need to be included, and often the pharmacokinetic parameters result in unusual estimates. A study reports the usage of blood flow limited PBPK model in the prediction of plasma time profile of quantum dots in mice. A novel parameter known as tissue distribution coefficient was used in place of the tissue blood partition coefficient in that study. The authors suggest that the partition coefficient might not exist for the nanoparticles.[281] In another study, using oral nanoparticles of estradiol, it was proposed that the mean absorption time of nanoparticles (calculated by subtracting intravenous mean residence time from oral) incorporates the drug release time.[282] These two examples present that some of the parameters used to represent drug pharmacokinetics might not be suitable for nanoparticles. Nano drug delivery systems often result in higher plasma maximum concentration (C_{max}) of the bioactives when delivered orally, which suggests improved absorption. However, nanoparticles in general result in increased AUC for the majority if not all bioactives, suggesting longer residence and slow release. As a general rule, lower concentrations in the serum represent lower toxicity of the drug, but nano delivery systems have been shown to be safe even at higher concentrations, which cannot be explained by the serum pharmacokinetics.[283] A general phenomenon to be observed with the majority of the nanoformulations in particular with polymers like PLGA given peroral is their delayed T_{max} and slow release that could be responsible for lower toxicity.[284] The understanding and modeling of the pharmacokinetics of nanoparticles is challenging and opens new opportunities in pharmacokinetics.

There is a great need to establish the in vitro assays, which can rapidly screen the quality, stability, and performance of the nanotherapeutics. In vitro in vivo correlations refer to the establishment of a relationship between in vitro property and in vivo response. The IVIVC of dissolution rate and the absorption rate have been widely used in the quality control and formulation development of oral formulations. IVIVC can also serve as surrogate for bioequivalence studies of formulations, saving time and money in the drug development

process. Successful IVIVC are linear mostly. However, nonlinear IVIVC are also reported to be useful, and they are called as in vitro in vivo relationships (IVIVR).[285]

2.4. Recommendations and Guidelines

It would be rather a very difficult call to make, if innovation in medicines/drug delivery is driven by the patients need or due to the market competition. The application of nanotechnology in medicines, particularly drug delivery, is thought to revolutionize the way medicines are administered and thereby healthcare. This was partially true to the extent that we force administering medicines by many unconventional routes/means for those drugs that is not possible, for example, insulin delivery peroral, nasal, skin, etc.; however, this has not made any significant difference to the patient. The industry started exploiting nano by milling the water-insoluble compounds/drugs with a view to improve their solubility by reducing the size, thereby surface area to volume ratio; however, this is restricted to selected compounds, and on the other hand improving solubility and bioavailability also would increase the toxicity of many compounds. Subsequently, the use of polymers/lipids to encapsulate the water-insoluble drugs with a view to improve the bioavailability and reduce the toxicity gained interest, resulted in the first generation nanoparticles. These nanoparticles being foreign bodies are cleared faster, and to keep them in circulation for longer durations, PEGylation was introduced that led to the invention of second generation nanoparticles. At this stage, the degree of complexity increased with the discovery of third and fourth generation nanoparticles that are site-specific nanosystems and theranostics (particles that can help detect the disease early and deliver the drugs to cure), respectively, that is still practiced at the academic level.

Significant progress is made in the materials front, but that is not well integrated with drugs that could evolve as drug delivery products, and the major reason for that is a drug is chosen for a fancy delivery device but not a delivery device constructed around a drug's requirement; an example to this extent is research on carbon nanotubes where efforts are concentrated on making the nondegradable and incompatible materials more biocompatible. On one hand, we say nanotechnology cannot fix everything, and at the same time, we generalize everything that is nano as toxic, which gave birth to "nanotoxicology". One would wonder that are we falling short of biocompatible materials that we aim at using an incompatible material making it compatible, which classifies under innovation? Further complexity is introduced into the system with the targeting approaches as well as diagnostics and delivery systems, without actually knowing the room for improvement that we can achieve with targeted systems. One should question at this stage when a drug that is discovered based on a specific receptor that in principle should not work on off targets, which is not the case. For example, aspirin once thought to be an analgesic and antipyretic is now used for almost every disease.

The crucial steps involved in making drug encapsulated nanoparticles using preformed polymers are emulsification, reduction of the droplet size, the evaporation of organic solvents, removal of free surfactants, drug, and freeze-drying to obtain the finished product that is stable under storage conditions. Centrifugation is often used as a means to purification, which would pose problems with respect to recovery, aggregation, and scale-up. Surface functionalizing these particles would require additional preparation and purification steps, which would further complicate the process, and freeze-drying for improved storage stability can also be an issue. The choice of organic solvent plays a crucial role in the overall product profile; however, there was no clear rationale described in the literature justifying the selection. The choice should be based on the physicochemical properties of the solvents such as interfacial tension, viscosity, vapor pressure, and water miscibility, and this in turn will help chose the surfactant.[172] The solvent and surfactant will have a significant influence on the drug loading and entrapment efficiency.[172,173,286] Extensive preformulation studies are required to identify appropriate combination of solvent, surfactant once a drug is identified to be formulated, and a further set of improvements with dose titration based on the pharmacokinetic data will be needed depending on the pathology that one intends to treat.[284,287,288] Freeze-drying (lyophilization) process needs a thorough understanding as it involves the choice of cryoprotectants based on the surface characteristic of the nanoparticles. Lyophilization involves three steps: freezing, primary drying, and secondary drying. The temperature and the rate at which the temperature is altered in each phase play an important role in the lyophilization.[247]

Formulating a drug substance is much more than just mixing a few ingredients/excipients. Although the traditional excipients are pharmacologically inert, every excipient has a role to play, and the selection is based on strong scientific rationale, which in turn depends on the drug's physicochemical properties, route and dose to be administered, and disease to be treated. However, the current generation delivery systems such as nanoparticles are designed with much less understanding of the drugs and the diseases to be treated with more emphasis laid on the chemistry of the carrier system. It is very important and crucial to understand the room for improvement for a drug that can be realized by a delivery system; for example, Abraxane a novel formulation of paclitaxel, was reported to have less or no benefits as compared to the generic Taxol.

3. ASSESSMENT

3.1. Promise versus Performance

This section reviews the number and volume of grants/projects, investment, publications, patents, products under development, and market based on nanoscale drugs and delivery systems with special focus on cancer research, which is one of the most heavily funded areas.[289,290] The factors affecting and reasons for (with examples of) success and failure stories[76] (spotlight products Doxil, Abraxane, Nanocrystal tech) shall be discussed. While every new technology and product promises revolution, we review what is the room for improvement in drug delivery and if the patients' concerns are being addressed. The advantages thought to be offered by nanotherapeutics are listed in Figure 9.

The "nanotech hype" becomes obvious in a study published by the U.S.-based venture capital firm luxcapital in which it was found that mentions of the term "nanotechnology" in press had increased by 2000% between 1995 and 2002 (luxcapital, 2003). The world market for nanotechnology in 2001 was estimated to be worth EUR 54 billion by DG Bank.[8] The global nanomedicines market alone is expected to reach $160 billion by 2015.[291,292] In 2004, the total sales of 38 identified nanomedicine products from all sectors of nanomedicine was calculated to be $6.8 billion.[82]

3.1.1. Hemolysis, Thrombogenecity, and Complement Activation. Hemolysis is the destruction of red blood

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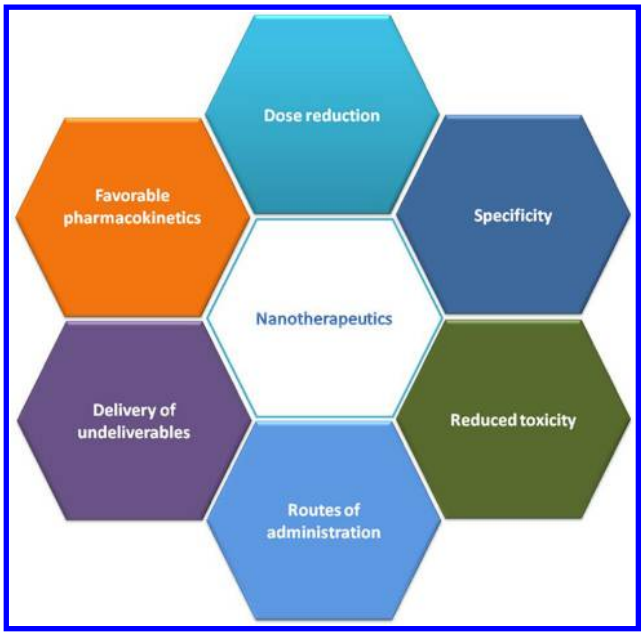

Figure 9. Unique advantages and promises of nanotherapeutics. Nanotherapeutics have been claimed to reduce the dose of the drugs by increasing the specificity of the drug molecules or targeting the drugs by using carriers. Nanomedicine is also very useful for the drugs, which are high dosed due to the poor bioavailability. Dose reduction might help in patient compliance due to the reduced frequency, especially for drugs that are administered by a health care professional. Some of the anticancer drugs are benefited from the favorable kinetics offered by the nano carriers, which have received a wide acceptance due to the reduced toxicity and increased efficacy. Poorly soluble compounds can be delivered efficiently by the "nano" approach. Reformulation utilizing another route of administration is also possible with nanotherapeutics. Because of the favorable kinetics, toxicity of the drugs can be reduced; however, formulation-related toxicities might appear.

cells that can lead to anemia and other pathological conditions. Many of the nano formulations incorporate surfactants, which might cause hemolysis. It is important to assess the safety of such formulations for hematocompatibility. Microemulsions utilizing pluronic surfactants showed no blood cell disruption.[293] Yet silica nanoparticles (mesoporous and nonporous) have been shown to induce the hemolysis in vitro. The hemolytic activity of mesoporous silica nanoparticles is lower than the nonporous silica nanoparticles. Size, porous order, and stability influence the hemolytic activity of the mesoporous silica nanoparticles. Surface modification with PEG can result in nonhemolytic activity but might be for short-term; long-term stability of the porous structure might be useful for safer mesoporous silica nanoparticles.[294]

Platelet aggregation might lead to vascular thrombosis and can result in stroke. Carbon nanotubes were shown to induce the thrombosis but not the fullerenes.[295] Silver nanoparticles have been thrombogenic in vitro and in vivo.[296] Contrary to this, antiplatelet actions of silver nanoparticles are also reported. Silver nanoparticles tend to accumulate in the platelets and reduce interplatelet proximity and are suggested for antithrombotic therapy.[297] Citric acid coated iron oxide nanoparticles have also shown antiplatelet activity, starch coated iron oxide particles were neutral to platelet aggregation, whereas gold nanoparticles represented pro-aggregatory response of platelets.[298,299]

Complement system protects the body from invasion by pathogens. It sometimes also produces inflammatory responses similar to pathogens in response to nanoparticle administration. The complement system in humans is composed of 35−40 proteins present in plasma, cell surfaces, and other body fluids. The concentration of these proteins is higher in plasma in comparison to other body fluids. Complement proteins when bound to foreign particles trigger proteolytic enzymes, which activate the C3 protein. The activated C3 undergoes chemical changes on the particle surface to form covalent bonds. This process is called the opsonization, and once this is done the particles adhere to the C3 binding sites on the phagocytic cells. The proteolytic enzymes also activate C5 protein, which is responsible for the membrane disruption of bacterial, viral, or liposomal boundaries. During the complement activation process, some inflammatory peptides are also released.

Complement system is the defense cascade of the innate immunity. Three pathways of activation are known to be involved in this cascade-classical, alternate, and lectin pathway.[300,301] Classical pathway involves the binding of circulating antibodies to specific pathogens or to other foreign or nonself antigens. It is initiated by the binding of immunoglobulin G (IgG) or immunoglobulin M (IgM) to the foreign antigens. The C1 complex, a multimeric complex consisting C1q, C1r, C1s molecules, binds to the Fc portion of the IgG or IgM immune complex. Binding of C1q activates the C1r and results in the cleavage of C1s. The activated C1s cleaves C4 and C2 to form C3 convertase C4b2a. This convertase splits C3 into C3a and C3b. C3b opsonizes pathogens and leads to phagocytosis. It also attaches to C3 convertase to form C5 convertase, which forms the last phase of the classical complement activation pathway and the initiation of the common pathway of all three activation pathways to form the membrane attack complex (MAC). Alternative pathway does not involve any antibodies and is due to the spontaneous hydrolysis of the C3 to form $C3(H_2O)$, which functionally resembles C3b. Factor B reversibly attaches to $C3(H_2O)$, while factor D cleaves the factor B. This event associated with small fragment Ba produces the C3 convertase of alternative pathway, $C3(H_2O)Bb$. This complex is stabilized by the plasma properdin, and the C3 convertase splits C3 to C3a and C3b of which C3b starts a new cycle to create C3 convertase again. Binding of C3b to C3 convertase creates the C5 convertase to start the formation of MAC. Lectin pathway is activated when mannose binding lectin (MBL) or ficolin bind to the carbohydrate moieties on the pathogens. Both MBL and ficolin are bound to MBL-associated serine proteases (MASP) and circulate in the blood. Binding to pathogens induces autoactivation of MASP2, which cleaves C4 and C2, in a similar way as it happens in the classical pathway, to give C3 convertase. Subsequently C3 is cleaved and C5 convertase is formed, which initiates the formation of MAC. In the process of MAC formation, only the C5 cleavage is enzymatic to give C5a and C5b. Accretion of C6 to the membrane bound C5b gives a hydrophilic complex, followed by conformational changes with the accretion of C7. Attachment of C8 to the complex by C8b induces the penetration of C8a-g into the lipid membrane of the organism. The final step is the formation of stable transmembrane pore with the binding of 10−15 C9 proteins. This pore causes the osmotic imbalance in the target cell and leads to the lysis. The small activation products C3a, C4a, and C5a are potent anaphylatoxins. Anaphylatoxins can induce multitude responses of the inflammation. They act as smooth muscle cell contractors,

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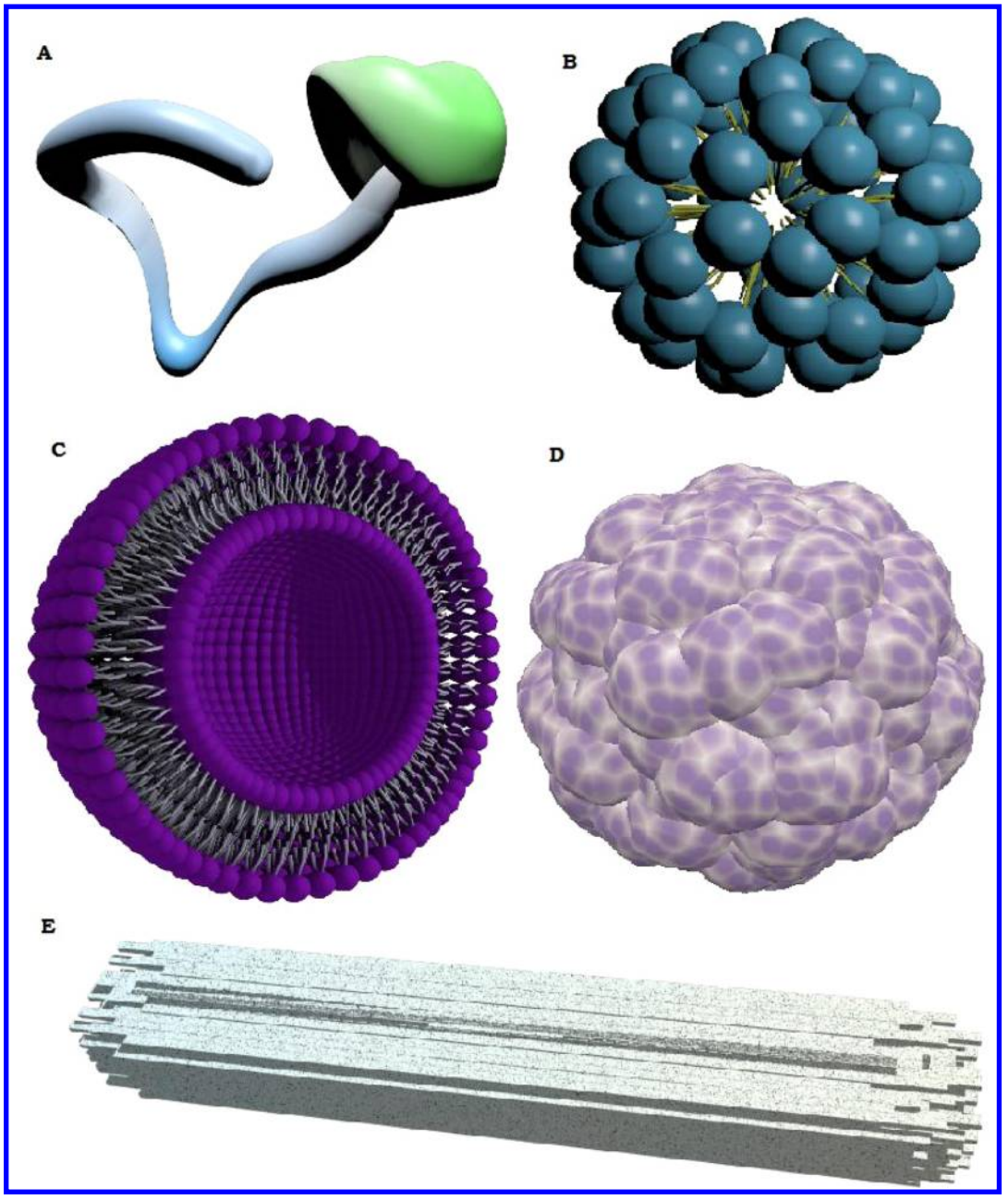

Figure 10. Artistic impression of the marketed formulations in nanoscale. (A) Polymer conjugates. (B) Micellar systems. (C) Liposomes. (D) Protein bound drugs. (E) Nanocrystal system. Polymer conjugates are therapeutic molecules linked covalently to a polymer. Micellar systems are made up of surfactants that can entrap the poorly soluble drug molecule. Liposomes are bilayered lipid membranes that can be used to encapsulate hydrophobic as well as hydrophilic molecules. Protein bound drugs are benefited by the natural properties of the protein like enhanced extravasation. The nanosized drug particle needs to be stabilized using sterical and/or electrostatical stabilizers. The nanosizing increases the surface area of the drug particles, which are generally manufactured using "top down", "bottom up", or a combination of both of these approaches. Increased surface area results in increased rate of dissolution and saturation solubility of the drug and thus increases bioavailability and sometimes effect of food on the absorption. BCS class II drugs are the choice for nanosizing for oral delivery.

cause histamine release from mast cells, and cause release of oxygen radicals from neutrophils. Other vasoactive substances like prostaglandins, kinins, and serotonin are also released as a consequence of complement activation. Thus, complement activation can induce all classical signs of inflammation including pain, hyperthermia, reddening, swelling, and impaired function.

Apart from the target cell lysis and inflammation, complement system also induces opsonization, which is another important defense mechanism. C3b, C4b, and C5b opsonize the surface of the foreign bodies and facilitate the phagocytosis. Additionally, after attachment of C3b and C4b to the soluble antigens, they are bound to complement receptor 1 on erythrocytes and are transported to the spleen and liver. C3 cleavage products also bridge the innate and adaptive immune systems by binding the opsonized antigens to the complement receptor 2 on the B-cells via the C3d fragment, which initiates the production of specific antibodies and differentiation of B cells.

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3.1.2. Review of Marketed Products and Technologies. Some kinds of nanotherapeutics are already in the market that includes polymer therapeutics, liposomes, micelles, proteinous systems, nanosized therapeutics, and viral vaccines (Figure 10). These nanotherapeutics were discussed in the following sections, and some of the marketed formulations are listed in Table 7. The types of nanotherapeutics that are in the market and pipeline are listed in Table 8.

Table 7. Some of the Nano Drug Delivery Products in the Market[a]

product	composition/nanotech component	indication
Abelcet	amphotericin B/lipid complex	fungal infections
Adagen	PEG-adenosine deaminase	immunodeficiency
Abraxane	paclitaxel protein bound nanoparticles	cancer
Amphotec	amphotericin B/lipid colloidal dispersion	fungal infections
Ambisome	liposomal amphotericin B	fungal infections
Cimzia	PEGylated antibody (Anti TNF Fab′)	rheumatoid arthritis
Cervarix	vaccine	cancer
Copaxone	copolymer of alanine, lysine, glutamic acid, and tyrosine	multiple sclerosis
DaunoXome	liposomal daunorubicin	Kaposi sarcoma
Doxil/Caelyx	liposomal doxorubicin	cancer, Kaposi sarcoma
Depocyt	liposomal cytarabine	cancer
Epaxal Berna	virosomal hepatitis vaccine	hepatitis A
Emend	nanosized aprepitant	antiemetic
Estrasorb	estradiol in micellar nanoparticles	menopausal therapy
Invega Sustenna	nanosized drug	atypical antipsychotic agent
Myocet	liposomal doxorubicin	breast cancer
Neulasta	PEG-G-CSF	febrile neutropenia
Oncaspar	PEG-asparaginase	leukemia
Pegasys	PEG-α-interferon 2a	hepatitis C
PEG-Intron	PEG-α-interferon 2b	hepatitis C
Macugen	PEGylated anti-VEGF aptamer	age-related macular degeneration
Mircera	PEGylated epoetin beta	anemia
Somavert	PEG-HGH	acromegaly
Renagel	cross-linked poly(allylamine) resin	chronic kidney disease
Emend	nanocrystalline aprepitant	antiemetic
MegaceES	nanocrystalline megesterol acetate	eating disorders
Rapamune	nanocrystalline sirolimus	immunosuppressant
Tricor Lyphantyl	nanocrystalline fenofibrate	lipid regulation
Triglide	nanocrystalline fenofibrate	lipid regulation
Visudyne	liposomal verteporfin	age-related macular degeneration

[a]Modified from refs 76 and 82.

3.1.2.1. Polymer Therapeutics. Since the discovery that small molecules can penetrate the silicone tubings at a controlled rate, polymeric materials are extensively used in the research laboratories and in industries for better delivery of drugs.[252,302] The ability to engineer the polymers to get the required properties for a specific use places them in a unique place for the development of drug delivery systems. Polymers are used as matrix material and coatings to control the rate of release of the encapsulated drugs. They are also used to achieve a desired effect at a specific time by making them bioreactive.

Table 8. Types of Nano Drug Delivery Systems

nano drug delivery system	description
micelles	spherical monolayered arrangement of polar molecules
vesicles	bilayered closed structures, often spherical, but can be of other shapes, uni- or multilamellar
dendrimers	branched spherical molecules
nanocrystals	crystals of drug with or without functional excipients
nanoparticles	hollow or solid particles made from inorganic material, natural/synthetic/semisynthetic polymers, lipids, metals
nanoemulsions	normally oil in water type
SNEDDS	self-nano emulsifying drug delivery system
liposomes	lipidic vesicles
niosomes	vesicles made of nonionic lipids
sphingosomes	vesicles made of sphingosine-based lipids
polymersomes	polymeric vesicles
dendrimersomes	dendrimer vesicles
polymer−drug conjugates	drug covalently bonded to a polymer or carrier peptide or nutrient/antigen/antibody substrate
protein-based nanoparticles	drugs bound to protein mass in nanosize
solid lipid nanoparticles	lipidic nanoparticles
virosomes	vaccine made up of viral capsule and lipids

Polymer drug conjugates are the hybrid molecules with a linker between the polymer and the therapeutic molecule, which are generally in the nanoscale. Polymer conjugates are being explored for delivering drug molecules for more than three decades. Polymer conjugates can increase the solubility of the hydrophobic drug molecules and allow the use of difficult to deliver molecules for the treatment. Especially, biomolecules like proteins and peptides that possess pharmacological activity but needed a delivery system for effective therapy are benefited mostly by the polymer conjugation. Later with the understanding of the EPR effect in tumors, conjugates found increased attention for cancer therapy because of their size. Polymer−drug conjugation promotes the passive tumor targeting by the EPR effect, and the endocytic uptake results in lysosomotropic delivery. These more accurately should be called new chemical entities rather than the drug delivery systems where the drug is associated with the carrier other than chemical bonds. These can be called as polymer therapeutics,[183] resembling a new class of chemical compounds that can be used for therapy. First generation products are already in the market for clinical usage. The first polymer therapeutic was approved in 1990.[303] Approval of polymer therapeutics for clinical usage has increased the interest in these nanomedicines among the researchers. Initially, polymer therapeutics were developed for the intravenous administration; however, subsequent developments resulted in oral as well as topical formulations.

Various types of polymer drug conjugates have been developed, but PEGylation only has given positive results. PEGylation is the covalent attachment of PEG to therapeutics agents. PEGylation is the most important approach in the development of protein conjugates with clinical success of the marketed products.[304,305] PEGylation offered nonimmunogenicity of the proteins of nonhuman origin and increased the circulation half-lives of the proteins. PEGylation slows the renal clearance by increasing the size of the biomolecule and renders nonimmunogenic by preventing the exposure of epitopes to antibodies and complement proteins. Even the proteins genetically engineered from human recombinant DNA sometimes show immunogenicity and are benefited from PEGyla-

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tion. Many new polymers have been developed for the purpose of increasing the half-life of therapeutics, but still there is no match to PEG.[306] The experience with PEG from the clinical usage, good biocompatibility, to the low cost propels the use of PEG.

PEG is a polyether backbone polymer synthesized by ring-opening polymerization of the ethylene oxide. PEGs with one end hydroxyl group or two end hydroxyl groups are generally obtained during the manufacturing process, which are alkoxy PEG hydroxyl and diol PEG, respectively. The molecular weights produced during the process show a Gaussian distribution pattern. Large molecular weight distributions are not suitable for conjugation. The hydroxyl groups produced can be modified to be reactive for conjugation with the drug or protein. Drug loading of the PEG does not increase with the increase in the molecular weight as the functional moieties are restricted to two and in some cases only one for a polymer molecule irrespective of the molecular weight. To overcome this drawback of PEG, branched and dendritic PEGs with multifunctionality have been developed.[306]

The polymer selection is based on its hydrophilicity, nonimmunogenicity, nontoxicity, biodegradability to safe compounds, and clearance. However, most of the marketed polymer therapeutics are based on the nonbiodegradable polymer PEG. Low molecular weight PEGs are known to be eliminated via the renal clearance and thus pose no risk to the body. Polymer−drug linkers can be covalently bound permanent linkers or reversible linkers. Reversible linkers are generally pH sensitive or peptidyl in nature.[307−309]

Proteins and peptide molecules are a major class of drugs being investigated for clinical use. The roadblocks in the development of proteins and peptides as therapeutics are the manufacturing and delivery of these molecules. The first polymer protein conjugate approved in 1990 is a conjugate of numerous strands of monomethoxy PEG of molecular weight 5000 covalently attached to enzyme bovine adenosine deaminase (Adagen). It is used as the replacement therapy of severe combined immunodeficiency disease associated with adenosine deaminase deficiency. PEGylated bovine adenosine deaminase has increased the half-life of the bioactive by 6.4 times in rodents.[310] Clinically this PEGylated enzyme is used intramuscularly. Oncaspar is another enzyme developed in a way similar to the Adagen using amide bond conjugation of monomethoxyPEG with the enzyme asparaginase for the treatment of acute lymphoblastic leukemia. Previously this disease was treated with asparginase from the bacteria *E. coli*, which caused severe hypersensitivity reactions in some cases. Attachment of PEG chains to the enzyme reduced the immunogenicity of the enzyme drastically.[305] The PEG modified α-interferon (PEGintron) is the single strand PEG attachment to α-interferon for the treatment of hepatitis-C. PEGintron is a mixture of positional isomers where PEG is attached to any one of the many available amine groups on the α-interferon.[311] The plasma half-life of PEGintron is 8 times higher than that of the native α-interferon. Pegasys is another conjugation molecule of α-interferon with a single branched PEG molecule with molecular weight of 40 kDa.[312] Neulasta is a PEG conjugate of granulocyte stimulating factor, which decreased the renal clearance of the native molecule.[313] All of these products contain the proteins covalently linked to the PEG chains permanently. Certolizumab Pegol is the first fab1 antibody fragment modified with 40 kDa PEG.[314] Pegaptanib is the first PEG conjugate of oligonucleotide in the market.[315] Mircera is a PEGylated epoetin, which displayed greater activity and increased half-life in contrast to erythropoietin.[316] Initially developed products contain PEG chains with smaller molecular weights, but recent approved products have PEG chains with higher molecular weights. All of the products marketed utilizing the advantage of PEGylation are large molecules. The pharmacology and chemistry of the PEGylation of marketed protein conjugates have been recently reviewed.[317,318] There is a significant body of literature on small molecule PEGylation, although this does not reflect the commercial products.[319,320]

Other polymer therapeutics marketed include Copaxone and Renagel.[321] Copaxone contains a polymer drug glatiramer acetate, which is identified by specific antibodies.[322] Renagel contains poly(allylamine hydrochloride) cross-linked with epichlorohydrin that is used in chronic kidney disease.[323]

Another important polymer conjugate is the SMANCS.[147] SMANCS is the acronym for poly(styrene-*co*-maleic acid) conjugated to neocarzinostatin. The major limitation of neocarzinostatin was due to the severe toxicity including bone marrow suppression. Additionally, the short half-life of about 2 min in mice indicated high doses of neocarzinostatin for clinical use. Conjugation with poly(styrene-*co*-maleic acid) resulted in improved plasma half-life, decreased immunogenicity, and importantly passive targeting to the tumor tissues. During the development of this conjugate, the landmark in passive targeting was unwrapped with the discovery of the EPR effect.[151,324]

3.1.2.2. Liposomes. The first description of liposomes was reported in 1965 when Bangham et al.[325] observed rapid association of phospholipids into bilayers in water. Liposomes are vesicles composed of outer lipid bilayer and an inner aqueous core with spherical shape. Lipids used in the liposomal preparation are generally nontoxic and nonimmunogenic; those usually represent natural and synthetic lipids and usually contain cholesterol to strengthen the structure. The liposomal formulation contains phospholipids that are amphiphilic and are composed of a three-carbon glycerol backbone, a polar headgroup attached to one carbon, and hydrophobic fatty acid chains attached to the other two carbon atoms. The hydrophobic chain can be saturated or unsaturated, and the lengths can vary. When added to water, these chains spontaneously arrange in bilayers with the polar head groups in contact with water molecules and the hydrophobic chains in between. Phospholipids undergo a transition change from gel form to the liquid crystalline form depending on their chain length and the unsaturation. The liquid crystalline form is leaky and not favorable for drug entanglement in the bilayer. Thus, the phospholipids that are stable at the body temperature are generally preferred for the development of liposomes. Polymer-conjugated lipids are also used in the preparation of the liposomes to impart nonimmunogenicity and stealth properties against the MPS and complement system in the blood circulation. Apart from this, other physicochemical properties like size, membrane fluidity, and charge density determine the interactions of the liposomes with the blood components.

Drugs with a wide range of lipophilicities can be encapsulated in liposomes. The drugs that need to be encapsulated can be located at the bilayer if hydrophobic and in the inner aqueous core if the drugs are hydrophilic. Depending on the number and size, liposomes can be multilamellar liposomes, large unilamellar liposomes, and small unilamellar liposomes. Other types include pH-sensitive liposomes, cationic liposomes, immunoliposomes, and long circulating liposomes. Various

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methods can be employed to obtain liposomes with different sizes and different characteristics. The simplest method is the thin film hydration method where a thin film of lipids is hydrated in an aqueous buffer at a temperature above the transition temperature of the lipids.[325] This method produces large multilamellar liposomes, which can be sonicated or filtered to obtain smaller liposomes. For the preparation of large unilamellar liposomes, solvent injection,[326] detergent dialysis,[327] calcium-induced fusion,[328] or reverse phase evaporation[329] is applied.

The physicochemical properties of the liposomes such as membrane fluidity, charge density, steric hindrance, and permeability determine the interaction of liposomes with the blood and biological components.[330] Liposomal encapsulation of drugs can increase the therapeutic index of the drugs, which otherwise are limited by the toxicity or rapid clearance. These carriers are being extensively studied for the clinical development of drugs that are considered undeliverable by other approaches due to the limited solubility or rapid metabolism and clearance.[331] The method of preparation is known to affect the drug loading efficiency and release of the drugs from the prepared liposomes.[332,333] Thus, it is necessary to screen the methods of liposomal preparation for a specific molecule to obtain optimum drug loading and required rates of release apart from the composition of the liposomes.

AmBisome is a liposomal formulation containing a single bilayer of lipids. The active ingredient amphotericin B is intercalated in the membrane. The liposomes (Ambisome) are less than 100 nm in diameter and intended for parenteral use to treat fungal infections. AmBisome showed increased safety in preclinical studies conducted in mice, rats, rabbits, and dogs.[334] The minimum inhibitory concentration for fungicidal action in vitro revealed that AmBisome has a potency similar to that of the amphotericin B.

Liposomes are the first kind of drug delivery systems, which are marketed to utilize the surface modification by PEGylation to increase the circulation times. This concept is known as "stealth" as the PEGylated liposomes delay the phagocytosis by MPS. Doxil is the PEGylated liposomal formulation, which is extensively used in cancers like Kaposi sarcoma, ovarian cancer, breast cancer, and multiple myeloma. PEG, which is known to increase the circulation time and reduce the immunogenicity of the protein therapeutics, was introduced to the liposomes during 1990s.[335] Conjugating the lipid anchor (distearoylphosphatidylethanolamine) to the PEG (MW 2 kDa) was shown to prolong the circulation half-life of the liposomes. PEGylation due to steric stabilization provides resistance to the uptake by the mononuclear phagocytic system resulting in increased circulation times. Because of this property, PEGylated liposomes are often called as stealth liposomes. Doxorubicin is hydrophilic, and thus it is located in the inner aqueous core of the liposomes, and the Doxil formulation contains these liposomes in the size range of 80−100 nm. Very high drug loading efficiencies are achieved in Doxil due to the manufacturing process, which employs the preparation of liposomes in ammonium sulfate buffer and raising the temperature of the liposomes over the phase transition temperature resulting in elongated crystals of doxorubicin in the liposomes, giving them a coffee bean shape in cryo-TEM.[146] Doxil alters the pharmacokinetics of the doxorubicin and results in reduced toxicities than those observed with free doxorubicin, but other mucocutaneous toxicities occur more with the Doxil. There are fewer liposomal marketed products than anticipated due to low drug loading, difficulty in sterilizing, stability, and poor batch to batch reproducibility.[336]

The success of liposomal drug delivery systems leads to the discovery of virosomes and polymersomes, which can be considered as the analogues of liposomes. Niosomes are a type of liposomes that utilize nonionic lipids during the formulation development. Surface functionalization of liposomes is a very active research area. Functionalization of the liposomes can be carried out during the preparation process or after the preparation of liposomes.[337]

3.1.2.3. Micellar Systems. Micelles are colloidal dispersions ranging from 5 to 100 nm.[338] Micelles are spontaneously formed from amphiphilic molecules or surface active agents at certain concentration and temperature. These molecules possess two distinct regions with opposite affinities to the solvent. At low concentrations, the amphiphilic or surfactant molecules stay separated; however, as the concentration increases to a critical level, aggregation takes place. The concentration that results in aggregates (the micelles) is known as the critical micellar concentration (CMC). The micelle formation is favored because the free energy of the system is decreased as the hydrophobic parts associate and move away from the aqueous phase. The hydrophobic part in the micelle forms the core, which is strengthened with the van der Waals forces, and the hydrophilic headgroup forms the shell. This arrangement decreases the free energy in the system, and the hydrophilic parts form hydrogen bonding with the water in the external medium. The pharmaceutical micelle delivery systems trap the nonpolar molecules in the core, polar molecules at the shell, and the molecules with intermediate polarity are placed between the core and shell. An ideal micellar system should spontaneously self-assemble to entrap the drug molecules and lie in the size range of 10−20 nm. The CMC value of the amphiphilic molecules should be in low millimolar concentrations. The first micellar formulation for drug delivery was approved in 1983 by the FDA.

3.1.2.4. Proteinous Systems. Advantages of proteins include the advantages of synthetic polymers and the advantages of absorbability and low toxicity of the degradation products.[222] Drugs can also be incorporated in the protein structures of around 100 nm. Abraxane that contains paclitaxel bound to human serum albumin was developed by Abraxis BioScience, which is now fully owned by Celgene Corp. The ProtoSphere technology is called nanoparticle albumin-bound (Nab) technology when specifically human serum albumin is used.

Paclitaxel is an insoluble compound that was delivered as an emulsified formulation in solvents polyoxyethylated castor oil (Cremophor EL) and ethanol for intravenous administration. This solvent-based paclitaxel has to be diluted 5−20 times with normal saline or 5% dextrose solution before being administered intravenously. The solvent used in the formulation is toxic and showed hypersensitivity reactions,[339] abnormal lipoprotein patterns, aggregation of erythrocytes,[340] and peripheral neuropathy.[341] Cremophor EL promotes the release of di(2-ethylhexyl) phthalate (DEHP) from the intravenous tubings containing polyvinyl chloride. DEHP releases histamine and causes hypersensitivity reactions including anaphylaxis and hepatotoxicity. To prevent the adverse effects of the formulation, a special infusion set has to be used for prolonged infusion, and premedication with corticosteroids, antihistamine, and H2-blocker is necessary while administering the solvent-based paclitaxel formulation.

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Even after these precautions, side effects persist in some patients.[342]

Albumin is a natural carrier of vitamins, hormones, and other hydrophobic substances in humans, which binds reversibly to these molecules and transports to different parts of the body.[343,344] Human serum albumin is very stable over pH 4−9 and temperature up to 60 °C as well as nonimmunogenic during clinical usage. Nab-paclitaxel is prepared by high pressure homogenization of paclitaxel in the presence of human serum albumin, which results in nanoparticles of 130 nm in size. The final nab-paclitaxel consists of a lyophilized powder, which is reconstituted in 0.9% sodium chloride solution before intravenous administration. 100 mg of paclitaxel contains 900 mg of albumin with it in the final formulation.[345] Nab-paclitaxel is taken up by the 60 kDa cell surface glycoprotein receptor (gp 60) on the endothelial cell membrane and internalized into caveola and subsequently delivers the drug to the tumor cell.[346] Selective accumulation of albumin in the tumor is demonstrated with the use of Evans blue that binds to the albumin.[154] Albumin bound dye is seen accumulating in the tumor due to the EPR effect, which suggests that the nab-paclitaxel can passively target the tumors. An added advantage over other nanotherapeutics for Nab-paclitaxel is the transcytosis across the vasculature through the gp 60 receptors.[170] Thus, permeation is increased by passive diffusion through the interstitial spaces and transcellular transport through the endothelial cells. Nab-paclitaxel was approved in 42 countries for the treatment of breast cancer. Although there were reports highlighting the superiority of Nab-paclitaxel over conventional taxol with respect to ease in administration, free of Cremophor EL, safety, and improved efficacy,[347] this form of paclitaxel drew a lot of criticism that it was able to shrink tumors but not extend patients' life expectancy with price 28 times higher than the generic.

3.1.2.5. Nanosizing. Nanosizing of the APIs is becoming a more and more popular approach to enhance the dissolution rate and solubility of the drugs. Nanosizing offers more benefits of increased surface area in comparison to micronization. This approach is used for the BCS class II (poorly soluble, permeable) and BCS class IV (poorly soluble, poorly permeable) drugs intended for oral delivery.[45] The greatest benefit of the nanosizing is observed for drugs that are less soluble, <200 μg/mL.[348] Drugs with high crystal energy (high melting point) and with high molecular weight generally benefit from nanosizing. The preferred chemical nature of the drug is neutral form rather than the salt forms. Although there is an example of a nanonized salt form of drug (MEGACE ES), salt forms in general result in the formation of free base during the nanonizing.[349]

Nanosizing many a times retained the crystal structure of the drug from its coarse particles.[350] Crystal defects can arise during the nanosizing, which result in the instability of the nanosuspensions. Sometimes it is also possible to get the drug particles with different form or amorphous nature. The nanosizing in comparison to the micronizing is a daunting task as the particles not only have to be reduced to nanosize but also need to be stabilized. Particle size reduction results in increased free energy, and without the stabilizers the nanoparticles tend to aggregate to reduce the free energy generated by the increased surface area. During nanomilling, optimum process time needs to be established for the manufacture of nanosuspensions, lesser times result in improper milling, and higher times cause the particles to aggregate resulting in nonhomogenous particle sizes. Hardness and crystalline structure of the drug majorly determine the achievable size reduction and shape of the nanoparticles. The shape can be with a small aspect ratio if it is a cube and large if it forms rod- or needle-like structures.[86]

The role of stabilizers generally comes into picture after the nanosizing of the drug particles during storage. These stabilizers play an important role in preventing the nanoparticles aggregation because of the interparticular forces. At the nanoscale, van der Waals interparticle attraction forces come into play as the distance between particles reduces. To counteract the van der Waals forces, steric stabilization or electrostatic stabilization of the nanoparticles was found to be very useful. Steric stabilization can be achieved by adsorbing polymers onto the surface of the nanoparticles and electrostatic stabilization by the adsorption of charged molecules to the surface. Typically both the stabilization approaches are adapted in nanosizing of APIs as steric stabilization alone may not provide enough force in preventing the flocculation of the nanoparticles.[349] Common steric stabilizers include the povidones, cellulosics, and pluronics. The polymer chain is selected such that the steric layer thickness is sufficient to provide stability and at the same time not to delay the dissolution of the drug. Nonionic surfactants are the choice of electrostatic stabilizers. Smaller surfactant molecules are not preferred as they can induce Ostwald ripening of the dispersion. Surfactants also help in the wetting and dispersion of the drug particles. Stabilizers play no role on the reduction of particle size during the milling process. Careful selection of stabilizers is necessary in the nanosizing of APIs to achieve greater stabilities. One type of stabilizers might not be suitable for all of the drugs. The selection of stabilizers for a drug and process is empirical rather than theoretical. Studies are underway to understand the physical and chemical interactions of the stabilizers with the drugs.[351]

Uniformity in the nanosuspensions can be very useful in preventing the Ostwald ripening and increasing the long-term physical stability. The polydispersity in particle sizes results in variations in the saturation solubility in the vicinity of the particles and concentrations of the drug in the dispersed systems, which is generally seen in suspensions. Ostwald ripening occurs through the dissolution of smaller particles to increase the size of the larger particles. Formulations containing nanosized API are typically solid dosage forms, and thus the nanosuspensions need to be freeze-dried after incorporating protectants or redispersants.[247]

Nanosizing of the drugs has been reported to improve the bioavailability and dose proportionality, reduce inter individual and fed/fast state variability, and enhance the rate of absorption.[177] Dissolution rate increase through the size reduction, up to nanoscale, increased the bioavailability of the drug aprepitant, which otherwise is affected by the presence of food.[89] This effect is largely dependent on the drug's properties and the dose. Increasing the dose of aprepitant did not show any improvements in the bioavailability. At low doses the absorption is dissolution rate limited, but at higher doses it is solubility limited for aprepitant. Thus, it is not always the case that monolithic drug nanoparticles show highest bioavailability. Sometimes other types of formulations depict the increased bioavailability than the monolithic nanoparticles; for example, a study with different formulations of spironolactone at dose of 50 mg/kg when administered orally to rats showed that the

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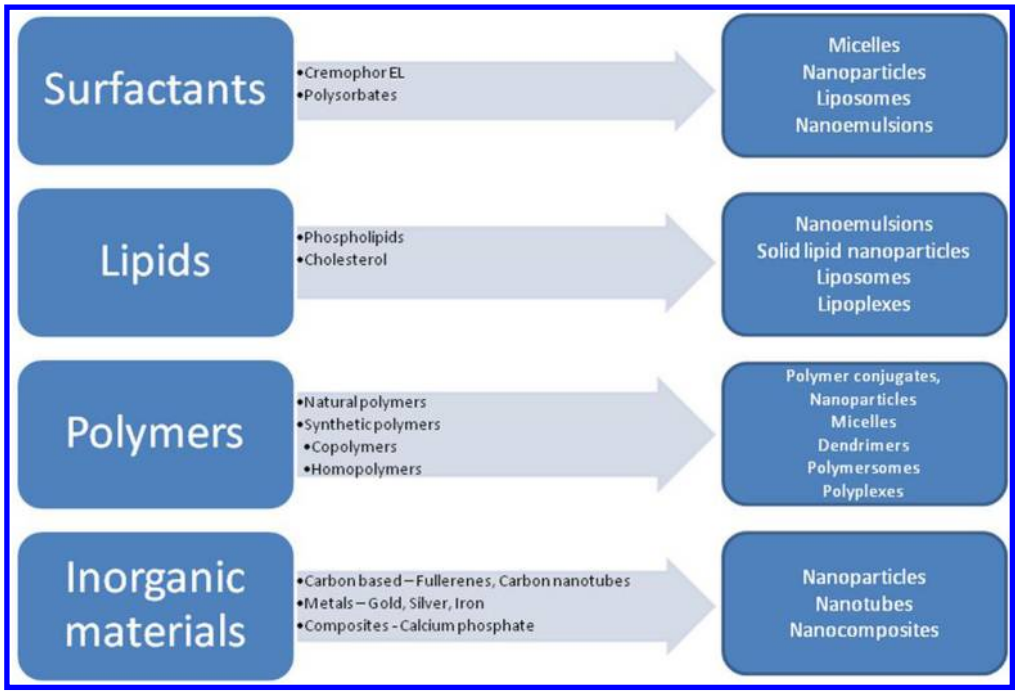

Figure 11. Materials used in different nano drug delivery systems.

solid lipid nanoparticles showed higher bioavailability in comparison to the nanosuspension formulations.[352]

Nanosize formulations are also used for intravenous delivery of drugs. Administration of micronized suspensions leads to the blockade of the capillaries, but the nanosuspensions can remain in circulation without blocking the capillaries. Nanosized drugs can be incorporated into many kinds of dosage forms such as solid, liquid, fast release, pulsatile, or controlled release forms and can be used through parenteral or oral routes. The size reduction approach cannot be applied to all drug candidates due to the harsh manufacturing process.

3.1.2.6. Viral Vaccines. Virosomes are composed of lipid vesicles with embedded virus envelop glycoprotein, with a diameter of 20–150 nm.[353] The virus enveloped glycoprotein can be obtained from influenza virus, Sendai virus, or vesicular stomatitis virus. Virosome production usually involves the dissolution of virus and removal of the capsid protein followed by the separation of envelop glycoprotein. This envelop glycoprotein is reconstituted in artificial lipid membranes like liposomes. Virosomes were initially developed for the delivery of DNA and drugs. Later, virosomes were developed as vaccines, which were proved to be very effective.[34,35] Virosomes prepared with influenza spike proteins resulted in high titers of influenza specific antibodies and have shown to be highly immunogenic and well tolerated in children. Virosomal hepatitis A vaccine is also developed in this manner, which has been shown to be very effective in humans.[354]

3.1.3. Research Pipeline. The following section discusses the most promising nanotherapeutics currently underway. The majority of the developments involve the use of polymers and lipids (Table 8), while inorganic materials appear to have been forced to deliver drugs (Figure 11).

3.1.3.1. Polymeric Nanoparticles and Nanocapsules. Polymeric nanoparticles constitute the largest group of nanotherapeutics under research, which promises the delivery of molecules that are considered undeliverable by other strategies and targeting. Polymeric nanoparticles, preferably biodegradable, are matrix particles in which the whole delivery system is in the solid form. The methods for the preparation of polymeric nanoparticles from preformed polymers include solvent displacement, emulsion–diffusion, salting out, dialysis, and supercritical fluid technology.[355–357] Solvent displacement method utilizes two phases, which can be both organic, both aqueous, or an organic phase and an aqueous phase. The two phases are a solvent phase and a nonsolvent phase. Solvent phase contains dissolved polymer and drug, while the nonsolvent phase is composed of a dissolved stabilizer. The solvent phase is added to the nonsolvent phase at once or in a controlled manner to instantaneously obtain nanoparticles. The solvent is evaporated generally under reduced pressure to complete the preparation process. The emulsion–diffusion method uses three phases, organic, aqueous, and a dilution phase. Drug and polymer are dissolved in the organic and the stabilizer in the aqueous phase similarly as in the above method.[172] To prepare the emulsion, the organic phase is added to the aqueous phase and stirred using a homogenizer and is diluted with water to facilitate the diffusion of the organic solvent into the aqueous phase resulting in the formation of nanoparticles. Single emulsification is sufficient to encapsulate the hydrophobic drug, but to encapsulate the hydrophilic drug, double emulsification[358] is generally employed. Using a cosolvent for the encapsulation of drugs, which have poor solubility in the routinely used organic solvents, is also very useful.[359–361] Solvent displacement method and emulsion–diffusion methods are extensively reviewed by Mora-Huertas and Elaissari.[357] The emulsion–diffusion–evaporation method is suitable for a wide range of molecules with diverse physicochemical properties; however, suitable stabilizer and solvent screening are necessary.[356] In the salting-out method, a completely water-miscible solvent is selected to dissolve the polymer and the drug.[362,363] High amounts of salts are added to the aqueous phase along with the stabilizer before the addition of polymer solution. The emulsion then is formed, and the solvent is diffused out in a similar way as in the case of the

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emulsion−diffusion method. In the dialysis method, the organic phase containing polymer is placed in the dialysis membrane with a specific molecular weight cutoff.[364,365] This is placed in a nonsolvent in which the organic phase is miscible but the polymer has limited solubility in it. As the concentration of the nonsolvent increases in the dialysis bag, precipitation takes place and the nanoparticles are formed. Use of supercritical fluid instead of the organic phase is thought to be a safer way of nanoparticles production as it does not use organic phases that might be toxic in some cases.[355] The polymer and drug are dissolved in the supercritical fluid, and then the fluid is passed through an orifice into air or a liquid medium, resulting in rapid expansion of the supercritical fluid to give the precipitates in nanoscale.

General methodology followed by the researchers is that the prepared nanoparticles are washed off the unbound or excessive stabilizer from the formulation and/or concentrating the nanoparticles. The typical final concentration obtained after the preparation of nanoparticles is about 50 mg of polymer in 20−50 mL, even higher volumes sometimes, of the aqueous phase. These concentrations are good for physicochemical characterization of the nanoparticles. However, to study the uptake in cell cultures or to study the loaded active agent's activity in animals, the formulations need to be concentrated sufficiently. This is carried out by using dialysis, ultracentrifugation, cross-flow filtration, filtration through 0.1 μm filter, or separation by gel filtration. These techniques are often not validated, and the precision in obtaining the same characteristics of the particles is often questionable. Nanoparticles prepared often constitute a wide size distribution range (at least 100 nm), and during the concentration process it is often possible to lose some of the prepared nanoparticles. Ultracentrifugation process many a times forms a hard cake of nanoparticles, and they are not easily redispersible. To disrupt the hard cake, sonication technique might be useful, but the stability and the loading efficiency of the drug in the nanoparticles have to be re-established to ensure that the physicochemical properties of the nanoparticles are not altered during the process of concentrating nanoparticles. Unfortunately, there were very few literature reports on the methods of concentrating nanoparticles and its effects on physicochemical properties of the nanoparticles. Most of the preclinical studies are carried out without understanding the effects of concentrating. Apart from these unknown effects, the concentrating process is time-consuming and cumbersome. A recent report suggests a novel method to concentrate the nanoparticles by hydrogen-bonding coacervate precipitation for PEG stabilized nanoparticles. Poly(ethylene oxide) forms a reversible coacervate with poly carboxylic acid compounds. The coacervate is easily filtered or centrifuged, and it can be reversed by changing the pH to 7. The nanoparticles then can be freeze-dried easily at lower volumes.[366]

Nanoparticles of the polymers can also be obtained directly from the monomers after polymerization. For this, the monomer and an initiator are used with or without a stabilizer. During the synthesis, formed polymers form aggregates and result in nanoparticles. High shears can also be used to get the particles with smaller sizes.

The above-discussed methods largely produce nanoparticles with spherical shape. Other shapes of the nanoparticles are also gaining importance because of the added advantages of shape. The approaches generally used for the fabrication of nanoparticles include self-assembly, photolithography, nonwetting template molding, micro fluids, and stretching of spherical particles. Some of these methods can be used for large-scale production, high throughput, and high precision in the shape.[367] The idea of using nonspherical particles for drug delivery comes from the different shapes of natural carriers and cells like red blood cells and platelets. It is also thought that the flow properties of nonspherical particles will provide added advantages in the circulation. DeSimone and colleagues[271,368] invented the PRINT (particle replication in nonwetting templates) technology, which enables one to manufacture the nanoparticles with size, shape, specific composition, and surface functionality. PRINT is a top down approach, which can be utilized to fabricate uniformly shaped monodisperse nanoparticles. Utilizing this approach polymeric, PEG hydrogel and proteinous nanoparticles were manufactured successfully. Surface functionalization of the PRINT particles can be done by matrix manipulation or post synthesis functionalization. Photolithography is also used in obtaining nano scaled fibers.[369] One of the other methods to produce monodisperse nanoparticles is the fabrication using a membrane emulsification process in which the dispersed phase is injected into the continuous phase through a membrane.[370]

Nanoparticles can be prepared using many kinds of polymers (Table 9). The most explored natural and synthetic polymers for the preparation of nanoparticles are chitosan[371] and poly(lactide-*co*-glycolic) acid (PLGA),[372] respectively. Sufficient hydrophobicity and high solubility of PLGA in the organic phases used for the nanoparticles preparation make it an ideal candidate. Apart from PLGA, poly(lactic) acid, poly(methyl methacrylate), and poly(caprolactone) are major synthetic polymers explored for the preparation of nanoparticles. Stabilizer plays a major role in the preparation of these nanoparticles. Lack of toxicity, irritancy and allergenicity, along with biodegradability are the desirable properties of an ideal polymer for the nanoparticle preparation. Stabilizer is thought to form a coat around the nanoparticles and gives steric and electrostatic stabilization to prevent aggregation and caking.[373]

Table 9. Most Popular Polymers Used in Nanoparticles[375]

type	material
synthetic homopolymers	poly(lactide)
	poly(lactide-*co*-glycolate)
	poly(ε-caprolactone)
	poly(isobutylcyanoacrylate)
	poly(hexylcyanoacrylate)
	poly(*n*-butylcyanoacrylate)
	poly(acrylate)
	poly(methacrylate)
	poly(methyl methacrylate)
copolymers	poly(lactide)-poly(ethylene glycol)
	poly(lactide-*co*-glycolide)-poly(ethylene glycol)
	poly(ε-caprolactone)-poly(ethylene glycol)
	poly(hexadecylcyanoacrylate-*co*-poly(ethylene glycol) cyanoacrylate)
protein-based polymers	albumin
	gelatin
natural polymers	chitosan
	alginate
	dextran

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Poly(vinyl alcohol) is the most widely used stabilizer for the manufacture of nanoparticles.

Drugs can be loaded by entrapment or adsorption, among which the former results in high loading efficiencies.[374] Drugs are thought to be released from the polymeric nanoparticles by degradation or erosion of the polymer, and desorption and diffusion of the drug, which can happen simultaneously.[356] Drug loading efficiency and release kinetics can be controlled by the selection of polymers and organic solvents for the preparation of the nanoparticles by the emulsion−diffusion−evaporation method.[172,173]

Nanoparticles are mostly used to entrap the anticancer agents. Probably the demand for advanced delivery systems in cancers is more than other diseases as the treatment is limited due to poor efficacy and toxicity. Anticancer drugs are encapsulated to make the delivery feasible and reduce nontarget tissue toxic effects. Another important reason is that the nanoparticles can passively target the tumors due to the EPR effect. However, none of the delivery systems offered significant improvements over the conventional forms with respect to life expectancy of the patient other than some marginal benefits with respect to compliance. Although many preclinical studies showed success in the delivery of these drug molecules, the clinical success is yet to be achieved for these nano drug delivery systems.[375] The clinical pipeline of new drug discovery is narrowing day by day. The importance of nanoparticles is evident in these circumstances where these delivery systems can help in improving the deliverability of many old drugs that have been discarded due to solubility problems or unfavorable pharmacokinetics and toxicity. Nanosystems by virtue of their route independent deliverability can offer many existing drugs the possibility of treating new nontarget diseases.

The majority of the preclinical studies encompassing polymeric nanoparticles have been studied intravenously. However, other routes of administration have also been considered seriously for the development of nanoparticles. Oral delivery is one of the important aspects of polymeric nanoparticles. Transdermal, pulmonary applications have also been discussed in the literature. Surface engineered polymeric nanoparticles have been studied in targeting the lymphatics, brain, tumors, liver, etc.

Nanocapsules are vesicular systems that exhibit core−shell structure. Drug can be located in the core or in the coating of the nanocapsule (Figure 12). The shell is a solid material, and the core could be either liquid or semisolid or solid. Initial nanocapsules encapsulated the oil cores, but recently methods to encapsulate hydrophilic molecules were also developed. High drug loading efficiency and low polymer content associated with the delivery system are the major advantages of the nanocapsules over nanoparticles (matrix type).[376] It acts like a drug reservoir and can be hydrophilic or lipophilic. Nanocapsules can be distinguished from the nanoparticles by the presence of two distinct regions, the rigid polymeric shell and core. Interfacial polymerization can yield hollow nanoparticles, which are generally made from the reaction of the monomers from aqueous and organic phases of the emulsion. The reaction rate can be controlled to get different sizes of nanoparticles with high efficiency of drug loading.[377,378]

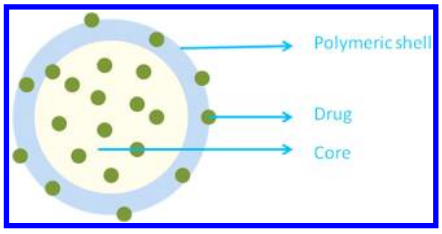

Figure 12. Artistic impression of a nanocapsule. Nanocapsules can be identified by the presence of core and shell structures, and the core can be liquid, solid, or semisolid, while the shell is polymeric. Drug can be entrapped in the core or can be adsorbed onto the shell.

3.1.3.2. Metal and Inorganic Nanoparticles. Gold, silver, iron oxide, silica, and carbon-based nanomaterials have been extensively researched for diagnostic and therapeutic purposes. The widespread usage of gold nanoparticles as nanomedicine is due to their unique tunable optical properties. Gold nanoparticle-mediated hyperthermia has been effective in preclinical studies, and clinical examination of these particles is underway.[379,380] Engineering the size, shape, and composition is possible to achieve desired light scattering or absorption by gold nanoparticles. Various modifications of the gold nanoparticles have been studied for improvements in the photothermal therapy by gold nanoparticles that include gold−silica nanoshells, gold nanorods, gold nanocages, gold−gold sulfide nanoparticles, and hollow gold nanoshells.

Nanoparticles can be made from magnetic materials in which drug is generally entrapped or adsorbed. Two types of iron oxide, maghemite (γ-Fe_2O_3) and magnetite (Fe_3O_4), are the most widely used materials for the manufacture of magnetic nanoparticles. Magnetic nanoparticles intended for drug delivery should be nontoxic, nonimmunogenic, biocompatible, and water-based. These magnetic nanoparticles contain superparamagnetic material, which means that they do not have any magnetism in the absence of magnetic field. The advantages of using magnetic material include easy detection, magnetic manipulation, and energy transfer.[269] As the organs and tissues of the body are nonmagnetic, detection of magnetic nanoparticles is easy. Using the magnetic field gradient, it is possible to drag the magnetic nanoparticles to the site of interest to increase the localization. The magnetic nanoparticles can then be used to induce the hyperthermia to destroy the tumor. In anticancer therapy, magnetic nanoparticles are very useful to kill the cancer cells by hyperthermia only at the tumor.

Some cancer cells are more susceptible to high temperatures than the normal cells; thus they can be destroyed thermally by increasing the temperature at the cancerous cells selectively up to 42 °C. This can be achieved by injecting the magnetic nanoparticles at the malignant tissue, after which an alternating magnetic field can be applied to make the magnetic nanoparticles absorb the energy and heat the tissue. Although this seems a plausible approach theoretically, practical problems pose a challenge. To achieve the hyperthermia, the size, shape, and physical properties of the nanoparticles should be optimized so that the nanoparticles will have suitable Curie temperature. Larger particles will be easier to localize at the target site with moderate magnetic field gradient. However, with larger particles, there is great chance of removal by the MPS from the circulation, affecting the concentrations at the tumor.[381] Thus, an optimization of the size and surface modification of the nanoparticles is necessary to target the tissues efficiently. The material used in the manufacture of magnetic nanoparticles must be biocompatible and nontoxic. It is confirmed that the iron oxide is biocompatible and eventually used in the formation of blood hemoglobin. However, this is not the case with cobalt or gold nanoparticles.

Magnetic nanoparticles for the therapy are generally in the form of ferrofluids, which are stable dispersions of magnetic beads in a solution. Particle solvent interactions and interparticle repulsions should be strong enough to overcome the prevailing van der Waals attractive forces and magnetic forces in cases of nanoparticles with permanent magnetic moment.[382]

Using the magnetic nanoparticles has the specific advantage of targeting. Targeting with magnetism can be considered as active targeting. As in other cases of targeting, the surface ligands with affinity to specific targets tend to accumulate in specific tissues. In contrast to these active targeting techniques, magnetic targeting utilizes external force for the targeting. Magnetic targeting can bring the nanoparticles to the sites of interest; however, to induce cellular internalization of nanoparticles, surface ligands might be useful. Thus, combining both approaches, it is possible to get precise targeting of the tissue of interest and reduce the exposure to nontarget tissues.[383] Foy and Labhasetwar in their recent lead opinion discussed the pros and cons of the use of iron in cancer therapy.[384]

3.1.3.3. Self-Nanoemulsifying Drug Delivery Systems. Self-nanoemulsifying drug delivery systems (SNEDDS) are described as the isotropic mixtures of oils, surfactants, and cosolvents with solubilized drug, which form microemulsion by diluting with aqueous medium or biological fluids. Microemulsion and miniemulsion represent the droplets with the sizes in nanoscale range. However, nowadays micro and miniemulsions are sometimes renamed as nanoemulsions.[385] Similarly, SNEDDS is used synonymously with self-microemulsifying drug delivery systems (SMEDDS). The term SNEDDS is introduced with the advent of the nanotechnology boom. SNEDDS concept was introduced to improve the oral delivery of hydrophobic drugs;[386,387] however, some parenteral and dermal applications are also reported in the literature.[388,389]

3.1.3.4. Solid Lipid Nanoparticles and Nanostructured Lipid Carriers. Solid lipid nanoparticles (SLN) are composed of lipids and present an efficient delivery system for the delivery of hydrophobic drugs.[390] These particles imbibe the advantages of polymeric nanoparticles in rigidity and structure while maintaining the biodegradability, biocompatibility, and ease of manufacturing advantages of liposomes.[391] The formulation includes biocompatible lipids such as hard fats, triglycerides, or lipid acids. They are stabilized with biocompatible stabilizers like lecithin or polysorbate 80. The lipids are melted for formulation of solid lipid nanoparticles, which are solid at body temperature. The advantages of SLNs include the avoidance of organic solvents during preparation, improved bioavailability of the encapsulated drugs, sustained release, and protection of the loaded drugs from degradation.[392] Nanostructured lipid carriers (NLC) contain the binary mixture of solid lipid and a liquid lipid. The major advantage of the NLCs is in increasing the drug loading. Nanocochleates are developed to encapsulate drugs between the lipid bilayers. These nanocochleates are being evaluated for improving the bioavailability of poorly soluble drugs.[393]

3.1.3.5. Protein Vaults. Protein vaults naturally occur in nearly all eukaryotic cells. These are hollow barrel-like particles with invaginated waist and caps on each end. Major vault protein (MVP) is thought to be present in 96 copies per vault constituting 75% of the vault protein mass. Using recombinant technology, protein vaults can be manufactured and can be engineered as drug carriers. Wild-type vaults consist of other proteins to complete the vault structure, but it was found that recombinant MVP alone can form the vault structure in the insect cells.[37] 96 copies of the major vault protein (MVP) can self-assemble to form protein vaults of 72.5 × 41 nm with a large hollow interior.[394,395]

Protein vaults can be engineered to encapsulate and release the drugs at a specific site,[396] and can also be engineered to target the cell surface receptors.[397] Recombinant vault nanoparticles can enter the cells via the endocytosis. Membrane lytic adenovirus protein can be fused to the interior of the vault nanoparticles to impart membrane penetrating ability.[398] Vault nanoparticles have also been investigated as vaccine delivery agents.[399]

3.1.3.6. Dendrimers. Dendritic polymers were successfully synthesized during the 1980s, which are now called dendrimers.[400,401] These are the result of a relatively new development in the polymer chemistry and represent more advanced nanoengineering method for drug delivery. Dendrimers are highly branched three-dimensional structures with a core, ordered branches and a surface. The structure of the dendrimers is characterized by layers or generations. Generation 0 represents the core, and additional attachments forming a layer are known as a generation. The branching increases with the branch generations in the dendrimer. These molecules have a unique identity among the polymers as they possess well-defined structure, size, and can be obtained with very low polydispersity. This is due to the stepwise synthesis of dendrimers in contrast to other polymer synthesis where the synthesized polymer chain size is polydisperse. Practically it is possible to obtain absolutely monodispersed dendrimers for low generations (1−3); however, for higher generations sometimes it becomes difficult to purify the perfect dendrimers from dendrimers with minor defects, slightly deviating from the absolute monodispersity. Utilizing different molecules, the functionality of the dendrimers can be tailor-made to suit the requirement of a specific case in drug delivery.[402]

Dendrimers can be synthesized either by the divergent or by the convergent method. The combination of these two approaches is also reported, which is known as the double exponential growth.[403,404] The selection of approach for the synthesis of dendrimers is based on the requirements and the suitability of chemical reactions. The majority of the dendrimers are synthesized employing covalent bonding.[405,406] Divergent approach can be used to synthesize large quantities of dendrimers and higher generation dendrimers. In the divergent approach, the synthesis starts from the core, and the size of the dendrimer increases gradually to result in the required generation dendrimer. The control and the quality of the prepared dendrimers are poor in the divergent method. In the convergent method, structural control can be achieved but with lower number of generations. Commercially poly(propyleneimine) (PPI) and poly(amidoamine) (PAMAM) dendrimers are prepared by the divergent method, and polyether dendrimers are prepared by the convergent method. In the convergent approach, the dendrimers are built from the periphery.[407] This method allows a better control over the dendrimer architecture than the divergent method.[408] In this method, the synthesis starts from the periphery and ends with joining the branches to the core.

With modifications in the design and composition, dendrimers can be synthesized to act like a micelle giving the name of unimolecular micelles.[409] The formed unimolecular micelles are independent of temperature and concentration unlike the other micellar structures. In an experiment, these

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amphiphilic unimolecular micelles increased the solubility of pyrene by 120-fold.

Dendrimers can be designed to entrap the drug molecules inside the cavities or can be covalently bound to the drugs to deliver them to the specific sites in the body. Dendrimers with lower generations (1−3) are dome shaped, and higher generations (4−7) are spherical in shape. The voids in the denrimer architecture can entrap the smaller drug molecules.[410] Various studies have demonstrated that a variety of molecules can be encapsulated in the cavities of dendrimers, which act as boxes for the molecules.[411,412] The number of molecules entrapped in the cavities is dependent on the shapes of the guest molecules and the cavities. The release of the encapsulated molecules is very slow under normal conditions, which illustrated that the shells need to be bioactive to release the drugs.[413] Dendrimers can be PEGylated to decrease the inherent toxicity and solubilize the poorly water-soluble drugs.[414−416] Drug loading decreased with the increase in the molecular weight of PEG from 2 kDa to higher molecular weights.[417] Dendrimers with surface functional groups are being prepared and offer covalent linkage for a variety of drug molecules to improve the delivery related problems.[418,419] The covalent processes have produced more than 100 different dendrimer interiors and 1000 types of surface chemistries. Apart from chemical synthesis, dendrimers can also be prepared from amphiphilic dendrons by supramolecular assembly.[420]

Biodegradable dendrimers have also been developed recently, which are generally synthesized by introducing an ester bond in the architecture. Ester bonds can be cleaved hydrolytically or enzymatically, imparting biodegradability to the dendrimers. The biodegradability of the polyester dendrimers is based on the chemical nature of the bonds, hydrophilicity of the monomers, and the size of the dendrimer.[421]Applications of dendrimers in photodynamic therapy, boron neutron capture therapy, and gene therapy are being explored.[422] Dendrimers are also investigated and engineered to possess stimuli responsive properties in drug delivery.[423,424]

One of the main advantages of dendrimers is their renal clearance due to the size smaller than the renal threshold.[425] Important concern in the usage of dendrimers is their toxicity. It is observed that the dendrimers can rip off the lipid bilayer from the cell surfaces and make a shell of the bilayer around the dendrimer leading to cellular toxicity.[426] The cytotoxicity was increased with the size and the generation of PAMAM dendrimers. Surface modifications of toxic dendrimers can yield safer dendrimers.[427] VivaGel is the first example of dendrimer formulations, which is under clinical trials presently, as a vaginal virucide for topical application. It is a lysine-based dendrimer with naphthalene disulphonic acid surface groups.

3.1.3.7. Polymeric Micelles and Polymersomes. Amphiphilic polymers can assemble to form micellar structures of nanosize that are known as polymeric micelles. The core is normally hydrophobic, and the shell is hydrophilic. Vice versa arrangement forming hydrophobic shell and hydrophilic core is also possible, which are known as reverse micelles. The amphiphilic nature of the polymers causes spontaneous aggregation resulting in the formation of micelles under aqueous environments. The driving force for the aggregation is their hydrophobicity. The amphiphilic polymers are generally obtained using hydrophobic poly esters or poly amino acids covalently linked to hydrophilic polymer blocks such as PEG, poly(*N*-vinyl-2-pyrrolidone), poly(2-ethyl-2-oxazoline), or poly(acrylic acid). Various types of amphiphilic polymers have been prepared and tested for drug delivery with different chain lengths of hydrophilic, hydrophobic blocks, and using different kinds of block combinations. These polymers can be diblock or triblock copolymers. In diblock copolymers, one block must be hydrophilic and the other hydrophobic, and in triblock copolymers one block can represent the hydrophobic part and the remaining two hydrophilic parts or vice versa. The particle sizes for micelles are normally less than 50 nm and are monodispersed.[428] These micelles represent core−shell organization and sometimes are referred to as capsular forms.[429] Polymeric micelles are reported to be much more stable than the surfactant micelles. The micellar structure is stable even when the polymeric micelles are diluted below the critical micellar concentration. Shell cross-linking can also be carried out for enhanced stability of the micelles. The critical micellar concentration is dependent on the length of the hydrophobic block in the amphiphilic polymer.[430] Engineering the shell of the polymeric micelles is possible to enhance the circulation times and to provide other desirable properties such as targeting.

Polymersomes are bilayered polymeric vesicles and are sometimes referred to as nanoscale bags by some scientists. Amphiphilic polymers can form bilayers in solutions similarly as liposomes are formed from phospholipids.[431] These polymers are mostly amphiphilic diblock copolymer-based. A ratio of 35 ± 10% of the hydrophilic part to the total mass of the amphiphile is necessary to form the polymersomes. Amphiphiles with higher values form micelles and lower values form inverted structures.[432] Apart from the hydrophilicity, copolymer structure, composition, and solubility also play an important role in the formation of polymersomes.[433] The polymersomes formed from polyethylethene-*block*-poly(ethylene glycol) are highly deformable but also possess enhanced toughness in comparison to the liposomes. The permeability of water through the polymer layer is found to be 10 times less than the liposomes.[434,435] Apart from the toughness, the thickness of the membrane forming the polymersome can also be manipulated to suit the requirement.

The surface of the polymersomes can be modified by chemical modification of the hydrophilic part of the amphiphile to inculcate desired surface functionalities.[436] Polymersomes can be made pH responsive to selectively release the entrapped drug at a specific pH.[437,438] Most of the polymersomes are taken up by endocytosis and are localized in lysosomes; however, polymersomes made up of poly(2-(methacryloyloxy)-ethyl-phosphorylcholine)-*co*-poly(2-(diisopropylamino)ethyl methacrylate) (PMPC−PDPA) are pH responsive and thus can escape lysosomal compartments.[439]

3.2. Development Issues

This section reviews what drives the development of nanoscience and technology based on emerging concepts and explores the role of drivers like human resources, funding, and recognition in its promotion.[440] While the reports look good on a lab setup, is enough being thought about scale-up issues, screening, safety evaluations,[441] clinical trials, and what approach should be employed by regulatory agencies who have to review the risk against benefit?[442] Industrial development of the nanomedicine is just picking up to start with the success of the nanotherapeutics already marketed. However, there is a big uncertainty about the kind of new strategies that might be implied in the clinical trials of the nanotherapeutics by the regulatory agencies.

3.2.1. Scale-Up of Available Technologies. Enormous research is being carried out in the area of nanotherapeutics development at lab scale. Scaling up of the laboratory technologies to industrial level is a prerequisite for economic production and success in clinical usage. Nanosizing of drugs is more advanced in this area in comparison to other processes for preparation of nanotherapeutics. Successful industrial technologies presently are available for nanosizing of the drugs.

Lack of large-scale economic production of the polymeric nanoparticles is thought to be hindering the market reach of these drug delivery systems. Polymeric nanoparticles at lab scale are produced employing emulsification techniques. The volumes of solvents used in these techniques are often high, and the separation of the solvents after preparation of nanoparticles is cumbersome.

3.2.2. Product Development and Characterization. In multicomponent systems such as drug encapsulated nanoparticles, appropriate selection of the raw material and the process variables play a crucial role in the product development. Even after 40 years of rigorous research in emulsion-based nanoparticulate drug delivery systems using either lipids or polymers as major excipients, not much emphasis was laid on other key ingredients such as organic solvents and surfactants, which also have significant influence on the product profile. So far the focus has been on the class of solvents to be used or not to be based on their toxicity profiles (class III being best) leaving aside the most crucial physicochemical properties of the solvents such as interfacial tension, water solubility, vapor pressure, and viscosity.[172] The solvent selection will in turn have a significant impact on the surfactant selection, which is primarily used to minimize the interfacial tension and stabilize the system. Not all solvents and surfactants are compatible with all of the drugs so the screening should be based on the drug,[285] as well as the delivery system.[173] Over 90% of the research using preformed polymers in particular polyesters like PLGA/PLA/PCL use class II solvents such as dichloromethane or chloroform with PVA being most common surfactant with almost every drug that was investigated. A thorough understanding of each excipient and the active ingredient is important to establish a well-characterized product that can be prepared in large quantities. These things can be kept simpler yet very useful; however, the intellectual property rights and the requirements to keep different from what is on the market is posing significant problems for developing genuine products.

Characterizing for size of the nanotherapeutics is the primary requisite to be claimed as a nanotherapeutic. Size is generally expressed as the diameter for most of the nanotherapeutics as they are spherical. These days, advancements in the technology are leading to the development of differently shaped nanotherapeutics. For these systems, height or length and width or thickness is measured.

There are very few techniques available for the determination of particle size of nanotherapeutics in solution. The most popular technique that measures the hydrodynamic diameter of the particles accurately is called dynamic light scattering, which is also known as photon correlation spectroscopy and quasielastic light scattering. Light scattering technique gives the hydrodynamic diameter considering the particles to be spherically shaped. In this method, solution containing nanoparticles is illuminated with monochromatic laser, and the scattered light is detected with a fixed or variable angle. The scattering intensity is time dependent in microsecond durations due to the Brownian motion. The scattering intensity fluctuation deflects the rate of particle diffusion. The fluctuations are captured using the autocorrelation method, in which the scattering intensity at time t is compared to itself at time $t + \tau$, where τ is correlation delay time. This process is repeated over the observation period and generates the correlation function with a range of τ values. The graphical description of the correlation is called the correlogram. From the decay of the correlation function, the translational diffusion coefficient is calculated. Using the translational diffusion coefficient, from the Stokes−Einstein equation, the hydrodynamic diameter of the particle can be calculated. Viscosity and temperature will have an influence on the measured particle size; thus it is important to know the medium or its viscosity and temperature at which the measurements are taken. The nanoparticles generally contain a large population with imperfections and natural conformational variations as the large number of atoms are involved in the manufacture of the nanoparticles; thus it is essential to know the variability in particle size. Dynamic light scattering technique gives dimensionless polydispersity index calculated from the correlogram. As stipulated by ISO 13321:1996 Annex A, presently the recognized standard method for the analysis of the autocorrelation data is only by cumulants method. Cumulants method gives the mean intensity-weighted size called the z-average size. The cumulants does not provide the polydispersity index directly, as it is calculated by assuming the variations in the mean size as a single mode Gaussian function and thus gives a hypothetical size distribution.

Electron microscopy, which is of two types, transmission electron microscopy (TEM) and scanning electron microscopy (SEM), can be used for the imaging of the nanoparticles. These can be used in conjunction with back scattering detector or energy dispersive X-ray spectroscopy detector to perform the elemental analysis. TEM and EDX coupled to SEM are also very useful tools to study the biodistribution of the nanoparticles. Very thin sections of the tissues (70−100 nm) are needed for imaging under the TEM so that the electrons can pass through the sample. TEM analysis allows the visualization of nanoparticles and fine cellular structures in the tissues. Elemental composition of the sample can be identified by the incorporation of an energy dispersive X-ray (EDX) spectrometer in the SEM. During the EDX analysis, the sample is bombarded with an electron, which displaces the electrons of the specimen from their energy levels. The emptied energy level can be filled by the high energy outer shell electron; during the process X-rays are emitted. The energy of the X-rays released from each atom is unique and thus can aid in the elemental analysis of the sample. This technique is especially useful for metal nanoparticles.

Atomic force microscopy utilizes a cantilever with a sharp probe to scan the sample surface. The mirrored surface deflects the laser beam to the split photodiode. As the cantilever moves on the sample surface, it experiences attractive or repulsive forces and leads to a deflection in the cantilever orientation, which can be traced by the laser beam reflected from the mirrored surface of the cantilever. On the basis of the nature of the sample interactions with the cantilever probe, different operating modes can be selected, contact mode, intermittent contact mode, and noncontact mode. In contact mode, the interaction between the cantilever probe and the sample is repulsive, and in noncontact mode, the tip interacts with the surface by the long-range surface forces. In intermittent contact mode, the tip is made to oscillate close to its resonance

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frequency perpendicular to the sample surface, which is also called the tapping mode. As the oscillating probe moves along the sample, probe can experience long-range surface forces or weak repulsive forces, and as a result the amplitude of the oscillation varies. Tapping mode has the advantage to scan the soft surfaces and is suitable for probing particles that are weakly adhered to the surface, and this is suitable for nanoparticles size measurements. AFM gives the three-dimensional surface profile of the particles. For a spherical particle, the height of the nanoparticle represents the size or diameter of the nanoparticle.

Electron microscopy and atomic force microscopy are also used for the size determinations. The advantage of using microscopic (nanoscopic) techniques is that the actual size of the sample can be measured, whereas with the light scattering hypothetical hydrodynamic diameter is measured. For nanotherapeutics with different aspect ratios, nanoscopic techniques are the best suitable methods; however, the sample size used for such measurements is very negligible, not necessarily taking into account the wide particle population. The use of both light scattering and the nanoscopic methods in conjunction should offer a better understanding of the sample population.

Zeta potential is generally measured for the nanoparticles, which gives the idea about the stability of the nanotherapeutics in a suspension form and in understanding the tendency to aggregate. Electrostatic stabilization can be explained by the Derjaguin−Landau−Verwey−Overbeek (DLVO) theory. According to the DLVO theory, two main forces act on the particles, which are electrostatic repulsive forces and van der Waals attractive forces. The repulsion is due the electrical double layer surrounding the particle surface. The double layer consists of the inner Stern layer and the outer Gouy layer. The Stern layer is composed of the counterions, and the Gouy layer is a diffusion layer of ions (Figure 13). The attractive forces act

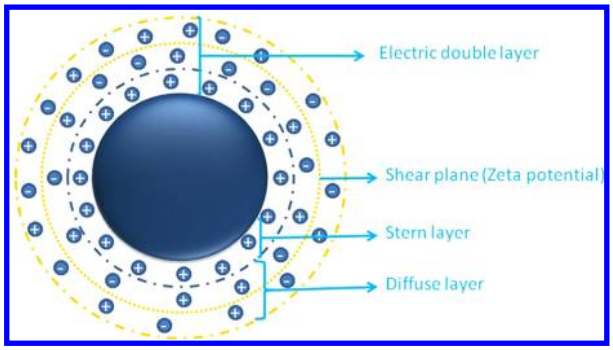

Figure 13. Particle surface depicted with electric double layer in the liquid medium.

on larger and very shorter distances between the particles, resulting in the energy minima, and the repulsive forces act at intermediate distances preventing the aggregation of particles. As the particles move due to Brownian motion, a distinction is made in the diffuse layer between the ions moving with the particle (the boundary of the surrounding liquid layer of the particle) and ions moving with the dispersant; this plane is known as the slipping plane or shear plane. The suspensions stability is generally measured by the zeta potential, which is the charge on the shear plane. Higher zeta potentials indicate a higher stability of the particles in the dispersion. In zeta potential measurements, an electrical potential is applied across the sample, and the electrophoretic mobility of the particles is measured by laser Doppler velocimetry. Utilizing the electrophorectic mobility, zeta potential is calculated from the Henry equation. Nanoparticles with zeta potentials in the range of ±10 mV are considered to be neutral, and higher than +30 mV and lower than −30 mV are considered strongly cationic and strongly anionic, respectively. Zeta potential is dependent on the pH and the conductivity of the medium, and thus it is necessary to accurately report them.

It is important to measure the particle size and zeta potential at various stages of the preparation process such as fresh, after removal of free surfactants/drugs, and after freeze-drying, and this will help build the product profile. For the monolithic nanotherapeutics intended for oral absorption, determination of crystalline status, rate of dissolution, and saturation solubility forms the part of the characterization.[177]

Analytical techniques have also advanced alongside of nanotechnology and nanoscience providing efficient tools for the characterization of complex multifunctional nanotherapeutics.[443] MALDI TOF can be very useful in characterizing the dendrimers. Drug loading efficiency is another important parameter to be calculated. The final concentration of the drug expressed as weight percentage in the formulation is helpful for estimating the excipients needed for a required dose as this will hold the key for the disease to be treated and the route. Characterization of the nanotherapeutics can be extended to confirm the location of the drug on the carrier. Stabilizers are the important components of most of the nanotherapeutics apart from the carrier material. Estimation of stabilizer associated with the final formulation is required as this would influence the absorption (depending on the route of administration), circulation times, tissue localization, as well as toxicity.

For most of the therapeutics, a single dose is sufficient to study the tissue distribution; however, as the nanotherapeutics utilize lower doses than the conventional drugs, it might be necessary to use multiple doses to study the tissue distribution of the nanotherapeutics. As the nanotherapeutics can be visualized using advanced microscopic techniques, qualitative information on the tissue distribution of the nanotherapeutics can be obtained from TEM. Environmental scanning electronic microscopy can also be used to avoid the stringent sample preparation process.

On the other hand, having a regulation in place that facilitates the approval of nanotherapeutics is becoming a challenge, and the challenges are manifold, with the real ones being mindset and availability of sufficient material. To test a drug in humans, regulatory approval is necessary, and in the application for first in man studies the investigators should present sufficient data supporting that the drug can be safely tested in humans. For small molecules there are well-defined criteria; however, for nanotherapeutics we often tend to highlight lack of rules and methods for conducting safety studies. The biggest realistic limitation is lack of material/bulk production of drug encapsulated nanoparticles to conduct chronic toxicity studies, as most of the nanoparticles drug delivery research is done at the academic level providing the proof of concept with emphasis on materials innovation and moving forward very quickly leaving behind many fundamental issues that need attention. It is worthwhile to think at this stage what changes do we intend to make to fundamental toxicology studies that are good enough to prove safety/tolerability of any drug or drug encapsulated delivery system; however, one might consider looking at more organs or more parameters than what is done for small molecules. On the other hand, we still follow

conventional in vitro release studies even though we do not follow the essential criteria of sink conditions, and we attempt to make an in vitro−in vivo correlation either conducting a low or high dose pharmacokinetic study. Currently, there is a lot of confusion with several of the issues mixed up, for example, occupational health hazards of bulk nanomaterial production versus safety of nanoparticles encapsulating drugs, and the former draws more attention shadowing the latter. One of the rules that can be implemented could be materials that are known to produce toxicity (e.g., carbon nanotubes, silica, etc.) are not permitted for use in drug delivery systems, irrespective of the surface compatibility solutions one would provide. The majority of the studies demonstrating the biocompatibility of such materials are done on a short-term basis, which is not sufficient to claim any benefits as the majority of these delivery systems are used to treat chronic disease conditions.

Equal efforts are in place to come up with different regulatory methodologies to assess the safety of nanoparticles because of the interference with the routine in vitro assays that are used to establish the safety of the pharmaceuticals. The varying reactivity of nanoparticles in the in vitro assays is due to their optical and catalytical properties. Bacterial endotoxin or lipopolysachride is a membrane component of Gram negative bacteria, which can cause fever, shock, and even death. FDA has set limits for the endotoxin units that may be present in the drugs, delivery systems, or medical devices. Limulus amoebocyte lysate (LAL) reacts with the endotoxin, and detection of the products of this reaction gives an estimate of the endotoxin present in the formulation. However, nanoparticles often interfere with the reactivity of endotoxin, which might give rise to underestimation or overestimation of the endotoxin present in the formulation. One of the objectives of the nanotechnology characterization laboratory (NCL) of USA is to develop and qualify the tests to accurately assess the safety and efficacy of nanoparticles.[444] However, it is not clear how much value addition these in vitro tests would offer as ultimately these systems need to be tested in vivo on a case by case basis.

Nanoparticles intended for systemic administration need to be tested for complement activation.[103] All three pathways of complement activation result in the breakage of C3 component. Measuring the C3 component breakage products can be used as a basis for the estimation of complement activation by nanoparticles. In this method, human plasma is exposed to nanoparticles and then analyzed by polyacrylamide gel electrophoresis followed by Western blot with anti-C3 antibodies. Anti-C3 antibodies recognize C3 component and its breakage products. The test samples are then compared to the negative (untreated plasma) and positive (cobra venom factor) control amounts. These assays use human plasma and C3 antibodies specific to humans. These antibodies provide a good estimation of the complement activation by the nanoparticles in plasma. However, sometimes in preclinical studies it becomes essential to study the localized complement activation in various tissues. Immunohistochemistry techniques are used to determine the tissue-specific complement activation, or the complement activation can be determined in the tissue homogenates.[445] Marketed liposomal preparations such as Doxil and Ambisome are reported to activate complement leading to hypersensitivity reactions (HSRs).[446,447]

Unlike the systemic complement activity, which might be deleterious, complement activation at local sites is necessary in enhancing the vaccine's efficacy. This is because of the fact that complement activation is also responsible for adaptive immunity. Complement activation is required in case of vaccine delivery.[110] Subcutaneous or intradermal administration of vaccine nanoparticles is beneficial in improving the efficacy of the vaccine.[103] Interestingly, the focus thus far has been only on the products that are intended for intravenous administration, and these assays or the groups doing these do not consider particles administered by alternate routes such as oral that resemble the intravenously administered particles when they are taken up intact into systemic circulation.

The science and technology administrators across the globe with the support of the academic and corporate leaders launched nanotechnology initiatives with a view to benefit society and mankind. All new developments take enormous time before they are accepted by wider communities, and history vouches for this. Nanotechnology is thought to be the biggest ever revolution that converges all areas of science, engineering, and medicine, demanding huge investments. The return on investments for such technological/industrial revolutions is always on long-term basis, and forcing early returns often causes problems and mistrust, and the best example to describe this is healthcare and in particular drug delivery. Undoubtedly, nanotechnology has huge potential in healthcare setting, in particular drug delivery; however, a systematic research without ignoring the fundamentals of drug delivery is of utmost importance if we have to benefit from this revolution. On the other hand, it would not be wrong to say that we have already achieved huge success with respect to human resource development by training young scientists in this fascinating area of science as measured by the number of Ph.D.'s and other technical graduates.

3.2.3. Clinical Trials. Presently, >200 nanotherapeutics are under the clinical development. Especially in cancer clinical trials, nanotherapeutics often prove to be safer at higher drug doses than the native forms.[448] Sometimes the drugs that are discarded in previous clinical trials, when formulated as nanotherapeutics, have been shown to improve the patient's condition and reduce the toxicity in human trials.[449] However, postmarket analyses most often contradict the prelaunch claims that need to be thoroughly addressed, for example, Abraxane.

Metal nanoparticles are extensively used to induce hyperthermia at the tumor site in clinical trials. Gold nanoparticles tagged with tumor necrosis factor-α (TNF-α) are being used in human trails to treat several types of cancer. Apart from metal nanoparticles, liposomal formulations are also being tested. For example, ThermoDox, a lysothermosensitive doxorubicin liposomal formulation, is under phase I/II clinical trial that utilizes local hyperthermia to specifically release the drug at the target site.[450]

Some of the results from the clinical trials of nanotherapeutics have been published, which are discussed in the following text. CPX-1, a novel liposomal formulation of irinotecan and floxuridin, was used in the phase I open label dose escalating study, and the results showed that the CPX-1 is well tolerated in humans and showed antitumoral activity in patients with advanced solid tumors.[451] MCC-465 is an immunoliposome encapsulating doxorubicin, and tagged with PEG and $F(ab')_2$ fragment of the human monoclonal antibody (goat antihuman), which reacts with stomach tumors more than 90% and have no reactivity with normal tissue. In preclinical studies, MCC-465 was shown to possess superior activity against human stomach cancerous cells in comparison

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Table 10. List of Terms with the Nano- Prefix

nanoactuator	nanoSQUID	nanophytoplankton	nanodispersion	nanocrystallography
nanoasperity	nanoswitch	nanoplatelet	nanodust	nanodiamond
nanobiometrics	nanotorus	nanopowder	nanoethics	nanodomain
nanobubble	nanotransport	nanomodule	nanofin	nanoelement
nanocap	nanovehicle	nanoneedle	nanofractal	nanofabrication
nanochannel	nanowaveguide	nanoparticle	nanogranule	nanoflake
nanocomponent	nanoantenna	nanolocalized	nanoisland	nanofragment
nanoconfinement	nanobarn	nanomesh	nanolithography	nanographene
nanocontact	nanoblend	nanomolecular	nanomembrane	nanojunction
nanocube	nanobunch	nanorefrigerator	nanoplankton	nanoobject
nanodielectric	nanocapacitor	nanosandwich	nanopolariton	nanopharmaceuticals
nanodosimetry	nanocircuitry	nanoshell	nanoprecipitate	nanoplasmonics
nanoemulsion	nanocomputer	nanostring	nanorelay	nanopolymer
nanofilament	nanoconjugate	nanosyringe	nanoscale	nanoprobe
nanofluid	nanocontainer	nanotoxicity	nanosize	nanoresistor
nanogap	nanocylinder	nanotriangle	nanostripe	nanoscroll
nanoimprint	nanodisc/nanodisk	nanovessel	nanotechnical	nanosphere
nanolayer	nanodot	nanowhisker	nanotrack	nanosurface
nanomachine	nanoequivalent	nanoaperture	nanotube	nanothermometer
nanometrology	nanofilm	nanobead	nanovoid	nanotransistor
nanomorphology	nanoform	nanobot	nanoarchitecture	nanovaccinology
nanooptoelectronics	nanograin	nanocable	nanobioelectronics	nanowall
nanophase	nanointerface	nanocatalyst	nanobridge	nanosystems
nanoplate	nanolevitation	nanocoating	nanocalorimeter	nanobiotechnology
nanopositioning	nanomanipulation	nanoconductor	nanoceria	nanomechanics
nanopyramid	nanomodification	nanoconstriction	nanocolumn	nanobiology
nanoroughness	nanomotor	nanoconverter	nanocone	
nanosensor	nanopattern	nanodiagnostics	nanoconstruct	

to doxorubicin or PEGylated liposomal doxorubicin. The phase I clinical trials showed that the formulation is well tolerated and allowed for the calculation of recommended dose for phase II.[452] Paclitaxel loaded polymeric micelles, polymer doxorubicin, and P-glycoprotein targeted micellar doxorubicin were in various stages of clinical trials.[453−455]

4. OPINION

4.1. What Is in a Name?

While many agree that nano is a case of fashionable rebranding, this section reviews the use and abuse of the prefix nano and its role in creating the public image of nanotechnology.

Table 10 lists the terms with nano prefix that have been coined or have received high attention in recent years. Once in a while when different technologies pop up with a promise of betterment of the health care, some new terms appear in the scientific literature, for example, gene therapy, high throughput screening, tissue engineering, etc.[74] These terms represent a new technology or approach in medicine. The terms associated with nano lack precise definitions and nonuniformity in usage of these terms are often resulting in confusion.

With the advent of nanotechnology, being majorly multidisciplinary, many new terms associated with nano are being created. The prefix "nano" means, in the metric system, a billionth (10^{-9}) part, and a nanometer is 10^{-9} m. The prefix nano is based on the Greek word meaning dwarf, which became a scientific word in 1960. Presently, the prefix nano is associated with materials, technologies, phenomena, and approaches. Among the words with the nano prefix, the most popularized term is nanotechnology. Nanomedicine was coined a decade ago and popularized with the extensive research in the diagnosis and therapy.[82] Since its introduction, this word has gained a lot of attention in the scientific community as well as in the public. The popularity can be understood from the results obtained by the popular Internet search engine Google, which yields more than 22 million results for nanotechnology and 2 million for nanomedicine. Nanotoxicology is another word gaining importance with growing concerns about the occupational hazards, environmental, health, and safety (EHS) concerns. The number of scientific journals with the "nano" title and focus has also drastically increased in the past decade. One notable achievement of the term nano is that it is helping many divergent fields of science to converge. In our opinion, nanotechnology has brought more interdisciplinary research than any other technology or scientific advancement in recent years.

There is an increasing tendency to call all of the nanotherapeutics as nanoparticles, which is not appropriate. Liposomes, polymer drug conjugates, do not fit in the "nanoparticles" definition, but can be called as nanotherapeutics. The scientific usage of the terms should be more logical than routine usage, giving an accurate meaning. There is a great influence of funding agencies and regulatory authorities on defining the newly invented terms and technologies. With the advent of the nanotechnology, polymer science for drug delivery is also brought under the umbrella of nanomedicine. Polymer conjugates, polymeric micelles, and dendrimers are presently considered as nanotherapeutics. Dendrimers are discovered after the inception of nanotechnology, but the polymer drug conjugates existed before the dawn of the "nano" world.

"Engineered nanomaterials" is another word used widely in the safety and toxicology reports of nanotechnology. Engineered nanomaterials are the materials purposefully

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created with at least one orthogonal dimension in the range of 1−100 nm. The size range is not so rigid for the engineered nanomaterials; for example, fullerenes, although smaller than 1 nm, are considered as engineered nanomaterials.

Although the current scenario with nano disciplines fits in very well with the famous proverb "make hay while the sun shines" that is diluting the overall impact of this technology, there is a huge potential for this technology in several areas, and drug delivery could be the front runner in healthcare.

4.2. Approach To Be Embraced for Further Development of Nano Drug Delivery

Nanotechnological advancements in the fields of engineering and physics need to be applied to the maximum extent to harness the potential of nano drug delivery. To achieve this daunting task of making nano drug delivery closer to the clinical use, close collaborations among scientists of the whole spectrum of disciplines are needed. Nanotechnology is not a mere miniaturization to nanoscale; it is a revolution in physical concepts, design, and materials manufacturing, which is yet to be explored for nanomedicine. The potentials of this technology need to be explored with a view of dealing an unexplored regime at nanoscale rather than simply applying the known phenomenon of bulk agents to these nanoscale agents. It should bring novel methods to stabilize the nanoparticles, achieve control over the size and shape, and explain the biological interactions of nanoparticles.

Traditional cell culture studies are designed majorly to study the effects of soluble compounds. With the advent of nanotechnology, many colloids have been studied using cell culture studies, but there are many important factors that need to be taken into consideration while interpreting the results of studies carried out using nanoparticles. The solid particles do not behave as soluble agents, thus making it difficult for the expression of the appropriate dose. The effect of diffusion and sedimentation of nanoparticles was studied using cells grown on a coverslip inverted in the culture well and a upright culture plate and found that the gold nanoparticles internalization in cells is sedimentation dependent mainly; however, at smaller particle sizes there was no significant difference in the uptake of cells grown on the inverted coverslips or normal culture plates.[456] Particles also tend to agglomerate in the culture media, and the intake is influenced by the extent of agglomeration.[238] In vitro assays employing nanoparticles should nullify the effects of sedimentation rate in efficacy or toxicity studies so as to compare with other related studies.

Uptake of nanoparticles is endocytosis driven and thus varies among cell types. To date, uptake studies of nanotherapeutics using cells were differentiated as phagocytic cells and nonphagocytic cells. Nanoparticles have to cross a lot of cellular barriers to reach the target site, depicting a greater need of the studies to be carried out in a series of cells in the way of nanotherapeutics reaching the target cells.

The selection of a nano drug delivery system for a specific application is not well-defined, and among each nano delivery system the formulation has to be optimized to suit the application. Experimental evaluation of a variety of nano drug delivery systems employing different strategies seems to be a viable approach; however, the large number of experiments makes it difficult in the selection of the right formulation. Theoretical methods offer the benefits of screening wide range of formulations quickly. Theoretical basis for many of the nano delivery systems is under rapid development that should be embraced to the development of nano drug delivery.

Factors other than formulation characteristics, for example, the role of hypoxia, vascular permeability,[457] alterations in the rate of endocytosis,[101] etc., also need to be studied for the better understanding of the pharmacokinetics of nanotherapeutics. Some of these external factors might also be used for the passive targeting of the nanoparticles in specific disease conditions.

The emulsion evaporation method is the most widely reported method for the preparation of polymeric nanoparticles from preformed polymers. Routinely, centrifugation is used as a means to separate excessive surfactant or to concentrate the nanoparticles. Methods such as coacervate precipitation[366] can be explored for easy separation of the nanoparticles from the solution. Literature suggests rapid developments in the preparation of nanoparticles using a variety of techniques. Some of these methods need to be scaled up or made economical to be used for the industrial production of the nanoparticles. Centrifugation may not be feasible at large scale, and alternatives need to be worked out such as ultra filtration or dialysis; however, these methods are limited to very specific surfactants and drugs.[458,459]

The characterization of the nanoparticles should be made feasible to understand the behavior of the nanoparticles in the biological system. Various properties of the nanoparticles might be altered during the administration, distribution, and finally while eliciting the response. Thus, for a given formulation, it is essential to know its fate during different phases of the nanoparticles delivery to ensure the safety of the formulation and to increase the efficacy. As the nanotherapeutics alter the pharmacokinetics and dynamics of the drug encapsulated or attached, a thorough preclinical characterization is also necessary for these formulations. There are some instances where the nanoparticles interfere with the assays of routine examination; in such cases, an alternative methodology needs to be approached to carry out the assay. Above all, the methods have to be simple, reproducible, and scalable at affordable costs.

4.3. Nanotoxicology

Toxicity studies are a fundamental part of healthcare, environmental monitoring, drug discovery, and development, but "nanotoxicology" has gained an unprecedented focus,[460] and negative hype threatens to outgrow the positive.[85,461,462] Nanotoxicology can be divided into two major areas, environmental nanotoxicology and nanotoxicology associated with pharmaceutical agents.

4.3.1. Environmental Nanotoxicology. Special focus on money, time, and efforts is being dedicated to this new field. The major culprit that rang the bell ever since nano was reinvented is the carbon particle emission from automobiles. Cigarette smoke contains over 4000 chemicals of which about 50 are known carcinogens. Alcohol consumption and high fat diet controversially have been known to cause potential risk to individuals, but they are still widely accepted and used. Why is nano considered as the biggest threat to mankind?

Carbon nanotubes represent a major class of nanomaterials, and the safety assessment of nanotubes is essential for their commercialization and usage. The similarity of carbon nanotube toxicity to the asbestos toxicity was raised more than a decade ago. There are certain similarities and dissimilarities of carbon nanotubes to the asbestos fibers. The nanotube fibers' diameter and length are similar to asbestos,

and the chemistry and surface properties are different. Asbestos is known to cause mesothelioma, a cancer of the lining of the lungs and abdominal cavity. Direct injection of multiwalled carbon nanotubes into the peritoneal cavity has been shown to induce inflammation, granulomas,[262] and malignant mesothelioma after repeated administrations.[463] These studies have employed nanotubes with lengths between 10 and 20 μm. The long tubes are not completely engulfed by the macrophages, resulting in persistent free radical production resulting in toxicity. Although these studies hint towards toxicity of carbon nanotubes, the risk cannot be established because the nanotube penetration into the mesothelial lining is not understood completely, there is insufficient data indicating the biopersistance of nanotubes, and the studies employed sensitive animals. The nanotubes are still under the research phases, and thus manipulating the nanotube chemistry to avoid potential risks is thought to be a viable approach to avoid the fear of nanotoxicity;[261] this is more toward an academic satisfaction. A pubmed search with terms "carbon nanotubes drug delivery" revealed 409 reports, whereas terms "carbon nanotube toxicity" led to 570 and "nanoparticles drug delivery" resulted in 10 290 reports (December 21, 2011). Even though the use of carbon nanotubes in drug delivery is marginal with just under 4%, the hype this created is phenomenal, which academics should be responsible for.

The main reason for the scare about the nanoparticles is because their behavior in vivo is not fully understood and due to the speculations about their compatibility and degradation without firm experimental evidence. Some of the products of nanotechnology have been identified to be toxic, raising concerns about the safety of all of the nanomaterials.

The possible routes of nanomaterial exposure are presented in Figure 14. Inhalation and dermal routes are the most possible routes of unintended nanomaterial exposure. Some of the engineered nanomaterials are reported to be toxic if one is exposed to them through inhalation. Polymeric nanoparticles, which are degradable, are proven to show no irritancy through the pulmonary route. Concerns are raised about the toxicity of titanium dioxide nanoparticles, but the majority of the studies reported that skin acts as an efficient barrier for the nanomaterial penetration. Biopersistance and insolubility can be related to the toxicity and other risks of engineered nanomaterials.

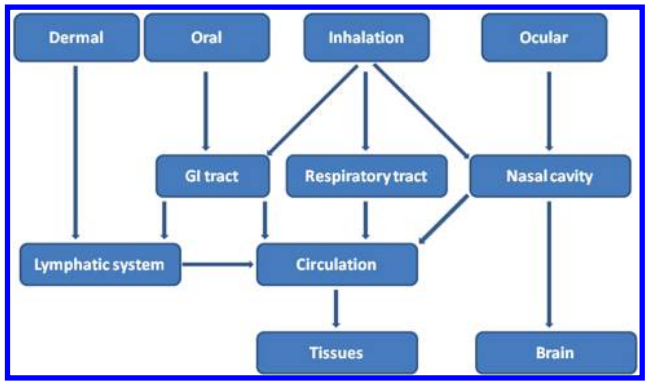

Figure 14. Possible routes of nanoparticle penetration and toxicity. Adapted from Yokel and Macphail.[464]

It is not fully understood how the nanoparticles can influence the human immune system. Some imagine nanoparticles as haptens, which can elicit immune response when attached to a protein; because of their small size, they might interact with proteins to modify their structure and impart antigenicity.[465] The long-term and subacute toxicity are other concerns that need to be addressed to establish the safety of the nanoparticles. For example, similarities of carbon nanotubes to asbestos are being identified raising the long-term toxicities associated with products of nanotechnology.[466]

It is very difficult to establish the animal models to address the environmental/occupational hazards of nanomaterials where a proper consideration of the dose, duration, and route of exposure is important. One of the ways to address this problem could be to compare the biochemical parameters such as blood markers (inflammation, oxidative stress, etc.) of those individuals who are exposed to such materials with those of the unexposed, while adapting appropriate precautions of using safety protection equipment such as masks, gloves, etc. that minimize the exposure if not completely avoid it. Maybe one should think of the people working in radioactive or chemical industries, where the risk is identified and known.

4.3.2. Nanotoxicology of Pharmaceuticals. It goes without saying that the medicines intended for treating diseases in living organisms should be safe, and safety is a relative term. In conventional medicines, the toxicity/safety is centric to the active principle ingredient with excipients being inert, and once safety/tolerance is established each time it is reformulated no safety studies are required. However, when an active principal ingredient is reformulated as nanomedicines or for that matter of fact any advanced delivery formulation that is intended for systemic/local administration and can alter the pharmacokinetics whether or not the excipients have an active role, they need to be proven safe.

The safety assessment guide for the small molecules is well established, and it basically covers the entire range of studies that can prove or disprove the safety of the compounds in question. The general toxicological studies include (i) short-term (nonregulatory) in rodents and nonrodents that include dosage range studies and maximum tolerated dose, the regulatory study is performed under the same conditions but for 1 month, and these data are to support the first clinical trial in humans, (ii) long-term (regulatory), which is typically up to 6 months in rodents and nonrodents to support phase II clinical trials in humans, and (iii) longer-term (regulatory) general toxicology studies typically up to 9 months in nonrodents and carcinogenicity studies for lifetime of rodents, to support phase III in humans. Genotoxicity in vitro and in vivo is also performed for the small molecules to detect relevant genetic changes leading to carcinogenic effects.

The safety assessment of nanomedicines is much easier and straightforward when compared to that of environmental/occupational hazard. If nanomaterials/nanomedicines are speculated to be too toxic or dangerous, and one would not risk going by conventional fashion of short-term, long-term, and longer-term, why not subject them to long-term and longer-term, which would establish their possible tolerance in humans and also warn if they are carcinogenic, which could be the biggest threat to humans? The biggest bottleneck for this as we see is the availability of the material.

The regulatory bodies are responding to academic speculations who do not favor the concept of nano, based on generalized concepts resulting in the uncertainty and chaos. Toxicity does not mean that the compound can pose a potential risk for environment or health of living beings.

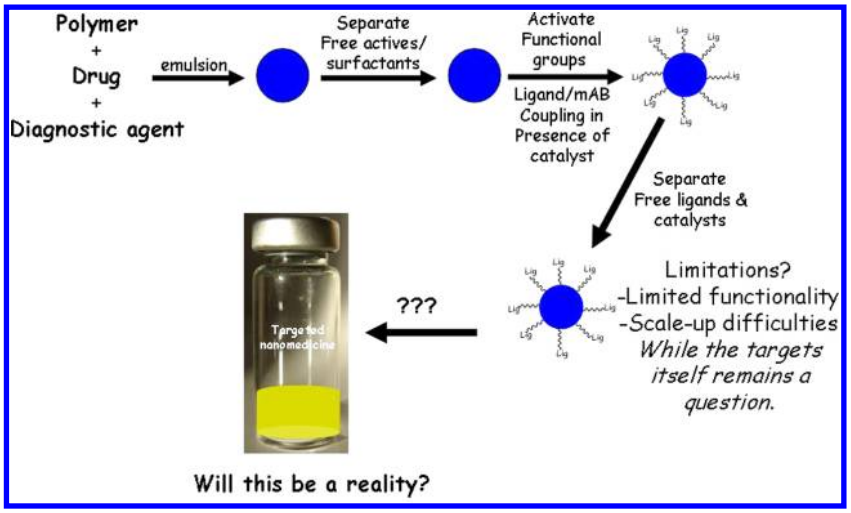

Figure 15. The current laboratory scale preparation scheme of targeted/theranostic polymer nanoparticles.

Toxicity is associated with any material at higher doses. It must be essential to understand the dose exposure of the material.

4.3.2.1. Do We Have Anything That Can Be Declared Completely Safe? The toxicity profile of the nanocarriers needs to be established in each case with a specific route or drug rather than general assumptions. It is necessary to determine the toxicity in the dose range, which is clinically relevant rather than using suboptimal or higher doses. It is not appropriate to classify a material as toxic or safe without mentioning the doses as there is no safe material over larger doses.

GRAS (generally recognized as safe) is a term designated to a specific material, for uses at specific doses and specific routes of administration by the FDA (food and drugs administration). Some of the materials that can be safely used orally or dermally might not be safer for intravenous use. Thus, the classification of a material toxic or safe without knowing the dose ranges and intended application might not make sense. Similarly, the toxicity or safety of nanomaterials should also have measures for size, shape, and surface properties of the materials. Above all, the same material used to formulate different drugs may also have different concentrations of the excipients associated with the dose given the loading and entrapment efficiency of the drug. So when everything in this world is relative and looked at risk versus benefit point of view, why is nano dealt with differently?

4.3.2.2. Criteria for Toxicology – Review of Types of Studies for Nano. The lack of suitable methods to assess the toxicity of nanoparticles has been identified by many scientists, and approaches to establish reliable techniques have been proposed. Establishing reliable techniques to make a new system of toxicology has been proposed. These include the assessment of currently available tools for nanomaterial testing, integrating various approaches to the testing strategies, and developing new tools for nanomaterials.[85,467]

Fear associated with nano is due to the fact that some of the novel nanomaterials persist for longer durations in the body and environment. Establishing the biodegradation times of the nanomaterials and classifying them might help understand the toxicity of nanomaterials. This might as well be size dependent for some materials.

Occasional attempts were made in the literature to understand the safety of the nanoparticles used in drug delivery, such as PLGA nanoparticles, but unfortunately those studies offer inadequate/no information on the safety, for one or more reasons: (i) dose not right as it is always decided by the drug and disease, (ii) duration not right, and (iii) using model compounds like dyes, or studies conducted by inhalation route using nanomaterials such as silver, which may not find application in drug delivery.[468,469]

Needless to say, realizing the potential of nanotechnology in medicines requires a multidisciplinary taskforce involving academia, industry, and regulatory agencies. These groups are realizing that many commonalities would exist in regulating conventional small molecule drugs products and nanomedicines.

4.4. Identity Crisis

Being different from others might offer better marketing strategies but not necessarily better therapeutic outcome, and this is witnessed by several products that are on market.

The important challenges encountered by the nanomedicine are not related to science or technology, but rather they are related to the communication, education, regulations, and business models.[15] The unique problem of nanotechnology is related to the linguistics. The scientific vocabulary in the field of nanotechnology is often confusing, and there is a great need for the legal terminology, which can be used for patenting and marketing these nanotherapeutics.[10,11,15] Maybe one can take this as an opportunity to revise the patenting laws as well by restricting the inventor's claims that can be substantiated by the data rather than speculations and covering a whole range of applications that may or may not be realized. This will save a lot of time and resources from being wasted in trying for more and more innovative solutions as everything has been covered by some existing inventions already.

Nanomedicine has started with overwhelming synthetic nanostructures without considering the biological consequences; however, the understanding of biocompatibility, biodegradability, and toxicity of the nanomaterials is increasing and leading the nanomedicine toward safer delivery systems. Degradable carbon nanotubes are also produced in this way, although the question of toxicity will still remain. While we still have not achieved considerable success with respect to first and second generation nanoparticles, we are moving at a rapid pace attempting to develop tissue or cell specific delivery systems, or theranostic particles that can detect and treat diseases, involving

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much more complicated preparation processes that are difficult to scale-up (Figure 15).

Environmental health and safety of nanomaterials from other fields of science and should not hinder the nanomedicine research. Nanomedicine can offer widespread benefits for the human health care, which might not be possible with other means.

Nanotechnology can indeed promise drug delivery; however, drug selection remains the key as not all drugs, and particularly those requiring high doses, can be delivered using nanoparticles. Understanding the pathological requirement is important in the design so that the delivery system meets the purpose, for example, sustained, controlled, pulsatile release, etc. A better understanding of the drug's physicochemical and biopharmaceutical properties will also help design a delivery system for alternative routes and indications. Therefore, it is very important to understand the drug and the disease well before a delivery system is designed, and this will hold the key to success. At this juncture, we better exploit the underexplored simple first and second generation nanosystems, before we get to the more complicated third and fourth generation nanosystems.

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REFERENCES

(1) Wang, Z. L. *Adv. Mater.* **2003**, *15*, 1497.
(2) de Jager, M.; van Noort, J. *Encyclopedia of Life Sciences*; John Wiley & Sons Ltd.: New York, 2007.
(3) Lövestam, G.; Rauscher, H.; Roebben, G.; Klüttgen, B. S.; Gibson, N.; Putaud, J.-P.; Stamm, H. *European Commission Joint Research Centre Reference Report*, 2010.
(4) Feynman, R. P. *Miniaturization*; Reinhold Publishing Corp.: New York, 1961.
(5) Bath, J.; Turberfield, A. J. *Nat. Nanotechnol.* **2007**, *2*, 275.
(6) Porter, A. L.; Youtie, J. *Nat. Nanotechnol.* **2009**, *4*, 534.
(7) Min, Y.; Akbulut, M.; Kristiansen, K.; Golan, Y.; Israelachvili, J. *Nat. Mater.* **2008**, *7*, 527.
(8) Schmid, G.; Decker, M.; Ernst, H.; Fuchs, H.; Grünwald, W.; Grunwald, A.; Hofmann, H.; Mayor, M.; Rathgeber, W.; Simon, U.; Wyrwa, D. *Graue Reihe* **2003**, *35*, 1.
(9) The British Standards Institution (BSi). *Publicly Available Specification (PAS)*, 2007; Vol. *136*, p 1.
(10) Toumey, C. *Nat. Nanotechnol.* **2011**, *6*, 393.
(11) Balogh, L. P. *Nanomedicine* **2010**, *6*, 397.

(12) Auffan, M.; Rose, J.; Bottero, J.-Y.; Lowry, G. V.; Jolivet, J.-P.; Wiesner, M. R. *Nat. Nanotechnol.* **2009**, *4*, 634.
(13) National Institutes of Health (NIH). *NIH Roadmap for Medical Research: Nanomedicine*, 2006; https://commonfund.nih.gov/nanomedicine/.
(14) Kreuter, J. *Encyclopedia of Pharmaceutical Technology*; Marcel Dekker: New York, 1994; p 165.
(15) Balogh, L. P. *Nanomedicine in Health and Disease*; Taylor & Francis Inc.: U.S., 2011.
(16) Glänzel, W.; Meyer, M.; Plessis, M. D.; Thijs, B.; Magerman, T.; Schlemmer, B.; Debackere, K.; Veugelers, R. *Nanotechnology-Analysis of an Emerging Domain of Scientific and Technological Endeavour*; Steunpunt O & O Statistieken, K. U. Leuven, 2003.
(17) Mogoutov, A.; Kahane, B. *Research Policy* **2007**, *36*, 893.
(18) Noyons, E.; Buter, R.; Raan, A. v.; Schmoch, U.; Heinze, T.; Hinze, S.; Rangnow, R. *Mapping Excellence in Science and Technology across Europe Nanoscience and Nanotechnology*; European Commission, 2003.
(19) Porter, A.; Youtie, J.; Shapira, P.; Schoeneck, D. *J. Nanopart. Res.* **2008**, *10*, 715.
(20) Huang, C.; Notten, A.; Rasters, N. *J. Technol. Transfer* **2010**, *36*, 145.
(21) Guan, J.; Ma, N. *Research Policy* **2007**, *36*, 880.
(22) Youtie, J.; Shapira, P.; Porter, A. *J. Nanopart. Res.* **2008**, *10*, 981.
(23) Kostoff, R.; Stump, J.; Johnson, D.; Murday, J.; Lau, C.; Tolles, W. *J. Nanopart. Res.* **2006**, *8*, 301.
(24) Kostoff, R.; Koytcheff, R.; Lau, C. *Scientometrics* **2007**, *70*, 565.
(25) Kostoff, R.; Koytcheff, R.; Lau, C. *J. Nanopart. Res.* **2007**, *9*, 701.
(26) Kostoff, R.; Koytcheff, R.; Lau, C. *J. Technol. Transfer* **2008**, *33*, 472.
(27) Chrzeszczyk, A.; Wishnia, A.; Springer, C. *Biochim. Biophys. Acta* **1977**, *470*, 161.
(28) Beese, L.; Stubbs, G.; Cohen, C. *J. Mol. Biol.* **1987**, *194*, 257.
(29) Claude, A.; Fullam, E. *J. Exp. Med.* **1945**, *81*, 51.
(30) Moore, P. *Biochemistry* **2001**, *40*, 3243.
(31) Schmidt, D. *Proteins*; Applied Science Publishers: London, 1982; p 61.
(32) Phadungath, C. *Songklanakarin J. Sci. Technol.* **2005**, *27*, 201.
(33) Hernell, O.; Staggers, J. E.; Carey, M. C. *Biochemistry* **1990**, *29*, 2041.
(34) Huckriede, A.; Bungener, L.; Daemen, T.; Wilschut, J. *Methods Enzymol.* **2003**, *373*, 74.
(35) Kaneda, Y. *Adv. Drug Delivery Rev.* **2000**, *43*, 197.
(36) Clardy, J.; Walsh, C. *Nature* **2004**, *432*, 829.
(37) Stephen, A.; Raval-Fernandes, S.; Huynh, T.; Torres, M.; Kickhoefer, V.; Rome, L. *J. Biol. Chem.* **2001**, *276*, 23217.
(38) Brunner, J. R. *Food Proteins*; AVI Publishing Co. Inc.: U.S., 1977; p 175.
(39) He, X. M.; Carter, D. C. *Nature* **1992**, *358*, 209.
(40) Pillai, O.; Dhanikula, A. B.; Panchagnula, R. *Curr. Opin. Chem. Biol.* **2001**, *5*, 439.
(41) Rosen, H.; Abribat, T. *Nat. Rev. Drug Discovery* **2005**, *4*, 381.
(42) Balmayor, E. R.; Azevedo, H. S.; Reis, R. L. *Pharm. Res.* **2011**, *28*, 1241.
(43) Tan, S. Y.; Grimes, S. *Singapore Med. J.* **2010**, *51*, 842.
(44) Lipinski, C.; Hopkins, A. *Nature* **2004**, *432*, 855.
(45) Merisko-Liversidge, E.; Liversidge, G. G. *Adv. Drug Delivery Rev.* **2011**, *63*, 427.
(46) Allen, T. M.; Cullis, P. R. *Science* **2004**, *303*, 1818.
(47) Kante, B.; Couvreur, P.; Lenaerts, V.; Guiot, P.; Roland, M.; Baudhuin, P.; Speiser, P. *Int. J. Pharm.* **1980**, *7*, 45.
(48) Couvreur, P.; Tulkens, P.; Roland, M. *FEBS Lett.* **1977**, *84*, 323.
(49) Moghimi, S. M.; Kissel, T. *Adv. Drug Delivery Rev.* **2006**, *58*, 1451.
(50) Hanes, J. *Nanomedicine* **2006**, *2*, 273.
(51) Alonso, M. J. *Biomed. Pharmacother.* **2004**, *58*, 168.
(52) Duncan, R. *Mater. Today* **2005**, *8*, 16.
(53) Gultepe, E.; Nagesha, D.; Sridhar, S.; Amiji, M. *Adv. Drug Delivery Rev.* **2010**, *62*, 305.
(54) Torchilin, V. P. *Nat. Rev. Drug Discovery* **2005**, *4*, 145.
(55) Farokhzad, O. C.; Langer, R. *ACS Nano* **2009**, *3*, 16.
(56) Zhang, J.; Wu, L.; Chan, H. K.; Watanabe, W. *Adv. Drug Delivery Rev.* **2011**, *63*, 441.
(57) Yanez, J. A.; Wang, S. W.; Knemeyer, I. W.; Wirth, M. A.; Alton, K. B. *Adv. Drug Delivery Rev.* **2011**, *63*, 923.
(58) Lebhardt, T.; Roesler, S.; Uusitalo, H. P.; Kissel, T. *Eur. J. Pharm. Biopharm.* **2011**, *78*, 90.
(59) Plapied, L.; Duhem, N.; des Rieux, A.; Préat, V. *Curr. Opin. Colloid Interface Sci.* **2011**, *16*, 228.
(60) Papakostas, D.; Rancan, F.; Sterry, W.; Blume-Peytavi, U.; Vogt, A. *Arch. Dermatol. Res.* **2011**, *303*, 533.
(61) Marie-Alexandrine, B.; Stephanie, B.; Yves, C. *WIREs Nanomed. Nanobiotechnol.* **2011**, *3*, 463.
(62) Prow, T. W.; Grice, J. E.; Lin, L. L.; Faye, R.; Butler, M.; Becker, W.; Wurm, E. M.; Yoong, C.; Robertson, T. A.; Soyer, H. P.; Roberts, M. S. *Adv. Drug Delivery Rev.* **2011**, *63*, 470.
(63) Csaba, N.; Garcia-Fuentes, M.; Alonso, M. J. *Expert Opin. Drug Delivery* **2006**, *3*, 463.
(64) Bangham, A. D.; Horne, R. W. *J. Mol. Biol.* **1964**, *8*, 660.
(65) Kreuter, J.; Speiser, P. P. *J. Pharm. Sci.* **1976**, *65*, 1624.
(66) Daniel, M.-C.; Astruc, D. *Chem. Rev.* **2003**, *104*, 293.
(67) Khanna, S. C.; Jecklin, T.; Speiser, P. *J. Pharm. Sci.* **1970**, *59*, 614.
(68) Khanna, S. C.; Speiser, P. *J. Pharm. Sci.* **1969**, *58*, 1114.
(69) Merkle, H. P.; Speiser, P. *J. Pharm. Sci.* **1973**, *62*, 1444.
(70) Birrenbach, G.; Speiser, P. P. *J. Pharm. Sci.* **1976**, *65*, 1763.
(71) Benacerraf, B.; Bilbey, D.; Biozzi, G.; Halpern, B. N.; Stiffel, C. *J. Physiol.* **1957**, *136*, 287.
(72) Scheffel, U.; Rhodes, B. A.; Natarajan, T. K.; Wagner, H. N., Jr. *J. Nucl. Med.* **1973**, *13*, 498.
(73) Kramer, P. A. *J. Pharm. Sci.* **1974**, *63*, 1646.
(74) Park, K. *J. Controlled Release* **2007**, *120*, 1.
(75) Shi, J.; Votruba, A. R.; Farokhzad, O. C.; Langer, R. *Nano Lett.* **2010**, *10*, 3223.
(76) Zhang, L.; Gu, F. X.; Chan, J. M.; Wang, A. Z.; Langer, R. S.; Farokhzad, O. C. *Clin. Pharmacol. Ther.* **2007**, *83*, 761.
(77) Davis, M. E.; Chen, Z.; Shin, D. M. *Nat. Rev. Drug Discovery* **2008**, *7*, 771.
(78) Riehemann, K.; Schneider, S. W.; Luger, T. A.; Godin, B.; Ferrari, M.; Fuchs, H. *Angew. Chem., Int. Ed.* **2009**, *48*, 872.
(79) Matsumura, Y.; Maeda, H. *Cancer Res.* **1986**, *46*, 6387.
(80) Romberg, B.; Hennink, W.; Storm, G. *Pharm. Res.* **2008**, *25*, 55.
(81) Harris, J. M.; Chess, R. B. *Nat. Rev. Drug Discovery* **2003**, *2*, 214.
(82) Wagner, V.; Dullaart, A.; Bock, A.-K.; Zweck, A. *Nat. Biotechnol.* **2006**, *24*, 1211.
(83) Hodes, G. *Adv. Mater.* **2007**, *19*, 639.
(84) Lidén, G. *Ann. Occup. Hyg.* **2011**, *55*, 1.
(85) Krug, H.; Wick, P. *Angew. Chem., Int. Ed.* **2011**, *50*, 1260.
(86) Müller, R. H.; Peters, K. *Int. J. Pharm.* **1998**, *160*, 229.
(87) Van Eerdenbrugh, B.; Vermant, J.; Martens, J. A.; Froyen, L.; Humbeeck, J. V.; Van den Mooter, G.; Augustijns, P. *Mol. Pharmaceut.* **2010**, *7*, 1858.
(88) Kakran, M.; Sahoo, N. G.; Shegokar, R.; Al Shaal, L.; Li, L.; Muller, R. H. *Eur. J. Pharm. Biopharm.* **2012**, *80*, 113.
(89) Wu, Y.; Loper, A.; Landis, E.; Hettrick, L.; Novak, L.; Lynn, K.; Chen, C.; Thompson, K.; Higgins, R.; Batra, U.; Shelukar, S.; Kwei, G.; Storey, D. *Int. J. Pharm.* **2004**, *285*, 135.
(90) Alexis, F.; Pridgen, E.; Molnar, L.; Farokhzad, O. *Mol. Pharmaceut.* **2008**, *5*, 505.
(91) Scherphof, G. *Adv. Drug Delivery Rev.* **1998**, *32*, 81.
(92) Chrastina, A.; Massey, K. A.; Schnitzer, J. E. *WIREs Nanomed. Nanobiotechnol.* **2011**, *3*, 421.
(93) Ma, X.; Wu, Y.; Jin, S.; Tian, Y.; Zhang, X.; Zhao, Y.; Yu, L.; Liang, X. J. *ACS Nano* **2011**, *5*, 8629.
(94) Gratton, S. E. A.; Ropp, P. A.; Pohlhaus, P. D.; Luft, J. C.; Madden, V. J.; Napier, M. E.; DeSimone, J. M. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 11613.

(95) Cartiera, M. S.; Johnson, K. M.; Rajendran, V.; Caplan, M. J.; Saltzman, W. M. *Biomaterials* **2009**, *30*, 2790.
(96) Iversen, T. G.; Skotland, T.; Sandvig, K. *Nano Today* **2011**, *6*, 176.
(97) Kelf, T.; Sreenivasan, V.; Sun, J.; Kim, E.; Goldys, E.; Zvyagin, A. *Nanotechnology* **2010**, *21*, 285105.
(98) Champion, J. A.; Mitragotri, S. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 4930.
(99) Champion, J. A.; Walker, A.; Mitragotri, S. *Pharm. Res.* **2008**, *25*, 1815.
(100) Sahay, G.; Alakhova, D.; Kabanov, A. *J. Controlled Release* **2010**, *145*, 182.
(101) Wang, Y.; Roche, O.; Yan, M. S.; Finak, G.; Evans, A. J.; Metcalf, J. L.; Hast, B. E.; Hanna, S. C.; Wondergem, B.; Furge, K. A.; Irwin, M. S.; Kim, W. Y.; Teh, B. T.; Grinstein, S.; Park, M.; Marsden, P. A.; Ohh, M. *Nat. Med.* **2009**, *15*, 319.
(102) Chow, A.; Brown, B.; Merad, M. *Nat. Rev. Immunol.* **2011**, *11*, 788.
(103) Dobrovolskaia, M. A.; Aggarwal, P.; Hall, J. B.; McNeil, S. E. *Mol. Pharmaceut.* **2008**, *5*, 487.
(104) Owens, D. E., III; Peppas, N. A. *Int. J. Pharm.* **2006**, *307*, 93.
(105) Moghimi, S. M.; Szebeni, J. *Prog. Lipid Res.* **2003**, *42*, 463.
(106) Almeida, J. P.; Chen, A. L.; Foster, A.; Drezek, R. *Nanomedicine* **2011**, *6*, 815.
(107) Moghimi, S.; Hunter, A.; Murray, J. *Pharmacol. Rev.* **2001**, *53*, 283.
(108) Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. *J. Controlled Release* **2000**, *65*, 271.
(109) Nishioka, Y.; Yoshino, H. *Adv. Drug Delivery Rev.* **2001**, *47*, 55.
(110) Reddy, S.; van der Vlies, A.; Simeoni, E.; Angeli, V.; Randolph, G.; O'Neil, C.; Lee, L.; Swartz, M.; Hubbell, J. *Nat. Biotechnol.* **2007**, *25*, 1159.
(111) Moghimi, S. M. *FEBS Lett.* **2003**, *540*, 241.
(112) Crater, J. S.; Carrier, R. L. *Macromol. Biosci.* **2010**, *10*, 1473.
(113) Gouyer, V.; Gottrand, F.; Desseyn, J.-L. *PLoS One* **2011**, *6*, e18761.
(114) Matsuo, K.; Ota, H.; Akamatsu, T.; Sugiyama, A.; Katsuyama, T. *Gut* **1997**, *40*, 782.
(115) Pullan, R. D.; Thomas, G. A.; Rhodes, M.; Newcombe, R. G.; Williams, G. T.; Allen, A.; Rhodes, J. *Gut* **1994**, *35*, 353.
(116) Lai, S. K.; Wang, Y.-Y.; Hanes, J. *Adv. Drug Delivery Rev.* **2009**, *61*, 158.
(117) Cone, R. A. *Adv. Drug Delivery Rev.* **2009**, *61*, 75.
(118) Florence, A. T. *Drug Discovery Today* **2005**, *2*, 75.
(119) Florence, A. T.; Hussain, N. *Adv. Drug Delivery Rev.* **2001**, *50*, S69.
(120) Eldridge, J. H.; Hammond, C. J.; Meulbroek, J. A.; Staas, J. K.; Gilley, R. M.; Tice, T. R. *J. Controlled Release* **1990**, *11*, 205.
(121) Awaad, A.; Nakamura, M.; Ishimura, K. *Nanomedicine* **2011**, *8*, 627.
(122) Jani, P.; Halbert, G. W.; Langridge, J.; Florence, A. T. *J. Pharm. Pharmacol.* **1990**, *42*, 821.
(123) Meaney, C.; O'Driscoll, C. *Eur. J. Pharm. Sci.* **1999**, *8*, 167.
(124) Lehr, C. M.; Lee, V. H. L. *Pharm. Res.* **1993**, *10*, 1796.
(125) Rosenthal, S. J.; Tomlinson, I.; Adkins, E. M.; Schroeter, S.; Adams, S.; Swafford, L.; McBride, J.; Wang, Y.; DeFelice, L. J.; Blakely, R. D. *J. Am. Chem. Soc.* **2002**, *124*, 4586.
(126) Roger, E.; Lagarce, F.; Garcion, E.; Benoit, J.-P. *Nanomedicine* **2010**, *5*, 287.
(127) Patton, J. S.; Byron, P. R. *Nat. Rev. Drug Discovery* **2007**, *6*, 67.
(128) Brain, J. D. *Diabetes Technol. Ther.* **2007**, *9*, S4.
(129) Bailey, M. M.; Berkland, C. J. *Med. Res. Rev.* **2009**, *29*, 196.
(130) Schmid, O.; Möller, W.; Semmler-Behnke, M.; Ferron, G.; Karg, E.; Lipka, J.; Schulz, H.; Kreyling, W.; Stoeger, T. *Biomarkers* **2009**, *14*, 67.
(131) Kreyling, W. G.; Semmler-Behnke, M.; Möller, W. *J. Aerosol Med.* **2006**, *19*, 74.
(132) Michinaka, Y.; Mitragotri, S. *J. Controlled Release* **2011**, *153*, 249.
(133) Dong, Q.; Hurst, D.; Weinmann, H.; Chenevert, T.; Londy, F.; Prince, M. *Invest. Radiol.* **1998**, *33*, 699.
(134) Sato, N.; Kobayashi, H.; Hiraga, A.; Saga, T.; Togashi, K.; Konishi, J.; Brechbiel, M. W. *Magn. Reson. Med.* **2001**, *46*, 1169.
(135) Kobayashi, H.; Brechbiel, M. *Adv. Drug Delivery Rev.* **2005**, *57*, 2271.
(136) Fang, C.; Shi, B.; Pei, Y. Y.; Hong, M. H.; Wu, J.; Chen, H. Z. *Eur. J. Pharm. Sci.* **2006**, *27*, 27.
(137) Moghimi, S. M.; Hedeman, H.; Muir, I. S.; Illum, L.; Davis, S. S. *Biochim. Biophys. Acta* **1993**, *1157*, 233.
(138) Liu, D.; Mori, A.; Huang, L. *Biochim. Biophys. Acta* **1992**, *1104*, 95.
(139) Sun, X.; Rossin, R.; Turner, J.; Becker, M.; Joralemon, M.; Welch, M.; Wooley, K. *Biomacromolecules* **2005**, *6*, 2541.
(140) Yang, Z.; Leon, J.; Martin, M.; Harder, J.; Zhang, R.; Liang, D.; Lu, W.; Tian, M.; Gelovani, J.; Qiao, A.; Li, C. *Nanotechnology* **2009**, *20*, 165101.
(141) Choi, H.; Liu, W.; Misra, P.; Tanaka, E.; Zimmer, J.; Itty Ipe, B.; Bawendi, M.; Frangioni, J. *Nat. Biotechnol.* **2007**, *25*, 1165.
(142) De Jong, W.; Hagens, W.; Krystek, P.; Burger, M.; Sips, A.; Geertsma, R. *Biomaterials* **2008**, *29*, 1912.
(143) Fang, J.; Sawa, T.; Maeda, H. *Adv. Exp. Med. Biol.* **2003**, *519*, 29.
(144) Iyer, A. K.; Khaled, G.; Fang, J.; Maeda, H. *Drug Discovery Today* **2006**, *11*, 812.
(145) Maeda, H. *Bioconjugate Chem.* **2010**, *21*, 797.
(146) Gabizon, A.; Shmeeda, H.; Grenader, T. *Eur. J. Pharm. Sci.* **2011**, *45*, 388.
(147) Maeda, H. *Adv. Drug Delivery Rev.* **2001**, *46*, 169.
(148) Folkman, J. *Nat. Med.* **1995**, *1*, 27.
(149) Folkman, J. *J. Natl. Cancer Inst.* **1990**, *82*, 4.
(150) Daruwalla, J.; Greish, K.; Malcontenti-Wilson, C.; Muralidharan, V.; Iyer, A.; Maeda, H.; Christophi, C. *J. Vasc. Res.* **2009**, *46*, 218.
(151) Maeda, H.; Bharate, G. Y.; Daruwalla, J. *Eur. J. Pharm. Biopharm.* **2009**, *71*, 409.
(152) McDonald, D. M.; Choyke, P. L. *Nat. Med.* **2003**, *9*, 713.
(153) Narang, A. S.; Varia, S. *Adv. Drug Delivery Rev.* **2011**, *63*, 640.
(154) Fang, J.; Nakamura, H.; Maeda, H. *Adv. Drug Delivery Rev.* **2011**, *63*, 136.
(155) Ahlstrom, H.; Christofferson, R.; Lorelius, L. E. *APMIS* **1988**, *96*, 701.
(156) Maeda, H. *J. Controlled Release* **1992**, *19*, 315.
(157) Yuan, F.; Dellian, M.; Fukumura, D.; Leunig, M.; Berk, D. A.; Torchilin, V. P.; Jain, R. K. *Cancer Res.* **1995**, *55*, 3752.
(158) Misra, R.; Acharya, S.; Sahoo, S. K. *Drug Discovery Today* **2010**, *15*, 842.
(159) Duncan, R. *Pharm. Sci. Technol. Today* **1999**, *2*, 441.
(160) Danhier, F.; Feron, O.; Preat, V. *J. Controlled Release* **2010**, *148*, 135.
(161) Greish, K.; Fang, J.; Inutsuka, T.; Nagamitsu, A.; Maeda, H. *Clin. Pharmacokinet.* **2003**, *42*, 1089.
(162) Seki, T.; Fang, J.; Maeda, H. *Cancer Sci.* **2009**, *100*, 2426.
(163) Torchilin, V. *Adv. Drug Delivery Rev.* **2011**, *63*, 131.
(164) Jain, K. K. *BMC Med.* **2010**, *8*, 83.
(165) Boerman, O. C.; Dams, E. T.; Oyen, W. J.; Corstens, F. H.; Storm, G. *Inflammation Res.* **2001**, *50*, 55.
(166) Simon, G. H.; von Vopelius-Feldt, J.; Fu, Y.; Schlegel, J.; Pinotek, G.; Wendland, M. F.; Chen, M. H.; Daldrup-Link, H. E. *Invest. Radiol.* **2006**, *41*, 45.
(167) Florence, A. T. *Pharm. Res.* **1997**, *14*, 259.
(168) Sakuma, S.; Hayashi, M.; Akashi, M. *Adv. Drug Delivery Rev.* **2001**, *47*, 21.
(169) Mathiowitz, E.; Jacob, J. S.; Jong, Y. S.; Carino, G. P.; Chickering, D. E.; Chaturvedi, P.; Santos, C. A.; Vijayraghavan, K.; Montgomery, S.; Bassett, M.; Morrell, C. *Nature* **1997**, *386*, 410.
(170) Desai, N.; Trieu, V.; Yao, R.; Labao, E.; De, T.; Soon-Shiong, P. *EJC Suppl.* **2004**, *2*, 182.

(171) Ruenraroengsak, P.; Cook, J. M.; Florence, A. T. *J. Controlled Release* **2010**, *141*, 265.
(172) Sahana, D.; Mittal, G.; Bhardwaj, V.; Kumar, M. N. V. R. *J. Pharm. Sci.* **2008**, *97*, 1530.
(173) Mittal, G.; Sahana, D. K.; Bhardwaj, V.; Kumar, M. N. V. R. *J. Controlled Release* **2007**, *119*, 77.
(174) Colvin, V. L. *Nat. Biotechnol.* **2003**, *21*, 1166.
(175) Dobrovolskaia, M.; Patri, A.; Simak, J.; Hall, J.; Semberova, J.; de Paoli Lacerda, S. *Mol. Pharmaceut.* **2012**, *9*, 382.
(176) Venuganti, V.; Sahdev, P.; Hildreth, M.; Guan, X.; Perumal, O. *Pharm. Res.* **2011**, *28*, 2246.
(177) Muller, R. H.; Jacobs, C.; Kayser, O. *Adv. Drug Delivery Rev.* **2001**, *47*, 3.
(178) Lai, S. K.; O'Hanlon, D. E.; Harrold, S.; Man, S. T.; Wang, Y. Y.; Cone, R.; Hanes, J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 1482.
(179) Choi, C. H. J.; Zuckerman, J. E.; Webster, P.; Davis, M. E. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 6656.
(180) Lynch, I.; Salvati, A.; Dawson, K. A. *Nat. Nanotechnol.* **2009**, *4*, 546.
(181) Foldvari, M.; Bagonluri, M. *Nanomedicine* **2008**, *4*, 183.
(182) Goldberg, M.; Langer, R.; Jia, X. *J. Biomater. Sci., Polym. Ed.* **2007**, *18*, 241.
(183) Duncan, R. *Curr. Opin. Biotechnol.* **2011**, *22*, 492.
(184) Shenoy, D.; Little, S.; Langer, R.; Amiji, M. *Pharm. Res.* **2005**, *22*, 2107.
(185) Murthy, K. S.; Ma, Q.; Remsen, E. E.; Wooley, K. L. *J. Mater. Chem.* **2003**, *13*, 2785.
(186) Toda, M.; Kitazawa, T.; Hirata, I.; Hirano, Y.; Iwata, H. *Biomaterials* **2008**, *29*, 407.
(187) Hirata, I.; Hioki, Y.; Toda, M.; Kitazawa, T.; Murakami, Y.; Kitano, E.; Kitamura, H.; Ikada, Y.; Iwata, H. *J. Biomed. Mater. Res., Part A* **2003**, *66*, 669.
(188) Takeoka, S.; Okamura, Y.; Teramura, Y.; Watanabe, N.; Suzuki, H.; Tsuchida, E.; Handa, M.; Ikeda, Y. *Biochem. Biophys. Res. Commun.* **2003**, *312*, 773.
(189) Lay, J.; Stang, M.; Fisher, P.; Yankaskas, J.; Bennett, W. *J. Aerosol Med.* **2003**, *16*, 153.
(190) Pardeike, J.; Müller, R. H. *Int. J. Pharm.* **2010**, *391*, 322.
(191) Tang, Y. J.; Ashcroft, J. M.; Chen, D.; Min, G.; Kim, C. H.; Murkhejee, B.; Larabell, C.; Keasling, J. D.; Chen, F. F. *Nano Lett.* **2007**, *7*, 754.
(192) Marchesan, S.; Da Ros, T.; Spalluto, G.; Balzarini, J.; Prato, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3615.
(193) Kim, J. S.; Kuk, E.; Yu, K. N.; Kim, J. H.; Park, S. J.; Lee, H. J.; Kim, S. H.; Park, Y. K.; Park, Y. H.; Hwang, C. Y.; Kim, Y. K.; Lee, Y. S.; Jeong, D. H.; Cho, M. H. *Nanomedicine* **2007**, *3*, 95.
(194) Li, W. R.; Xie, X. B.; Shi, Q. S.; Zeng, H. Y.; Ou-Yang, Y. S.; Chen, Y. B. *Appl. Microbiol. Biotechnol. Adv.* **2010**, *85*, 1115.
(195) Irache, J. M.; Merodio, M.; Arnedo, A.; Camapanero, M. A.; Mirshahi, M.; Espuelas, S. *Mini-Rev. Med. Chem.* **2005**, *5*, 293.
(196) Weber, C.; Coester, C.; Kreuter, J.; Langer, K. *Int. J. Pharm.* **2000**, *194*, 91.
(197) Arnedo, A.; Espuelas, S.; Irache, J. M. *Int. J. Pharm.* **2002**, *244*, 59.
(198) Zong, Y.; Guo, J.; Ke, T.; Mohs, A. M.; Parker, D. L.; Lu, Z. R. *J. Controlled Release* **2006**, *112*, 350.
(199) Auguste, D. T.; Armes, S. P.; Brzezinska, K. R.; Deming, T. J.; Kohn, J.; Prud'homme, R. K. *Biomaterials* **2006**, *27*, 2599.
(200) Vivero-Escoto, J. L.; Slowing, I.; Wu, C. W.; Lin, V. S. *J. Am. Chem. Soc.* **2009**, *131*, 3462.
(201) Roser, M.; Fischer, D.; Kissel, T. *Eur. J. Pharm. Biopharm.* **1998**, *46*, 255.
(202) Xiao, K.; Li, Y.; Luo, J.; Lee, J. S.; Xiao, W.; Gonik, A. M.; Agarwal, R. G.; Lam, K. S. *Biomaterials* **2011**, *32*, 3435.
(203) He, C.; Hu, Y.; Yin, L.; Tang, C.; Yin, C. *Biomaterials* **2010**, *31*, 3657.
(204) Kim, B.; Han, G.; Toley, B. J.; Kim, C. K.; Rotello, V. M.; Forbes, N. S. *Nat. Nanotechnol.* **2010**, *5*, 465.
(205) Stylianopoulos, T.; Poh, M. Z.; Insin, N.; Bawendi, M. G.; Fukumura, D.; Munn, L. L.; Jain, R. K. *Biophys. J.* **2010**, *99*, 1342.
(206) Slowing, I.; Trewyn, B. G.; Lin, V. S. Y. *J. Am. Chem. Soc.* **2006**, *128*, 14792.
(207) Bye, E.; Davies, R.; Griffiths, D. M.; Gylseth, B.; Moncrieff, C. B. *Br. J. Ind. Med. Chem.* **1984**, *41*, 228.
(208) Roelofs, F.; Vogelsberger, W. *J. Phys. Chem. B* **2004**, *108*, 11308.
(209) Borm, P.; Klaessig, F. C.; Landry, T. D.; Moudgil, B.; Pauluhn, J.; Thomas, K.; Trottier, R.; Wood, S. *Toxicol. Sci.* **2006**, *90*, 23.
(210) Parsons, G. E.; Buckton, G.; Chatham, S. M. *Int. J. Pharm.* **1992**, *83*, 163.
(211) Lee, J.; Choi, J. Y.; Park, C. H. *Int. J. Pharm.* **2008**, *355*, 328.
(212) Mishra, P. R.; Al Shaal, L.; Muller, R. H.; Keck, C. M. *Int. J. Pharm.* **2009**, *371*, 182.
(213) Baba, K.; Pudavar, H. E.; Roy, I.; Ohulchanskyy, T. Y.; Chen, Y.; Pandey, R. K.; Prasad, P. N. *Mol. Pharmaceut.* **2007**, *4*, 289.
(214) Tang, B. C.; Dawson, M.; Lai, S. K.; Wang, Y. Y.; Suk, J. S.; Yang, M.; Zeitlin, P.; Boyle, M. P.; Fu, J.; Hanes, J. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 19268.
(215) Bures, P.; Huang, Y.; Oral, E.; Peppas, N. A. *J. Controlled Release* **2001**, *72*, 25.
(216) Yoncheva, K.; Gómez, S.; Campanero, M. A.; Gamazo, C.; Irache, J. M. *Expert Opin. Drug Delivery* **2005**, *2*, 205.
(217) Wang, Y. Y.; Lai, S. K.; Suk, J. S.; Pace, A.; Cone, R.; Hanes, J. *Angew. Chem., Int. Ed.* **2008**, *47*, 9726.
(218) Mert, O.; Lai, S. K.; Ensign, L.; Yang, M.; Wang, Y. Y.; Wood, J.; Hanes, J. *J. Controlled Release* **2012**, *157*, 455.
(219) Kingshott, P.; Griesser, H. J. *Curr. Opin. Solid State Mater. Sci.* **1999**, *4*, 403.
(220) Pereverzeva, E.; Treschalin, I.; Bodyagin, D.; Maksimenko, O.; Langer, K.; Dreis, S.; Asmussen, B.; Kreuter, J.; Gelperina, S. *Int. J. Pharm.* **2007**, *337*, 346.
(221) Zucchi, R.; Danesi, R. *Curr. Med. Chem.: Anti-Cancer Agents* **2003**, *3*, 151.
(222) Elzoghby, A. O.; Samy, W. M.; Elgindy, N. A. *J. Controlled Release* **2012**, *157*, 168.
(223) Russell, L. D.; Russell, J. A. *Am. J. Anat.* **1991**, *192*, 142.
(224) Engstrom, J. D.; Tam, J. M.; Miller, M. A.; Williams, R. O., III; Johnston, K. P. *Pharm. Res.* **2009**, *26*, 101.
(225) Sharma, G.; Valenta, D. T.; Altman, Y.; Harvey, S.; Xie, H.; Mitragotri, S.; Smith, J. W. *J. Controlled Release* **2010**, *147*, 408.
(226) Champion, J. A.; Katare, Y. K.; Mitragotri, S. *J. Controlled Release* **2007**, *121*, 3.
(227) Moghimi, S. M.; Hamad, I.; Andresen, T. L.; Jorgensen, K.; Szebeni, J. *FASEB J.* **2006**, *20*, 2591.
(228) Jeon, S. I.; Lee, J. H.; Andrade, J. D.; De Gennes, P. G. *J. Colloid Interface Sci.* **1991**, *142*, 149.
(229) Moghimi, S. M.; Hunter, A. C.; Murray, J. C. *FASEB J.* **2005**, *19*, 311.
(230) Mishra, V.; Mahor, S.; Rawat, A.; Gupta, P. N.; Dubey, P.; Khatri, K.; Vyas, S. P. *J. Drug Targeting* **2006**, *14*, 45.
(231) Ulbrich, K.; Hekmatara, T.; Herbert, E.; Kreuter, J. *Eur. J. Pharm. Biopharm.* **2009**, *71*, 251.
(232) Kreuter, J. *Adv. Drug Delivery Rev.* **2001**, *47*, 65.
(233) Mittal, G.; Carswell, H.; Brett, R.; Currie, S.; Kumar, M. N. V. R. *J. Controlled Release* **2011**, *150*, 220.
(234) Yoo, J. W.; Irvine, D. J.; Discher, D. E.; Mitragotri, S. *Nat. Rev. Drug Discovery* **2011**, *10*, 521.
(235) Sacanna, S.; Irvine, W. T. M.; Chaikin, P. M.; Pine, D. J. *Nature* **2010**, *464*, 575.
(236) Kwok, P.; Tunsirikongkon, A.; Glover, W.; Chan, H.-K. *Pharm. Res.* **2011**, *28*, 788.
(237) Wu, L.; Zhang, J.; Watanabe, W. *Adv. Drug Delivery Rev.* **2011**, *63*, 456.
(238) Limbach, L. K.; Li, Y.; Grass, R. N.; Brunner, T. J.; Hintermann, M. A.; Muller, M.; Gunther, D.; Stark, W. J. *Environ. Sci. Technol.* **2005**, *39*, 9370.

(239) Hackley, V. A.; Ferraris, C. F. U.S. Government Printing Office, 2001; Special Publication 960-3, p 6.
(240) Murdock, R.; Braydich-Stolle, L.; Schrand, A.; Schlager, J.; Hussain, S. *Toxicol. Sci.* **2008**, *101*, 239.
(241) Van Eerdenbrugh, B.; Van den Mooter, G.; Augustijns, P. *Int. J. Pharm.* **2008**, *364*, 64.
(242) Tam, J. M.; Engstrom, J. D.; Ferrer, D.; W., R. O., III; Johnston, K. P. *J. Pharm. Sci.* **2010**, *99*, 3150.
(243) Tam, J. M.; McConville, J. T.; Williams, R. O., III; Johnston, K. P. *J. Pharm. Sci.* **2008**, *97*, 4915.
(244) Greulich, C.; Kittler, S.; Epple, M.; Muhr, G.; Koller, M. *Langenbecks Arch. Surg.* **2009**, *394*, 495.
(245) Gruber, P.; Longer, M. A.; Robinson, J. R. *Adv. Drug Delivery Rev.* **1987**, *1*, 1.
(246) Tirosh, B.; Rubinstein, A. *J. Pharm. Sci.* **1998**, *87*, 453.
(247) Abdelwahed, W.; Degobert, G.; Stainmesse, S.; Fessi, H. *Adv. Drug Delivery Rev.* **2006**, *58*, 1688.
(248) Lee, M.; Kim, M.; Kim, S.; Lee, J. *J. Pharm. Sci.* **2009**, *98*, 4808.
(249) Searles, J.; Carpenter, J.; Randolph, T. *J. Pharm. Sci.* **2001**, *90*, 860.
(250) Beirowski, J.; Inghelbrecht, S.; Arien, A.; Gieseler, H. *J. Pharm. Sci.* **2011**, *100*, 1958.
(251) Beirowski, J.; Inghelbrecht, S.; Arien, A.; Gieseler, H. *J. Pharm. Sci.* **2011**, *100*, 4471.
(252) Grund, S.; Bauer, M.; Fischer, D. *Adv. Eng. Mater.* **2011**, *13*, B61.
(253) Chipman, S. D.; Oldham, F. B.; Pezzoni, G.; Singer, J. W. *Int. J. Nanomed.* **2006**, *1*, 375.
(254) Duncan, R.; Gilbert, H. R.; Carbajo, R. J.; Vicent, M. J. *Biomacromolecules* **2008**, *9*, 1146.
(255) Besheer, A.; Hertel, T. C.; Kressler, J.; Mader, K.; Pietzsch, M. *J. Pharm. Sci.* **2009**, *98*, 4420.
(256) Zhang, R.; Jain, S.; Rowland, M.; Hussain, N.; Agarwal, M.; Gregoriadis, G. *J. Diabetes Sci. Technol.* **2010**, *4*, 532.
(257) Calderon, M.; Quadir, M. A.; Sharma, S. K.; Haag, R. *Adv. Mater.* **2010**, *22*, 190.
(258) Kostarelos, K.; Bianco, A.; Prato, M. *Nat. Nanotechnol.* **2009**, *4*, 627.
(259) Allen, B.; Kichambare, P.; Gou, P.; Vlasova, I.; Kapralov, A.; Konduru, N.; Kagan, V.; Star, A. *Nano Lett.* **2008**, *8*, 3899.
(260) Bianco, A.; Kostarelos, K.; Prato, M. *Chem. Commun.* **2011**, *47*, 10182.
(261) Kane, A.; Hurt, R. *Nat. Nanotechnol.* **2008**, *3*, 378.
(262) Poland, C.; Duffin, R.; Kinloch, I.; Maynard, A.; Wallace, W.; Seaton, A.; Stone, V.; Brown, S.; Macnee, W.; Donaldson, K. *Nat. Nanotechnol.* **2008**, *3*, 423.
(263) Ganta, S.; Devalapally, H.; Shahiwala, A.; Amiji, M. *J. Controlled Release* **2008**, *126*, 187.
(264) Gassmann, P.; List, M.; Schweitzer, A.; Sucker, H. *Eur. J. Pharm. Biopharm.* **1994**, *40*, 64.
(265) Chen, X.; Young, T. J.; Sarkari, M.; Williams, r., R.O.; Johnston, K. P. *Int. J. Pharm.* **2002**, *242*, 3.
(266) Hu, J.; Johnston, K. P.; Williams, r., R. O. *Eur. J. Pharm. Sci.* **2003**, *20*, 295.
(267) Rogers, T. L.; Nelsen, A. C.; Hu, J.; Brown, J. N.; Sarkari, M.; Young, T. J.; Johnston, K. P.; Williams, r., R. O. *Eur. J. Pharm. Biopharm.* **2002**, *54*, 271.
(268) Chan, H.-K.; Kwok, P. *Adv. Drug Delivery Rev.* **2011**, *63*, 406.
(269) Varadan, V. K.; Chen, L.; Xie, J. *Nanomedicine: Design and Applications of Magnetic Nanomaterials, Nanosensors and Nanosystems*; Wiley: Chichester, 2008; p 8.
(270) Keck, C.; Müller, R. *Eur. J. Pharm. Biopharm.* **2006**, *62*, 3.
(271) Perry, J.; Herlihy, K.; Napier, M.; Desimone, J. *Acc. Chem. Res.* **2011**, *44*, 990.
(272) Adler-Moore, J. *Bone Marrow Transplant.* **1994**, *14*, 7.
(273) Clemons, K. V.; Sobel, R. A.; Williams, P. L.; Pappagianis, D.; Stevens, D. A. *Antimicrob. Agents Chemother.* **2002**, *46*, 2420.
(274) Knezevic, I.; Griffiths, E. *Biologicals* **2011**, *39*, 252.
(275) van der Walle, C.; Sharma, G.; Kumar, M. N. V.R. *Expert Opin. Biol. Ther.* **2009**, *6*, 177.
(276) Van der Walle, C.; Olejnik, O. *Peptide and Protein Delivery*; Academic Press: New York, 2011; p 1.
(277) Kalra, S.; Kalra, B.; Agrawal, N. *Diabetes, Metab. Syndr.* **2010**, *2*, 66.
(278) Prokop, A.; Davidson, J. *J. Pharm. Sci.* **2008**, *97*, 3518.
(279) Nestorov, I. *Clin. Pharmacokinet.* **2003**, *42*, 883.
(280) Li, M.; Al-Jamal, K.; Kostarelos, K.; Reineke, J. *ACS Nano* **2010**, *4*, 6303.
(281) Lin, P.; Chen, J.-W.; Chang, L.; Wu, J.-P.; Redding, L.; Chang, H.; Yeh, T.-K.; Yang, C.; Tsai, M.-H.; Wang, H.-J.; Kuo, Y.-C.; Yang, R. *Environ. Sci. Technol.* **2008**, *42*, 6264.
(282) Mittal, G.; Kumar, M. *J. Pharm. Sci.* **2009**, *98*, 3730.
(283) Heinemann, V.; Bosse, D.; Jehn, U.; Kahny, B.; Wachholz, K.; Debus, A.; Scholz, P.; Kolb, H. J.; Wilmanns, W. *Antimicrob. Agents Chemother.* **1997**, *41*, 1275.
(284) Ankola, D.; Wadsworth, R.; Kumar, M. N. V. R. *J. Biomed. Nanotechnol.* **2011**, *7*, 300.
(285) Polli, J.; Crison, J.; Amidon, G. *J. Pharm. Sci.* **1996**, *85*, 753.
(286) Shaikh, J.; Ankola, D. D.; Beniwal, V.; Singh, D.; Kumar, M. N. V. R. *Eur. J. Pharm. Sci.* **2009**, *37*, 223.
(287) Grama, C. N.; Ankola, D. D.; Kumar, M. N. V. R. *Curr. Opin. Colloid Interface Sci.* **2011**, *16*, 238.
(288) Ankola, D. D.; Battisti, A.; Solaro, R.; Kumar, M. N. V. R. *J. R. Soc., Interface* **2010**, *7*, S475.
(289) Peer, D.; Karp, J. M.; Hong, S.; Farokhzad, O. C.; Margalit, R.; Langer, R. *Nat. Nanotechnol.* **2007**, *2*, 751.
(290) Nie, S.; Xing, Y.; Kim, G. J.; Simons, J. W. *Annu. Rev. Biomed. Eng.* **2007**, *9*, 257.
(291) Global Information Inc., http://www.giiresearch.com/.
(292) *Nanomedicine: A global market report*; Global Industry Analysts, Inc., 2009.
(293) Morey, T. E.; Varshney, M.; Flint, J. A.; Seubert, C. N.; Smith, W. B.; Bjoraker, D. G.; Shah, D. O.; Dennis, D. M. *J. Nanopart. Res.* **2004**, *6*, 159.
(294) Lin, Y.-S.; Haynes, C. *J. Am. Chem. Soc.* **2010**, *132*, 4834.
(295) Radomski, A.; Jurasz, P.; Alonso Escolano, D.; Drews, M.; Morandi, M.; Malinski, T.; Radomski, M. W. *Br. J. Pharmacol.* **2005**, *146*, 882.
(296) Jun, E.-A.; Lim, K.-M.; Kim, K.; Bae, O.-N.; Noh, J.-Y.; Chung, K.-H.; Chung, J.-H. *Nanotoxicology* **2011**, *5*, 157.
(297) Shrivastava, S.; Bera, T.; Singh, S.; Singh, G.; Ramachandrarao, P.; Dash, D. *ACS Nano* **2009**, *3*, 1357.
(298) Deb, S.; Raja, S.; Dasgupta, A.; Sarkar, R.; Chattopadhyay, A.; Chaudhuri, U.; Guha, P.; Sardar, P. *Blood Cells, Mol., Dis.* **2012**, *48*, 36.
(299) Deb, S.; Patra, H.; Lahiri, P.; Dasgupta, A.; Chakrabarti, K.; Chaudhuri, U. *Nanomedicine* **2011**, *7*, 376.
(300) Sarma, J. V.; Ward, P. A. *Cell Tissue Res.* **2011**, *343*, 227.
(301) Ehrnthaller, C.; Ignatius, A.; Gebhard, F.; Huber-Lang, M. *Mol. Med.* **2011**, *17*, 317.
(302) Folkman, J.; Long, D. M., Jr.; Rosenbaum, R. *Science* **1966**, *154*, 148.
(303) USFDA, 2011.
(304) Davis, F. F. *Adv. Drug Delivery Rev.* **2002**, *54*, 457.
(305) Greenwald, R. B.; Choe, Y. H.; McGuire, J.; Conover, C. D. *Adv. Drug Delivery Rev.* **2003**, *55*, 217.
(306) Zacchigna, M.; Cateni, F.; Drioli, S.; Bonora, G. M. *Polymers* **2011**, *3*, 1076.
(307) Duncan, R. *Nat. Rev. Drug Discovery* **2003**, *2*, 347.
(308) Duncan, R. *Nat. Rev. Cancer* **2006**, *6*, 688.
(309) Filpula, D.; Zhao, H. *Adv. Drug Delivery Rev.* **2008**, *60*, 29.
(310) Davis, S.; Abuchowski, A.; Park, Y. K.; Davis, F. F. *Clin. Exp. Immunol.* **1981**, *46*, 649.
(311) Wang, Y. S.; Youngster, S.; Bausch, J.; Zhang, R.; McNemar, C.; Wyss, D. F. *Biochemistry* **2000**, *39*, 10634.
(312) Monfardini, C.; Schiavon, O.; Caliceti, P.; Morpurgo, M.; Harris, J. M.; Veronese, F. M. *Bioconjugate Chem.* **1995**, *6*, 62.

(313) Holmes, F. A.; O'Shaughnessy, J. A.; Vukelja, S.; Jones, S. E.; Shogan, J.; Savin, M.; Glaspy, J.; Moore, M.; Meza, L.; Wiznitzer, I.; Neumann, T. A.; Hill, L. R.; Liang, B. C. *J. Clin. Oncol.* **2002**, *20*, 727.
(314) Patel, A. M.; Moreland, L. W. *Expert Rev. Clin. Immunol.* **2010**, *6*, 855.
(315) Ng, E. W.; Shima, D. T.; Calias, P.; Cunningham, E. T., Jr.; Guyer, D. R.; Adamis, A. P. *Nat. Rev. Drug Discovery* **2006**, *5*, 123.
(316) McGahan, L. *Issues Emerg. Health. Technol.* **2008**, *113*, 1.
(317) Fishburn, C. S. *J. Pharm. Sci.* **2008**, *97*, 4167.
(318) Alconcel, S. N. S.; Baas, A. S.; Maynard, H. D. *Polym. Chem.* **2011**, *2*, 1442.
(319) Veronese, F. M.; Pasut, G. *Drug Discovery Today* **2005**, *10*, 1451.
(320) Canal, F.; Sanchis, J.; Vicent, M. J. *Curr. Opin. Biotechnol.* **2011**, *22*, 894.
(321) USFDA.
(322) Francis, D. A. *Int. J. Clin. Pract.* **2001**, *55*, 394.
(323) von Appen, K. *IDrugs* **1999**, *2*, 146.
(324) Maeda, H. *Adv. Enzyme Regul.* **2001**, *41*, 189.
(325) Bangham, A. D.; Standish, M. M.; Watkins, J. C. *J. Mol. Biol.* **1965**, *13*, 238.
(326) Hope, M. J.; Bally, M. B.; Mayer, L. D.; Janoff, A. S.; Cullis, P. R. *Chem. Phys. Lipids* **1986**, *40*, 89.
(327) Matsumoto, S.; Kohda, M.; Murata, S. I. *J. Colloid Interface Sci.* **1977**, *62*, 149.
(328) Papahadjopoulos, D.; Vail, W. J.; Newton, C.; Nir, S.; Jacobson, K.; Poste, G.; Lazo, R. *Biochim. Biophys. Acta* **1977**, *465*, 579.
(329) Szoka, J., F.; Papahadjopoulos, D. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 4194.
(330) Sharma, A.; Sharma, U. S. *Int. J. Pharm.* **1997**, *154*, 123.
(331) Allen, T. M.; Moase, E. H. *Adv. Drug Delivery Rev.* **1996**, *21*, 117.
(332) Fan, M.; Xu, S.; Xia, S.; Zhang, X. *J. Agric. Food Chem.* **2007**, *55*, 3089.
(333) Maestrelli, F.; Gonzalez-Rodriguez, M. L.; Rabasco, A. M.; Mura, P. *Int. J. Pharm.* **2006**, *312*, 53.
(334) Adler-Moore, J.; Proffitt, R. T. *J. Antimicrob. Chemother.* **2002**, *49*, 21.
(335) Woodle, M. C.; Lasic, D. D. *Biochim. Biophys. Acta* **1992**, *1113*, 171.
(336) Bawarski, W.; Chidlowsky, E.; Bharali, D.; Mousa, S. *Nanomedicine* **2008**, *4*, 273.
(337) Jølck, R.; Feldborg, L.; Andersen, S.; Moghimi, S.; Andresen, T. *Adv. Biochem. Eng. Biotechnol.* **2011**, *125*, 251.
(338) Torchilin, V. *Pharm. Res.* **2007**, *24*, 1.
(339) Rowinsky, E. K.; Cazenave, L. A.; Donehower, R. C. *J. Natl. Cancer Inst.* **1990**, *82*, 1247.
(340) Gelderblom, H.; Verweij, J.; Nooter, K.; Sparreboom, A. *Eur. J. Cancer* **2001**, *37*, 1590.
(341) Authier, N.; Gillet, J. P.; Fialip, J.; Eschalier, A.; Coudore, F. *Brain. Res.* **2000**, *887*, 239.
(342) ten Tije, A. J.; Verweij, J.; Loos, W. J.; Sparreboom, A. *Clin. Pharmacokinet.* **2003**, *42*, 665.
(343) Curry, S.; Mandelkow, H.; Brick, P.; Franks, N. *Nat. Struct. Biol.* **1998**, *5*, 827.
(344) Herve, F.; Urien, S.; Albengres, E.; Duche, J. C.; Tillement, J. P. *Clin. Pharmacokinet.* **1994**, *26*, 44.
(345) Gradishar, W. J. *Expert Opin. Pharmacother.* **2006**, *7*, 1041.
(346) Tiruppathi, C.; Song, W.; Bergenfeldt, M.; Sass, P.; Malik, A. B. *J. Biol. Chem.* **1997**, *272*, 25968.
(347) Yamamoto, Y.; Kawano, I.; Iwase, H. *OncoTargets Ther.* **2011**, *4*, 123.
(348) Merisko-Liversidge, E.; Sarpotdar, P.; Bruno, J.; Hajj, S.; Wei, L.; Peltier, N.; Rake, J.; Shaw, J. M.; Pugh, S.; Polin, L.; Jones, J.; Corbett, T.; Cooper, E.; Liversidge, G. G. *Pharm. Res.* **1996**, *13*, 272.
(349) Kesisoglou, F.; Panmai, S.; Wu, Y. *Adv. Drug Delivery Rev.* **2007**, *59*, 631.
(350) Liu, P.; Rong, X.; Laru, J.; van Veen, B.; Kiesvaara, J.; Hirvonen, J.; Laaksonen, T.; Peltonen, L. *Int. J. Pharm.* **2011**, *411*, 215.
(351) Verma, S.; Huey, B. D.; Burgess, D. J. *Langmuir* **2009**, *25*, 12481.
(352) Langguth, P.; Hanafy, A.; Frenzel, D.; Grenier, P.; Nhamias, A.; Ohlig, T.; Vergnault, G.; Spahn-Langguth, H. *Drug Dev. Ind. Pharm.* **2005**, *31*, 319.
(353) Manchester, M.; Singh, P. *Adv. Drug Delivery Rev.* **2006**, *58*, 1505.
(354) Ambrosch, F.; Finkel, B.; Herzog, C.; Koren, A.; Kollaritsch, H. *Infection* **2004**, *32*, 149.
(355) Rao, J. P.; Geckeler, K. E. *Prog. Polym. Sci.* **2011**, *36*, 887.
(356) Bhardwaj, V.; Hariharan, S.; Bala, I.; Lamprecht, A.; Kumar, N.; Panchagnula, R.; Kumar, M. N. V. R. *J. Biomed. Nanotechnol.* **2005**, *1*, 235.
(357) Mora-Huertas, C. E.; Fessi, H.; Elaissari, A. *Adv. Colloid Interface Sci.* **2011**, *163*, 90.
(358) Ayoub, M.; Ahmed, N.; Kalaji, N.; Charcosset, C.; Magdy, A.; Fessi, H.; Elaissari, A. *J. Biomed. Nanotechnol.* **2011**, *7*, 255.
(359) Bala, I.; Bhardwaj, V.; Hariharan, S.; Sitterberg, J.; Bakowsky, U.; Kumar, M. N. V. R. *Nanotechnology* **2005**, *16*, 2819.
(360) Ratnam, D. V.; Chandraiah, G.; Sonaje, K.; Viswanad, B.; Bhardwaj, V.; Ramarao, P.; Ravikumar, M. N. V. *J. Biomed. Nanotechnol.* **2008**, *4*, 33.
(361) Meena, A. K.; Ratnam, D. V.; Chandraiah, G.; Ankola, D. D.; Rao, P. R.; Kumar, M. N. V. R. *Lipids* **2008**, *43*, 231.
(362) Allemann, E.; Gurny, R.; Doelker, E. *Int. J. Pharm.* **1992**, *87*, 247.
(363) Ganachaud, F.; Katz, J. L. *ChemPhysChem* **2005**, *6*, 209.
(364) Lee, J.; Cho, E. C.; Cho, K. *J. Controlled Release* **2004**, *94*, 323.
(365) Akagi, T.; Kaneko, T.; Kida, T.; Akashi, M. *J. Controlled Release* **2005**, *108*, 226.
(366) D'Addio, S. M.; Kafka, C.; Akbulut, M.; Beattie, P.; Saad, W.; Herrera, M.; Kennedy, M. T.; Prud'homme, R. K. *Mol. Pharmaceut.* **2010**, *7*, 557.
(367) Champion, J. A.; Katare, Y. K.; Mitragotri, S. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 11901.
(368) Petros, R. A.; Desimone, J. M. *Nat. Rev. Drug Discovery* **2010**, *9*, 615.
(369) Tao, L.; Zhao, X.; Gao, J.; Hu, W. *Nanotechnology* **2010**, *21*, 95301.
(370) Yanagishita, T.; Fujimura, R.; Nishio, K.; Masuda, H. *Langmuir* **2009**, *26*, 1516.
(371) Kumar, M. N. V. R.; Muzzarelli, R. A.; Muzzarelli, C.; Sashiwa, H.; Domb, A. J. *Chem. Rev.* **2004**, *104*, 6017.
(372) Lu, J. M.; Wang, X.; Marin-Muller, C.; Wang, H.; Lin, P. H.; Yao, Q.; Chen, C. *Expert Rev. Mol. Diagn.* **2009**, *9*, 325.
(373) Sahoo, S. K.; Panyam, J.; Prabha, S.; Labhasetwar, V. *J. Controlled Release* **2002**, *82*, 105.
(374) Alonso, M. J.; Losa, C.; Calvo, P.; Vila-Jato, J. L. *Int. J. Pharm.* **1991**, *68*, 69.
(375) Vauthier, C.; Bouchemal, K. *Pharm. Res.* **2009**, *26*, 1025.
(376) Mora-Huertasa, C. E.; Fessi, H.; Elaissari, A. *Int. J. Pharm.* **2010**, *385*, 113.
(377) Couvreur, P.; Barratt, G.; Fattal, E.; Legrand, P.; Vauthier, C. *Crit. Rev. Ther. Drug Carrier Syst.* **2002**, *19*, 99.
(378) Pinto Reis, C.; Neufeld, R.; Ribeiro, A.; Veiga, F. *Nanomedicine* **2006**, *2*, 8.
(379) Hirsch, L.; Stafford, R.; Bankson, J.; Sershen, S.; Rivera, B.; Price, R.; Hazle, J.; Halas, N.; West, J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13549.
(380) Kennedy, L.; Bickford, L.; Lewinski, N.; Coughlin, A.; Hu, Y.; Day, E.; West, J.; Drezek, R. *Small* **2011**, *7*, 169.
(381) Koltsov, D.; Perry, M. *Phys. World* **2004**, *17*, 31.
(382) Connolly, J.; T. G., S. P. *J. Magn. Magn. Mater.* **2001**, *225*, 156.
(383) Arruebo, M.; Fernández-Pacheco, R.; Ricardo Ibarra, M.; Santamaría, J. *Nano Today* **2007**, *2*, 22.
(384) Foy, S. P.; Labhasetwar, V. *Biomaterials* **2011**, *32*, 9155.
(385) Shah, P.; Bhalodia, D.; Shelat, P. *Syst. Rev. Pharm.* **2010**, *1*, 24.
(386) Shah, N. H.; Carvajal, M. T.; Patel, C. I.; Infeld, M. H.; Malick, A. W. *Int. J. Pharm.* **1994**, *106*, 15.

(387) Lei, Y.; Lu, Y.; Qi, J.; Nie, S.; Hu, F.; Pan, W.; Wu, W. *Int. J. Nanomed.* **2011**, *6*, 795.
(388) Lee, J. M.; Park, K. M.; Lim, S. J.; Lee, M. K.; Kim, C. K. *J. Pharm. Pharmacol.* **2002**, *54*, 43.
(389) Shukla, A.; Krause, A.; Neubert, R. *J. Pharm. Pharmacol.* **2003**, *55*, 741.
(390) Müller, R. H.; Mäder, K.; Gohla, S. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 161.
(391) Wissing, S. A.; Kayser, O.; Muller, R. H. *Adv. Drug Delivery Rev.* **2004**, *56*, 1257.
(392) Muller, R. H.; Radtke, M.; Wissing, S. A. *Adv. Drug Delivery Rev.* **2002**, *54*, S131.
(393) Campo, S.; Arseni, B.; Rossi, S.; D'Alessio, V.; Lu, R.; Ngoje, J.; Ahl, P.; Bonitz, S.; Mannino, R.; Di Mitri, D.; Battistini, L.; Carminati, P.; De Santis, R.; Ruggiero, V. *Transplantation* **2008**, *86*, 171.
(394) Kickhoefer, V. A.; Garcia, Y.; Mikyas, Y.; Johansson, E.; Zhou, J. C.; Raval-Fernandes, S.; Minoofar, P.; Zink, J. I.; Dunn, B.; Stewart, P. L.; Rome, L. H. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4348.
(395) Mikyas, Y.; Makabi, M.; Raval-Fernandes, S.; Harrington, L.; Kickhoefer, V. A.; Rome, L. H.; Stewart, P. L. *J. Mol. Biol.* **2004**, *344*, 91.
(396) Yu, M.; Ng, B.; Rome, L.; Tolbert, S.; Monbouquette, H. *Nano Lett.* **2008**, *8*, 3510.
(397) Kickhoefer, V.; Han, M.; Raval-Fernandes, S.; Poderycki, M.; Moniz, R.; Vaccari, D.; Silvestry, M.; Stewart, P.; Kelly, K.; Rome, L. *ACS Nano* **2009**, *3*, 27.
(398) Lai, C. Y.; Wiethoff, C. M.; Kickhoefer, V. A.; Rome, L. H.; Nemerow, G. R. *ACS Nano* **2009**, *3*, 691.
(399) Champion, C.; Kickhoefer, V.; Liu, G.; Moniz, R.; Freed, A.; Bergmann, L.; Vaccari, D.; Raval-Fernandes, S.; Chan, A.; Rome, L.; Kelly, K. *PLoS One* **2009**, *4*, e5409.
(400) Tomalia, D. A.; Baker, H.; Dewald, J.; Hall, M.; Kallos, G.; Matertin, S.; Roeck, J.; Ryder, J.; Smith, P. *Polym. J.* **1985**, *17*, 117.
(401) Newkome, G. R.; Yao, Z.; Baker, G. R.; Gupta, V. K. *J. Org. Chem.* **1985**, *50*, 2003.
(402) Lee, C.; MacKay, J.; Fréchet, J.; Szoka, F. *Nat. Biotechnol.* **2005**, *23*, 1517.
(403) Kawaguchi, T.; Walker, K. L.; Wilkins, C. L.; Moore, J. S. *J. Am. Chem. Soc.* **1995**, *117*, 2159.
(404) Medina, S.; El-Sayed, M. *Chem. Rev.* **2009**, *109*, 3141.
(405) Newkome, G. R.; Moorefield, C. N.; Voegtle, F. *Dendrimers and Dendrons: Concepts, Syntheses, Applications*; Wiley-VCH: Weinheim, 2001.
(406) Frechet, J.; Tomalia, D. *Dendrimers and Other Dendritic Polymers*; Wiley: Chichester, UK, 2001.
(407) Hawker, C. J.; Frechet, J. M. J. *J. Am. Chem. Soc.* **1990**, *112*, 7638.
(408) Qiu, L.; Bae, Y. *Pharm. Res.* **2006**, *23*, 1.
(409) Hawker, C. J.; Wooley, K. L.; Frechet, J. M. J. *J. Chem. Soc., Perkin Trans. 1* **1993**, *12*, 1287.
(410) Naylor, A. M.; Goddard, W. A., III; Kiefer, G. E.; Tomalia, D. A. *J. Am. Chem. Soc.* **1989**, *111*, 2339.
(411) Jansen, J. F. G. A.; de Brabander van den Berg, E. M. M.; Meijer, E. W. *Science* **1994**, *266*, 1226.
(412) Bhadra, D.; Yadav, A.; Bhadra, S.; Jain, N. *Int. J. Pharm.* **2005**, *295*, 221.
(413) Jansen, J. F. G. A.; Meijer, E. W.; de Brabander van den Berg, E. M. M. *J. Am. Chem. Soc.* **1995**, *117*, 4417.
(414) Bhadra, D.; Bhadra, S.; Jain, S.; Jain, N. K. *Int. J. Pharm.* **2003**, *257*, 111.
(415) Sarkar, K.; Yang, H. *Drug Delivery* **2008**, *15*, 343.
(416) Kojima, C.; Kono, K.; Maruyama, K.; Takagishi, T. *Bioconjugate Chem.* **2000**, *11*, 910.
(417) Yang, H.; Lopina, S. T.; DiPersio, L. P.; Schmidt, S. P. *J. Mater. Sci.: Mater. Med.* **2008**, *19*, 1991.
(418) Majoros, I. J.; Myc, A.; Thomas, T.; Mehta, C. B.; Baker, J. R. J. *Biomacromolecules* **2006**, *7*, 572.
(419) Majoros, I. J.; Thomas, T. P.; Mehta, C. B.; Baker, J. R. J. *J. Med. Chem.* **2005**, *48*, 5892.
(420) Rosen, B. M.; Wilson, D. A.; Wilson, C. J.; Peterca, M.; Won, B. C.; Huang, C.; Lipski, L. R.; Zeng, X.; Ungar, G.; Heiney, P. A.; Percec, V. *J. Am. Chem. Soc.* **2009**, *131*, 17500.
(421) Morgan, M.; Nakanishi, Y.; Kroll, D.; Griset, A.; Carnahan, M.; Wathier, M.; Oberlies, N.; Manikumar, G.; Wani, M.; Grinstaff, M. *Cancer Res.* **2006**, *66*, 11913.
(422) Baker, J. R. J. *Hematol. Am. Soc., Hematol. Educ. Program* **2009**, *1*, 708.
(423) Hui, H.; Fan, X. D.; Cao, Z. L. *Polym. J.* **2005**, *46*, 9514.
(424) Lai, P. S.; Lou, P. J.; Peng, C. L.; Pai, C. L.; Yen, W. N.; Huang, M. Y.; Young, T. H.; Shieh, M. J. *J. Controlled Release* **2007**, *122*, 39.
(425) Gillies, E. R.; Frechet, J. M. *Drug Discovery Today* **2005**, *10*, 35.
(426) Roco, M. C. *Biomedical Nanotechnology*; Taylor and Francis: Boca Raton, FL, 2005.
(427) Jevprasesphant, R.; Penny, J.; Attwood, D.; McKeown, N.; D'Emanuele, A. *Pharm. Res.* **2003**, *20*, 1543.
(428) Soussan, E.; Cassel, S.; Blanzat, M.; Rico-Lattes, I. *Angew. Chem., Int. Ed.* **2009**, *48*, 274.
(429) van Dongen, S. F. M.; de Hoog, H. P. M.; Peters, R.; Nallani, M.; Nolte, R. J. M.; van Hest, J. C. M. *Chem. Rev.* **2009**, *109*, 6212.
(430) Lu, W.; Li, F.; Mahato, R. *J. Pharm. Sci.* **2011**, *100*, 2418.
(431) Letchford, K.; Burt, H. *Eur. J. Pharm. Biopharm.* **2007**, *65*, 259.
(432) Rodríguez-Hernández, J.; Chécot, F.; Gnanou, S.; Lecommandoux, S. *Prog. Polym. Sci.* **2005**, *30*, 691.
(433) Du, J. Z.; Reilly, R. Z. *Soft Matter* **2009**, *5*, 3544.
(434) Discher, B. M.; Won, Y.-Y.; Ege, D. S.; Lee, J. C.-M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143.
(435) Discher, B. M.; Bermudez, H.; Hammer, D. A.; Discher, D. E.; Won, Â. Y. Y.; Bates, F. S. *J. Phys. Chem. B* **2002**, *106*, 2848.
(436) Onaca, O.; Enea, R.; Hughes, D. W. *Macromol. Biosci.* **2009**, *9*, 129.
(437) Kim, M. S.; Lee, D. S. *Chem. Commun.* **2010**, *46*, 4481.
(438) Chen, W.; Meng, F. H.; Cheng, R.; Zhong, Z. Y. *J. Controlled Release* **2010**, *142*, 40.
(439) Lomas, H.; Canton, I.; MacNeil, S.; Du, J.; Armes, S. P.; Ryan, A. J.; Lewis, A. L.; Battaglia, G. *Adv. Mater.* **2007**, *19*, 4238.
(440) Roco, M. *J. Nanopart. Res.* **2008**, *10*, 11.
(441) Borm, P. A.; Kreyling, W. J. *Nanosci. Nanotech.* **2004**, *4*, 521.
(442) Seaton, A.; Tran, L.; Aitken, R.; Donaldson, K. *J. R. Soc., Interface* **2009**, *2*, 2.
(443) Adams, F. *Talanta* **2011**, *85*, 1230.
(444) McNeil, S. E. *WIREs Nanomed. Nanobiotechnol.* **2009**, *1*, 264–271.
(445) Harboe, M.; Thorgersen, E.; Mollnes, T. *Adv. Drug Delivery Rev.* **2011**, *63*, 976.
(446) Szebeni, J.; Bedöcs, P.; Rozsnyay, Z.; Weiszhár, Z.; Urbanics, R.; Rosivall, L.; Cohen, R.; Garbuzenko, O.; Báthori, G.; Tóth, M.; Bünger, R.; Barenholz, Y. *Nanomedicine* **2012**, *8*, 176.
(447) Chanan-Khan, A.; Szebeni, J.; Savay, S.; Liebes, L.; Rafique, N. M.; Alving, C. R.; Muggia, F. M. *Ann. Oncol.* **2003**, *14*, 1430.
(448) Vasey, P. A.; Kaye, S. B.; Morrison, R.; Twelves, C.; Wilson, P.; Duncan, R.; Thomson, A. H.; Murray, L. S.; Hilditch, T. E.; Murray, T.; Burtles, S.; Fraier, D.; Frigerio, E.; Cassidy, J. *Clin. Cancer Res.* **1999**, *5*, 83.
(449) http://nano.cancer.gov/objects/pdfs/CaNanoPlan.
(450) Borys, N. *2010 American Society of Clinical Oncology annual meeting*, Chicago, IL, June 4–8, 2010.
(451) Batist, G.; Gelmon, K. A.; Chi, K. N.; Miller, W. H. J.; Chia, S. K.; Mayer, L. D.; Swenson, C. E.; Janoff, A. S.; Louie, A. C. *Clin. Cancer Res.* **2009**, *15*, 692.
(452) Matsumura, Y.; Gotoh, M.; Muro, K.; Yamada, Y.; Shirao, K.; Shimada, Y.; Okuwa, M.; Matsumoto, S.; Miyata, Y.; Ohkura, H.; Chin, K.; Baba, S.; Yamao, T.; Kannami, A.; Takamatsu, Y.; Ito, K.; Takahashi, K. *Ann. Oncol.* **2004**, *15*, 517.
(453) Matsumura, Y. *Adv. Drug Delivery Rev.* **2008**, *60*, 899.
(454) Seymour, L. W.; Ferry, D. R.; Kerr, D. J.; Rea, D.; Whitlock, M.; Poyner, R.; Boivin, C.; Hesslewood, S.; Twelves, C.; Blackie, R.; Schatzlein, A.; Jodrell, D.; Bissett, D.; Calvert, H.; Lind, M.; Robbins, A.; Burtles, S.; Duncan, R.; Cassidy, J. *Int. J. Oncol.* **2009**, *34*, 1629.

(455) Valle, J. W.; Armstrong, A.; Newman, C.; Alakhov, V.; Pietrzynski, G.; Brewer, J.; Campbell, S.; Corrie, P.; Rowinsky, E. K.; Ranson, M. *Invest. New Drugs* **2010**, *29*, 1029.
(456) Cho, E. C.; Zhang, Q.; Xia, Y. *Nat. Nanotechnol.* **2011**, *6*, 385.
(457) Wood, J. G.; Johnson, J. S.; Mattioli, L. F.; Gonzalez, N. C. *J. Appl. Physiol.* **2000**, *89*, 1561.
(458) Italia, J. L.; Sharp, A.; Carter, K. C.; Warn, P.; Kumar, M. N. V. R. *PLoS One* **2011**, *6*, e25744.
(459) Italia, J. L.; Kumar, M. N. V. R.; Carter, K. C. *J. Biomed. Nanotechnol.* **2012**, *8*, 695.
(460) Medina, C.; Santos-Martinez, M. J.; Radomski, A.; Corrigan, O. I.; Radomski, M. W. *Br. J. Pharmacol.* **2007**, *150*, 552.
(461) Dobrovolskaia, M. A.; McNeil, S. E. *Nat. Nanotechnol.* **2007**, *2*, 469.
(462) Winkler, D. A.; Mombelli, E.; Pietroiusti, A.; Tran, L.; Worth, A.; Fadeel, B.; McCall, M. *J. Toxicology* **2012**, DOI: 10.1016/j.tox.2012.11.005.
(463) Takagi, A.; Hirose, A.; Nishimura, T.; Fukumori, N.; Ogata, A.; Ohashi, N.; Kitajima, S.; Kanno, J. *J. Toxicol. Sci.* **2008**, *33*, 105.
(464) Yokel, R. A.; Macphail, R. C. *J. Occup. Med. Toxicol.* **2011**, *6*, 7.
(465) Donaldson, K.; Stone, V.; Tran, C. L.; Kreyling, W.; Borm, P. J. *Occup. Environ. Med.* **2004**, *61*, 727.
(466) Chan, V. S. *Regul. Toxicol. Pharmacol.* **2006**, *46*, 218.
(467) Hartung, T. *Nature* **2009**, *460*, 208.
(468) Semete, B.; Booysen, L.; Lemmer, Y.; Kalombo, L.; Katata, L.; Verschoor, J.; Swai, S. S. *Nanomedicine* **2010**, *6*, 662.
(469) Sung, J. H.; Ji, J. H.; Park, J. D.; Yoon, J. U.; Kim, D. S.; Jeon, K. S.; Song, M. Y.; Jeong, J.; Han, B. S.; Han, J. H.; Chung, Y. H.; Chang, H. K.; Lee, J. H.; Cho, M. H.; Kelman, B. J.; Yu, I. J. *Toxicol. Sci.* **2009**, *108*, 452.