3 Models Used for Biopharmaceutical Evaluation of Nanoparticulate Drug Delivery System (NPDDS)

Seema Kohli and Sumeet Dwivedi

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Abstract

Nanoparticle drug delivery systems are engineered technologies that use nanoparticles for the targeted delivery and controlled release of therapeutic agents. The modern form of a drug delivery system should minimize side effects and reduce both dosage and dosage frequency. Nanoparticle drug delivery focuses on maximizing drug efficacy and minimizing cytotoxicity. Fine-tuning nanoparticle properties for effective drug delivery involves addressing the following factors. The surface-area-to-volume ratio of nanoparticles can be altered to allow for more ligand binding to the surface. Model-based methods are increasingly used in almost every area of biopharmaceutical process technology. It can be applied in the field of experimental design, process characterization, process design, monitoring, and control. Benefits of these methods are lower experimental effort, process transparency, clear rationality behind decisions, and increased process robustness. Biopharmaceutical modeling has become integral to the design and development of new drugs. Influencing key aspects of the development process, including drug substance design, formulation design, toxicological exposure assessment, and biopharmaceutical modeling, is now seen as the linchpin to a drug's future success. And while there are a number of commercially available software programs for drug modeling, there has not been a single resource guiding pharmaceutical. In the present chapter, these entire models used for biopharmaceutical evaluation of NPDDS have been highlighted.

Keywords

Nanoparticle · Biopharmaceutical · Models · Evaluation

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1 Introduction to Biopharmaceuticals

Biopharmaceuticals are sophisticated medicines made from living cells or creatures and are frequently produced utilizing cutting-edge biotechnological techniques. A biopharmaceutical (biological or biologic) is a therapeutic product generated from biological sources such as humans, animals, or microorganisms and consists of carbohydrates, proteins, nucleic acids, living cells, or tissues. Unlike traditional pharmaceuticals, which are made through chemical methods, biopharmaceutical goods are made by biological processes such as the extraction of living systems or the synthesis of r-DNA technology. Transgenic species, such as plants, animals, or bacteria that have been genetically modified, could be used to create biopharmaceuticals [1].

Recombinant human insulin (trade name "Humulin") was the first biopharmaceutical to be licensed for human medical purposes for marketing in 1982. Vaccines, entire blood (or blood components), immunosera, antigens, hormones, cytokines, enzymes, allergenics, cell treatments, gene therapies, tissues, and monoclonal antibodies are all examples of biopharmaceuticals in use today. Human treatments based on cells, genes, or tissue engineering are referred to as "advanced therapy medical products" (ATMPs) by the European Medicines Agency (EMA). CTPs are biomedicines that contain cells/tissues that have been modified to change their biological features and can be utilized to treat, prevent, or diagnose diseases. Gene therapy products (GTPs) are therapeutic agents used to enhance genetics by repairing, deleting, inserting, or replacing defective genes or making site-specific alterations for target therapies. Tissue engineering is the use of a combination of cell, engineering, and material approaches, as well as appropriate components, to improve, repair, or replace a portion or the entire biological system [2, 3].

Biosimilars, also known as "follow-on biologics," are biologic medicinal products that are almost identical to copies of original products developed by various pharmaceutical companies. Despite small changes in therapeutically inactive components, it is quite identical to a licensed reference product. In terms of safety, purity, and potency, there are no clinically relevant differences between biosimilars and reference products. In terms of dosage, safety, strength, administration, quality, performance, and intended uses, a generic medicine is identical to a brand name drug. To ensure that the generic drug can be used in place of the brand name drug, a series of stringent tests must be completed [4–6].

A generic drug must have the same active pharmaceutical ingredients (APIs) as the brand name product and must be proven to be bioequivalent to the brand name drug.

The scientific evaluation of generic drug substitutability or therapeutic equivalence is required. If a generic drug is determined to be therapeutically equivalent to a brand name product, it has the same effects and is less expensive. When the patent on the original "innovator" product expires, biosimilars, like generic medications, can be created and are formally approved replicas of the original products. A generic medicine and a biosimilar, on the other hand, have a lot of distinctions. Biosimilars have the same therapeutic impact as generic medications, but they are only comparable to the original "innovator" drugs because they have been validated. Unlike generic medications, where the APIs are same, biosimilars will not be identical to the reference pharmaceuticals. Despite this diversity, all generic pharmaceuticals and biosimilars must maintain a constant level of quality and efficacy throughout their life cycles [7].

2 Nanoparticulate Drug Delivery System (NPDDS)

Nanotechnology and nanoscience advancements have brought up new possibilities in medical science. Both the corporate and public sectors have increased their interest and investment in nanotechnology research and implementation for a variety of applications in the biological sciences. Nanoscience and nanotechnology have infiltrated the pharmacy industry, opening up new avenues

for enhanced drug delivery and therapy. Although nanotechnology holds a lot of promise, it also poses new concerns in terms of safety and ethics. Because traditional dose forms have a number of drawbacks, formulation pharmacists are constantly working on new medication delivery strategies [8].

Despite the fact that new-generation medications have potent activity, the majority of them have limitations such as poor water solubility, low gastrointestinal permeability, high first pass metabolism, poor stability, nonselective distribution, and others. As a result, in recent years, new particulate drug delivery systems (PDDS), either polymer- or lipid-based, have been explored as agents to treat a variety of diseases. Nanoparticulate systems (NS) have shown to be effective in overcoming these issues, with the potential to improve therapeutic outcomes. Traditional drug delivery techniques, particularly in cancer chemotherapy, are hampered by biological barriers. Drug NPs or NS, on the other hand, have shown enhanced membrane permeability, leading to improved therapy not just in cancer but also in the treatment of other diseases [9].

NPs are well known for increasing drug solubility and bioavailability, but they can also be utilized to target medications, especially peptide-based therapies, to specific areas or organs, as well as control drug release. Nasal delivery of nanoparticulate insulin was observed to result in increased insulin absorption. Within 10 years, it is estimated that 50% of all drug delivery and design will be automated. NPDDS' ultimate goal is to develop clinically relevant formulations that will improve therapy and patient quality of life. Chemotherapy is one area where NS has shown tremendous promise, as nonselective distribution of cytotoxic medicines in conventional dose forms resulted in severe adverse effects. According to the existing literature, the NPDDS are not only useful in chemotherapy but can also be used to deliver other types of medications to their sites of action via various routes of administration. Because NPs can be kept at the application site and prolong drug release to the eye, they may be a preferable alternative for ophthalmic administration. The TB treatments based on NPs are widely used [10, 11].

2.1 Types of Nanoparticles for Drug Delivery

NPs for drug delivery can be classified into solid lipid nanoparticles (SLNs), nanospheres, nanocapsules, liposomes, and polymersomes and micelles, based on the methods of preparation as shown in Fig. 3.1 [12]:

- Nanospheres are matrix systems in which the drug is incorporated throughout the solid polymers, whereas SLNs are solid nanoparticles made by integrating drug in lipid.
- Nanocapsules are vesicular systems in which a single polymeric membrane surrounds the drug alone or the drug restricted to an aqueous or oily drop. Lipophilic medications are generally encapsulated in nanocapsules.
- A polymersome is defined as a polymeric membrane with many layers. Various phospholipids (saturated and unsaturated) have been employed in nanosized liposomal systems, with promising results. To avoid buildup of the polymer matrix after repeated treatment, polymeric NPs are often made from biodegradable polymers.

2.2 Method of Preparation of Nanoparticles

In general, NPs are prepared by processes, such as solvent evaporation, solvent diffusion/displacement, reverse salting-out and droplet gelation, emulsification and polymerization, dispersion polymerization, interfacial condensation polymerization, and interfacial complexation [12, 13]:

- The SLN is prepared by emulsification and solvent evaporation techniques. The simplest method of NP preparation is nanoprecipitation. PLA, PLGA, and poly-e-caprolactone (PCL) NPs can be prepared by emulsification

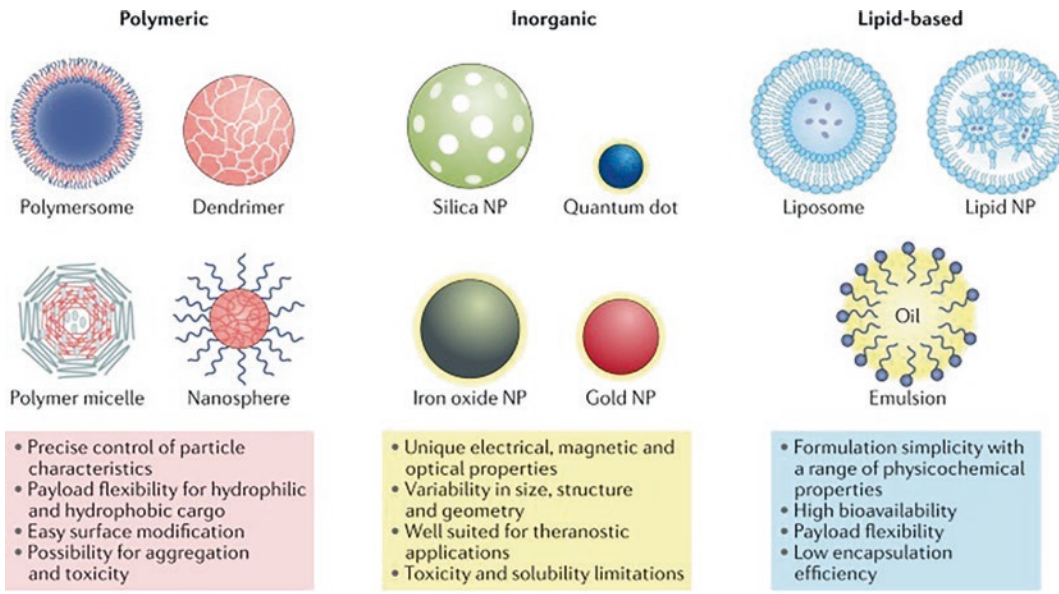

Fig. 3.1 Different types of nanoparticles for drug delivery

and solvent evaporation process, with average particle size of 250 nm and above. Amphiphilic block copolymer NPs can be prepared by using this method with polymers such as PEG-PCL, PEG-PLGA, PEG-PLA, and PEG-PACA. Though this method is used for preparing lipophilic drug-loaded NPs extensively, even hydrophilic drugs can also be encapsulated by this process. Solvents used for this method are methylene chloride, chloroform, ethyl acetate, etc.

- Another method used for preparation of NPs is emulsification and solvent diffusion/displacement process. As the name implies, diffusion of organic solvent into the aqueous phase is the key step in emulsion solvent displacement method. The organic solvent should be partially soluble in water and is selected from a wide range of solvents such as benzyl alcohol, 2-butanone, methyl acetate, propylene carbonate, ethyl acetate, isopropyl acetate, methyl acetate, methyl ethyl ketone, and isovaleric acid. This method can be used to prepare NPs of size around 150 nm for poorly water-soluble drugs.
- Nanocapsules can be prepared by the same method just by adding a small amount of oil into the organic phase.
- In emulsification-reverse salting-out method, water-miscible acetone is emulsified with aqueous phase containing high concentration of salts or sucrose. Magnesium chloride, calcium chloride, and magnesium acetate salts are preferably used, because of their high salting-out effect in aqueous phase. When acetone is added to aqueous phase, the miscibility of water to acetone decreases, due to the presence of the large quantity of electrolyte which holds water molecules, resulting in emulsion droplet formation. The precipitation of polymer from the emulsion is induced by adding excess water, which results in a sudden drop of the salt or sucrose concentration in the continuous phase of the emulsion, and hence inducing the organic solvent to migrate out of the emulsion droplets; this process is called reverse salting-out. The gelling property of the polymers is used to prepare the NPs from the emulsion, the polymers used being agarose, alginate, and pectin. This method of preparation is called emulsion droplet gelation.
- NPs prepared by in situ polymerization use monomer (alkylcyanoacrylates) to produce polymerization of alkylcyanoacrylate while forming NPs. The NPs are synthesized by an in situ spontaneous polymerization reaction.

This polymer can encapsulate both lipophilic and hydrophilic drugs and is used to prepare nanospheres and nanocapsules containing an aqueous or oily core. The NPs prepared by in situ method follow anionic polymerization reaction mechanism, and the reaction is spontaneously initiated by hydroxyl groups of water or any nucleophilic groups.

- Nanoprecipitation is the simplest, fastest, and most reproducible and economical method of preparing NPs especially for lipophilic drugs. In this method, the polymer, drug, and lipophilic surfactant are dissolved in a semipolar water-miscible solvent such as acetone, ethanol, dimethylformamide, and dimethylsulfoxide. The basic requirement of the selection of solvent is miscibility with aqueous phase, and the aqueous phase has to be a nonsolvent of the polymer. Once the organic solvent is added to aqueous phase, NPs form instantaneously because of the rapid diffusion of water-miscible solvent into the aqueous phase. Because of the instantaneous process, the nanoprecipitation method provides very fine particles (about 200 nm) with a narrow size distribution. This method can also be used to encapsulate hydrophilic drugs. The SLNs are composed of physiological lipid, dispersed in water or a solution of aqueous surfactant. The lipid matrices used in the preparation of SLNs are Acidan N 12, B-CD21C6, cetyl palmitate, Dynasan 114 (trimyristin), Dynasan 116 (tripalmitin), glyceryl behenate, glycerol monostearate, monostearin, stearic acid, tristearin, tricaprin, Witepsol E 85, and Precirol ATO 5. The proposed advantages of these polymeric NPs are increased drug stability, high drug payload (both hydrophilic and lipophilic drugs), no biotoxicity of the carrier, ease of scaleup, and sterilization.
- The nanoemulsion is prepared mainly by the spontaneous emulsification or titration method. In this method, the nanoemulsion is prepared by blending oil, water, surfactant, and cosurfactant in the appropriate proportions with mild agitation. Nanoemulsions are thermodynamically stable preparations with a particle size of less than 100 nm. Nanoemulsions are mainly used to improve the transdermal and dermal delivery of drugs. The various approaches and methods of NP preparation are depicted in Fig. 3.2.

2.3 Methods of Concentrating NPs

The prepared NPs require concentrating to reduce the volume of administration; this reduces the systemic overexposure of excipients. The concentration process plays an important role in final particle size and its aggregation in the final formulations. There are several methods for concentrating NPs, such as centrifugation, lyophilization, evaporation, and dialysis. Concentrating to the desired volume by evaporation is usually performed by rotary evaporation. Based on the solvent and polymer, the temperature and vacuum are optimized. During this process, the polymer layer of the NPs is also solidified. Using lyophilization, the NPs are transformed into a free flowing dry powder, and this approach also helps to avoid microbiological degradation, premature polymer degradation, physicochemical instability, and loss of drug activity. To avoid damage to NPs during the freezing and lyophilization process, special excipients, for cryoprotectant (to overcome freezing stress) and lyoprotectant (to overcome drying stress) actions, are added to the nanosuspension before freezing. Some of the very frequently used cryo- or lyoprotectants are glucose, sucrose, lactose, mannitol, sorbitol, poly(vinyl pyrrolidone), glycerol, poly(vinyl alcohol), and dextran. Other methods of concentrating NPs are centrifugation and ultracentrifugation. Normal centrifugation, performed at low g forces, can remove aggregates and large particles from the polymeric nanoparticle suspension, but this method will not guarantee removal of all particles above the nanometer size in the formulation. Ultracentrifugations can sediment particles with slightly higher density than water. Ultracentrifugation is performed at 100,000–110,000 g for 30–45 min to form pellet of NPs. These pellets can be reconstituted to the desired volume of dispersion medium. NP concentration by dialysis can be performed using dif-

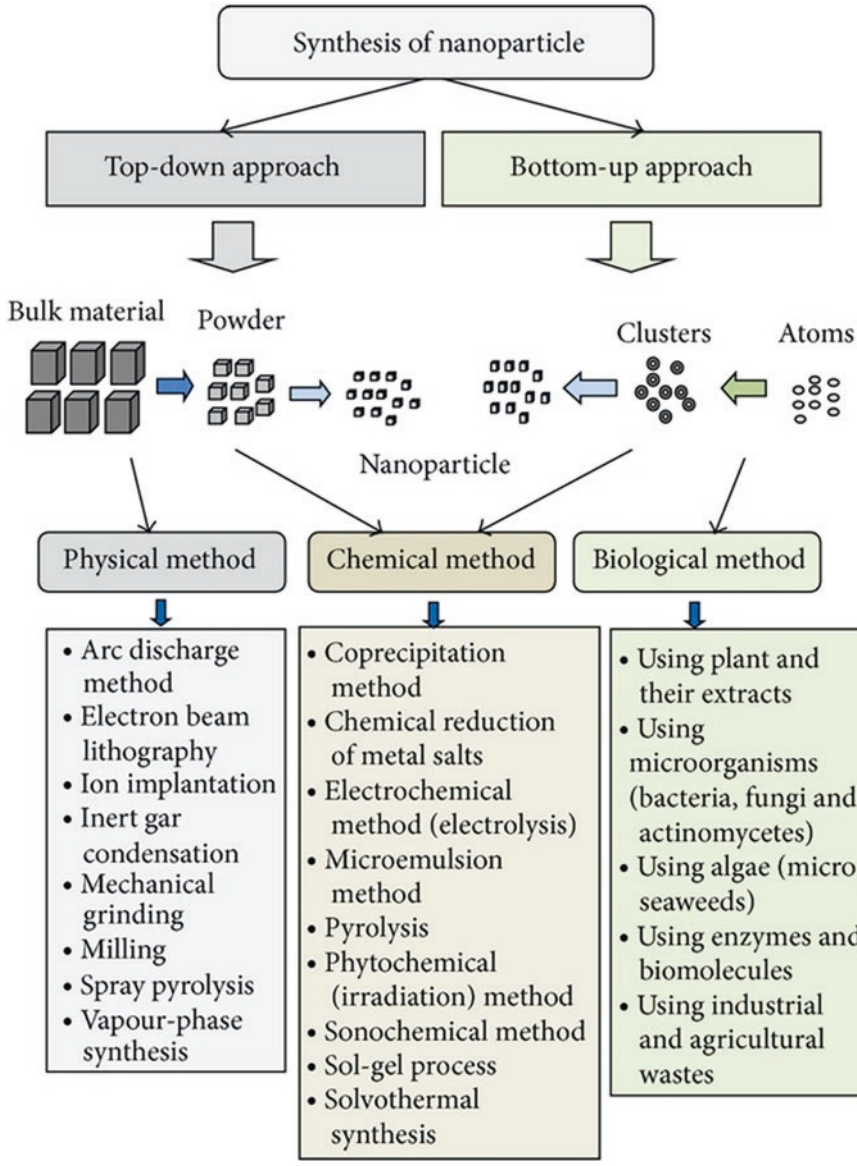

Fig. 3.2 Approaches and methods for nanoparticles

ferent cellulose membranes with various molecular weight cut-offs. In the simple dialysis method, the concentration of the suspension is performed against a polymer solution. This causes an osmotic stress producing a displacement of water from the nanosuspension toward the counter-dialysis solution. The dialysis method results showed that the amount of water removed can be controlled and the process is reproducible [12, 13].

2.4 Models for Biopharmaceutical Evaluation of NPDDS

Biopharmaceutical modeling has become integral to the design and development of new drugs. Influencing key aspects of the development process, including drug substance design, formulation design, and toxicological exposure assessment, biopharmaceutical modeling is now seen as the linchpin to a drug's future success.

And while there are a number of commercially available software programs for drug modeling, there has not been a single resource guiding pharmaceutical professionals to the actual tools and practices needed to design and test safe drugs.

2.5 Molecular Modeling

Molecular modeling can be seen as the sum of two components: a molecular model and a computational technique to properly characterize the behavior of the molecules.

Building a suitable molecular model, that is, how the system under investigation is rationalized and represented in the framework of a meaningful simulation, is the first fundamental step. In this framework, molecular models can be essentially divided into two categories; on the one hand, full atomistic models provide the highest level of detail since all atoms (considered as the smallest constitutive units of the model) are explicitly accounted for. On the other hand, coarse-grained (CG) models summarize the atomic detail by enclosing groups of atoms into beads that lump the main peculiarities (in terms of charge, polarity, etc.) of the atoms that they embed. This simplification is unavoidable for complex systems whose atomistic representation would be prohibitive from a computational point of view, in terms of the system size and/or time and length scales needed to investigate the phenomena of interest. Despite the loss of detail, a CG model that retains the main features of the system is able to provide meaningful insights at a reasonable computational cost (vide infra). For the sake of completeness, there exist more detailed representations where electrons are the smallest constitutive units and are explicitly included. Such models are treated with quantum chemistry methods, which are not considered or discussed here since their application in the field of nanomedicine is hindered by their computational inefficiency.

In a broader sense, a molecular model also includes unavoidable simplifications that allow for the simulation of complex systems, either at a full atomistic or CG level of detail, which could not be treated otherwise. The simulation of protein adsorption on a microparticle surface, for example, is unfeasible because of the system size. Such a system is usually simplified by adopting a molecular model that involves the adsorption of a protein on a flat surface with a suitable thickness. This approach is reasonable since the phenomena of interest are restricted to the solvent/particle interface; in addition, since protein size is much smaller than microparticle radius, curvature effects can be reasonably neglected.

The second component of molecular modeling is constituted by suitable computational methods that allow the characterization of the dynamics, energetics, and conformational sampling of the system of interest. Full atomistic models are usually treated with molecular dynamics, while other techniques such as CG molecular dynamics and dissipative particle dynamics (DPD) are employed along with CG models [14–16].

2.6 Full Atomistic Models

In molecular dynamics simulations, atoms are represented as spheres that interact with each other by virtue of a potential energy function, usually called the force field (FF). Molecular coordinates and velocities as a function of simulation time can be evaluated by solving Newton's equation of motion with a suitable numerical integration scheme, as shown in Eq. 3.1:

$$m_i \frac{d^2 r_i}{dt^2} = F_i = -\nabla U(r) \tag{3.1}$$

where m_i is the mass of the ith atom, r_i are the spatial coordinates of the ith atom, t is time, F_i is the force acting on the ith atom, and $U(r)$ is the potential energy (i.e., the FF), which is a function of the coordinates of all atoms present in system r. Such an approach essentially implies a couple of assumptions, as follows. First, the motion of electrons can be reasonably described by the dynamics of the corresponding nuclei (Born–Oppenheimer approximation). Second, the motion of the atomic nuclei (which are heavier

than electrons) can be described as point particles that follow classical mechanics; this is an acceptable approximation when quantum effects are not important. Generally speaking, a FF takes into account both intramolecular and intermolecular interactions, in terms of bonds, angles, dihedrals, and long-range interactions, namely, van der Waals and electrostatic.

FFs contain several parameters that are computed in order to reproduce the conformational energies and minimum energy structures obtained from high-level quantum mechanics calculations and/or experimental data, such as hydration enthalpies or structural parameters from NMR experiments. There are "general-purpose" FFs, usually employed to describe small ligands, as well as FFs specifically tailored for given categories of molecules, like proteins, nucleic acids, carbohydrates, and lipids. The choice and the quality of the FF cannot be underestimated, since they strongly affect the reliability of the simulation outcome.

MD simulations do not explicitly consider electrons, so chemical reactions and excited states cannot be investigated; however, they constitute the ideal tool for those systems that are mainly governed by non-covalent interactions, like electrostatic and van der Waals forces. MD also allows environmental conditions to be included through the addition of explicit solvent molecules, ions, and other solute molecules into the system. The main outputs from an MD simulation are molecular trajectories, the post-processing of which can provide structural information (binding poses, protein conformation) as well as energetic information such as interaction energies [17–19].

2.7 Enhanced Sampling Methods

The characteristic time and length scales of MD simulations are in the tens to hundreds of nanoseconds (up to 1000 ns) and tens of nanometers (up to 20 nm), respectively. However, many phenomena of interest (e.g., molecular binding, protein unfolding) need large time scales to occur (up to minutes), and their investigation through MD would be in principle unfeasible; this is due to the presence of metastable states separated by high free energy barriers. A way to overcome this issue is to use enhanced sampling methods, which allow enhancement of the transitions between different metastable states separated by energy barriers higher than the thermal energy k_BT, which would not be crossed in a standard simulation at temperature T (where k_B is the Boltzmann constant and T is absolute temperature). As recently reviewed, there are three different suitable approaches: (i) increasing the temperature T, (ii) changing the potential $U(r)$, and (iii) adding an external bias potential $V(r)$. Each approach has its own methods, the discussion of which (along with their theoretical basis) is well beyond the purpose of this review; the interested reader is referred to ad hoc reviews. Some of the popular enhanced sampling techniques are replica exchange (RE, first approach) and well-tempered metadynamics (WTM), which belongs to the third group. In particular, WTM and its variant forms allow the free energy of the system under investigation to be recovered by adding an external bias on a selected number of degrees of freedom, commonly referred to as collective variables (CVs). CVs are generally functions of atomic coordinates and can range from simple quantities, such as distances and dihedral angles, to more complicated variables, like the number of hydrogen bonds/hydrophobic contacts, alpha-helix content in a protein, or Debye–Hückel interaction energy. CVs must be chosen so that they can discriminate between metastable states and can be representative of the transition mechanism. Typical applications of WTM and WTM-based methods are the study of protein conformations (also in the presence of denaturants), the binding poses of small ligands to target proteins, and the conformation and self-assembly of polymeric and supramolecular systems. Some phenomena, such as protein folding, require a relevant number of CVs to perform meaningful simulations. Although conceptually feasible, running a WTM simulation with many CVs introduces some issues such as a drop in computational efficiency and a nontrivial analysis of the results obtained. In order to overcome this issue, some

WTM variants have been proposed, discussed, and validated in literature (mainly for protein folding), namely, parallel tempering metadynamics (PTMD), parallel tempering metadynamics in the well-tempered ensemble (PTMD-WTE), and bias exchange metadynamics (BEMD) [20–21].

2.8 CG Models

The aim of CG models is to perform meaningful simulations of systems whose analysis would be challenging or unfeasible with full atomistic MD methods by building simplified representations that allow the main physical/chemical features (like the interplay between hydrophobic and hydrophilic effects) to be retained.

In the coarse-graining procedure, groups of atoms are enclosed into "beads" or "interaction sites" that are representative of the embedded atoms in terms of charge, size, hydrophobicity/hydrophilicity, etc. Beads interact with each other by virtue of a potential energy function, which takes into account both bonded interactions (i.e., bond, angles, and dihedrals) and nonbonded interactions and which is parameterized in order to optimally reproduce some experimental properties (like water/octanol partition) or the behavior of more detailed full atomistic simulations [22, 23].

Trajectories can be computed by integrating Newton's equation of motion and also adding other components to the force such as friction due to the solvent (if implicit solvent methods are used) (vide infra).

It is worth mentioning that the coarse-graining procedure can be performed to different extents, since a bead can enclose a group of atoms (three to four heavy atoms), a group of monomers (or amino acids), an entire protein, or an entire microparticle, according to the aim of the simulation. In this review, the term "CG models" is employed for all those approaches where there is a loss of degrees of freedom with respect to a full atomistic description.

A common drawback of CG models is that parameterization is strictly tailored for the system under investigation and in principle should be repeated for every new system; in other words, parameters are not transferable. In this regard, the MARTINI FF (Marrink et al., 2007) attracted a lot of interest due to its reliability and straightforward coarse-graining procedure. Beads (which include groups of three to four heavy atoms) still interact with each other through a simple potential energy function, as described for MD (vide supra). MARTINI offers a library of parameterized beads, mainly divided into four categories: polar, nonpolar, apolar, and charged; in addition, each group includes subgroups representative of polarity and hydrogen bond capability. Parameters for bonded interactions (bonds, angle, dihedrals) must be determined from detailed MD simulations, while nonbonded interactions are tuned in order to reproduce thermodynamic properties like free energy of hydration, free energy of vaporization, and partitioning between water and different solvents. Explicit water and ions can also be added (a MARTINI water bead is representative of four water molecules) [24, 25].

Bead parameterization can be further refined by the user in order to improve agreement with full atomistic simulations. Even with simulations based on the MARTINI FF, some phenomena of interest can be still characterized at a time scale that is not accessible.

Another widely employed method with CG models is DPD. Bead trajectories are still obtained by means of Newton's equation of motion, assuming that each *i*th particle is subjected to three pair-additive forces that arise from the interactions with the other *j*th particles: a conservative force, a dissipative force, and a random force.

$$m_i \frac{d^2 r_i}{dt^2} = f_i = \sum_{j \neq i} F_{ij}^c + F_{ij}^d + F_{ij}^r$$

The conservative force F^c is due to the interaction potential of particles and accounts for both bonded and long-range interactions through an elastic force and a soft repulsion force, respectively. F^d is a dissipative force that damps the relative motion between particles, and F^r is a random force directed along the line that connects bead centers. Dissipative and random forces are

momentum-conserving and represent the minimal model that takes into account viscous forces and thermal noise between particles [26–28].

3 Conclusion

Biopharmaceuticals' recent breakthrough success has transformed the treatment of a variety of disorders. However, there are still issues with formulation and administration. Colloidal nanocarriers may be a viable solution for overcoming these obstacles. Nanotechnology not only provides novel technologies for biopharmaceutical manufacturing but also suggests noninvasive, safe, and targeted decontamination solutions. Furthermore, the application of nanotechnology could improve the accessibility of biopharmaceuticals to target locations for the treatment of specific clinical disorders. Biological and technological obstacles make clinical translation and commercialization for biopharmaceutical delivery questionable, despite nanocarriers' great qualities. Scaling up nanocarrier formulations and conducting quality control to manage their physicochemical qualities takes a lot of work. The efficacy and short- and long-term toxicity of the nanocarrier-based biopharmaceuticals used in a given therapy must be determined. Overall, nanocarrier-based biopharmaceutical delivery has a lot of promise for successful treatment of diseases.

References

1. Walsh G. Biopharmaceutical benchmarks 2018. Nat Biotechnol. 2018;36(12):1136–45.
2. Chen YC, Cheng HF, Yeh MK. Cell therapy regulation in Taiwan. Cell Transplant. 2017;26(3):483–92.
3. Gonçalves GAR, Paiva RMA. Gene therapy: advances, challenges and perspectives. Einstein (Sao Paulo). 2017;15(3):369–75.
4. Calo-Fernández B, Martínez-Hurtado JL. Biosimilars: company strategies to capture value from the biologics market. Pharmaceuticals. 2012;5(12):1393–408.
5. Nick C, The US. Biosimilars act: challenges facing regulatory approval. Pharm Med. 2012;26(3):145–52.
6. Camacho LH, Frost CP, Abella E, Morrow PK, Whittaker S. Biosimilars 101: considerations for U.S. oncologists in clinical practice. Cancer Med. 2014;3(4):889–99.
7. Declerck P, Farouk-Rezk M, Rudd PM. Biosimilarity versus manufacturing change: two distinct concepts. Pharm Res. 2016;33(2):261–8.
8. Vert M, Doi Y, Hellwich KH, Hess M, Hodge P, Kubisa P, Rinaudo M, Schué FO. Terminology for biorelated polymers and applications (IUPAC recommendations 2012). Pure Appl Chem. 2012;84(2):377 410.
9. Vert M, Doi Y, Hellwich K-H, Hess M, Hodge P, Kubisa P, Rinaudo M, Schué F. Terminology for biorelated polymers and applications (IUPAC recommendations 2012). Pure Appl Chem. 2012;84(2):377–410.
10. Khan I, Saeed K, Khan I. Nanoparticles: Properties, applications and toxicities. Arab J Chem. 2019;12(7):908–31.
11. Reiss G, Hutten A. Magnetic Nanoparticles. In: Sattler KD, editor. Handbook of nanophysics: nanoparticles and quantum dots. CRC Press; 2010. p. 2 1. ISBN 9781420075458.
12. Crucho CIC, Barros MT. Polymeric nanoparticles: A study on the preparation variables and characterization methods. Mater Sci Eng C. 2017;80:771–84.
13. Pal SL, Utpal Jana PK, Manna GP, Mohanta RM. Nanoparticle: an overview of preparation and characterization. J Appl Pharm Sci. 2011;01(06):228–34.
14. Bochicchio D, Pavan GM. Molecular modelling of supramolecular polymers. Adv Phys X. 2018;3:315–37. https://doi.org/10.1080/23746149.2018.1436408.
15. Bruno A, Scrima M, Novellino E, D'errico G, D'ursi, A. M., and Limongelli, V. The glycan role in the glycopeptide immunogenicity revealed by atomistic simulations and spectroscopic experiments on the multiple sclerosis biomarker CSF114(Glc). Sci Rep. 2015;5:9200. https://doi.org/10.1038/srep09200.
16. Camilloni C, Pietrucci F. Advanced simulation techniques for the thermodynamic and kinetic characterization of biological systems. Adv Phys X. 2018;3:1477531. https://doi.org/10.1080/23746149.2018.1477531.
17. Deighan M, Bonomi M, Pfaendtner J. Efficient simulation of explicitly solvated proteins in the well-tempered ensemble. J Chem Theory Comput. 2012;8:2189–92. https://doi.org/10.1021/ct300297t.
18. Deighan M, Pfaendtner J. Exhaustively sampling peptide adsorption with metadynamics. Langmuir. 2013;29:7999–8009. https://doi.org/10.1021/la4010664.
19. Dickson CJ, Hornak V, Pearlstein RA, Duca JS. Structure-kinetic relationships of passive membrane permeation from multiscale modeling. J Am Chem Soc. 2017;139:442–52. https://doi.org/10.1021/jacs.6b11215.
20. Frenkel D, Smit B. Understanding molecular simulation: from algorithms to applications. San Diego: Academic Press; 2002.
21. Bochicchio D, Pavan GM. Effect of concentration on the supramolecular polymerization mechanism via

implicit-solvent coarse-grained simulations of water-soluble 1,3,5-benzenetricarboxamide. J Phys Chem Lett. 2017;8:3813–9. https://doi.org/10.1021/acs.jpclett.7b01649.

22. Wei S, Ahlstrom LS, Brooks CL. Exploring protein-nanoparticle interactions with coarse-grained protein folding models. Small. 2017;13:1603748. https://doi.org/10.1002/smll.201603748.
23. Lopez H, Lobaskin V. Coarse-grained model of adsorption of blood plasma proteins onto nanoparticles. J Chem Phys. 2015;143:243138. https://doi.org/10.1063/1.4936908.
24. Yu GB, Zhou J. Understanding the curvature effect of silica nanoparticles on lysozyme adsorption orientation and conformation: a mesoscopic coarse-grained simulation study. Phys Chem Chem Phys. 2016;18:23500–7. https://doi.org/10.1039/C6CP01478J.
25. Marrink SJ, Risselada HJ, Yefimov S, Tieleman DP, De Vries AH. The MARTINI force field: coarse grained model for biomolecular simulations. J Phys Chem B. 2007;111:7812–24. https://doi.org/10.1021/jp071097f.
26. Marrink SJ, Tieleman DP. Perspective on the Martini model. Chem Soc Rev. 2013;42:6801–22. https://doi.org/10.1039/c3cs60093a.
27. Utesch T, Daminelli G, Mroginski MA. Molecular dynamics simulations of the adsorption of bone morphogenetic protein-2 on surfaces with medical relevance. Langmuir. 2011;27:13144–53. https://doi.org/10.1021/la202489w.
28. Valsson O, Tiwary P, Parrinello M. Enhancing important fluctuations: rare events and metadynamics from a conceptual viewpoint. Annu Rev Phys Chem. 2016;67:159–84. https://doi.org/10.1146/annurev-physchem-040215-112229.