Chapter 28

Copaxone® in the Era of Biosimilars and Nanosimilars

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28.1 Introduction

28.1.1 Nanoterminology and the Issue of Size in Drug Delivery

Rapid advances and product development in nanomedicine are in full swing as it continues to influence the pharmaceutical, device and biotechnology industries [1a, 1b, 2]. Nanomedicine is driven by collaborative research, patenting, commercialization, business development and technology transfer within diverse areas such as biomedical sciences, chemical engineering, biotechnology, physical sciences, and information technology.

Although various "nano" terms, including "nanotechnology," "nanopharmaceutical," "nanodrug," "nanotherapeutic," "nanomaterial," and "nanomedicine," are widely used, there is confusion, disagreement and ambiguity regarding their definitions. In fact, there is no precise definition of nanotechnology as applied to pharmaceuticals or in reference to drug delivery. This haunts regulators, patent offices, policy-makers, drug formulation scientists, pharma executives, and legal professionals [3–9]. In particular, regulatory agencies and governmental entities such as US Food and Drug Administration (FDA), the European Medicines Agency (EMA), Environmental Protection Agency (EPA), Centers for Disease Control and Prevention (CDC), National Institute for Occupational Safety and Health (NIOSH), International Organization for Standardization (ISO) Technical Committee on Nanotechnology (ISO/TC229), ASTM International, the Organization for Economic Co-operation and Development (OECD) Working Party on Manufactured Nanomaterials (OECD WPMN) and the US Patent and Trademark Office (PTO) continue to grapple with this critical issue [3].

The term nanotechnology is a bit misleading given that it is not one technology, but an umbrella term encompassing several technical/scientific fields, processes and properties at the nano/micro scale [3, 6–9]. Various definitions of nanotechnology have sprung up over the years [3]. Some label it as the manipulation, precision placement, measurement, modeling or manufacture of matter in the sub-100 nm range [10], or in the 1–200 nm range [11, 12]. Some definitions omit a lower range, others refer to sizes in one, two or three dimensions while others require a size plus

special/unique property or *vice versa*. Others point to a size ranging from 1 to 1000 nm in both nanotechnology and pharmaceutical science [13–16]. This latter definition may be the most appropriate from a drug delivery perspective. The FDA, which has not adopted any "official" regulatory definition in this regard, now uses a loose definition for products that involve/employ nanotechnology and stretched the upper limit to 1000 nm. This underscores the urgency of establishing a uniform "nano" terminology. The need for an internationally agreed definition for key terms like nanotechnology, nanoscience, nanomedicine, nanobiotechnology, nanodrug, nanotherapeutic, nanopharmaceutical and nanomaterial is critical [3]. This is important for harmonized regulatory governance, accurate patent searching and prosecution, standardization of procedures, assays and manufacturing, quality control, safety assessment, and more.

Given this backdrop, and the fact that there is no international scientifically accepted nomenclature or uniform regulatory definition pertaining to nanotechnology [6–9], the following widely-accepted definition unconstrained by an arbitrary size limitation has been previously proposed [3, 9]:

The design, characterization, production, and application of structures, devices, and systems by controlled manipulation of size and shape at the nanometer scale (atomic, molecular, and macromolecular scale) that produces structures, devices, and systems with at least one novel/ superior characteristic or property.

This definition above has four key features [3]:

- First, it recognizes that the properties and performance of the synthetic, engineered "*structures, devices, and systems*" are inherently rooted in their nanoscale dimensions. The definition focusses on the unique physiological behavior of the "*structures, devices, and systems*" that is occurring at the nanoscale; it does *not* focus on any shape, aspect ratio, specific size or dimensionality.
- Second, the focus of this flexible definition is on "technology" that has commercial potential from a consumer perspective, not "nanoscience" or basic R&D conducted in a lab-setting that almost certainly lacks commercial implications.
- Third, the "*structures, devices, and systems*" that result must be "*novel/superior*" compared to their bulk, conventional counterparts.

- Fourth, the concept of "*controlled manipulation*" as compared to "self-assembly" is critical to the definition.

Size limitation below 100 nm is frequently touted as the basis of novel properties of nanopharmaceuticals. However, this is simply not true or critical to a drug company from a formulation, delivery, or efficacy perspective because the desired or novel physicochemical properties (e.g., improved bioavailability, reduced toxicities, lower dose or enhanced solubility) may be achieved in a size outside this arbitrary range. For example, the surface plasmon-resonance (SPR) in gold or silver nanoshells/nanoprisms that imparts their unique property as anticancer thermal drug delivery agents also generally operates at sizes greater than 100 nm. Similarly, at the tissue level, the enhanced permeability and retention (EPR) effect that makes passive nanoparticle drug delivery an attractive option operates in a wide range, with nanoparticles of 100–1000 nm diffusing selectively (extravasation and accumulation) into the tumor. At the cellular level, the size range for optimal nanoparticle uptake and processing depends on many factors but is often beyond 100 nm. Liposomes in a size range (diameter) of about 150–200 nm have been shown to have a greater blood residence time than those with a size below 70 nm. In fact, there are numerous FDA-approved and marketed nanonanodrug products where the particle size does not fit the sub-100 nanometer profile: Abraxane (~120 nm), Myocet (~190 nm), DepoCyt (10–20 micometer), Amphotec (~130 nm), Epaxal (~150 nm), DepoDur (10–20 μm), Inflexal (~150 nm), Lipo-Dox (180 nm), Oncaspar (50–200 nm), etc.

This does not imply that any size will do for delivering nanopharmaceuticals. For example, submicron sizes are generally considered essential for biological distribution of biopharmaceuticals for safety reasons [17]. Particles greater than 5 μm can often cause pulmonary embolism following intravenous injection [18]. Therefore, submicron particle size is preferred for all parenteral formulations. In ophthalmic applications, the optimal particle size is less than 1 μm because microparticles around 5 μm can cause a scratchy feeling in the eyes [19].

Just like nanotechnology, there is no universally accepted definition of nanomedicine. The European Science Foundation [20] correctly defines nanomedicine as:

> *[T]he science and technology of diagnosing, treating, and preventing disease and traumatic injury, of relieving pain, and of preserving and improving human health, using molecular tools and molecular knowledge of the human body.*

The National Institutes of Health (NIH) Roadmap for Medical Research in Nanomedicine [21] defines nanomedicine as:

> *[A]n offshoot of nanotechnology, refers to highly specific medical interventions at the molecular scale for curing disease or repairing damaged tissues, such as bone, muscle, or nerve.*

Although numerous nanodrugs have been routinely used in medicinal products for decades without any focus or even awareness of their nano-character, it is only within the past two decades that they have been highlighted due to their potential of revolutionizing drug delivery [3, 22]. Obviously, the "Holy-Grail" of any drug delivery system is to deliver the correct dose of a particular active agent to a specific disease site while minimizing toxic side effects and optimizing therapeutic benefit. This is often not achievable via traditional drugs [22–26]. However, the potential now exists via engineered nanopharmaceuticals.

Many liquid nanopharmaceuticals are colloidal drug delivery systems of 1–1000 nm [3, 26]. Their nano-character—functional complexity and application potential—is related to one or more of the following properties [25–26]:

- nano-scale dimensions/small size (high surface area-to-volume ratio)
- reduced toxicity
- controlled-release property
- altered/modified pharmacokinetics
- enormous compositional range and variety of therapeutics and carriers that can be formulated/packaged
- superior biological distribution and targeting capabilities due to the ability to attach specific targeting moieties
- various delivery route potential (oral, topical, intravenous, subcutaneous, etc.)
- variety of shapes/geometries (Fig. 28.1)
- crystallinity
- aspect ratio
- surface charge

There are numerous potential advantages of nanopharmaceuticals, and harnessing them depends on various factors such as

their mode of delivery and specific class employed [25–29]. Some of these advantages include:

- increased bioavailability due to enhanced water solubility of hydrophobic drugs because of the large specific surface area;
- ability to protect biologically unstable drugs from the hostile bioenvironment of use/delivery/release (e.g., against potential enzymatic or hydrolytic degradation);
- extended drug residence time at a particular site of action or within specific targeted tissue and/or extended systemic circulation time;
- controlled drug release at a specific desired site of delivery;
- endocytosis-mediated transport of drugs through the epithelial membrane;
- bypassing or inhibition of efflux pumps such as P-glycoprotein;
- targeting of specific carriers for receptor-mediated transport of drugs;
- enhanced drug accumulation at the target site so as to reduce systemic toxicity;
- providing biocompatibility and biodegradability;
- offering a high drug-loading capacity;
- providing long-term physical and chemical stability of drugs; and
- improved patient compliance.

In this chapter, the following equivalent terms are used interchangeably: nanodrug, nanotherapeutic, nanomedicine and nanopharmaceutical. Again, there is no formal definition for a nanotherapeutic. In this chapter, we will employ Dr. Bawa's definition [3] for a nanotherapeutic formulation (or nanodrug product) as being*: (1) a formulation, often colloidal, containing therapeutic

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*This definition parallels that proposed by numerous experts and disregards that presented by US federal agencies like the NNI. See also:

Bogunia-Kubik, K., Sugisaka, M. (2002). From molecular biology to nanotechnology and nanomedicine. *BioSystems*, **65**, 123–138.

Junghanns, J.-U. A. H., Müller, R. H. (2008). Nanocrystal technology, drug delivery and clinical applications. *Int. J. Nanomed.*, **3**(3), 295–310.

Ledet, G., Mandal, T. K. (2012). Nanomedicine: Emerging therapeutics for the 21st century. *U.S. Pharm.*, **37**(3), 7–11.

McDonald, T. O., Siccardi, M., Moss, D., Liptrott, N., Giardiello, M., Rannard, S., Owen, A. (2015). The application of nanotechnology to drug delivery in medicine. In: P. Dolez, ed. *Nanoengineering: Global Approaches to Health and Safety Issues*, Elsevier, pp. 173–223.
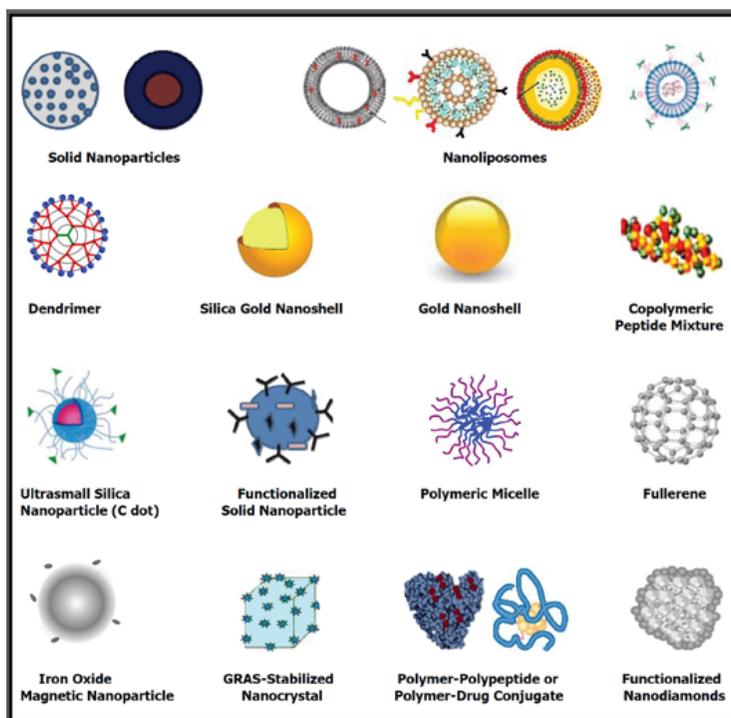
Figure 28.1 Schematic Illustrations of Nanoscale Drug Delivery System Platforms (Nanotherapeutics or Nanodrug Products). Shown are nanoparticles (NPs) used in drug delivery that are either approved, are in preclinical development or are in clinical trials. They are generally considered as first or second generation multifunctional engineered NPs, generally ranging in diameters from a few nanometers to a micron. Active biotargeting is frequently achieved by conjugating ligands (antibodies, peptides, aptamers, folate, hyaluronic acid) tagged to the NP surface via spacers or linkers like PEG. NPs such as carbon nanotubes and quantum dots, although extensively advertised for drug delivery, are specifically excluded from the list as this author considers them commercially unfeasible for drug delivery. Non-engineered antibodies and naturally occurring NPs are also excluded. Antibody-drug conjugates (ADCs) are encompassed by the cartoon labelled "Polymer-Polypeptide or Polymer-Drug Conjugate." This list of NPs is not meant to be exhaustive, the illustrations are not meant to reflect three dimensional shape or configuration and the NPs are not drawn to scale. Abbreviations: NPs: nanoparticles; PEG: polyethylene glycol; GRAS: Generally Recognized As Safe; C dot: Cornell dot; ADCs: Antibody-drug conjugates. (Copyright © 2016 Raj Bawa. All rights reserved).

particles (nanoparticles) ranging in size from 1–1,000 nm; *and* (2) either (a) the carrier(s) is/are the therapeutic (i.e., a conventional therapeutic agent is absent), or (b) the therapeutic is directly coupled (functionalized, solubilized, entrapped, coated, etc.) to a carrier. There are a number of FDA-approved, commercialized nanopharmaceuticals [7, 8, 25–27] (Fig. 28.1) for intravenous use as well as for non-intravenous delivery. However, numerous nanopharmaceuticals are still at the development or clinical trial phase. This chapter focuses on Copaxone®, a drug developed and marketed by Teva Pharmaceutical Industries, Ltd., Israel (NYSE: TEVA) and indicated for the treatment of patients with relapsing forms of multiple sclerosis (MS) [30].

28.1.2 Copaxone® (Glatiramer Acetate) and Multiple Sclerosis

Copaxone® is a non-biologic (synthetic) complex drug ("NBCD") and first-generation nanomedicine composed of an uncharacterized mixture of immunogenic polypeptides in a colloidal solution. The active ingredient in Copaxone®—glatiramer acetate—is a heterogeneous synthetic mixture of polypeptides comprising four amino acids (L-glutamic acid, L-alanine, L-lysine, and L-tyrosine) in a defined molar ratio [37]. Glatiramer acetate has immunomodulatory effects on innate and acquired immunity and is indicated for the treatment of patients with relapsing forms of multiple sclerosis (MS) [30]. Copaxone® is currently available as a daily 20 mg subcutaneous injection (approved in the United States in 1996). A 40 mg subcutaneous injection, administered three times a week was approved in 2014 in the United States and Europe.

MS is a chronic degenerative autoimmune disease in which inflammatory infiltrates damage the myelin sheath and central axons, impeding neuronal conduction [60]. MS affects an estimated 2.3 million people worldwide, a majority of whom are women [61]. MS typically strikes young adults; most patients are diagnosed between ages 20–50 [61]. Symptoms of MS include visual disturbances, ataxia, weakness, fatigue, cognitive impairment, depression, sexual dysfunction, lack of bladder control and spasticity [60, 62]. The cause of MS is unknown; however, a combination of several factors are implicated [63]. It is generally

accepted that MS involves an immune-mediated process directed against the myelin in the central nervous system [63].

Until the development of disease-modifying therapies (DMTs) such as Copaxone®, only symptomatic therapy was available to MS patients. Currently there are several DMTs approved in the EU, Canada, and US for the treatment of RRMS. However, Copaxone® is unique in several ways. Copaxone® is the first drug to show proven therapeutic efficacy in MS and the first product to have a copolymer of amino acids as its active ingredient [64]. Copaxone® is not a single molecular entity, rather a it is heterogeneous mixture of potentially millions of distinct, synthetic polypeptides of varying lengths, some containing up to 200 amino acids with structural complexity comparable to that of proteins, or even more complex than proteins [37]. It is presently impossible to isolate and identify its pure components even via the most technologically sophisticated multidimensional separation techniques [37]. The complexity of glatiramer acetate is amplified by several aspects [37, 46, 65]: (1) the active moieties in glatiramer acetate are unknown; (2) the mechanisms of action are not completely elucidated; (3) pharmacokinetic testing is not indicative of glatiramer acetate bioavailability; (4) pharmacodynamic testing is not indicative of therapeutic activity and there are no biomarkers available as surrogate measures of efficacy; and (5) small changes in the glatiramer acetate mixture can change its immunogenicity profile. Therefore, analogous to biological products, synthetic glatiramer acetate is defined, in large part, by its well-controlled manufacturing process that has been used by Teva for more than 20 years.

28.2 Non-Biologic Complex Drugs and Regulatory Pathway for Follow-On Products

Medicinal products can be broadly divided into three classes: (1) small-molecule drugs, (2) biologic drugs, and (3) non-biological (synthetic) complex drugs or NBCDs. NBCDs have been defined as medicinal products that are not biological medicines. In NBCDs, the active agent or therapeutic moiety is not a homo-

molecular structure but consists of different yet closely related and often nanoparticulate structures that cannot be isolated, fully quantitated, and/or characterized via standard analytical or physicochemical techniques [66] (Fig. 28.2). It is also unknown which structural elements might affect their therapeutic performance [66]. As stated earlier, Copaxone® is a NBCD. In addition to Copaxone®, other NBCDs include certain liposomes and iron-carbohydrate drugs. NBCDs typically use multiple starting components and the final product(s) represents the result of a complex and often proprietary manufacturing process. In this regard, if one of the starting components or final products exhibit nano-dimensions or nano-characteristics, the term nanomedicine is often employed.
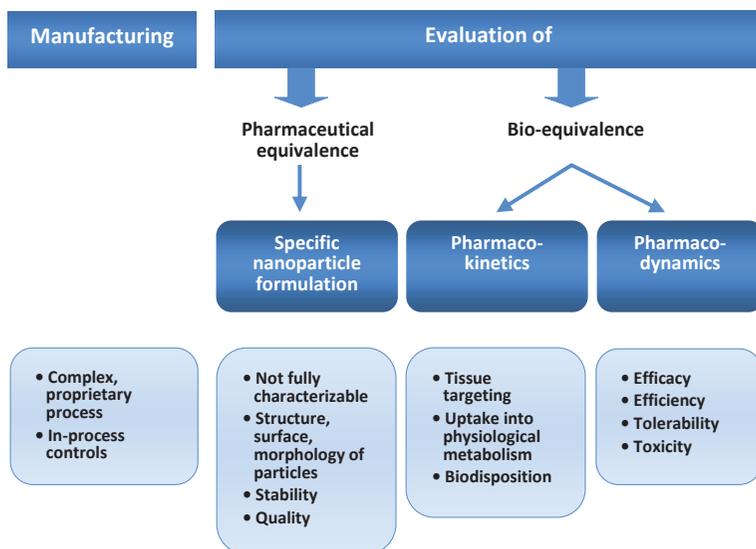
Figure 28.2 NBCDs therapeutic equivalence from manufacturing to safety and efficacy. Reproduced with permission from [66].

Both the FDA and the EMA regulate new drugs and biologics for approval and licensure. In the US, small-molecule drugs are regulated under the Food, Drug and Cosmetics Act ("FDCA" or "FD&C Act"). However, biologics are currently regulated under both the Public Health Service Act ("PHSA") and FDCA because some products also fall within the older FDCA approval route of "drugs."

Although the PHSA uses the term "biologics" when referring to biological products, there are other interchangeably equivalent terms in this regard: "biopharmaceuticals," "biomolecular drugs," "biologic drugs," and "protein products." Branded biologics are referred to as "pioneer," "branded" or "reference" biologics. Small molecule drugs approved by the FDA are known as New Chemical Entities (NCEs) while approved biologics are referred to as New Biological Entities (NBEs). As a result, a new drug application for an NCE is known as a New Drug Application (NDA), whereas a new drug application for an NBE is called a Biologic License Application (BLA). Note that prior to the 1980s there were very few marketed biologics, so the very term "pharmaceutical" or "drug" implied a small molecule drug.

The classic generic pathway for a small molecule drug relies on the therapeutic equivalence to the innovator or reference listed drug (RLD). This entails that it be pharmaceutically equivalent (i.e., identical active substance) and bioequivalent (i.e., comparable pharmacokinetics) as established in a small volunteer study that does not require formal clinical efficacy or safety studies. The acceptance intervals must show that for bioequivalence the logarithm transformed AUC and C_{max} ratios must lie within an acceptance range of 0.80 to 1.25 for the 90% confidence intervals [68]. This classic generic approval approach has been successful for many well-defined, small, low-molecular weight drugs where the analytical testing fully characterized the product.

The pathway to a biosimilar product is different. The concept of similarity was introduced in the general biosimilar guidelines published by the EMA in 2005 [68, 69a]. They were revised in 2014 [69b]. To become authorized as a biosimilar in the European Union (EU) the applicant needs to show similarity in quality, safety and efficacy [69a]. A biosimilar needs a full quality dossier comparable with that of an original biological product. The application should also contain a comparison in physicochemical and other *in vitro* characteristics showing no clinically relevant differences between the biosimilar and innovator. Full toxicity programs need not be repeated and animal pharmacokinetic (PK) and pharmacodynamic (PD) studies may be used to demonstrate similarity with the original. To show clinical similarity, it is not necessary to present equivalent efficacy in all clinical endpoints for every indication for which the

original product is registered. Using a biological endpoint that is sensitive to show possible differences in clinical activity between products is allowed.

In 2010, the Biosimilars Act was enacted into law that established an approval route for biosimilars in the US.[†] The FDA published draft guidances for biosimilars (also known as follow-on biologics or subsequent entry biologics) in 2012 which state that biosimilar applicants should include analytical studies that (i) demonstrate the biological product as being highly similar to the reference product notwithstanding minor differences in clinically inactive components; (ii) include animal studies including the assessment of toxicity; and (iii) provide a clinical study or studies (including the assessment of immunogenicity and PK/PD) that are sufficient to demonstrate safety, purity, and potency in one or more appropriate conditions of use for the reference product is licensed and intended to be used and for which licensure is sought for the biological product [70a]. The agency also stated that it has

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[†]*See*: Biosimilars, Available at: http://www.fda.gov/Drugs/DevelopmentApproval Process/HowDrugsareDevelopedandApproved/ApprovalApplications/ TherapeuticBiologicApplications/Biosimilars/default.htm (accessed on October 6, 2015):

The Patient Protection and Affordable Care Act (Affordable Care Act), signed into law by President Obama on March 23, 2010, amends the Public Health Service Act (PHS Act) to create an abbreviated licensure pathway for biological products that are demonstrated to be "biosimilar" to or "interchangeable" with an FDA-licensed biological product. This pathway is provided in the part of the law known as the *Biologics Price Competition and Innovation Act* (BPCI Act). Under the BPCI Act, a biological product may be demonstrated to be "biosimilar" if data show that, among other things, the product is "highly similar" to an already-approved biological product.

A biosimilar product is a biological product that is approved based on a showing that it is highly similar to an FDA-approved biological product, known as a reference product, and has no clinically meaningful differences in terms of safety and effectiveness from the reference product. Only minor differences in clinically inactive components are allowable in biosimilar products.

An *interchangeable* biological product is biosimilar to an FDA-approved reference product and meets additional standards for interchangeability. An interchangeable biological product may be substituted for the reference product by a pharmacist without the intervention of the health care provider who prescribed the reference product.

FDA requires licensed biosimilar and interchangeable biological products to meet the Agency's rigorous standards of safety and efficacy. That means patients and health care professionals will be able to rely upon the safety and effectiveness of the biosimilar or interchangeable product, just as they would the reference product.

the discretion to determine that an element described above is unnecessary [70a]. An updated version of this guidance was published in 2015 [70b]. The FDA approved the first biosimilar in March 2015. The product developed by Sandoz and called Zarxio (filgrastim-sndz) is the less expensive alternative to Amgen's Neupogen® (filgrastim), a biologic that boosts leucocytes in cancer patients. In addition, a generic version of Remicade® is poised to receive FDA approval later in 2015. The FDA currently has four applications under regulatory review.

The therapeutic equivalence for the third class of follow-on synthetic complex drugs, or NBCDs, has been a hot topic of conversation for the last few years. A workshop on NBCDs was held in Leiden, Netherlands in 2009. The goal of this workshop was to collaborate with various stakeholders, including manufacturers of original products as well as generics and biosimilars, to produce a consensus paper about the scientific issues involved in showing therapeutic equivalence of NBCDs to support the development of harmonized regulatory pathways for NBCD follow-on products [67]. Additional critical discussions on this topic have been held at various workshops and conferences:

- the FIP Centennial Congress held in Amsterdam, Netherlands in 2012;
- the New York Academy of Sciences conference held in New York City in 2012 [73];
- the EUFEPS Regulatory Science Network Workshop held in Ankara, Turkey in 2012;
- the AAPS Meeting held in San Antonio, Texas, USA in 2013;
- the New York Academy of Sciences conference held in New York City in 2013 [74];
- the FDA Public Hearing in 2014 on "Challenges for Non-Biological Complex Drugs" held in Silver Spring, Maryland, USA; and
- the International Symposium on Scientific and Regulatory Advances in Complex Drugs held in Budapest, Hungary in 2014.

The consensus of these meetings is similar: (i) NBCDs are a diverse group of products that cannot be fully characterized because they are extremely complex; (ii) NBCDs cannot be evaluated by any of the existing regulatory pathways developed by EMA and

FDA, i.e., regulatory guidelines for small molecules and biologics (generic drugs and biosimilars respectively) cannot be extrapolated to NBCDs; and (iii) no dedicated regulatory pathways for NBCD follow-on versions exist [68]. Due to heterogeneity and complexity, although NBCDs may share certain features with biologicals, they are much more complex. This is especially true for Copaxone® which contains many "biological-like" constituents; however, it cannot be copied (on the other hand, the sequences of biologicals and biosimilars are identical). NBCD mixtures cannot be fully defined via physico-chemical analysis, and their biological and clinical characteristics are highly dependent upon the specific manufacturing process [68] (Fig. 28.2). In contrast, biosimilars are better understood and the pharmaceutical ingredients in those products are better characterized than the NBCD mixtures. Even then, at least the same basic regulatory guidelines/principles that are used for biosimilars should be used for NBCDs, such as the need for animal and/or clinical data and the need to show similarity in quality, safety, and efficacy. The requirements for follow-on NBCDs should be based on the biosimilar approach with specific requirements based on the science of the individual product. In summary, the new regulatory pathways developed for biologics may serve as the basis for regulating (with case-by-case adjustments) follow-on NBCD products [68]. The reader is directed to an outstanding recent text that discusses various scientific aspects and the regulatory landscape of NBCDs [76].

28.3 Nanomedicines and Regulatory Pathway for Nanosimilars

Glatiramoids are NBCDs [77]. Copaxone® (glatiramer acetate), the first and most studied glatiramoid, is a first-generation nano-medicine that comprises a nano-sized polypeptide mixture with molecules and molecular structures ranging from 1.5 to 550 nm in size. The data demonstrating the colloidal properties of Copaxone® and the nanomedicine attributes are discussed in detail in Section 28.4.

Currently the FDA, EMA, and other regulatory agencies examine each new nanomedicine product on a product-by-product basis [71]. There generally is a lack of recognition that nanomedicine products need their own therapeutic category or regulatory

pathway [71]. However, as the first generation nanomedicine products are coming off patent, regulatory agencies will need to determine what the requirements are for a follow-on nanomedicine product or a nanosimilar product. As many of the NBCDs are also nanomedicines, the requirements for follow-on NBCDs are facing the same lack of regulatory clarity.

The EMA in 2013 published a paper on next-generation nanomedicines and nanosimilars [72]. This paper notes that in order to demonstrate similarity, there is a need for stepwise comparability studies to generate evidence substantiating the similar nature, in terms of quality, safety, and efficacy of the nanosimilar and the originator/innovator nanomedicine [72]. As nanomedicines differ significantly in their complexity, a case-by-case or product/class specific approach for their evaluation may be necessary [72]. This paper [72] also stresses that any drug developed for comparison to the innovator product must demonstrate equivalence in terms of quality, safety and efficacy prior to grant of market authorization. In addition, given the degree of complexity of many nanomedicine products, special scientific considerations may be required to ensure this equivalence of performance [72]. The EMA has also published reflection papers on intravenous liposomal products developed with reference to an innovator product and nano-sized colloidal iron-based preparations developed with reference to an innovator product. To date, there is no reflection paper from the EMA or guidance document from the FDA for glatiramer acetate.

The FDA, on the other hand, continues to state that it will regulate nanomedicine products under its current regulatory regime and that this framework is sufficiently robust and flexible [42], a view not shared by most experts [7, 8, 26].

28.4 Colloidal and Nanomedicine Properties of Copaxone®

Copaxone® is produced using well-established solution polymerization techniques [52]. The nanoscale size of glatiramer acetate molecules is an intrinsic process-related property associated with its chemical nature. The consistent manufacturing process employed by Teva Pharmaceutical Industries, Inc. creates a mixture of glatiramer acetate polypeptides with an average

molecular weight (MW) ranging from 5000–9000 Daltons (the MW distribution of the glatiramer acetate components spans a range of 2500–20,000 Daltons) [37]. The polypeptides in glatiramer acetate appear to range from approximately 20 to 200 amino acids in length, with an average polypeptide length of about 60 amino acids [53]. The theoretical length of glatiramer acetate molecules ranges from 3 to 30 nm, with an average of about 8 nm for the peptide of 7000Da MW. However, as described further below, the molecules and molecular associations in glatiramer acetate appear to reach up to 550 nm.

The FDA has defined the term "colloid" for regulatory purposes as "a chemical system composed of a continuous medium (continuous phase) throughout which are distributed small particles, 1 to 1000 nm in size (disperse phase), that do not settle out under the influence of gravity; the particles may be in emulsion or in solution." [54]. While this definition, which appears to be derived from Dorland's Medical Dictionary for Health Consumers [55], is generally considered accurate, a more precise, scientific definition is as follows [56]:

> *A colloid, or disperse phase, is a dispersion of small particles of one material in another. In this context, "small" means something less than about 500 nm in diameter (about the wavelength of visible light). In general, colloidal particles are aggregates of numerous atoms or molecules, but are too small to be seen with an ordinary optical microscope. They pass through most filter papers, but can be detected by light scattering and sedimentation.*

Regardless of the definition applied, Copaxone® unquestionably is a colloidal solution. Glatiramer acetate nanoparticles are within the typical colloidal size range of 1 to 1000 nm (1 μm) (denoted as radius (r) in Stoke's law) and are uniformly suspended in a continuous medium (mannitol solution). The mannitol solution is a "true" solution, i.e. it is a homogenous solution in which the ratio of solute to solvent remains constant and in which all of the solute particles have diameters less than 10^{-7} centimeters (<10 nm), and the mannitol in solution cannot be centrifuged or filtered from the solution. As such, the aqueous mannitol solution constitutes a continuous medium. The glatiramer acetate nanoparticles dispersed in the mannitol solution do not precipitate under the influence of normal gravitational forces, even when stored at 2–8°C for up to 2 years; thus, Copaxone® is stable under these conditions.

The results of traditional colloidal assessments capable of distinguishing compositional features of Copaxone® at the molecular level further confirm the colloidal nature of Copaxone® [57]. These experiments, which included ultracentrifugation, DLS, AFM, cryogenic temperature, transmission electron microscopy, and zeta potential testing, demonstrate the following:

- Copaxone® is composed of two, distinct populations of polypeptides, both of which are within the size range for colloids (i.e. 1 to 1000 nm).
- The glatiramer acetate polypeptides are stable and distributed uniformly throughout the aqueous mannitol medium.
- Copaxone® constituents can be separated into layers by ultracentrifugation and then easily reconstituted, indicating that Copaxone® is a lyophilic colloidal solution in which the dispersed particles are well-solvated and stabilized rather than a true solution in which the dispersed particles are dissolved.
- Copaxone® has a high zeta potential, suggesting that it is highly stable and resists flocculation and settling under normal gravitational forces.

The results of this testing are discussed in more detail below:

Separation by Ultracentrifugation and Resuspension

Stable colloids do not "settle out under the influence of" normal gravitational forces. However, they will potentially exhibit separation of the disperse phase under increased gravitational forces, such as ultracentrifugation. The stability of colloidal solutions is characterized by, among other things, Stoke's law ($dx/dt = 2r^2(d_c - d_p)g/9h$). By increasing gravity (g) through ultracentrifugation where ($d_c - d_p$) is negative, dx/dt can be increased sufficiently to separate the suspended particles in the stable colloidal solutions.

To show that Copaxone® is not a "true" solution, a Copaxone® sample was ultracentrifuged for 24 h at 4°C under 530,000g ("treated sample"). The sample was segregated into a concentrated layer of higher MW polypeptide moieties (a whitish, dispersed phase in the lower layer of the centrifuged sample) and a layer of lower MW polypeptide moieties (a more translucent upper layer). The upper and the lower layers of the treated sample were tested

for glatiramer acetate concentrations, which were measured using size exclusion chromatography and compared with the untreated Copaxone® sample (Fig. 28.3). The concentration of glatiramer acetate in the upper layer of the treated sample was about 1/10 of the concentration in the lower layer.
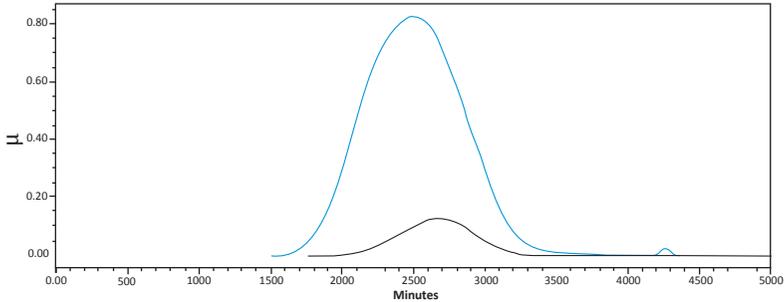

Figure 28.3   Size exclusion chromatography. Relative concentrations and molecular weight distribution profiles of the upper layer (shown in black), and lower layer (shown in blue) of the Copaxone® sample after ultracentrifugation.

The treated sample layers were then re-mixed by vortexing, and the concentration of glatiramer acetate in the reconstituted solution was measured again. The concentration of the reconstituted sample was equivalent to that of the original untreated sample (Fig. 28.4).
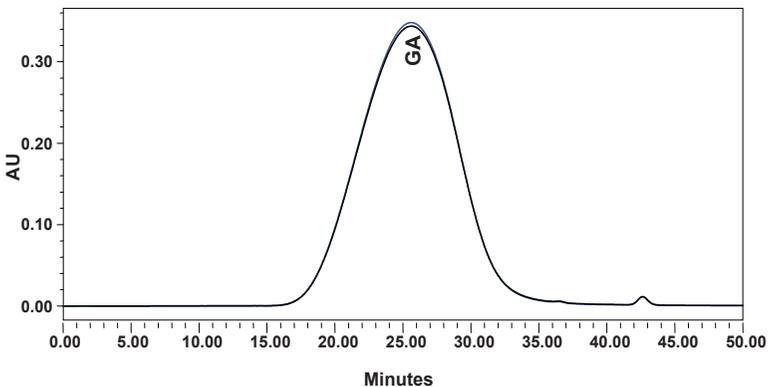

Figure 28.4   Size exclusion chromatography overlaid profiles of the untreated (shown in black) and reconstituted (shown in blue) Copaxone® samples.

This testing demonstrates that Copaxone® constituents can be concentrated under strong centrifugal force, and the resulting concentrate can be easily reconstituted to its original composition. In other words, Copaxone® can be reversibly re-suspended, a property expected only of a colloidal solution, not that of a true solution.

Dynamic Light Scattering (DLS)

DLS determines particle size in solution by measuring their diffusion rate (Brownian motion). Small molecules diffuse more quickly than large molecules. Molecules of different sizes scatter light at different intensities. DLS measures intensity as a function of particle sizes; however, it is important to note that DLS results are qualitative and not quantitative. The capacity of a large molecule to scatter light is significantly higher than that of a small molecule; therefore, a single large molecule can scatter light more intensely than a large population of small particles. Thus, the results of DLS should be evaluated accordingly: the area under the peak does not correlate with the number of particles (population size) represented by that peak.

Scientists at Teva developed and optimized operational DLS conditions for the glatiramoid class of compounds. Measurements were sensitive to particle sizes in the nm range (1–1000 nm). Robust manufacturing process was demonstrated by the reproducibility of results of multiple measurements on many different Copaxone® batches manufactured at varying time periods. DLS measurements were performed on Copaxone® diluted with a 20 mM NaCl solution and filtered through a 1.2 µm disc filter prior to analysis, and on samples obtained by ultracentrifugation at different G-forces (the upper layers and the constituents concentrated at the bottom).

DLS analysis shows that the untreated Copaxone® mixture consists of two main polypeptide populations. The first population is characterized by a distribution of particle sizes in the range of 1.5 to 15 nm, with the most abundant size being approximately 5.6 nm. The second population contains particles in the range of 20 to 550 nm, with the most abundant size being approximately 111 nm (Fig. 28.5). The first population likely represents "mono-particles," or separated molecules, which comprise the most abundant

fraction; whereas the second population can be attributed to larger entities (e.g., labile intermolecular associates) that may be formed by interactions between amino acid sequences on the polypeptide chains.
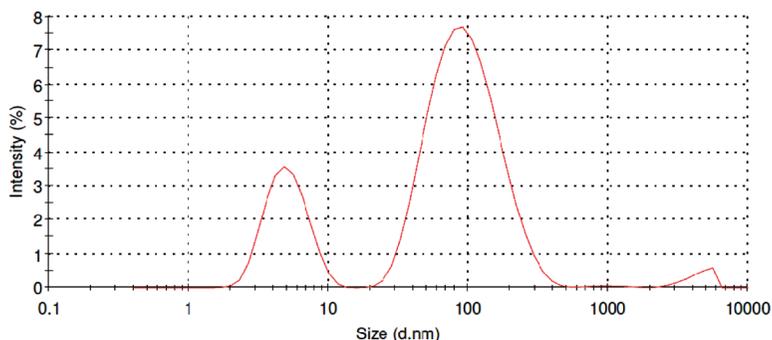

Figure 28.5 A typical (untreated) Copaxone® DLS scan.

The dispersed Copaxone® solution was exposed to ultracentrifugation at different G-forces. As mentioned above, ultracentrifugation resulted in a clear upper layer and a viscous whitish fraction. The upper layer and the material concentrated at the bottom were then tested by DLS (after reconstitution in water). At 290,000g, the upper layer still contained both "light" and "heavy" peaks (Fig. 28.6a), whereas the lower fraction contained the "heavy" peak only (Fig. 28.6b), Ultracentrifugation at higher G-force (650,000g) resulted in a more effective concentration of the heavy peak (Fig. 28.6c,d).

Thus, after ultracentrifugation, there was a change in the profile of the suspended glatiramer acetate nanoparticles with regard to their size distribution, i.e. the larger molecularly associated nanoparticles segregated at the bottom, whereas the smaller nanoparticle associates remained in the upper layer. The extent of concentration of the larger associates at the bottom was proportional to the applied G-force value. This separation would not have been observed if Copaxone® was a true, homogenous solution.

In summary, the Copaxone® solution was successfully separated by ultracentrifugation into several populations of constituents according to their sizes, as determined by DLS.
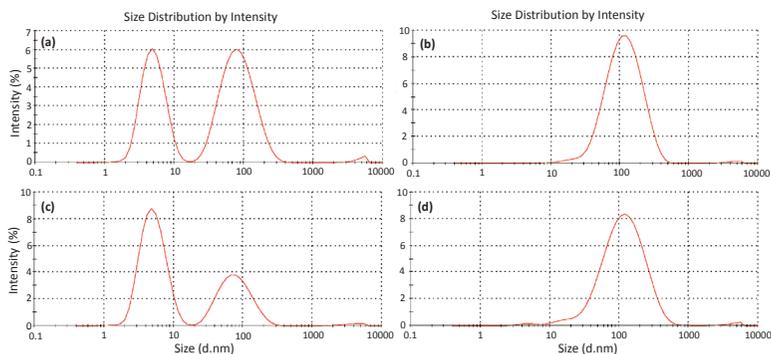
Figure 28.6 DLS results of the Copaxone® sample after ultracentrifugation at different G-forces. The Copaxone® sample was subjected to ultracentrifugation at the conditions described in panels a–d. (a) Upper layer after 2 h ultracentrifugation at 290,000g: two populations are observed; the relative amount of smaller particles is increased compared with non-treated Copaxone®. (b) Bottom fraction after 2 h ultracentrifugation at 290,000g re-suspended in water: only the larger size components are observed. (c) Upper layer after 2 h ultracentrifugation at 650,000g: the relative amount of larger particles is even more reduced due to further separation under higher G-force. (d) Bottom fraction after 2 h ultracentrifugation at 650,000g re-suspended in water: only the higher size constituents are observed.

It is noteworthy that re-solution in water of the larger molecularly-associated nanoparticles from the material obtained at the bottom of the tube resulted in the observation of only the larger particles. This indicates that the larger particles are labile intermolecular associates of several nano-sized "mono"-molecules arranged in a thermodynamically preferable disposition and thus are relatively stable. The observation of the two distinct populations of particles in the original analysis further supports this conclusion. If the solution were merely a mix of agglomerates, one would have expected a continuum of particle size distribution over the range. The appearance of two distinct populations of particles shows that Copaxone® is a colloidal system that is more complex than a mere solution of agglomerated particles and actually comprises a unique microstructure of two particulate populations. In other words, this DLS testing demonstrates that Copaxone® comprises thermodynamically stable, nano-sized association complexes.

Atomic Force Microscopy (AFM)

AFM is a type of scanning tunneling microscopy. AFM produces images of the surface ultrastructure of a substance with molecular resolution under physiological conditions. The samples are dried with nitrogen prior to scanning. The resolution of this technique varies from about 0.1 nm to the sub-micron range. Via AFM, the size (length, width, and height) of individual particles can be measured and the results can be visualized in three dimensions.

A typical topographic image of a Copaxone® dried sample is shown in Fig. 28.7. A Copaxone® aliquot from a syringe and a placebo sample from an identical syringe were dried with nitrogen on a flat support, and then scanned to produce the surface ultrastructure with molecular resolution. AFM analysis of Copaxone® samples revealed dispersed "dot-like" and "string-like" components. In the placebo syringes, no such entities were apparent, indicating that the particles observed in the Copaxone® samples did not originate from mannitol or from any part of the syringes (i.e., they are characteristic of glatiramer acetate).
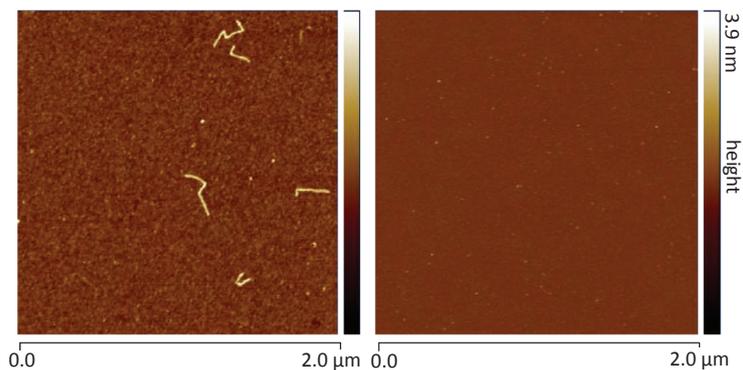
Figure 28.7 Typical topographic image of Copaxone® and placebo bulk solutions. AFM analysis of Copaxone® samples (left) from a bulk solution in a syringe revealed dispersed "dots" and "strings" particles. In the placebo syringes (right), no such entities were visible, indicating that the particles observed in the Copaxone® samples (left) did not originate from mannitol or from any part of the syringes (they are characteristic of glatiramer acetate).

The DLS study above indicated that the population of larger sized particles under the peak on the right (Fig. 28.3) (about 110 nm

average size distribution) can be concentrated at the bottom of a tube by ultracentrifugation. In order to characterize the strings detected by the AFM technique in the Copaxone® sample and to investigate correlations between the results of the DLS scans and the AFM images, samples described in the DLS study above (the bottom fractions) were diluted with water, dried with nitrogen and analyzed.

AFM images for those fractions from a Copaxone® batch (at a higher resolution) are shown in Fig. 28.8. The concentrated "heavy" material appeared to contain the same string-like entities as seen in the untreated Copaxone® samples (Fig. 28.7, left), although, as expected, at a higher concentration. No round shaped particles were detected in the lower layer. By contrast, analysis of the upper layer revealed none of the string-like particles present in the lower fraction. This, correlates with data obtained via DLS testing indicating two subpopulations of particles with different average sizes, and supports the assumption that the strings are the larger sized particles in the Copaxone® sample.
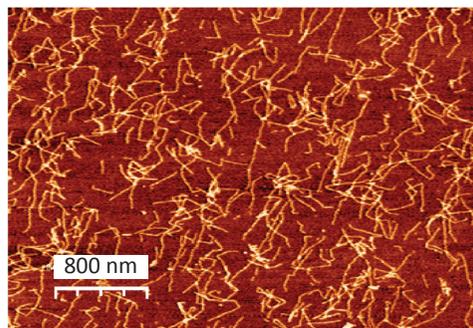
Figure 28.8 Topographic image for "heavy" fraction from Copaxone®, separated by ultracentrifugation. Results shown in this figure complement results of the DLS study, in that AFM established that the heavier population of Copaxone® polypeptides consists of string-like entities of variable sizes.

The DLS and AFM data above taken together confirms the colloidal characteristics of Copaxone®, namely, that it maintains a homogenous appearance throughout its 2-year shelf life (i.e., it contains polypeptide particles of different sizes that do not precipitate under gravity) but it can be separated into subpopulations under ultracentrifugation. These techniques also demonstrate

the presence of stable polypeptide particles within the colloidal size distribution range (1–1000 nm).

Cryogenic Temperature Transmission Electron Microscopy (Cryo-TEM)

Cryo-TEM is a method of obtaining high-resolution, direct images of molecules or molecular assemblies in their native environment. Thus, it can elucidate the nature of the basic building blocks that make up a sample, covering a wide range of length scales from a few nm to several microns. Rapid freezing of the sample prevents alterations in the sample and eliminates potential structural changes, redistribution of elements, and/or the washing away or evaporation of substances originally present in the sample [58]. This technique was used to confirm results of DLS and AFM testing, and to eliminate the impact (if any) of sample preparation on the size, shape, and type of Copaxone® molecular assemblies.

A drop of Copaxone® was placed onto a TEM copper grid (to prevent the formation of ice crystals) and analyzed at –170°C. Samples were analyzed in different locations on the grid, using variable magnification, in an attempt to detect the potential existence of both larger and smaller structures. A placebo (mannitol solution) was used as control (Fig. 28.9).
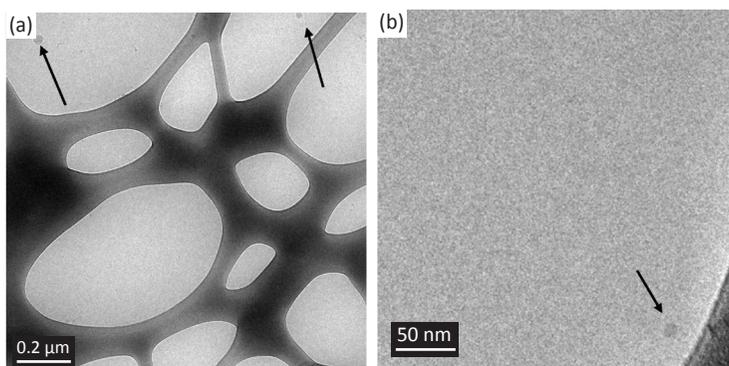

Figure 28.9 Typical Cryo-TEM results for a placebo (mannitol solution) sample: (a) Moderate magnification, (b) high magnification. The sample contained globule particles, with varying sizes of 30 ± 5 nm, as indicated by the black arrows. These globules most likely originated from the silicon oil droplets present in the syringe.

Copaxone® samples tested under the same conditions appear quite different (Fig. 28.10). They largely contain three populations of particles dispersed in the continuous mannitol solution: fibers (or strings) of 60–300 nm length, spherical particles of ~4 nm, and globules of ~30 nm; the latter are consistent with the globules in the placebo sample.
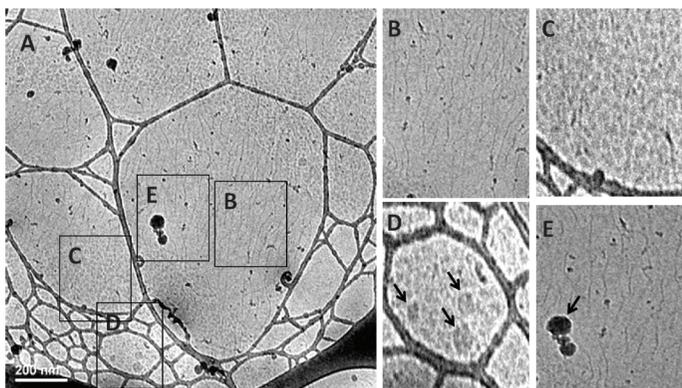

Figure 28.10 (A) Cryo-TEM image of typical structures present in Copaxone® samples. Images B through E are enlarged areas of image A. (B) Fibers of 60–300 nm length and width of 6 ± 1 nm. (C) Spherical particles of ~4 nm in diameter. (D) Globules of ~30 nm in diameter (also detected in placebo samples, see Fig. 28.5 above); (E) Black frost particles (not related to the sample).

Results of Cryo-TEM analysis support results of DLS and AFM testing. Examination of native structural features of the Copaxone® sample, as in the DLS and AFM experiments, revealed two populations of glatiramer acetate nanoparticles dispersed in the aqueous mannitol phase:

- One population of Copaxone® nanoparticles were spherical with sizes being 4 ± 2 nm (Fig. 28.10C), which correspond to the smaller polypeptide moieties shown on the DLS scans (Fig. 28.5, peak on left), and with the "dot-like" structures on topographic AFM images (Fig. 28.7, left).
- The second population of polypeptides appeared as "strings" with lengths of ~60 to 300 nm (Fig. 28.10B), which correspond with the DLS peak indicating larger moieties (Fig. 28.5, peak on right) and the topographic images from

the AFM analysis showing elongated fibers (Figs. 28.7, left and 28.8).

The polypeptide's particle size distribution and their dispersion in the continuous mannitol aqueous phase shown in the Cryo-TEM study provide additional evidence of the colloidal nature of Copaxone®.

Zeta Potential

The stability of colloidal solutions "is determined by the balance of attractive and repulsive forces between individual particles. The repulsive force prevents two particles from approaching one another and adhering together" [57]. If the repulsive force is sufficiently high, the colloidal solution "will resist flocculation and the colloidal system will be stable" [57].

Zeta potential is a measure of the electrokinetic potential in colloidal systems. The magnitude of the zeta potential gives an indication of the stability of a colloidal system. If all the particles have a large negative or positive zeta potential then they will tend to repel each other and, as noted above, there is no tendency to flocculate. However, if the particles have low zeta potential values then there is no force to prevent the particles from coming together and flocculating. The dividing line between stable and unstable is generally taken at either +30 mV or −30 mV. Particles with zeta potentials more positive than +30 mV or more negative than −30 mV are normally considered stable [59].

Representative zeta potential results for three Copaxone® batches are shown in Fig. 28.11 and results are summarized in Table 28.1. Placebo (mannitol solution) exhibited a zeta potential that was about 5 mV.

Table 28.1   Summary of Zeta potential results.

| Sample	Batch	Zeta potential (mV)	
		Average	STDV
Copaxone®	1	36.5	2.3
	2	37.4	2.0
	3	34.5	1.0
Placebo	—	5.5	
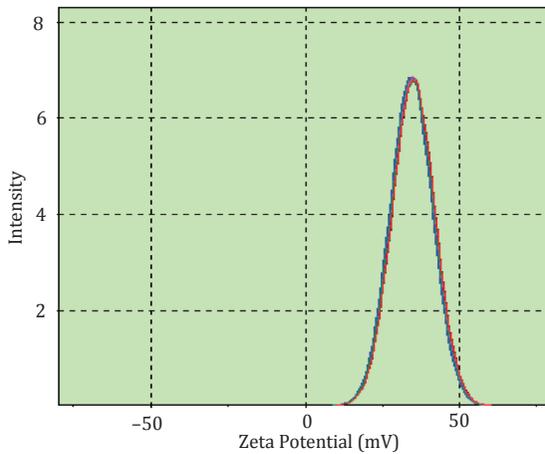
Figure 28.11 Zeta potential of three batches of Copaxone®.

As shown in Table 28.1, the zeta potentials are approximately 34–37 mV, indicating the stability of the colloidal solution through strong electrostatic repulsion of Copaxone® moieties, which prevents their flocculation. Zeta potential results confirm the physical stability of the Copaxone® colloidal solution.

Summary of Test Results

The results of these studies—ultracentrifugation and reconstitution, DLS, AFM, Cryo-TEM, and zeta potential—complement each other and, taken together, confirm that Copaxone® is a stable, lyophilic colloidal solution. These studies show that under adequate centrifugal force, Copaxone® can be separated into layers exhibiting different concentrations that are easily re-dispersed back to the original concentration upon vortexing. They demonstrate the presence of solvated, stable, nano-sized molecules and associations dispersed homogenously within the aqueous mannitol solution. The sufficiently high zeta potential values attest to the stability of the colloidal solution caused by electrostatic forces in the product. The dual population of small and large nanoparticles observed in the analysis is supportive of the unique and distinct microstructure of the Copaxone® product.

28.5 Immunogenicity

There is one aspect of Copaxone® that raises special safety and effectiveness concerns that merit heightened vigilance with respect to the approval of any potentially interchangeable follow-on glatiramer acetate product. *In particular, glatiramer acetate is an immunomodulator*. In other words, Copaxone® is intended to achieve its therapeutic effects by interacting with and modulating a patient's immune system over an extended period of time. For this reason, Copaxone®'s package insert warns that chronic use has the potential to alter healthy immune function as well as induce pathogenic immune mechanisms, although no such effects have been observed with Copaxone® [30].

As discussed above, due to the complexity and inexorable link between the manufacturing process and quality, any follow-on product almost certainly will differ from Copaxone®'s structure and composition of active ingredients because it will be made using a different manufacturing process than that used by Teva. Although it is not possible to fully characterize and compare these complex mixtures, differences are revealed via sophisticated analytical techniques. Purported generic glatiramer acetate so-called generic products have been approved in India, Argentina, and Mexico. A variety of physicochemical tests have been done on these products and they have been proven to be similar to Copaxone® in some basic features. However, they are different in the bulk composition of constituents when analyzed via methods for analysis of complex closely related molecules. In this regard, a widely used analytical tool for characterization of complex mixtures of biologics in the context of biosimilars is the ion mobility mass spectrometry (IMMS) [75]. The ion mobility method applies multidimensional separation techniques based on size, shape, charge and mass of the molecules in the sample mixture and is capable of separating isomeric peptides that chromatographic techniques cannot. The analysis produces a three-dimensional heat map to highlight intensity differences of peptides at various mass/charge and drift times. The difference between the intensities of heat maps for the generics as compared to Copaxone® (result of subtraction of generic heat map from that of Copaxone®) show highlighted areas indicating different polypeptide populations compared to those of Copaxone® lots tested (Fig. 28.12).

A quantitative assessment of these differences in heat maps was used that integrates the intensity values within these highlighted areas to produce a total intensity value (TIV). If the composition of a sample was exactly the same as the reference Copaxone®, the heat map would theoretically have no highlighted areas and a TIV = 0. Conversely, heat maps with more highlighted areas signify greater difference to the reference Copaxone® and will have a higher TIV. As shown in Fig. 28.13, the TIVs of Copaxone® batches are within a narrow range of its inherent batch-to-batch variability, whereas the generics were 8–13 fold higher (Fig. 28.13). Clearly, these results indicate a profound difference in size, shape and charge of the constituent polypeptides in Copaxone® as compared to the purported generic products.
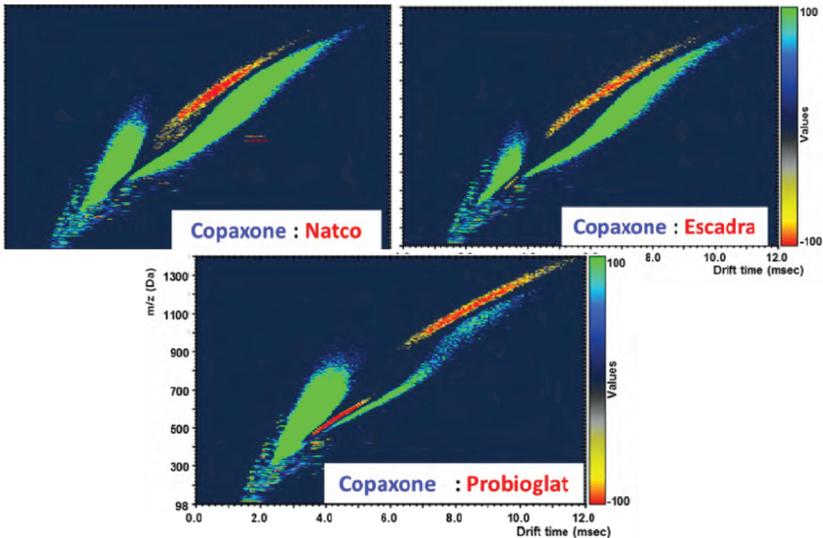
Figure 28.12 IMMS heat map: Copaxone® versus generics.

Because Copaxone® is an immunomodulator, a follow-on product characterized by different constituent population could have significant and unpredictable differences from Copaxone® in its immunological mechanisms, raising major safety and efficacy concerns. The potential risks associated with such follow-on products include increased immunogenicity, immunotoxicity, induction of additional autoimmune disorders, lack of efficacy,

and exacerbation of the MS disease processes. Moreover, because of the nature of both RRMS and Copaxone®, these risks may not develop for months or years and, once apparent, may be irreversible. *It is thus critical to ensure that any proposed follow-on product has a long-term immunogenicity profile that is comparable to Copaxone®'s before approval. This can only be done based upon data from appropriate clinical testing.*
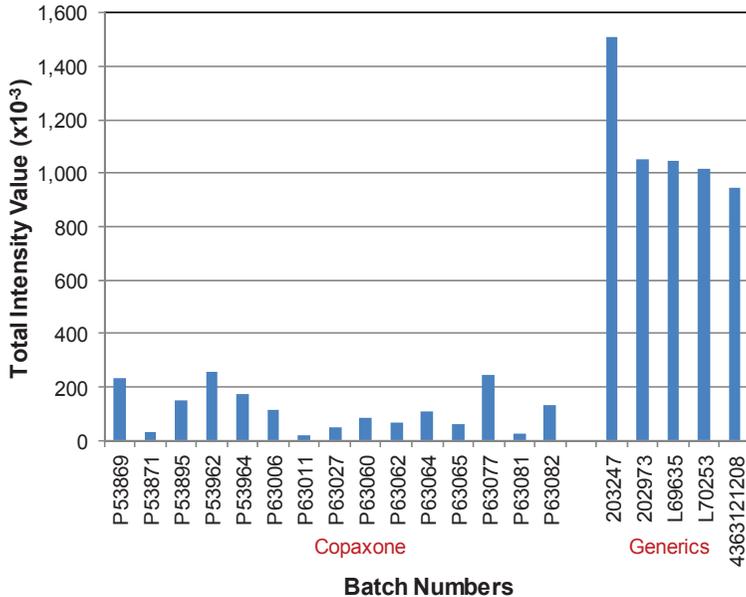
Figure 28.13 Total intensity values (TIV) obtained from comparison of heat maps of various Copaxone® batches and generics.

Glatiramer acetate is a highly immunogenic antigen-based therapy, and anti-glatiramer acetate antibodies are detected in all treated patients and animals [31]. These antibodies, however, do not neutralize biological activity or clinical efficacy and are not associated with local or systemic adverse effects in RRMS patients receiving chronic treatment [32–34]. In fact, some evidence suggests that anti-glatiramer acetate antibodies may enhance the biological activity of Copaxone® [35].

The anti-glatiramer acetate antibody profile (titers and isotypes) changes with repeated glatiramer acetate administration, resulting in a unique response profile over time. In RRMS patients,

anti-glatiramer acetate antibody levels peak between 3 and 6 months of treatment initiation, and then gradually decline [30]. Anti-glatiramer acetate antibodies are mainly of the IgG class. Studies conducted by various groups report that, initially, anti-glatiramer acetate IgG-2 antibodies predominate but with continued treatment, antibodies gradually shift to the IgG-1 isotype, which is consistent with the glatiramer acetate-reactive T cell shift from a Th1 to a Th2 phenotype [31]. After months of treatment, anti-glatiramer acetate IgG-4 antibodies become evident [34].

Evidence of anti-glatiramer acetate IgE antibodies is equivocal. Investigations by Teva had indicated that anti-glatiramer acetate IgE antibodies were infrequent and at low levels, not dose-related, and their detection was not associated with clinical adverse events or hypersensitivity reactions [36].

For proposed follow-on glatiramer acetate products, the risk of unwanted immunogenicity is significant. Even if a follow-on glatiramer acetate product has very similar properties to Copaxone® and is produced by a method that is basically similar to the Teva process, the efficacy, safety, and the immunogenicity of the follow-on product can still differ markedly from those of Copaxone® [37]. Several product-related factors can influence its immunogenicity profile. Potential immunogenic risks associated with antibodies to a proposed follow-on glatiramer acetate product include: (1) formation of immune-complexes; (2) development of drug neutralizing antibodies; (3) hypersensitivity reactions; and (4) induction of additional autoimmune disorders.

Anti-glatiramoid antibodies with a different repertoire than that of anti-glatiramer acetate antibodies could lead to formation of immune complexes. Immune-complex deposition in the glomeruli can cause kidney damage over time, becoming clinically evident only after long-term use of the drug [38–39]. In preclinical studies of chronic glatiramer acetate administration to rats and monkeys, there was marginal and only equivocal signs of glatiramer acetate and complement localization in the glomeruli [30]. Localization of immune complexes in the kidney was not found in a longer, 2-year bioassay [36]. Immune-complex disease, presenting as glomerulonephritis, was not seen in clinical studies of glatiramer acetate and was reported only once during extensive post-marketing experience with Copaxone® [36]. In contrast, in a toxicity study of chronic administration of the higher molecular weight

glatiramoid TV-5010 to rats, dose-dependent glomerulonephritis attributed to immune-complex deposition was observed at all tested TV-5010 dose levels [40]. This finding reinforces the importance of conducting chronic toxicity studies of proposed follow-on glatiramer acetate products, as longer-term adverse effects such as glomerulonephritis may not be apparent in short-term studies.

While anti-glatiramer acetate antibodies do not inhibit (but may enhance) the therapeutic activity of Copaxone® [31], slight variations in the primary, secondary, and tertiary structure of the active ingredient in a proposed follow-on product may result in the induction of anti-drug antibodies with neutralizing activity. Experience with different recombinant tumor necrosis factor alpha (TNF-α) antagonist drugs demonstrates the variety of antibody profiles possible for individual agents within the same drug class. Anti-infliximab antibody level is associated with decreased therapeutic response, whereas, anti-etanercept antibody level does not appear to influence drug effectiveness or adverse events [40]. The influence of anti-adalimumab antibodies on drug efficacy and adverse events is controversial; serum adalimumab concentrations can be dramatically lower in patients with anti-adalimumab antibodies, possibly because of increased drug clearance due to immune-complex formation [40].

Antibodies to many therapeutic peptides have been reported to induce hypersensitivity. NBI 5788, like glatiramer acetate, is an altered peptide ligand (APL) of myelin basic protein (MBP). NBI 5788 caused a relatively high rate (9%) of hypersensitivity reactions in MS patients in a phase II clinical trial, leading to early discontinuation of the study [41]. Hypersensitivity reactions can be immediate and are usually mediated by specific IgE antibodies, which trigger clinical signs and symptoms of variable severity, from benign urticaria to life-threatening bronchospasm, angioedema, or anaphylactic shock. Importantly, long-term follow-up of patients who received NBI 5788 showed that even short-term therapy with an APL can induce long-term persistence of altered responses to both the APL and the native protein/peptide [45].

Pathogenic antibodies and T cells can induce autoimmune reactions. When an epitope on an exogenous peptide or protein bears similarities to amino acid sequences on an endogenous protein in body constituents (molecular mimicry), anti-exogenous-

protein antibodies can neutralize the biological activity of the endogenous protein, leading to severe adverse events [43–44]. Glatiramer acetate originally was designed to mimic/resemble the encephalitogen, MBP [46] and preclinical and *in vitro* studies show that glatiramer acetate is cross-reactive with MBP at the cellular and humoral levels [31]. Despite this cross-reactivity, glatiramer acetate is not encephalitogenic (the encephalitogenic potential of Copaxone® batches is routinely tested by Teva). Rather than induce MBP-specific T cells, *in vitro* and *ex vivo* studies have shown the opposite: glatiramer acetate inhibits expansion and induces anergy of MBP-specific T cells [47–49]. Similarly, although monoclonal anti-glatiramer acetate antibodies can cross-react with MBP, polyclonal anti-glatiramer acetate antibodies from treated patients do not, and glatiramer acetate does not induce auto-reactive antibodies [31].

Since the active amino acid sequences in the glatiramer acetate mixture responsible for its efficacy are unknown, it is impossible to predict whether follow-on products will have the same efficacy as Copaxone®. They could have a weaker anti-inflammatory effect and/or enhance a pro-inflammatory environment, further exacerbating MS pathogenic processes. A reduced anti-inflammatory effect may provide less effective control of MS relapses, which would be difficult to detect in the post-marketing environment because MS relapses and progression of disability are not completely abolished by any MS therapy. On the other hand, creation or amplification of a pro-inflammatory environment would likely increase relapse rate and progression of disability or worse (e.g. have a profound encephalitogenic effect). Clinical trials of non-glatiramoid APLs of MBP in MS patients have shown that very strong responses to the APL can augment disease-related immune responses to the native antigen or produce intolerable immune-mediated secondary effects, including hypersensitivity reactions [41, 45].

Clinical experience with CGP77116, which, like Copaxone®, is an APL of MBP, exemplifies this risk. CGP77116 was associated with unexpected pro-inflammatory encephalitogenic effects, inducing brain inflammation that necessitated early termination of a study in RRMS patients [50]. In contrast to results of *in vitro* studies, CGP77116 *in vivo* caused substantial expansion of CGP77116-specific T cells that were cross-reactive with native MBP—a

necessary prerequisite for "bystander suppression." However, rather than stimulating an anti-inflammatory T-cell phenotype, the majority of activated CGP77116-specific T cells were of a pro-inflammatory (Th0/Th1) phenotype. Moreover, in some patients, worsening disease could be linked to CGP77116-induced expansion of MBP-specific T cells, which likely exacerbated pathogenic demyelination [50].

Finally, the potential for the development of cross-reactive neutralizing antibodies must be assessed before any regulatory authority approves any follow-on glatiramer acetate product intended to be used interchangeably with Copaxone®. Switching between two complex polypeptide products with subtle differences in structure and/or composition may increase the chance of cross-reactivity, a phenomenon that has been observed with interferon beta products [51]. Upon switching from Copaxone® to a follow-on product or using them interchangeably, antibodies formed against Copaxone® may neutralize the activity of the proposed generic product and *vice versa*. If this were the case, patients would be left without any effective treatment. Again, there is no evidence that progression of neurologic disability associated with untreated MS can ever be reversed.

Although Copaxone® is not currently regulated as a "biological product" in the US or Europe, it nevertheless shares many of the same characteristics as biological products, including a large and complex molecular structure and concomitantly complex interactions with the immune system. Consequently, the same scientific justifications for requiring data on the risks of switching "interchangeable" biological products, on a product-by-product basis, should apply equally to proposed generic glatiramer acetate products that are intended to be used interchangeably with Copaxone®. Indeed, because Copaxone® is intended to be used chronically, and because its effects on the immune system appear to evolve over time, there is no way to predict the effect of a "switch" or for that matter, multiple switches, on safety or effectiveness without conducting adequate and well-controlled clinical trials.

28.6 Conclusions and Future Prospects

The complexity of Copaxone® raises safety and effectiveness concerns that merit heightened vigilance with respect to the

approval of potentially interchangeable follow-on glatiramer acetate versions. Currently there is no defined mechanism for follow-on versions of NBCDs such as certain liposomal drugs, glatiramoids like Copaxone® and iron-sugar complexes. As discussed earlier, this is because the classical paradigm for abbreviated authorizations of conventional small molecules is not appropriate or valid because NBCDs lack a homo-molecular structure that cannot be fully quantitated or characterized via conventional physicochemical analytical tools and their composition and quality generally depends upon the manufacturing process and controls. As a result, originator NBCDs are not fully characterizable, some are not amenable to therapeutic bioequivalence testing, and comprehensive regulatory evaluation and guidelines for follow-on versions of NBCDs or nanosimilars are currently not developed. Consequently, they present a challenge to regulatory bodies like the FDA and EMA, manufacturers, physicians, and pharmacists.

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J. Michael Nicholas received his PhD in pharmacology from the University of Tennessee Center for Health Sciences. After postdoctoral work at the University of Mississippi in Jackson he joined Mylan Pharmaceuticals in Morgantown, WV, as Director of Scientific Affairs. While at Mylan, he later served as Director of Scientific and Regulatory Affairs and was involved with the development and approval of both generic and branded products. Following Mylan, he accepted a position with Marion Laboratories in Kansas City in the Regulatory Affairs department. Over the years, Dr. Nicholas has been involved with all aspects of regulatory matters including product development and approval. While at Marion Laboratories, Marion Merrell Dow and Hoechst Marion Roussel, Dr. Nicholas served as the Director of Product Approval within the Regulatory Affairs Department and directed the submission of INDs, NDAs, and their subsequent approval. Prior to his current position, he was vice president, US Regulatory Affairs and Compliance, Marketed Products for Aventis Pharmaceuticals and was responsible for regulatory matters for approved products. Currently, Dr. Nicholas is vice-president of Specialty Life Cycle Initiatives for Teva Brand Pharmaceuticals, Overland Park, Kansas, USA and is responsible for product life cycle planning.

Vera Weinstein received her PhD in synthetic organic chemistry from the Hebrew University of Jerusalem, Israel (HUJI). After several years at HUJI as a senior lecturer, she joined Portman Pharmaceutical Industries as chief research chemist and was involved with the development of branded products. In 1996, Dr. Weinstein accepted a position with Teva as an Analytical Team Leader, and later as CMC Program Leader in Innovative R&D. At her present position,

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