



An expanding horizon of complex injectable products: development and regulatory considerations

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Abstract

There has been a constant evolution in the pharmaceutical market concerning the new technologies imbibed in delivering drug substances for various indications. This is either market-driven or technology-driven to improve the overall therapeutic efficacy and patients' quality of life. The pharmaceutical industry has experienced rapid growth in the area of complex injectable products because of their effectiveness in the unmet market. These novel parenteral products, viz, the nanoparticles, liposomes, microspheres, suspensions, and emulsions, have proven their worth as "Safe and Effective" products. However, the underlying challenges involved in the development, scalability, and characterization of these injectable products are critical. Moreover, the guidelines available do not provide a clear understanding of these complex products, making it difficult to anticipate the regulatory requirements. Thus, it becomes imperative to comprehend the criticalities and develop an understanding of these products. This review discusses various complexities involved in the parenteral products such as complex drug substances, excipients, dosage forms, drug administration devices like pre-filled syringes and injector pens, and its different characterization tools and techniques. The review also provides a brief discussion on the regulatory aspects and associated hurdles with other parenteral products.

Keywords Complex injectables · Liposomes · Multivesicular liposome · Microspheres · Controlled release · USFDA

Abbreviations

| | |
|-------|--------------------------------------|
| AIDS | Acquired immunodeficiency syndrome |
| API | Active pharmaceutical ingredient |
| CNS | Central nervous system |
| EMA | European Medicines Agency |
| GDUFA | Generic Drug User Fee Amendments/Act |
| I.M. | Intramuscular |

| | |
|-------|--|
| I.V. | Intravenous |
| LAR | Long-acting release |
| LAIF | Long-acting injectable formulation |
| MVL | Multi-vesicular liposome |
| NBCD | Non-biological complex drug |
| NDA | New drug application |
| PFS | Pre-filled syringe |
| PSG | Product specific guidance |
| rDNA | Recombinant DNA |
| RLD | Reference Listed Drug |
| S.C. | Subcutaneous |
| USFDA | United States Food and Drug Administration |
| WFI | Water for injection |

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Introduction

In the last few decades, the pharmaceutical industry has experienced rapid growth in the sterile product market, leading to diverse dosage forms. The advances in sterile product formulations like suspensions, emulsions, liposomes, and micro- and nano-particles have reshaped the design

of therapeutic systems with better safety and efficacy perspective [1, 2]. Due to the complexity in the design and development of these drug products, they are referred to as “Complex Injectable Products” that require extensive studies and exclusive approval pathways. As per the United States Food and Drug Administration Generic Drug User Fee Act II (USFDA GDUFA II) commitment letter, complex injectable products comprise of a complex active pharmaceutical ingredient (API) (e.g., Copaxone[®]; Glatiramer acetate injection; marketed by Teva Pharmaceuticals USA, Inc.) or a complex formulation (e.g., Sandostatin LAR[®] Depot; Octreotide acetate for injectable suspension; marketed by Novartis Pharmaceuticals Corp.). Additionally, it may also consist of complex drug-device combinations where the drug substance is pre-loaded in the product-specific device (e.g., EpiPen[®]; epinephrine pre-filled auto-injector; Marketed by Mylan Specialty LP) (Fig. 1) [3, 4]. These products have proven to improve patient compliance and provide effective treatment modalities and good market position to pharmaceutical companies with unique intellectual property [1].

Developing new drug molecules for mitigating and curing various diseases is an ongoing and continuous process. Most of these new chemical entities developed today have a poor aqueous solubility and several undesirable physicochemical properties like short half-life, extensive degradation, high protein binding, first-pass metabolism, and poor intestinal permeability [5]. To overcome such challenges, these molecules are being formulated using advanced delivery systems like liposomes, polymer- or lipid-based nanoparticles, microspheres, and nanocrystals [6]. There has been a considerable emphasis on the targeted and site-specific delivery of therapeutics, which is possible with the development of

nano-formulations. These novel formulations are primarily designed and developed for parenteral applications for better therapeutic outcomes, which serve advantages like improved pharmacokinetic/pharmacodynamic behavior of the drug with reduced dosing frequency and minimal adverse effects [7]. Additionally, sustained-release dosage forms such as long-acting injectable products facilitate reformulation of existing conventional pharmaceutical products for improved clinical application [8]. These complex drug delivery systems also impart in vivo stability to the small and large drug molecules, which are prone to degradation in physiological conditions [9]. The commercial applications of complex injectables have been explored widely, ranging from cancer treatment to long-acting depot injection for central nervous system disorders [10, 11]. The historical turnaround in the last few decades dictates the success of drug products belonging to the category of complex injectables, thereby leading to a soaring interest of pharmaceutical industries and regulatory agencies in this area (Fig. 2).

Pharmaceutical companies across the globe are gradually shifting their focus towards the development of complex injectable products that help deliver more therapeutic value to the patients and provide the opportunity for a sustainable market position due to less competition. However, the development and approval of these products are challenging due to complex manufacturing/operation, requirement of highly skilled human resources and subject matter experts, high capital investment, and time-consuming translational activity from the laboratory to the market. Scale-up processes are tedious due to complicated multi-unit operation/manufacturing processes and the usage of uncommon equipment (like high-pressure homogenizer, lipid extruder, and

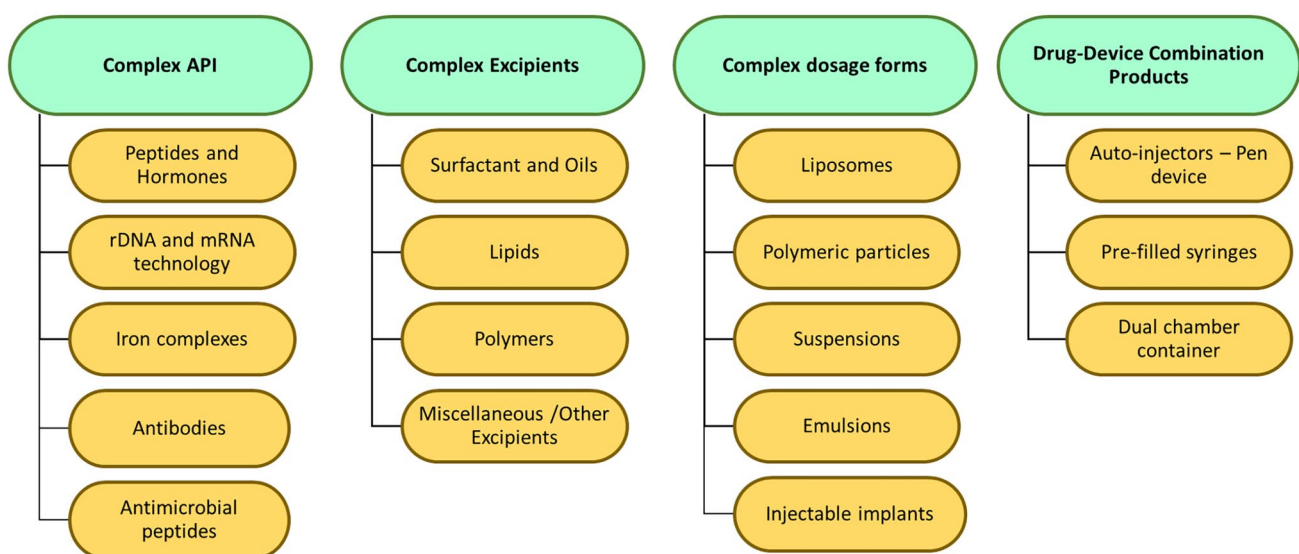
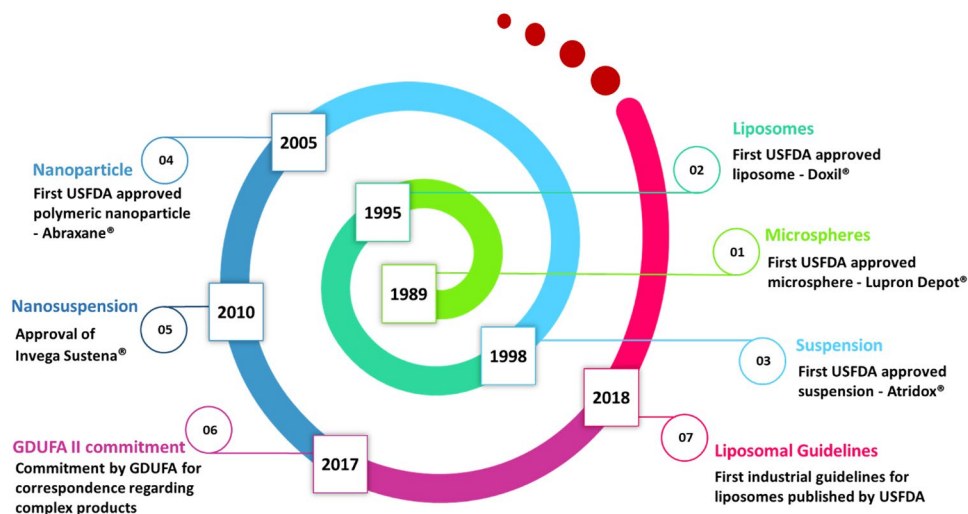


Fig. 1 Classification of complex injectable products

Fig. 2 Historic turning points in the advancement of complex injectables



the rest). Additionally, the filing pathways for approval of complex injectables are also perplexing due to specific quality attributes, non-conventional testing requirements, and a lack of clarity in regulatory requirements. The ambiguity in the regulatory filing persists because of the usage of complex formulation and excipients, complicated characterization of the drug products/drug substances/excipients, ill-defined in vitro drug release assay methods, and scarce models to establish in vivo pharmacokinetics [12]. The common technical challenges, along with their causes and consequences, are summarized in Table 1.

For facile translation of these products from lab to market, it becomes imperative to understand the hurdles at various stages like the development, characterization, scale-up, and regulatory filing. Considering the clinical and commercial importance, we have reviewed the possibilities and obstacles associated with the development of complex injectable products. In this review, various types of complex injectable products and their associated problems are addressed in detail. It also includes various bioanalytical tools/techniques

required for the successful characterization of these products. These scientific details will be beneficial to the pharmaceutical researchers and industries working in this area.

Complex injectable product

Complex injectable products are generally categorized based on the complexities associated with the dosage form, specialized excipients, drug substances, and drug-device combinations [13]. Information on various commercially approved complex injectable products is summarized in Table 2.

Complex active ingredient—complex mixtures of API, polymeric compounds, and peptides

There has been a constant requirement for novel therapeutics to treat chronic conditions with better safety and efficacy. Hence, this necessity urges the development of APIs. USFDA defines complex active ingredients as peptides,

Table 1 Challenges associated to complex injectables — Cause and Consequence

| S. no | Causes | Consequences |
|-------|---|--|
| 1. | Unavailability of comprehensible regulatory guidelines | Unclear road map for product development, consecutively minimizing regulatory approval |
| 2. | Discrepancy/deviation in manufacturing, processing, storage and administration | Instability and variability in dosage affecting therapeutic efficacy, and site-specific or systemic toxicity |
| 3. | Inappropriate selection and improper quality and grade of excipients and raw material | Physical and chemical incompatibility and instability leading to product degradation |
| 4. | Complexity involved in manufacturing process | Undesirable characteristics viz, poor entrapment efficiency, particle size and PDI, etc |
| 5. | Thermolabile, radiation-susceptible, larger size particles in formulation | Insufficient sterilization |
| 6. | Multi-component complex system | Difficulty in characterization thus requiring specific analytical, in vitro and in vivo tools and equipments |

Table 2 List of complex injectables approved by USFDA

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|-----------------|--------------|--------------------|-------------------------------|--------------------------------|--------------------------------------|---|--|------------------|--|---------------|--------------------------|------|
| Solution | | | | | | | | | | | | |
| 1. | Copaxone® | Glatiramer acetate | Peptide | Solution Pre-filled syringe | 20 mg/mL, 40 mg/mL | Mannitol | Relapse from multiple sclerosis | S.C. | For subcutaneous injection only. 20 mg/mL (per day) 40 mg/mL (thrice per week) | Feb 12, 2002 | Teva Pharmaceuticals USA | [14] |
| 2. | EpiPen® | Epinephrine | Small molecule | Solution Pen device | 0.3 mg/delivery, 0.15 mg/delivery | Sodium chloride, sodium metabisulfite, WFI | Treatment of type I allergic reaction including anaphylaxis | I.M., S.C. | Patient greater than or equal to 30 kg: EpiPen 0.3 mg Patient (15–30 kg): EpiPen Jr. 0.15 mg | Dec 22, 1987 | Mylan Specialty LP | [15] |
| 3. | Feraheme® | Ferumoxytol | Iron complex (elemental iron) | Solution | Equation 30 mg iron/mL | Elemental iron, mannitol | Iron deficiency anemia in adult patients with chronic kidney disease | I.V. (injection) | Initial dose of 510 mg followed by a second 510 mg dose 3 to 8 days later | Jun 30, 2009 | Amag Pharmaceuticals | [16] |
| 4. | Forteo™ | Teriparatide | Peptide | Solution Pen device | 0.25 mg/mL | Glacial acetic acid, sodium acetate (anhydrous), mannitol, meta cresol, WFI | Postmenopausal women with osteoporosis | S.C. | Recommended dose of 20 mcg subcutaneous per day | Nov 26, 2002 | Eli Lilly and Co | [17] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|--------|--------------|-------------------------|----------------------|---------------------|---------------|---|--|------------------|--|---------------|---------------------|------|
| 5. | Injectafer® | Ferric carboxymaltose | Iron complex | Solution | 50 mg iron/mL | Ferric carboxymaltose in WFI | Iron deficiency anemia in adult patients | I.V. (injection) | For patients weighing 50 kg or more: two doses separated by at least 7 days (750 mg each dose) For patients weighing less than 50 kg: two doses separated by at least 7 days (15 mg/kg of body weight in each dose) | Jul 25, 2013 | American Regent Inc | [18] |
| 6. | Saxenda® | Liraglutide recombinant | rDNA therapy | Solution Pen device | 6 mg/mL | Disodium phosphate dihydrate, propylene glycol, phenol, WFI | Chronic weight management in adults | S.C. | Recommended dose is 3 mg daily. Initiate at 0.6 mg per day for one week. In weekly intervals, the dose can be increased to 3 mg. Dose for pediatric patients to be reduced to 2.4 mg daily | Dec 23, 2014 | Novo Nordisk Inc | [19] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|--------|-------------------|--------------------|--------------------------------|-----------------------------|---|---|---|------|---|---------------|--------------------------|------|
| 7. | Somatuline® Depot | Lanreotide acetate | Synthetic cyclical octapeptide | Solution Pre-filled syringe | 60 mg/0.2 mL, 90 mg/0.3 mL, 120 mg/0.5 mL | Acetic acid, WFI | Acromegaly, gastroenteropancreatic neuroendocrine tumor and carcinoid tumor treatment | S.C. | Recommended dosage Acromegaly: 90 mg every 4 weeks for 3 months GEP-NETs: 120 mg every 4 weeks Carcinoid syndrome: 120 mg every 4 weeks | Aug 30, 2007 | Ipsen Pharma Biotech Sas | [20] |
| 8. | Sublocade® | Buprenorphine | Small molecule | Solution Pre-filled syringe | 100 mg/0.5 mL, 200 mg/mL | 50:50 PLGA, N-methyl-2-pyrrolidone | Opioid dependence treatment | S.C. | 300 mg as initial dose (for 2 month) followed by 100 mg maintenance dose | Nov 30, 2017 | Indivior Inc | [21] |
| 9. | Tymlos® | Abaloparatide | Hormone related peptide | Solution Pen device | 2 mg/mL | Sodium acetate trihydrate, phenol, acetic acid, WFI | Postmenopausal women with osteoporosis | S.C. | 80 µg (once)/day (monthly) | Apr 28, 2017 | Radius Health Inc | [22] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|--------|--------------|-------------------------|----------------------|---------------------|------------------------|---|--|------------------|--|---------------|---------------------|------|
| 10. | Venofer® | Ferric oxyhydroxide | Iron complex | Solution | Equation 20 mg iron/mL | Iron sucrose, WFI | Iron deficiency anemia in patients with chronic kidney disease | I.V. (Injection) | In adult patients—hemodialysis-dependent chronic kidney disease (HDD-CKD): 100 mg Non-dialysis-dependent chronic kidney disease (NDD-CKD): 200 mg Peritoneal dialysis Dependent chronic kidney disease (PDD-CKD): 300 or 400 mg In pediatric patients—HDD-CKD, PDD-CKD, or NDD-CKD: 0.5 mg/kg Must be selected according to desired dosing schedule for adults | Nov 6, 2006 | American Regent Inc | [23] |
| 11. | Victoza | Liraglutide recombinant | rDNA therapy | Solution Pen device | 6 mg/mL | Sodium phosphate dihydrate, propylene glycol, phenol, WFI | For controlling glucose level in type 2 diabetes mellitus | S.C. | 0.5 mg/kg Must be selected according to desired dosing schedule for adults | Jan 25, 2010 | Novo Nordisk Inc | [24] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|---------------------------|--------------|---|----------------------|---------------------------------------|---|--|--|------------------|---|---------------|-------------------------|------|
| Lyophilized powder | | | | | | | | | | | | |
| 12. | Advate® | Antihemophilic factor (recombinant, plasma/albumin-free method) | rDNA therapy | Lyophilized powder for reconstitution | 250, 500, 1000, 1500, 2000, and 3000 IU | Mannitol, trehalose, sodium chloride, histidine, Tris, calcium chloride, polysorbate 80, glutathione | Control and prevention of bleeding episode | I.V. (injection) | 20–40 IU per kg every other day (3–4 times 37 weekly) | July, 2003 | Baxter Health-care Corp | [25] |
| 13. | Eligard® | Leuprolide acetate | Peptide | Powder for reconstitution | 7.5 mg, 22.5 mg, 30 mg, 45 mg | Leuprolide acetate dispersed in PLGH or PLG, NMP | Prostate cancer | S.C. | One injection/month containing 7.5 mg of API | Jan 23, 2002 | Tolmar Therapeutics Inc | [26] |
| 14. | Vfend® | Voriconazole | Small molecule | Powder for reconstitution | 200 mg/vial | Sulfobutyl ether beta-cyclodextrin sodium, WFI | Invasive aspergillosis fungal infection, candida infection | I.V. (infusion) | Dosage in adults— invasive aspergillosis, candida infections, scedosporiosis, and fusariosis: loading dose of 6 mg/kg every 12 h for first 24 h followed by maintenance dose | May 24, 2002 | PfPrism Cv | [27] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|-----------------------|--------------|-----------------------|----------------------|---|---------------------------------|--|--------------------------|-----------------|--|---------------|------------------------|------|
| Nanosuspension | | | | | | | | | | | | |
| 15. | Abraxane® | Paclitaxel | Small molecule | Suspension (particle size: 130 nm) | 100 mg/vial | Paclitaxel: albumin (1:9) | Metastatic breast cancer | I.V. (infusion) | Metastatic breast cancer (MBC): 260 mg/m ² Non-small-cell lung cancer: 100 mg/m ² Pancreatic adenocarcinoma: 125 mg/m ² | Jan 7, 2005 | Abraxys Bioscience LLC | [28] |
| 16. | Aristada® | Aripiprazole lauroxil | Small molecule | Extended release suspension Pre-filled syringe | 441 mg, 662 mg, 882 mg, 1064 mg | Sorbitan monolaurate, polysorbate 20, sodium chloride, sodium phosphate dibasic anhydrous, sodium phosphate monobasic, WFI | Schizophrenia | I.M. | 441 mg, 662 mg, or 882 mg monthly, 882 mg dose every 6 weeks, or 1064 mg dose every 2 months | Oct 5, 2015 | Alkermes Inc | [29] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|--------|--------------------|---------------------------|----------------------|--|---|--|----------------------------|------|---|---------------|--|------|
| 17. | Invega Hafyera® | Paliperidone palmitate | Small molecule | Extended release suspension Pre-filled syringe | 1092 mg/3.5 mL, 1560 mg/5 mL | Polysorbate 20, PEG 4000, citric acid monohydrate, sodium dihy- drogen phos- phate monohy- drate, sodium hydroxide, WFI | Schizophrenia in adults | I.M. | Gluteal injection once every 6 months. If needed, dos- age adjust- ment can be made every 6 months between the dose of 1092 to 1560 mg based on individual response and tolerability | 2006 | Janssen Pharma- ceuticals Inc | [30] |
| 18. | Invega Sustena® | Paliperidone palmitate | Small molecule | Extended release suspension Pre-filled syringe | 39 mg/0.25 mL, 78 mg/0.5 mL, 117 mg/0.75 mL, 156 mg/mL, 234 mg/1.5 mL | Polysorbate 20, PEG 4000, citric acid monohy- drate, sodium dihydrogen phosphate monohydrate, disodium hydrogen phosphate anhydrous, sodium hydroxide, WFI | Schizophrenia | I.M. | Schizophrenia and schizoaffective disorder— initiation dosing at day 1: 234 mg; day 8: 156 mg. The recommended maintenance dose is 117 mg | Jul 21, 2009 | Janssen Pharma- ceuticals Inc | [31] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|--------|---------------------|------------------------------------|----------------------|--|---|--|---|------|--|-----------------|--|------|
| 19. | Invega Trimza® | Paliperidone palmitate | Small molecule | Extended release suspension Pre-filled syringe | 273 mg/0.88 mL, 410 mg/1.32 mL, 546 mg/1.75 mL, 819 mg/2.63 mL | Polysorbate 20, PEG 4000, citric acid monohydrate, sodium dihy- drogen phos- phate monohy- drate, sodium hydroxide, WFI | Schizophrenia | I.M. | Once every 3 months after they have been treated with Invega Sustenna® for atleast 4 months | May 18, 2015 | Janssen Pharma- ceuticals Inc | [32] |
| 20. | Trelstar® | Triptorelin pamoate | Hormone | Suspension | Equation 3.75, 11.25, 22.5 base/ vial | PLGA, mannitol, Na-CMC, poly- sorbate 80 | Prostate cancer treatment | I.M. | 3.75 mg every 4 weeks; 11.25 mg every 12 weeks; 22.5 mg every 24 weeks. Must be selected according to desired dosing schedule | Jun 15, 2000 | Verity Pharma- ceuticals Inc | [33] |
| 21. | BydureonB- Cise® | Exenatide | Peptide | Extended release suspension Pen device | 2 mg/0.85 mL | 50:50 PLGA, sucrose, MCT | Type 2 diabetes (diabetes mellitus) | S.C. | 2 mg once/ week | Oct 20, 2017 | AstraZeneca Ab | [34] |
| 22. | Cabenuva | Cabotegravir and Rilpivirine | Small molecule | Extended release suspension | 2 mg/mL cabotegravir and 3 mg/mL rilpivirine | Mannitol, polyethylene glycol 3350, polysorbate 20, WFI | Treatment of HIV-1 infection in adults | I.M. | Initiate the injections with 600 mg of cabote- gravir and 900 mg of rilpivirine | Jan 21, 2021 | ViiV Health- care | [35] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|---------------------|----------------------------|--------------------|----------------------|---|-------------------------------|---|-----------------------------------|------|--|---------------|----------------------|------|
| Microspheres | | | | | | | | | | | | |
| 23. | Lupaneta [®] Pack | Leuprolide acetate | Peptide | Microparticle Pre-filled dual chamber syringe | 3.75 mg/vial | Purified gelatin, D-mannitol Diluent: Na-CMC, D-mannitol polysorbate 80, WFI | Painful symptoms of endometriosis | I.M. | Single injection every month for up to six injections | Dec 14, 2012 | Abbvie Endocrine Inc | [36] |
| 24. | Lupron Depot [®] | Leuprolide acetate | Peptide | Microparticle Pre-filled dual chamber syringe | 7.5 mg, 22.5 mg, 30 mg, 45 mg | Purified gelatin, D-mannitol, PLGA Diluent: Na-CMC, D-mannitol polysorbate 80, WFI | Advanced prostatic cancer | I.M. | 7.5 mg for 1-month administration; 22.5 mg for 3-month administration; 30 mg for 4-month administration; 45 mg for 6-month administration. Must be selected according to desired dosing schedule | Jan 26, 1989 | Abbvie Endocrine Inc | [37] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|--------|--|---------------------|----------------------|------------------------------|---|--|-----------------------------------|------|--|---------------|-------------------------------|------|
| 25. | Risperdal [®] Costa [®] | Risperidone | Small molecule | Extended release microsphere | 12.5 mg/vial, 25 mg/vial, 37.5 mg/vial, 50 mg/vial | Risperidone micronecapsulated in PLGA Diluent: polysorbate 20, Na-CMC, disodium hydrogen phosphate dihydrate, citric acid anhydrous, sodium chloride, sodium hydroxide, WFI | Schizophrenia, bipolar I disorder | I.M. | 25 mg I.M. every 2 weeks. The maximum dose should not exceed 50 mg every 2 weeks | Oct 29, 2003 | Janssen Pharmaceuticals Inc | [38] |
| 26. | Sandostatin LAR [®] Depot | Octreotide acetate | Peptide | Microparticle | Equation 10, 20 or 30 mg base/vial | Octreotide distributed within PLGA copolymer; mannitol Diluent: Na-CMC, mannitol, poloxamer 188, WFI | Acromegaly, carcinoma tumor | I.M. | Initial dosing is usually 50 mcg administered twice or 3 times daily. Upward dose titration is frequently required | Nov 25, 1998 | Novartis Pharmaceuticals Corp | [39] |
| 27. | Signifor [®] LAR | Pasireotide pamoate | Peptide | Microparticle | Equation 10, 20, 30, 40, and 60 mg base/vial | Pasireotide pamoate distributed within PLGA copolymer Diluent: Na-CMC, mannitol, poloxamer 188, WFI | Acromegaly, Cushing's disease | I.M. | Initial dosing is 40 mg once every 4 weeks. Dose should be adjusted based on biochemical response and tolerability | Dec 15, 2014 | Recordati Rare Diseases Inc | [40] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|-----------------|--------------|-----------------|----------------------|---|-------------|--|-------------------------|-----------------|--|---------------|----------------------------|------|
| 28. | Vivitrol® | Naltrexone | Small molecule | Extended release microsphere | 380 mg/vial | Naltrexone microencapsulated in 75:25 PLG Diluent: Na-CMC, sodium chloride, polysorbate 20, WFI | Alcohol dependency | I.M. | 380 mg every 4 weeks through I.M. route | Apr 13, 2006 | Alkermes Inc | [41] |
| Liposome | | | | | | | | | | | | |
| 29. | Ambisome® | Amphotericin B | Small molecule | Liposome (globule size: less than 100 nm) | 50 mg/vial | Amphotericin B intercalated to liposomal membrane composed of HSPC, cholesterol, DSPC, alpha tocopherol and sucrose, disodium succinate hexahydrate buffer | Fungal infection | I.V. (infusion) | Empirical therapy: 3 mg/kg/day Systemic fungal infections: 3–5 mg/kg/day Cryptococcal meningitis in HIV-infected patients: 6 mg/kg/day | Aug 11, 1997 | Astellas Pharma US Inc | [42] |
| 30. | Depocyt® | Cytarabine | Small molecule | Multivesicular liposome | 10 mg/mL | Cytarabine encapsulated in MVL composed of cholesterol, triolein, DOPC, and DPPG; suspended in 0.9% w/v sodium chloride in WFI | Lymphomatous meningitis | I.T. | Maintenance dose: 50 mg in every 28 days for 4 doses (weeks 17, 21, 25, and 29) | Apr 1, 1999 | Pacira Pharmaceuticals Inc | [43] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|--------|----------------------|---------------------------|----------------------|--|----------|---|---|------------------|--|---------------|----------------------------|------|
| 31. | Depodur [®] | Morphine sulfate | Small molecule | Multivesicular liposome (globule size: 17–23 µm) | 10 mg/mL | Morphine sulfate encapsulated in MVL composed of cholesterol, triolein, tricaprylin, DOPC, and DPPG; suspended in 0.9% w/v sodium chloride solution | Pain | Epidural | Depending on the criticality of the surgery, recommended dose is between 10 and 20 mg | May 18, 2004 | Pacira Pharmaceuticals Inc | [44] |
| 32. | Doxil [®] | Doxorubicin hydrochloride | Small molecule | Liposome (globule size: 100 nm) | 2 mg/mL | Doxorubicin encapsulated in liposome composed of HSPC, cholesterol, mPEG-DSPE | Ovarian cancer, multiple myeloma, AIDS-related Kaposi's Sarcoma | I.V. (injection) | Ovarian cancer: 50 mg/m ² IV every 4 weeks AIDS-related Kaposi's Sarcoma: 20 mg/m ² IV every 3 weeks Multiple Myeloma: 30 mg/m ² IV on day 4 following bortezomib | Nov 17, 1995 | Baxter Healthcare Corp | [45] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|--------|--------------|--------------------------|----------------------|--|-------------------------|--|--|------------------|--|---------------|------------------------------|------|
| 33. | Exparel® | Bupivacaine | Small molecule | Multivesicular liposome (globule size: 24–31 µm) | 13.3 mg/mL | Bupivacaine encapsulated in MVL composed of cholesterol, tricaprylin, DEPC, and DPPG; suspended in 0.9% w/v sodium chloride solution | Inducing postsurgical analgesia | I.V. (injection) | | Oct 28, 2011 | Pacira Pharmaceuticals Inc | [46] |
| 34. | Onivyde® | Irinotecan hydrochloride | Small molecule | Liposome (globule size: 110 nm) | Equation 4.3 mg base/mL | Irinotecan encapsulated in liposome composed of cholesterol, DSPC, mPEG-2000-DSPE, HEPES, sodium chloride | Metastatic adenocarcinoma of the pancreas (combined therapy) | I.V. (infusion) | 70 mg/m ² infusion over 90 min in every 2 weeks | Oct 22, 2015 | Ipsen Biopharmaceuticals Inc | [47] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|---------------------|--------------|---------------------|----------------------|---------------------------------|------------|---|--|------------------|---|---------------|------------------------|------|
| 35. | Marqibo® | Vincristine sulfate | Small molecule | Liposome (globule size: 100 nm) | 0.16 mg/mL | Vincristine encapsulated in liposome composed of sphingomyelin and cholesterol (60:40); mannitol, sodium citrate, citric acid, sodium phosphate, ethanol, sodium chloride | Acute lymphoblastic leukemia treatment | I.V. (injection) | At a dose of 2.25 mg/m ² over 1 h once in every 7 days | Aug 9, 2012 | Acrotech Biopharma LLC | [48] |
| Nanoemulsion | | | | | | | | | | | | |
| 36. | Cinvanti® | Aprepitant | Small molecule | Emulsion | 7.2 mg/mL | Egg lecithin, dehydrated alcohol, sodium oleate, soybean oil, sucrose, WFI | Nausea and vomiting | I.V. (infusion) | 130 mg on day 1, infusion over 30 min prior to chemotherapy (in adults) | Nov 9, 2017 | Heron Therapeutics Inc | [49] |
| 37. | Cleviprex | Clevidipine | Small molecule | Emulsion | 0.5 mg/mL | Soybean oil, glycerine, purified egg yolk phospholipids, oleic acid, disodium edentate, sodium hydroxide | High blood pressure | I.V. (infusion) | Initiate I.V. infusion at 1–2 mg/h. Further dose need to be titrated based on the decrease in blood pressure. Maintenance dose of 4–6 mg/h is recommended | Aug 1, 2008 | Chiesi USA Inc | [50] |

Table 2 (continued)

| Sr. no | Product name | Drug substances | Drug substances type | Dosage form | Strength | Composition | Therapeutic indication | ROA | Dosage regimen | Approval date | Marketed by | Ref |
|----------------|--------------|-------------------|----------------------|-------------|-------------------------|---|--|-----------------|--|---------------|--------------------------|------|
| 38. | Diprivan® | Propofol | Small molecule | Emulsion | 10 mg/mL | Soybean oil, glycerol, egg lecithin, disodium edetate anhydrous | Inducing general anesthesia | I.V. (infusion) | For healthy adults of less than 55 years old: 40 mg in every 10 s until induction onset (2 to 2.5 mg/kg) | Oct 2, 1989 | Fresenius Kabi USA LLC | [51] |
| Implant | | | | | | | | | | | | |
| 39. | Zoladex® | Goserelin acetate | Hormone | Implant | Equation 3.6 mg of base | Dispersion of goserelin acetate in PLGA copolymer matrix | Prostate cancer, breast cancer, endometriosis management | S.C. | 3.6 mg is recommended to be administered every 28 days | Dec 29, 1989 | TerSera Therapeutics LLC | [52] |

polymeric compounds, naturally derived complex mixtures, or complex mixtures of APIs, including semi-synthetic mixtures and other complex substances like iron-carbohydrate complexes and synthetic nucleotides [53, 54]. Drug substances such as the highly potent APIs are also considered complex because of their high-risk profile and the requirement of enhanced containment [55]. Despite their synthetic origin, specific drug molecules classified under non-biological complex drugs (e.g., high molecular weight synthetic compounds) attribute intricacies with respect to structure, insufficient quantification and characterization techniques, and lack of tools for physicochemical analysis [56]. Recombinant DNA (rDNA) technology, which involves molecular cloning of foreign DNA, produces human proteins in microorganisms. This technology is widely used to produce biopharmaceutical proteins and gene therapy [57, 58]. However, the risk of generation of impurities during manufacturing, discrepancies in gene replication technique, etc., contributes to the complex nature of this product [59].

For the development and approval of complex API-based generic products, it is mandatory to prove pharmaceutical equivalence (inclusive of the API sameness) of the test product with a reference product [60]. This is usually established by tracking the source of API followed by its thorough characterization, which is a strenuous task. Moreover, these complex APIs possess unique properties (like structure conformities, particle size, and shape) which can only be characterized by specific and advanced techniques. Thus, satisfying API sameness and keeping up with the manufacturing hurdles amidst usage of advanced characterization tools and techniques builds up the overall intricacy of the product approval.

Peptides and hormones

Peptides and hormones are generally used for numerous therapeutic modalities such as the hormonal replacement therapy wherein they add to or supplement if the endogenous levels of peptide hormones are inadequate or absent. Despite their benefits, the challenge lies in combating its half-life and its successful incorporation into the human body, as these are naturally occurring substances [61]. Copaxone®, glatiramer acetate injection, is marketed by Teva Pharmaceuticals Inc. for treatment of multiple sclerosis (MS via subcutaneous administration) [14]. Glatiramer acetate is a mixture of synthetic polypeptides having unique anti-inflammatory and immunomodulatory activities [62]. Along with polypeptide-based composition, finished product availability as a pre-filled syringe makes Copaxone® a complex drug product. For better clarity and guidance of generic product applicants, USFDA has published product-specific guidance (PSG) for glatiramer. In this guidance, the USFDA recommends few criteria which are to be fulfilled in

order to demonstrate API sameness such as equivalence of fundamental reaction scheme, physicochemical properties including composition, structural signatures for polymerization and depolymerization, and results of biological assays [17]. Additionally, related substance characterization like peptide-related impurity analysis and non-clinical immunogenicity assessment of impurities, along with functionality testing of packaging components and other routine testings, is also required for the successful filing of generic glatiramer prefilled syringe-based drug product [63]. On the similar grounds, goserelin acetate, a decapeptide, has been formulated into PLGA microspheres by AstraZeneca and is commercially available as Zoladex[®]. It is a gonadotropin-releasing hormone indicated for the treatment of prostate cancer, endometriosis and in palliative therapy for breast cancer [52].

There are various hormone-based injectable products approved by the USFDA such as Aved[™] and Humulin R. Aved[™] is a testosterone undecanoate-containing sterile oil-based injection used for intramuscular administration. It was developed by Endo Pharms Inc. and sought USFDA approval in 1953. Humulin R is a Lilly product approved by the USFDA in the year 1982. It is a regular human insulin produced by the rDNA technology for the treatment of diabetes and is administered subcutaneously or intravenously. Despite these established products in the market, hurdles at multiple facets from manufacturing to clinical setting and marketing make the entire development process of new generation peptide and hormonal therapy very challenging [64, 65].

rDNA and mRNA technology

The rDNA and mRNA technology is used in gene therapy to cure/treat/prevent diseases by replacing the defective gene with a normal one [58]. It additionally enables the defined manufacturing of antibodies and proteins with accurate specificity and uniformity, but the process itself contributes to the complications in this technology [66]. Eli Lilly and Company's Forteo[™] is an rDNA origin teriparatide subcutaneous injection approved in the year 2002. It contains a recombinant human parathyroid hormone with an identical sequence to the human parathyroid hormone [17]. Similarly, a product containing PEGylated interferon alpha-2a is developed using rDNA technology by Hoffmann-La Roche Inc. and is marketed as Pegasys[®]. It is an antiviral drug product prescribed for the treatment of chronic hepatitis C [67]. Comirnaty[®] developed by Pfizer-BioNTech is the first mRNA vaccine approved by the USFDA in 2021 for the treatment of COVID-19. It is a sterile lyophilized suspension which is to be diluted with sterile 0.9% sodium chloride injection, USP before intramuscular administration. The active moiety is a nucleoside-modified mRNA encoding

the viral spike (S) glycoprotein of SARS-CoV-2 virus which elicits an immune response to the S antigen thereby protecting against COVID-19 [68]. There are various characterization challenges of rDNA and mRNA being used as APIs. Establishing the identity of such oligonucleotides, purity profiling, and analyzing their impurity for related substances are a challenge in developing such products [63].

Iron complexes

The iron sucrose product of AM Regent, Venofer[®], is available as an intravenous injection. This product was approved as an NDA in 2000 for its use as a hematinic agent. Venofer[®] is a complex of polynuclear iron (III)-hydroxide in sucrose [23]. For generic product approval, PSG recommends to prove the API sameness of the test product with the reference product. The guidance recommends evaluation of physicochemical properties of this drug product by characterization of the iron core, composition of carbohydrate shell and its surface properties, particle morphology, and determination of labile iron under relevant physiological conditions [69]. Another iron product, Feraheme[™] injection composed of active ingredient ferumoxytol, was launched by Covis Pharma and was approved in 2009 [16]. Feraheme[™] infusion is indicated to treat iron deficiency anemia in adult patients with chronic kidney disease [16]. The semi-synthetic carbohydrate shell confers the complexity of the product-coated super-paramagnetic iron oxide nanoparticles. These are further suspended in an isotonic, neutral pH solution administered at a relatively high dose by rapid intravenous injection [70]. A recently approved iron complex by AM Regent is Injectafer[®] which got approval in 2013 [18]. The active ingredient of this product, ferric carboxymaltose, is a colloidal iron (III) hydroxide in complex with carboxymaltose. This carbohydrate polymer helps in the release of iron [71]. These complexes are characterized for their X-ray diffraction, X-ray crystal structure, and NMR and IR spectroscopy. Also, cyclic voltammetry is used to examine the electronic property of the synthesized product [72].

Antibodies

Monoclonal antibodies (mAbs) are synthetically developed antibodies resembling the endogenous moieties. These antibodies seek out and bind to specifically selected proteins in the body and bring about the immunogenic effect [73]. Kimmtrak[®], a mAb product developed by Immunocore Ltd., recently got the USFDA approval in 2022. The active moiety Tebentafusp-tebn is a bispecific gp100 peptide-HLA-directed T cell receptor CD3 T cell engager employed for the indication of uveal melanoma. Similarly, Cinqair[®] is a humanized interleukin-5 antagonist mAb (IgG4 kappa) indicated for the treatment asthma. This Teva Respiratory

LLC product, composed of reslizumab, produced by rDNA technology, sought USFDA approval in the year 2016. Both Kimmtrak[®] and Cinqair[®] are supplied as a solutions in single-dose vial and are administered intravenously as infusion [74, 75].

Antimicrobial peptides

Antimicrobial peptides (AMPs) are oligopeptides used against a broad spectrum of organisms. They vary in number from about five to over hundreds of amino acids. AMPs are generally classified based on their target viz, antiviral peptides, antibacterial peptides, antifungal peptides, and antiparasitic peptides. Fuzeon[®] developed by Roche is one such example of antiviral peptides. Enfuvirtide is the active pharmaceutical ingredient of this injectable product. It is composed of naturally occurring L-amino acid residues and is a linear 36-amino acid synthetic peptide. It is a lyophilized powder which is to be reconstituted with sterile water for injection prior to subcutaneous administration [76]. Similarly, Vancocin is a vancomycin-based injection for intravenous use. The product developed by Baxter Healthcare is composed of tricyclic glycopeptide antibiotic drug derived from *Amycolatopsis orientalis* and is categorized under antibacterial peptides [77].

Complex excipients

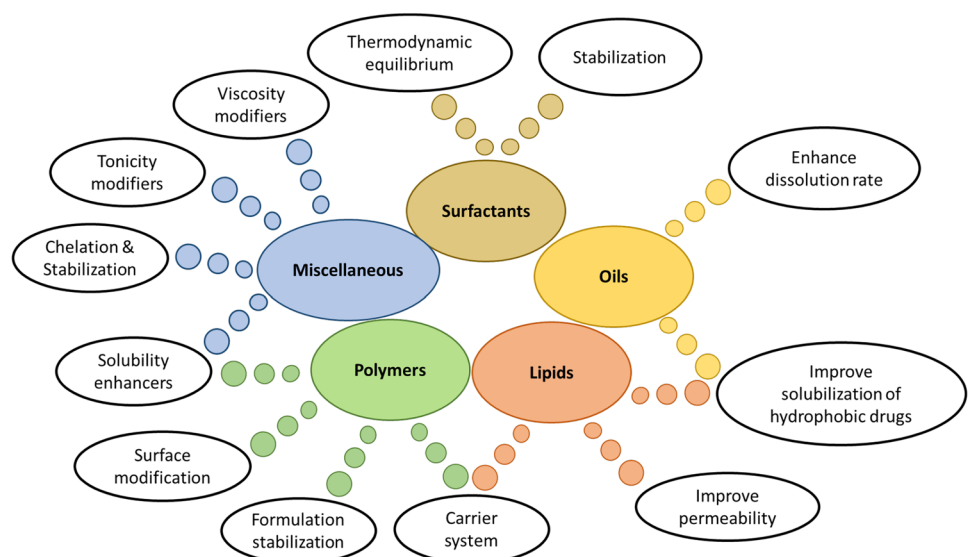
Excipients are a critical component of any drug product development as they are meant to provide desirable characteristics. In the last few decades, technological advancement has led to the development and usage of various functionalized complex excipients to provide special physicochemical properties to the finished products. These excipients enhance

the drug release, pharmacokinetic profiles, drug solubility, formulation stability, and likewise (Fig. 3). However, the formulation containing such excipients needs additional characterization tests to investigate the functionality of the dosage form. Regulatory agencies are also keen to review information about the toxicity of these novel specialized excipients [78].

Surfactant and oils

Surfactants and oils are extensively used in pharmaceutical formulations for varied reasons, such as improving drug solubility, stability, and the rest. Surfactants are amphiphilic molecules having hydrophilic and hydrophobic functional groups (hydrocarbon chain). Nanosuspension-based drug products employ surfactants to stabilize the nanosized drug particles in the colloidal dispersions [79]. Surfactants and oils also play a critical role in formulating injectable emulsions. The oils enhance the solubility and dissolution rate of poorly soluble drugs, whereas surfactants help to achieve thermodynamic equilibrium between water and oils forming oil–water interface [80]. Surfactant molecules help stabilize the colloidal system by reducing the interfacial tension and creating an interface with minimum free energy. Moreover, use of ionic surfactants increases the stability because of electrostatic repulsion induced by the surface charge [81]. Similarly, self-emulsifying systems are composed of oils, surfactants, and co-surfactants, which form an emulsion on mixing with water and need little to no energy input [82]. Various oils like Miglyol 812, Captex 355, Labrafac, cottonseed oil, soybean oil, and corn oil are commonly used for preparing emulsions and self-emulsifying systems. Similarly, compounds like sulfates or ester sulphonates,

Fig. 3 Multifaceted viewpoints involved in the evaluation of complex excipients for the development of complex injectables



quaternary ammonium salts, ethylene, propylene oxide, sorbitan esters, and ethoxylates are widely used as surfactants [83].

Paliperidone palmitate is an atypical antipsychotic drug indicated for the treatment of schizophrenia. Various paliperidone-loaded extended-release suspension drug products are approved by Janssen Pharmaceutica NV with different dosage regimens, i.e., once in a month (Invega Sustenna[®]) [31], once in 3 months (Invega Trinza[®]) [32], and once in 6 months (Invega Hafyera[™]) [30]. These products are administered through the intramuscular route (I.M.) and contain polysorbate 20 as a surfactant. Polysorbate 20 plays a pivotal role in stabilizing these suspensions, comprising of poorly aqueous soluble API dispersed in an aqueous phase [84, 85].

Lipids

In the last few decades, various lipidic formulations like liposomes, solid lipid nanoparticles, lipid emulsions, and cubosomes have gained attention among researchers and the pharmaceutical industry for effective drug delivery. These formulations require lipids as a potential functional excipient to impart desirable properties to the product. Lipidic excipients enhance the solubility of lipophilic drugs with high log P, which are poorly water-soluble. These excipients have also been reported to improve permeability of hydrophilic drugs by incorporating them in lipid matrices [86]. These lipids are endogenous as they are structural and functional components of cell membranes. Common phospholipids like egg phosphatidylcholine, hydrogenated soybean phosphatidylcholine (HSPC), glycerophosphocholine, soybean lecithin, and likewise are used to develop lipid-based injectable products (Fig. 4). These lipids are

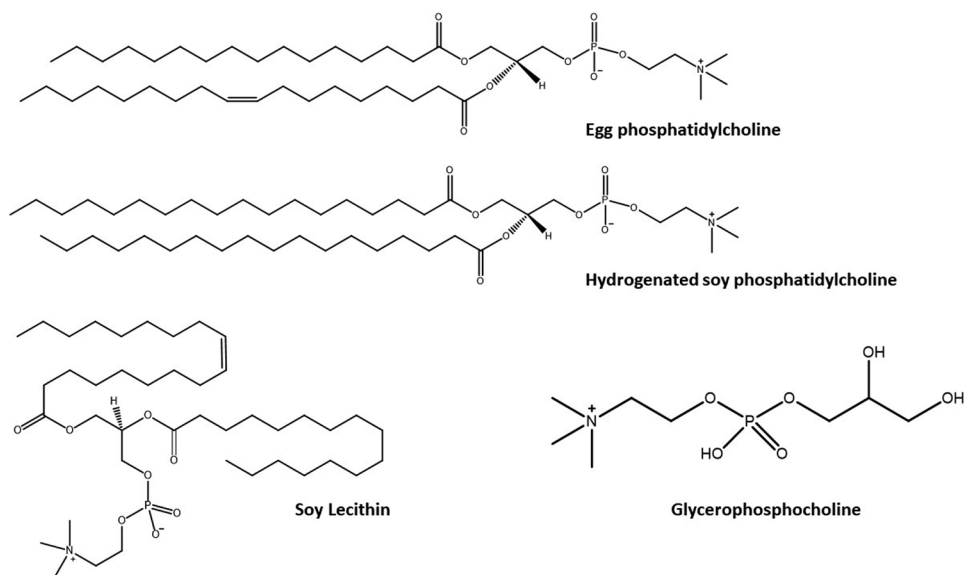
primarily composed of various fatty acids such as palmitic acid, stearic acid, oleic acid, linoleic acid, and linolenic acid [87]. As the selection of various lipidic combinations and their purity directly affects the product's quality and performance, the FDA expects the applicant to provide detailed information about the origin, distribution, and content of the lipid component in the drug product [88]. For example, in the Product Specific Guidance issued by the USFDA for Doxil[®], the generic drug manufacturer has to develop the drug product by obtaining the lipids from the same category of synthesis route. The lipids used in the manufacturing of Doxil[®] are HSPC, N-(carbonyl-methoxypolyethylene glycol 2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (MPEG-DSPE), and cholesterol [45].

Polymers

A wide range of polymers has proven their application in the development of pharmaceutical products due to specific functions. Polymers act as solubility enhancers, formulation stabilizers, and coating agents, which helps in improving the overall effectiveness of the formulation. Moreover, few polymers also provide an opportunity for functionalizing targeting ligands on their surface for the site-specific targeted delivery of therapeutic moieties. Attributed to these properties, polymers are used in numerous novel drug formulations imbuing desirable properties [89]. The long-acting formulations of various polymers benefit by administering lower drug doses relative to the daily oral regimen, improving patient compliance and convenience.

Formulations composed of poly (lactic-co-glycolic acid) (PLGA), a biodegradable co-polymer consisting of lactic acid and glycolic acid, provide a long-term drug release with reduction of dosing frequency. It is one of the most widely

Fig. 4 Structure illustration of various lipids used in complex injectables products



used type of polymer and is available in a linear form and as a star-shaped glucose (PLGA-Glu) moiety that is structurally non-linear. The linear arrangement of PLGA is highly hydrophobic and has a low loading capacity in its linear form [90]. Compared to the linear form, the star-shaped form has a three-dimensional branched structure. It possesses less solution viscosity, smaller hydrodynamic radius, higher drug loading, and drug encapsulation efficiency, encouraging its usage in formulation development (Fig. 5). For example, in manufacturing of Sandostatin long-acting release injectable formulation, PLGA-Glu polymer is utilized having a L/G ratio of 55:45 [91]. However, the characterization of non-linear PLGA with respect to its molecular weight, lactide:glycolide ratio, and morphology of PLGA is tedious due to shortcomings in analytical methods, contributing to the complexity of using the polymer [92, 93].

Other polymers such as N-(2-hydroxypropyl) methacrylamide, polyamidoamine, polyethylene glycol (PEG), poly(glutamic acid), dextrin, dextran, PEI, chitosan, poly(aspartamides), and poly(l-lysine) are employed as carriers for conjugation with therapeutic moiety to improve its pharmacodynamic and pharmacokinetic properties [94]. These are used to conjugate either with small molecules or with proteins and peptides. Naturally occurring biological polymers like albumin are also used for developing formulations of various therapeutic moieties. Abraxane[®], paclitaxel nanosuspension approved in 2005, is one such complex injectable product where albumin is chemically bound to paclitaxel and showed improved therapeutic responses as compared to conventional product (Taxol[®] injection) [28].

Additionally, certain amphiphilic polymers like cetyldimethicone copolyol (Abil EM 90), polysiloxane, and poly-glycerol-poly-ricinolate are employed as stabilizers for various emulsion, double emulsions, and suspensions. These polymers adsorb on the external interface of the globule and cover them leading to steric hindrance between the globules and thereby stabilizing the system. Also, polymers such as

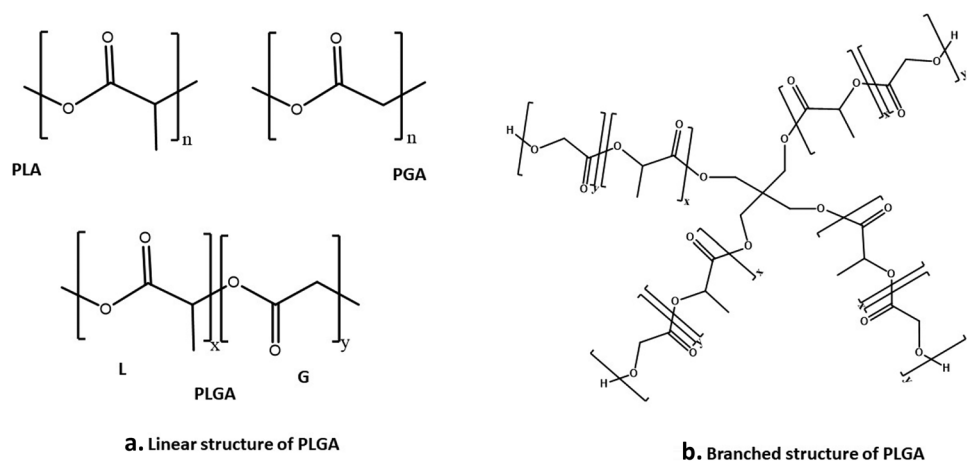
PEG are used to modify surfaces of nanocarriers to further improve its pharmacokinetic property and enhance its therapeutic efficacy [95].

Miscellaneous/other excipients

Various other specialized excipients, such as chelating agents, stabilizers, and likewise, are also categorized under complex excipients as they impose unique characteristics to finished products [78]. Also maintaining the physiological properties such as tonicity, osmolality, and pH, should be considered while formulating/reconstituting parenteral products. Excipients such as tonicity agents help keep up the tissue compatibility at the site of administration. Tonicity agents are either admixed during I.V. infusion or are added to the formulation if it is to be administered prior to dilution in a suitable isotonic solution. Sodium chloride, mannitol, dextrose, sucrose, etc. are some of the commonly used tonicity agents. These agents are also used to reconstitute powder for injection when required. Other than maintaining physiological conditions, excipients are also employed to modify the release profile and improve physical stability of the drug products. The release profile of the drug from the injectable dosage forms depends on the viscosity of the formulation. The intermolecular forces between drug and different excipients can further modify the overall viscosity of the formulation; hence, it becomes critical to account for this parameter. Various viscosity modifiers like sodium CMC, xanthan gum, and dipicolinic acid are used to serve this purpose [96].

Vfend[®] is a powder for solution for infusion of voriconazole developed by PF Prism CV in 2002. Voriconazole is slightly soluble in aqueous conditions over an acceptable pH range. Therefore, Vfend[®] was developed using novel cyclodextrin-based excipient, sulfobutyl ether beta-cyclodextrin (SBE- β -CD), which enhances the aqueous solubility of voriconazole. Inclusion complex formation of voriconazole

Fig. 5 Frame work of PLGA copolymer. **a** Linear structure and **b** branched structure



with SBE- β -CD helps achieve the desired solubility, which is usually not possible with conventional pharmaceutical approaches [97]. OptiMARK™, manufactured by Liebel-Flarsheim Company LLC, is a solution for injection of gadoversetamide, which is a contrast medium for magnetic resonance imaging. Gadoversetamide is a chelate of gadolinium and a novel excipient versetamide which acts as a stabilizer [98].

Since these novel excipients are used to address specific formulation issues, the developed products require extensive characterization to demonstrate drug product effectiveness and excipient functionality for successful regulatory submission.

Complex dosage forms

Most of the drugs existing in market possess undesirable properties like low solubility, short half-life, high protein binding, and extensive first-pass metabolism, thereby limiting their therapeutic activity and overall pharmaceutical application. In the last few decades, advanced drug delivery systems like nano-suspensions, nano-emulsions, and other carrier systems like liposomes and microspheres have been explored by pharmaceutical researchers to improve clinical outcomes of therapeutic agents through parenteral route of administration [99] (Fig. 6). Approval of the first nanotechnology-based drug product, Doxil®, in 1995, and its

commercial success paved the way for these dosage forms in clinical applications. The drug substances delivered through these multifunctional carrier system provide controlled drug release with better clinical outcomes [100]. However, there exists a major gap in scalability of these products because of the complexities involved in its design, characterization, and large-scale manufacturing. Thus, these products require special attention during regulatory approval to ascertain their safety and efficacy.

Another challenge related to the manufacturing of complex drug products intended for parenteral route is the sterility of the finished dosage form. Aseptic processing facilitates the manufacturing of sterile parenteral drug product wherein the components, such as polymers, lipids, organic solvents, and aqueous buffers, used are sterilized by filtering them through 0.22- μ pore size filters. Subsequently, these components are utilized for the preparation of drug products on the working stations located in the clean rooms (class-100 area). Aseptic filling should be critically monitored for potential sources of contaminants such as primary packaging material, operating personnel, environmental air contamination, and water drainage systems. Considering the unique chemical composition and physicochemical properties of the components involved in manufacturing of complex injectables, commonly used sterilization techniques (like autoclaving, gamma radiation, and ionizing radiation) could be detrimental to the stability of finished drug products [101]. The

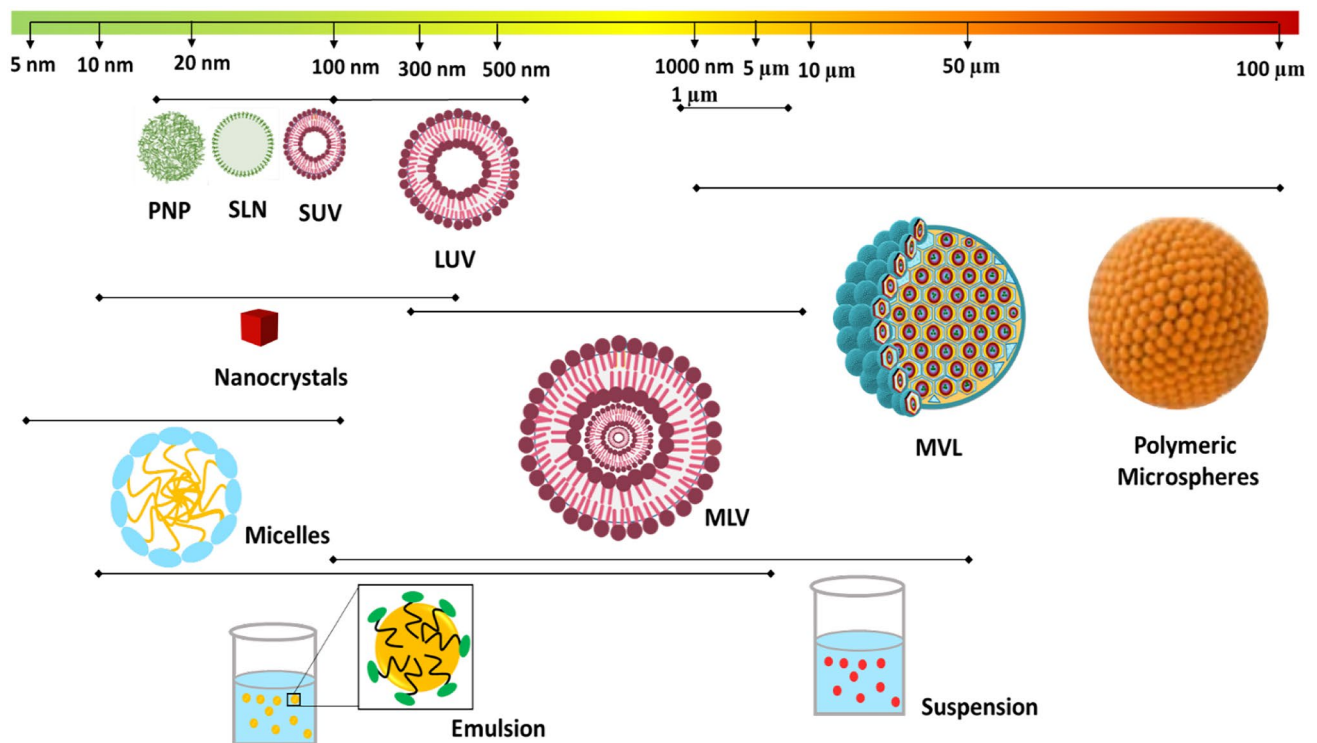


Fig. 6 Length scale showing the size of the various complex injectable dosage forms

manufacturing of these products often involve the use of biomaterials such as lipids or polymers in combination with surfactants and certain ligands for targeted delivery. These excipients should be biodegradable, biocompatible, and suitable with respect to hemocompatibility, histocompatibility, carcinogenicity, genotoxicity, and cytotoxicity.

Liposomes

Liposomes are novel carrier systems for drugs and are composed of one or more lipid bilayer membranes surrounding discrete aqueous compartments. These carrier systems can encapsulate the hydrophilic drugs in the aqueous compartment and lipid-soluble drugs within the lipid bilayer membrane. Liposomes serve the advantage of improving the pharmacokinetic properties of the drug molecule leading to lower doses and dosing frequency, site-specific drug delivery, and thus improved patient compliance towards the therapy [102]. Various technologies have been developed for the preparation of clinically and commercially viable liposomal-based products with particle sizes ranging from 50 nm to 50 μm [103, 104]. Electron microscopic images demonstrating the morphological structure of different technology-based commercially approved liposomal products are shown in Fig. 7.

Stealth[®] technology utilizes the conjugation of nano liposomes with globule size of 100–200 nm with polymers like PEG to provide nano-sized vesicular systems with prolonged blood circulation. Doxorubicin-loaded PEGylated liposome, marketed as Doxil[®] by Janssen Pharmaceuticals Inc., was the first nanotechnology-based product approved

in 1995. This product based on the Stealth[®] technology is proven to be highly effective for treating ovarian cancer, AIDS-related Kaposi's sarcoma, and multiple myeloma, and minimizing cardiotoxicity associated with doxorubicin. The improved therapeutic effect can be attributed to its globule size and surface characteristics, which facilitate passive targeting of drug to the tumor site [105]. For the development of the generic version of this liposomal formulation, USFDA has published a PSG, which provides information on in vitro, in vivo, and physicochemical characterization as needed for its successful filing and approval. Some of the critical physicochemical characteristics as recommended in Doxorubicin PSG are liposome composition, state of encapsulated drug, internal environment, morphology, number of liposomal lamellae, lipid bilayer phase transitions, size distribution, grafted PEG at the liposome surface, electrical surface potential or charge and in vitro leakage, and so forth [106]. Few generic companies like Sun Pharmaceutical Industries Ltd., Dr. Reddy's Laboratories Ltd., and Zydus Worldwide DMCC have already received the approval of generic doxorubicin liposomes. In recent years, few more products based on Stealth[®] Technology are either under development/approval stage or have sought approval for commercial purpose. Ipsen Biopharmaceuticals Inc. received a USFDA approval in 2015 for the irinotecan liposomal injection, Onivyde[®], which is also based on similar technology [47].

In the last couple of decades, DepoFoam[®] technology-based liposomes, i.e., multivesicular liposomes (MVL), are also gaining scientific attention for sustained delivery of therapeutics. This delivery system contains a non-concentric

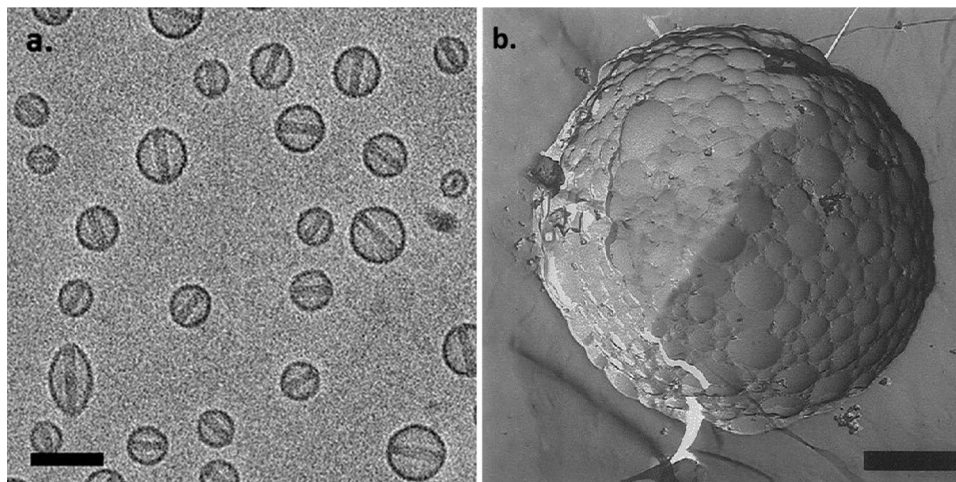


Fig. 7 Electron microscopy of liposomes. **a** Unilamellar Nanoliposomes (scale=100 nm) (Reprinted from International Journal of Pharmaceutics, 2018, 547(1–2), Damari SP, Shamrakov D, Varenik M, Koren E, Nativ-Roth E, Barenholz Y, Regev O, Practical aspects in size and morphology characterization of drug-loaded nanoliposomes, Pages No. 648–655. Copyright © 2018, with permission

from Elsevier). **b** Multivesicular Liposomes (scale=2 μm) (Reprinted with permission from Langmuir, 1996, 12(20), Spector MS, Zasadzinski JA, Sankaram MB, Topology of Multivesicular Liposomes, a Model Biliquid Foam, Pages No. 4704–4708. Copyright © 1996 American Chemical Society)

core that facilitates the controlled release of drugs at the site of action. Usually, these delivery systems are in the micron size range, with a median diameter of 20–30 μm [107]. Depofoam[®] technology has been successfully translated for commercial application by Pacira Pharmaceuticals with products like Depocyt[®], DepoDur[®], and Exparel[®], for sustained delivery of cytarabine, morphine and bupivacaine, respectively [108, 109]. Based on the commercial applications of MVL, various products have been developed to deliver small and large molecules, which are under the pre-clinical and clinical trials [110]. MVL preparation is a highly complex multistep process with adequate control of quality attributes like globule size, internal volume, drug encapsulation, and desired release characteristics. MVL is composed of synthetically modified naturally occurring lipids that are biocompatible and biodegradable in nature. This drug delivery system is different from unilamellar/multilamellar liposomes in that it consists of honeycomb-like internal structure of non-concentric lipid bilayers encapsulating a significantly higher amount of drug. The drug release from the MVL is initiated by the breakdown of the outermost vesicles leading to the redistribution of the drug within the

MVL. The gradual erosion of the vesicles within the MVL over a period of time results in diffusion of the drug for a prolonged period (Fig. 8). Manufacturing of MVL in aseptic conditions aggravates the complications in developing this product. USFDA has published PSG for the generic applicant of Exparel[®], which recommends establishing sameness of API and lipid excipients. It also highlights the agency's expectation of performing bioequivalence studies as a two-way crossover in vivo experiment for the test product in comparison to reference product. Other physicochemical properties like liposomal composition, internal aqueous environment, globule structure and morphology, and in vitro drug release rates are some of the critical parameters which are to be characterized for successful development of generic bupivacaine liposomes [107]. The thermosensitivity of this product can clearly be understood from the stability testing studied by the innovator where the testing was conducted at $5 \pm 2^\circ\text{C}$ (long-term) and $25 \pm 2^\circ\text{C}$ (accelerated) along with non-conventional intermediate conditions of 15°C . Because of thermo-sensitivity, this product comes with a temperature indicator in its packaging to indicate any thermal degradation during shipment [111].

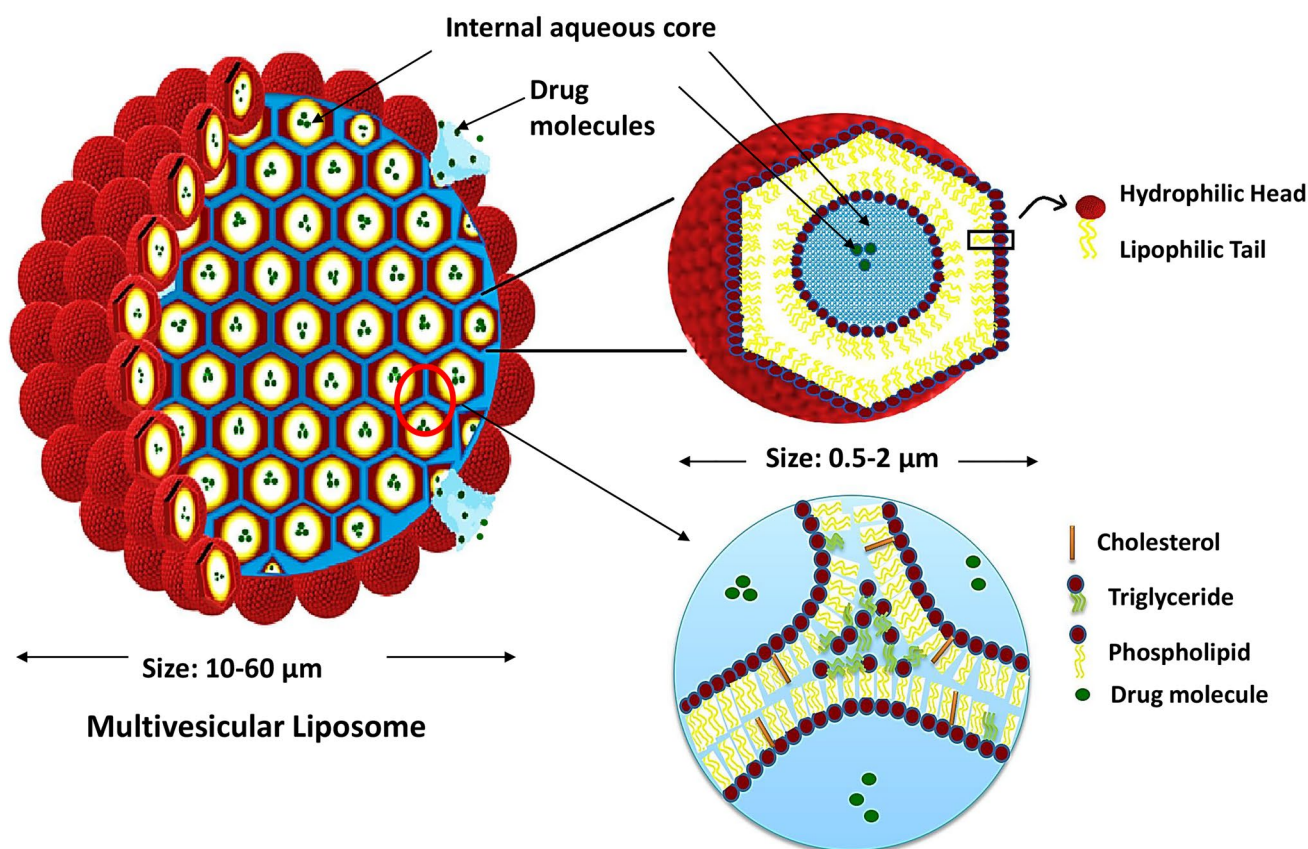


Fig. 8 Schematic representation of multivesicular liposome (MVL) structure (Reprinted by permission from Drug Delivery and Translational Research, 2021, Chaurasiya A, Gorajiya A, Panchal K, Katke

S, Singh AK, A review on multivesicular liposomes for pharmaceutical applications: preparation, characterization, and translational challenge. Copyright © 2021, with permission from Springer Nature)

Polymeric particles

In the last few decades, PLGA-based products have proven their application in the sustained delivery of various drugs by the parenteral route of administration. Octreotide is a long-acting octapeptide with pharmacologic properties similar to natural hormone somatostatin. An injectable suspension composed of octreotide microspheres comprises a biodegradable glucose star polymer, and D- and L-lactic and glycolic acid co-polymer. This formulation, marketed as Sandostatin LAR[®] Depot by Novartis Pharmaceuticals Corp., received its USFDA approval on Nov 25, 1998. For its generic product development and regulatory filing, USFDA has published PSG highlighting the requirements for single-dose, parallel design in vivo bioequivalence study of test product in comparison to reference product [112]. Similarly, Risperdal Consta[®], a long-acting injection of risperidone PLGA (75:25) microspheres for intramuscular administration, is employed for the treatment of schizophrenia and bipolar I disorder. Janssen Pharmaceuticals Inc. received approval from the USFDA for this pharmaceutical product in 2003 [38, 113, 114]. In a different product, a synthetic nonapeptide analog of naturally occurring gonadotropin-releasing hormone (GnRH), leuprolide acetate, was formulated into lyophilized PLGA microspheres and is commercially marketed by AbbVie Inc. as Lupron Depot[®] (depot suspension). Besides the use of synthetic polymers for preparation of intravenous particulate systems, there have been a few which are formulated using naturally occurring polymers/proteins like the albumin. Abraxane[®] is one such particulate product composed of paclitaxel bound to albumin protein with a mean particle size of about 130 nm. This Abraxis Bioscience product attained USFDA marketing approval in the year 2005. Abraxane[™] is available as a sterile lyophilized powder which is to be reconstituted with 0.9% sodium chloride injection, USP, and is administered as intravenous suspension for the treatment of breast cancer [28].

Suspensions

Suspensions are dosage forms used to deliver drugs which are less soluble or insoluble in water or a solvent system suitable for administration as a solution. Also, when the use of solubilizing agents for such drugs does not improve its solubility, suspensions become the choice of formulation to deliver these active moieties [115]. Invega[®] Sustenna[™] is a pharmaceutical injectable suspension developed by Janssen Pharms for the delivery of paliperidone palmitate. This product got its USFDA approval in the year 2006 for the treatment of schizophrenia. Another recently approved extended release suspension is Aristada[™]. This aripiprazole lauroxil-containing product developed by Alkermes Inc. secured its USFDA approval in the year 2015 for the

indication of schizophrenia. Both Invega[®] Sustenna[™] and Aristada[™] are sterile aqueous-based extended-release injectable suspensions available as pre-filled syringes for intramuscular administration [29, 31].

Nanosuspensions are colloidal dispersions having particle size ranging from few nanometers to 5 μm . They can be manufactured in aseptic and non-aseptic conditions based on particle size and sensitivity towards sterilization process. Most of these nanosuspension products are thermolabile in nature which limits the choice of sterilization method. As per sterilization decision tree published by the European Medicine Agency, autoclaving is the most preferred method of sterilization of injectable products followed by sterile filtration and aseptic processing/filling [116]. Sterilization using filtration technique is not feasible always as certain products have particle size of more than 0.2 μm . In such cases, the only option left to render sterility in finished product is aseptic processing and filling. In aseptic processing, all manufacturing activities starting from compounding/mixing with the formulation vehicle, particle size reduction, final volume make-up, filling, and sealing into the primary packaging are carried out under sterile conditions. Additionally, manufacturing of these products in aseptic condition need more considerations with respect to selection of excipients, method, and scale-up activities. Quality control testing of these products include assay, particle size, impurity determination, density, viscosity, bioburden, sterility, dissolution, etc.

Emulsions

The injectable emulsion-based formulation has proven to be a successful approach for hydrophobic drug delivery. Many products are developed and have received regulatory approval for commercial and clinical applications [117]. Propofol injection (Diprivan[®]) is oil-in-water emulsion marketed by Fresenius Kabi USA LLC as a general anesthetic by intravenous administration [51]. It is prepared using a combination of high shear and high-pressure homogenization methods. Similar to propofol, clevidipine shows poor aqueous solubility and is formulated as an injectable formulation for I.V. administration. Clevidipine emulsion for injection is marketed as Cleviprex[®] by Chiesi USA Inc. [50]. For successful filing and approval of such emulsion-based products, it is essential to develop a robust process to manufacture uniform-sized globules. Characterization with respect to globule size, surface charge, emulsion stability, drug content, and so on is required to be carried out in a meticulous manner.

The PSG of propofol recommends two-way crossover design bioequivalence studies for the development and filing of its generic product. The USFDA confers a provision of bio-waiver for qualitative and quantitative (Q1/Q2) similar

products with acceptable physicochemical characterization, population bioequivalence studies, and in vitro release between the test and reference product [118].

Injectable implants

In last few decades, injectable implants have gained attention as they possess several advantages like controlled and continuous release of drugs up to several weeks/months. Biodegradable polymers could be utilized to develop injectable implants because of their biocompatible nature and natural metabolic pathways. Of the various biodegradable polymers identified, PLGA has been widely explored to develop drug-loaded injectable implants. A commercially available injectable implant Zoladex[®] is a sterile, biodegradable product containing goserelin acetate dispersed in PLGA, administered by subcutaneous route [52].

Drug-device combination products

Delivery of drugs through parenteral routes such as intramuscular (I.M.), subcutaneous (S.C.), and intravenous (I.V.) is chosen when the drug molecules show degradation in the gastro-intestinal milieu or nasal tissues. However, parenteral routes of drug delivery suffer from disadvantages such as the requirement of skilled healthcare professionals for the administration of the drug product. Moreover, frequently dosing drugs with a short half-life (small molecules/peptides) for treating chronic diseases generate serious patient non-compliance [119]. Therefore, efforts are being made by the pharmaceutical industries for the development of drug delivery devices to improve patient compliance and overcome the barriers in parenteral drug therapy. Drug device systems such as pre-filled syringes (PFS), injector pens, and similar have made parenteral drug administration relatively easy and patient-friendly [120]. To ensure the safety, convenience, reduction of dosage errors and waste, proper labeling, drug product sterility, and stability, most regulatory agencies have laid down specific requirements and expectations for the approval of such drug-device combination products [121]. Products such as EpiPen[®] and EpiPen Jr[®] are examples of pre-filled auto-injectors which are indicated for emergency treatment of allergic reactions and anaphylaxis. Also, Invega[®] Sustenna[™] is provided in a prefilled syringe (cyclic-olefin-copolymer) with a plunger stopper and tip cap (bromobutyl rubber) [15, 31].

Regulators like USFDA and EMA have issued a guidance document describing the scientific and technical information to be considered by the manufacturer when developing a pen, jet, or related injector device meant for administration of a drug or a biological formulation [122]. The regulatory requirements for the injectors or pen devices differ depending on their intended use, product characteristics, proposed

labeling, and packaging. Here are some specific requirements needed to be considered by applicants for the successful development and approval of these complex drug-device combination products.

General information

The applicant must include usage information of the proposed drug-device combination, including the site and depth of injection, and type of use (single, reusable, disposable, or refillable injector). Detailed description about conditions of use should be mentioned, including the injection method (manual, piston, spring load, gas, jet), details about the dose such as single dose or multiple and adjustable dose, environmental storage conditions, and handling of the pre-filled injectors [123].

Device design

The design features of the pen device or auto-injector should be included in the submission documents. Detailed information on the technical specifications of the injector and its characteristics is a prerequisite [123]. The applicant needs to generate data comparing the design features of their injector to the parts of the injector in a similar approved product. The applicant is also expected to provide drawings of the engineering components, injector photographs, and stages showing the drug product delivery. Figure 9 illustrates the basic design and components of these devices. The drug products are stored in barrel/cartridges made up of compatible material like glass, polypropylene, cyclic olefin copolymer, and packed with plunger. The FDA recommends providing information on procedures for setting the required dose using mechanisms such as a dial, assembling the injector, preparing and positioning for an injection, resetting the quantity after use, and changing or disposing of the needle. These requirements are meant to assure the ability of the injector to be reliable and reproducible for delivering the drug product with desired injection volume to the target tissue. The guidance also suggests using graduation marks and fill lines to help the user set the correct dose or verify the set dose along with a feature of visual inspection of any particulate matter within the product.

Drug-device interaction

FDA expects the manufacturer to include information about the materials used in the construction and manufacturing of the injector devices. During the development of drug-device combination products, the interaction between the material of construction of the injector with the drug or biological product needs to be evaluated and supported with stability data [123].

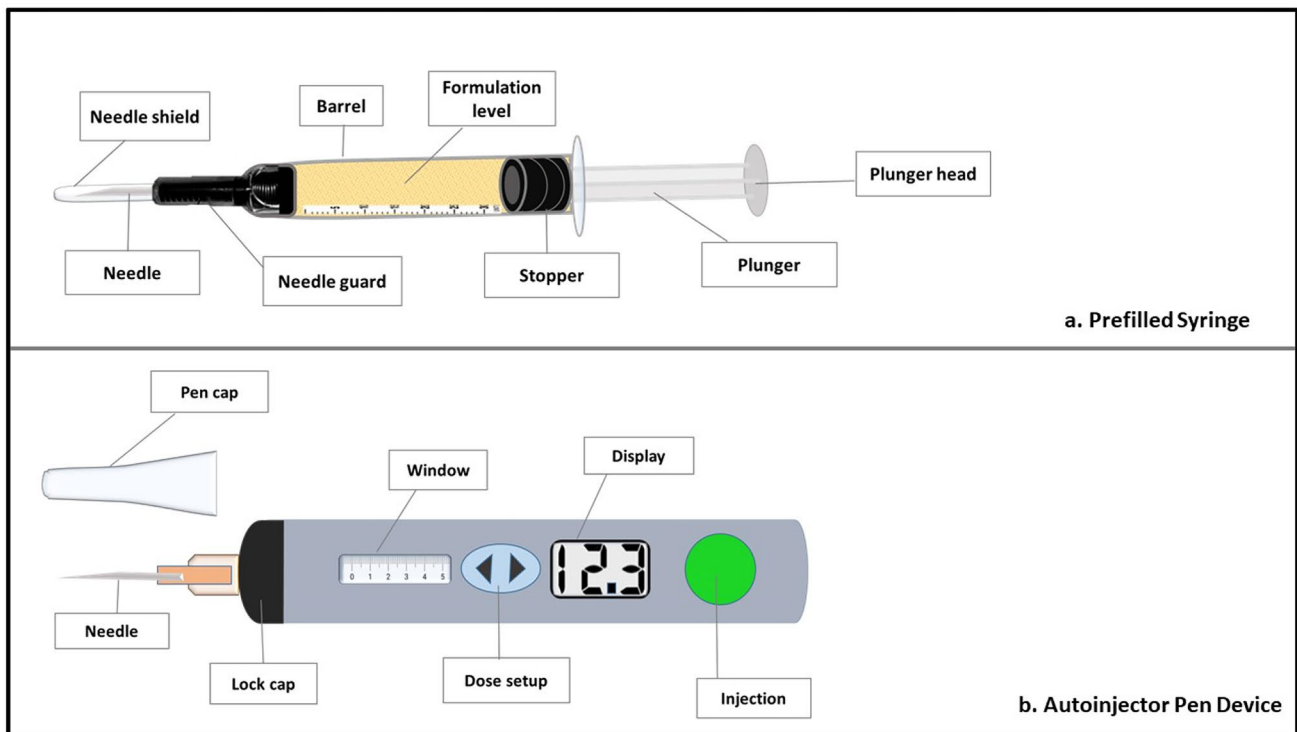


Fig. 9 Various components of injection devices. **a** Auto-injector pen device; **b** pre-filled syringes

Device safety features

To prevent injury and ensure accurate dosing, it is recommended to incorporate certain safety features into the injector device. These safety features may include audible, visual, and tactile notifications, switches, or mechanical protection. The manufacturer should also consider human factors while designing the injector device, such as the age of the target population, tissue characteristics at the site of administration, limitations due to visual impairment, or manual dexterity.

Device performance testing

As a component of general testing, FDA recommends the applicant to conduct performance testing of the devices. For the submission, general testing includes the test for dose accuracy, demonstration of the depth of needle penetration, and accurate dispersion of drug product at the target site. Special tests for evaluation of extractables, leachables, and absorbables, along with tests for container-closure integrity and shelf-life dating, are recommended to be submitted [123]. FDA has also provided a guidance document for the assurance of sterility of injectors [124]. Table 3 summarizes the list of various tests as needed for seeking regulatory approvals of prefilled syringe-based products.

Labeling requirement

Pen, jet, or injector devices are meant for direct use by the patients, caregivers, or healthcare professionals. The USFDA recommends developing the labels in consideration with the end-users and suggests including package insert for the instructions on how to use the injector safely and effectively [123].

Characterization tools for complex injectables

Typically, complex injectable products are comprised of unique physicochemical properties which facilitate their functionality. Therefore, to successfully develop and translate complex injectable formulations, it is necessary to identify and use suitable characterization tools/techniques to evaluate their physicochemical properties. Proper characterization ensures the therapeutic efficacy and safety profile of these developed formulations. Generally, these formulations are characterized for particle size, morphology, surface characteristics, drug content, entrapment efficiency, and others [125]. In this section, various tools and techniques commonly used to evaluate the characteristics of complex injectable products are discussed. For better understanding,

Table 3 Compendial tests for pre filled syringes (PFS) as per ISO 11040–4

| Sr. no | Test | Rationale |
|--------|--|--|
| 1. | Flange breakage resistance | While delivering the dose, most of the force is applied on plunger and barrel flange. Thus, it should be rigid to withstand the force |
| 2. | Luer cone breakage resistance | Luer cone is the assembly, where the needle is fixed by applying some torque. This test thus proves the ability of the luer to withstand the torque which is applied while attaching the needle before dose administration |
| 3. | Break loose and glide force | Break loose: it is the initial max force applied during administration Glide force: it is the average force required for completing the dose administration |
| 4. | Needle penetration test and Needle pull out force | For any stacked needle PFS, while administration practitioner has insert PFS into the vial stopper. It takes some force to penetrate and pull out. This force should be within the limit of manufacturer and end user requirements |
| 5. | Luer lock adaptor collar pull-off force | Luer lock adapter is fixed on PFS barrel tip which requires certain force to be removed. Limits are thus correspondingly fixed based on the manufacturer and end user |
| 6. | Closure system liquid leakage test | This test is performed to confirm sterility of injectable products |
| 7. | Luer lock adaptor collar torque resistance | Torque is applied while applying/removing the needle to luer lock. Thus, the adaptor should stay stiff and not be rotated while applying torque during screwing and unscrewing |
| 8. | Luer lock rigid tip cap unscrewing torque | Torque is applied while applying/removing the needle to luer lock. Thus, the adaptor should stay stiff and not be rotated while applying torque during screwing and unscrewing |
| 9. | Pull-off force of the tip cap or the needle shield | For PFS stacked needles, those were supplied with plastic rigid cap with elastomer |
| 10. | Dye solution tightness test | This test is performed to confirm sterility of injectable products |

we have summarized common characteristics required to be evaluated along with the tools in Table 4.

Size and shape/morphology

Particle size and shape/morphology have a significant role in determining the stability of the particulate formulation (micro- or nano-size range) [151]. They also affect the pharmacokinetics and pharmacodynamic behavior of the drug substances [152]. Particle size measurement is needed to conduct population bioequivalence for complex generic product development and subsequent approvals [106]. These measurements can be done by various available techniques with their different principle for the diverse range of the complex injectable products, as discussed below.

Dynamic light scattering

Dynamic light scattering (DLS) is one of the widely used light scattering techniques for particle size measurement of different formulations whose diameter ranges from nano- to sub-micron. Measurement of intensity fluctuation due to the Brownian movement of particles present in a colloidal dispersion system is the basic principle of the DLS technique [153]. Alternatively, it is also well-known as photon correlation spectroscopy or quasi-elastic light scattering technique [154]. By DLS, average hydrodynamic diameter and distribution of particle size range can be accessed. This technique

is commonly used to measure particle size (z-average, D10, D50, and D90) and polydispersity index (PDI) of various nanosized formulations like liposomes, nanosuspensions, nano-emulsions, and other similar products. Commercially available instrument for the measurement of particle size through DLS technique is a Zetasizer suitable for measurement of particle size ranging from more than 1 nm to less than 1 μm . Zetasizer instrument is developed and marketed by the company Malvern Panalytical Ltd.

Laser diffraction analysis

Laser diffraction analysis, or laser diffraction spectroscopy technique, is based on the measurement of angular changes in the intensity of scattered light [155]. The degree of scattering of light and particle size is inversely related to each other. Red and violet lasers are used to analyze large and sub-micron-sized particles, respectively [156]. Mastersizer, marketed by Malvern Panalytical Ltd., is based on this technique and is commercially available for the measurement of particle size ranging from submicron to millimeter.

Field-flow fractionation

Field-flow fractionation (FFF) techniques measure the size distribution and relative molecular mass of samples [157]. FFF is basically a separation technique where semi-permeable membrane filter-based channel wall

Table 4 Characterization of Complex Injectable Products

| Sr. no | Characteristics | Characterization tools | Complex products | Ref |
|--------|-------------------------------------|---|--|-----------------|
| 1 | Size and shape | Dynamic light scattering Laser diffraction analysis Field-flow fractionation Electron microscopy Differential centrifugal sedimentation Nanoparticle tracking analysis Small-angle X-ray scattering | Liposomes, nanoparticles, microspheres, emulsions, suspensions | [126–132] |
| 2 | Surface charge and surface analysis | Zeta potential Tunable resistive pulse sensing Atomic force microscopy Brunauere Emmette Teller Auger electron spectroscopy | Liposomes, nanoparticles, suspensions, emulsions | [133–137] |
| 3 | Morphology and topography | Scanning electron microscopy Transmission electron microscopy | Liposomes, nanoparticles, microspheres, emulsions, suspension | [138, 139] |
| 4 | Material characterization | X-ray diffraction Raman spectroscopy Atom probe tomography | Drug substance, peptides, liposomes, nanoparticles, microspheres | [140–142] |
| 5 | Thermodynamic characterization | Differential scanning calorimetry Differential thermal analysis Thermal gravimetric analysis | Drug substance, liposomes, emulsions | [143–145] |
| 6 | Lamellarity analysis | ³¹ P nuclear magnetic resonance | Liposomes | [146] |
| 7 | Assay/excipient content analysis | High-performance liquid chromatography Thin layer chromatography | Drug substance, lipid-based product, polymer product | [147, 148] |
| 8 | In vitro drug release | USP dissolution apparatus type 2 USP dissolution apparatus type 4 Bottle rotating apparatus Dialysis Bag Franz Diffusion Cell, etc | Liposomes, nanoparticles, microspheres, emulsions, suspensions | [106, 149, 150] |

are used to determine the size distribution of particles [158]. Commercially available instrument for the FFF technique is Eclipse™, developed and marketed by Wyatt Technology.

Electron microscopy

Electron microscopy is used to visualize the micron/nano-size particles, which helps to study their morphological characteristics [159]. Regulatory agencies recommend evaluation of particles' external/internal structures like internal volume, porosity, and coating thickness. These characteristics can have a direct/indirect effect on various mechanisms like drug release, entrapment, stability, and the rest. Scanning electron microscopy and transmission electron microscopy (TEM) are commonly used techniques that facilitate understanding surface morphology and the internal structure of particles, respectively [160].

Various types of electron microscopes are commercially available from companies like Hitachi, Philips, Bruker, and a few others.

Atomic force microscopy

Atomic force microscopy (AFM), also called scanning force microscopy, is used for evaluating the surface topographic image of the particles. In this method, the sample surface interacts with the instrument's cantilever and probe (sharp tip) assembly to provide a three-dimensional high-resolution image of a sample [161]. This method can determine particle shape, size, surface texture, and other topographic information. Various types of AFM models are commercially available by manufacturers like Bruker, Nanosurf, and Zurich Instruments. Based on the requirements and features, appropriate model of the instrument can be used for analysis.

Nanoparticle tracking analysis

Nanoparticle tracking analysis (NTA) is a relatively new technique that can measure particle size (30–1000 nm) of both mono- and poly-dispersed systems [131]. NTA is based on both light scattering and Brownian movement principle. It additionally also uses the Stokes–Einstein equation to measure the particle's average hydrodynamic diameter [162,

[163]. Nanoparticle tracking analyzers are commercially marketed by Malvern Panalytical Ltd., Microtrac Retsch GmbH, and Horiba Scientific.

Small-angle X-ray scattering

Small-angle X-ray scattering (SAXS) is a flexible and non-invasive analytical technique used for structural characterization. It measures the intensities of the scattered X-ray beam by the sample as a function of the scattering angle. It can measure particles having sizes of 1–1000 nm [164]. SAXS is also used to determine the structure of a drug inside the carrier system if it is in precipitated form like Doxil® [105]. Commercially available models for SAXS analysis are provided by Rigaku, Bruker, Malvern Panalytical Ltd., etc. Users can select the appropriate model based on specific requirements and applications.

Surface charge

The surface charge of particle-based injectable products helps in maintaining the physical stability and interaction with the biological membranes. Therefore, surface charge measurement of these particles helps determine their physical, chemical, and biological stability and behavior.

Role of zeta potential

The degree and nature of the interaction between particles can be accessed by the zeta potential (ZP) value. Zeta or electrokinetic potential represents the potential at the shear plane, which directly impacts the stability of the drug product [165]. Usually, a system with a higher ZP value (positive or negative) is electrically stable, but a low ZP value leads to instability [166]. Various instruments based on the principle of zeta potential measurement (like zeta sizer, litesizer, etc.) are available commercially and used for characterization of particulate formulations like liposomes, nanoparticles, nanosuspension, and other formulations. Zetasizer (Malvern Panalytical Ltd.) is a commercially available instrument for the measurement of zeta potential through electrophoretic mobility.

Tunable resistive pulse sensing

Tunable resistive pulse sensing (TRPS), also called as scanning ion occlusion sensing method works on the “Coulter counter” principle [167]. In this method, particles are allowed to pass through pores of thin membranes, which causes blockage in the ionic current flow for a short time and leads to the generation of “resistive pulse.” These pulses are detected, and particle population is characterized accordingly. TRPS can be used to measure the physicochemical

properties of nanoparticles, like concentration, size, and surface charge. This technique can detect particles of size ranging from about 50 nm to a few micrometers. Commercially available instruments for TRPS analysis are provided by Izon Science Ltd.

Physicochemical properties

Various complex drug substances and excipients possess unique physicochemical properties, which are majorly responsible for showing desired response and overall performance of the complex product. There are different sophisticated tools/techniques which can evaluate their physicochemical properties.

X-ray diffraction

X-ray diffraction (XRD) is a non-destructive technique used to characterize crystalline materials. It provides valuable information regarding the arrangement of atoms in the crystal lattice, crystal defect, electronic configuration, and so on [168]. XRD can help understand various characteristics of solid powder (drug substance/excipients) like polymorphism, degree of crystallinity, amorphous character, and a few other properties. These can directly affect parameters like drug release, entrapment, and stability. Commercially, various models of XRD instrument are provided by manufacturers like Bruker, Rigaku, Malvern Panalytical Ltd., and Anton Paar. Based on the applications and requirements, the users can select the appropriate model.

Raman spectroscopy

Raman spectroscopy is a rapid non-destructive technique used to characterize drug formulations’ structural, electrical, and chemical properties [169]. It is based on shifting of the wavelength of the inelastically scattered radiation [170]. It can be used to evaluate the chemical composition and structure of the sample. This technique helps generate a two-dimensional chemical image of the sample and visualizes the distribution of drug and excipient in complex formulations. Commercially available models for Raman spectroscopic analysis are provided by manufacturers such as Anton Paar, Horiba, and Mettler Toledo.

Thermal characterization

Thermodynamic characterization is an essential study during the design and development of any dosage form since it provides vital information regarding the phase transition, which affects product stability.

Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a thermoanalytical technique that measures the heat difference required to raise the temperature of the sample compared to reference with the progress of temperature. DSC is used to measure the thermal behavior of complex excipients (primarily lipids, polymers). The phase transition event, i.e., melting and crystallization (physical transformation) of the sample, is measured as it undergoes exothermic or endothermic reaction [171]. DSC is also used to measure the glass transition temperature (T_g) of a bulk solution intended for lyophilization recipe development. Several models of DSC analyzers are commercially available by manufacturers such as Shimadzu, Mettler Toledo, Malvern Panalytical, Linseis, and PerkinElmer. Based on the user requirements and applications, appropriate models can be selected.

Differential thermal analysis

Differential thermal analysis (DTA) is also a thermoanalytical technique, where measurement is based on the temperature difference between sample and reference. DTA can provide information on various parameters like phase transition temperature, crystallinity, purity, oxidative, and thermal stability [172]. Commercially, DTA analyzers are provided by manufacturers like TA Instruments, Shimadzu, and Mettler Toledo.

Thermal gravimetric analysis

The thermal gravimetric analysis (TGA) technique is used to evaluate thermal stability of sample as a function of the change in its mass at a constant heating rate. TGA can help determine information on the physical phenomenon (i.e., phase transition, adsorption, absorption, desorption), chemical phenomenon (i.e., thermal decomposition, chemisorption), and solid-gas reaction (i.e., oxidation, reduction) [173]. Numerous models of TGA analyzers are commercially available by manufacturers such as Shimadzu, TA Instruments, and Mettler Toledo.

Lamellarity analysis

Lamellarity refers to the number of lipid bilayers present in the liposomes. It influences entrapment efficiency and drug release kinetics [174]. Lamellarity can be determined by various techniques like ^{31}P nuclear magnetic resonance (^{31}P NMR) and Cryo-TEM [175, 176].

Lipid/polymer content analysis

The analysis of specialized excipients like polymers, lipids, and other excipients is important to predict the stability of the finished product. Various techniques like HPLC, TLC, and gel permeation chromatography are used for detecting and quantifying levels of these excipients in finished products [177, 178].

In vitro drug release

The in vitro drug release profile of complex formulations is a prerequisite to predict the release pattern in vivo and helps establish the IVIVC model. Various methods and instruments are available in order to conduct in vitro release studies. Generally, USP dissolution apparatus II and IV are commonly used depending on the type of formulation; e.g., formulation of octreotide employs the use of USP dissolution apparatus IV. Additionally, bottle rotating apparatus, an unconventional method, is also used to determine the release pattern of liposomes, nanoparticles at various temperature, and pH conditions [179, 180].

Commercial equipment used in the manufacturing of complex injectable products

The manufacturing of complex injectable drug products is a complicated process which include puzzled manufacturing scale-up activities and regulatory requirements. Due to the rapidly growing research in the field of complex injectables, innovation in the field of equipment development and manufacturing is taking a good pace considering the robustness and reproducibility of the output. In this section, we have discussed a few commonly used equipment utilized by the pharmaceutical industries for the manufacturing of complex injectables drug products.

Tangential flow filtration assembly

Tangential flow filtration (TFF), also known as “cross-flow filtration,” is commonly used for concentrating and removing of salts from dissolved molecules such as proteins, nucleic acids, peptides, and other biomolecules such as carbohydrates. In this, the stream of feed passes parallel to the membrane where one portion passes through it (as permeate) while the remainder is recirculated back to the feed reservoir (as retentate) with the help of circulating pumps. TFF is also used for exchanging buffers and gross fractionation which is used in manufacturing of drug products such as liposomal formulation of Doxil[®] [181]. Commercially, various manufacturers like Pall corporation, Millipore, Sartorius,

and others provide TFF assemblies based on the industrial requirements.

High-pressure homogenizer

High-pressure homogenizer (HPH) has been used in pharmaceutical industries for the purpose of particle size reduction in micro- to nano-range along with stabilization and mixing of formulation components during the manufacturing of complex injectable products. It serves characteristic advantages as it is a scalable and versatile processing method and is being employed in the preparation of different drug delivery systems such as nanoparticles, nanoemulsions, nanosuspensions, nanostructured lipid carriers, and others [182].

HPH typically consists of a high-pressure pump and homogenization valve. The high-pressure pump is of positive displacement type which is also suitable for highly viscous fluids that are usually used in formulating complex injectables. The homogenization valve increases the velocity of the fluid creating a turbulence leading to breakdown of the component fluid. HPH belongs to a new generation of homogenizers wherein it can be operated at pressure levels of up to 400 MPa. Commercially, HPHs are provided by various manufacturers like Microfluidizer® Processors, Avestin Inc., GEA, and Stansted Fluid Power Ltd.

In-line high shear homogenizer

In-line high shear mixers/homogenizers are of particular importance during large-scale manufacturing of various products like microspheres, suspensions, emulsions, and similar formulations [183]. In-line homogenizers work on rotor–stator technology and the particle size depends on the homogenization speed. Commercially, Kinematica, IKA, Silverson, etc. provide various designs of high shear homogenizer machines suitable for a varied production requirements and batch volumes.

Lipid extruder

Extrusion is a technique where the liposomal suspension is passed under applied pressure through polycarbonate membranes [184]. The polycarbonate membranes are of a particular pore size designed to obtain unilamellar liposomes of homogenous size from a population of heterogeneous multilamellar liposomes. LIPEX® Flow by Evonik manufactures a range of liposome extruders meant for seamless scale-up of the unit operation process from research laboratory to commercial manufacturing.

Regulatory aspects and challenges

Injectables administer the active moiety directly into the systemic circulation or target tissue invading the primary defense layer using a hollow needle and a syringe. The entire procedure carries considerable risk to patients concerning sterility breach, particle contaminations, toxic/allergic reactions, etc. Considering these risks, regulatory agencies take utmost care in approving injectable products [185]. As discussed earlier, complex injectable products carry surplus toil for its development and manufacturing, which raises more concerns for their regulatory approval. Therefore, regulatory agencies expect additional studies during its development, manufacturing, and characterization to mitigate the associated risks and approve products of the intended quality [186]. Some commercially approved complex injectable products include liposomes, nanoparticles, peptides, microspheres, emulsions, suspensions, and a few others [187]. USFDA has taken specific initiatives to create guidelines for these complex products to make the regulatory filing process facile and streamlined. These guidelines give the manufacturers an overview of the approach in order to develop these products, specific tests, and exhaustive characterization involved in the product development and means of overcoming existing challenges and concerns.

The complex nature of liposomes due to their constituents, thermodynamic nature, and sterility involved is not unknown. Therefore, the USFDA has published a guideline for the development and manufacturing of liposomal drug products [188]. This guideline provides the expectations of USFDA on the data needed to be submitted for the approval of the liposomal-based product. Table 5 summarizes the requirement for approval of these products by the USFDA. Doxil® (1995), Daunoxome® (1996), Ambisome® (1997), Depocyt® (1999), Myocet® (2000), Mepact® (2004), Advate® (2009), Exparel® (2011), Marquibo® (2012), Lipodox® (2013), Onivyde® (2015), etc. are some of the marketed injectable liposomal products. The USFDA has also published PSGs to provide clarity to generic applicants for the development of these products. The PSGs provide the agency's recommendation on characterization studies and bioequivalence studies for the approval of these generic products [189]. A similar regulatory approach has been devised for injectable formulations like nanosuspensions and microspheres. A range of these products exhibit a plethora of applicability in parenteral administration. Various nanoparticle-based products like Abraxane® (paclitaxel nanoparticles) and Invega Sustenna® (paliperidone palmitate injectable suspension), and microsphere-based depot formulations like Risperdal Consta®, Sandostatin® LAR Depot, Vivitrol®, and Byetta® have already proven clinical and commercial benefits and thereby are of high interest for pharmaceutical manufacturers and regulatory agencies [187].

Table 5 USFDA guidelines for injectable liposome preparation

| A. Chemistry, manufacturing and control of the product: | |
|---|---|
| a Detailed description on product composition | API used and its quantity, lipid components, non-lipid components, buffer components, quantity of each lipid component used in the final formulation, etc |
| b Physicochemical properties of the drug product | Morphology, particle size, surface characteristics, net charge, viscosity, phase transition temperature of the product, etc |
| c Critical quality attributes | As per Q8 (R2) ICH guidelines |
| d Description of manufacturing process and process controls | |
| e Control of lipid components | |
| f Specification of drug product | |
| g Stability data | Physical, chemical, and microbiological |
| h Post approval changes in manufacturing | |
| B. Human pharmacokinetics: bioavailability and bioequivalence: | |
| a Study on clinical pharmacology | Pharmacokinetic and mass balance studies for liposomal drug products, comparison clinical pharmacology studies with non-liposomal drug product |
| b Biopharmaceutical study | Drug release pattern, IVIVC, use of validated bioanalytical method, protein-liposome interaction |
| C Labeling: | |
| a Non-proprietary name for the drug product as per FD&C act | |
| b Description section | |
| c Dose and route of administration | |

However, generic product development and filing are challenging tasks due to the high complexity involved in its manufacturing and bioequivalence studies. The stringent regulatory expectation and lack of proper guidance concerning development, manufacturing, and detailed dosage form/polymer characterization add more complications to the overall development. Additionally, there is a requirement for specific toxicological studies like mutagenicity and genotoxicity, which further complicates the filing process. Regulatory agencies like the USFDA and EMA are very keen on providing clear expectations on the development of complex injectables for fastening the approval process. Providing PSGs is one of the major initiatives taken by the USFDA, but that is only limited to the generic applicant, and many times the information provided is also not sufficient. Considering the issues faced by the applicants, there is an immediate need to devise an automated system that would help them resolve queries during the complicated process of product development and approval [190, 191].

Conclusions and future perspectives

Complex injectable products involve multi-unit operations, critical advanced equipment, and highly skilled trained human resources for the successful development and approval from the regulatory bodies. These products face obstacles right from the selection of API and excipients to

the tedious manufacturing processes involved. Moreover, the intricacies involved in their accurate characterization add to the developmental challenges of complex products. Therefore, multistep manufacturing processes, non-conventional quality attributes, and unique characterization tools/techniques enhance the complexities. Thus, tapping the sameness and bioequivalence to the RLD as per the regulatory requirements becomes very critical. Despite these obstacles, there have been many commercially successful products, which pave the way for using these technologies in filing innovator and generic products. The agencies do acknowledge the complexities involved in the formulation and encourage the development of these products. Regulatory agencies like the USFDA and EMA are creating a support database for filing and approval of such products. They are also trying to create more transparent and meaningful guidelines, organizing product workshops and training sessions to educate industry professionals with real-world evidence and regulatory expectations. Product-specific guidelines published by the USFDA help the generic applicants streamline their development and filing processes. It also gives them a provision to waive off in vivo bioequivalence studies based on sound in vitro experiments. In our opinion, complex injectable products have sparked the pharmaceutical industry with possibilities of improving drug delivery techniques with enhanced therapeutic outcomes and clinical benefits. Researchers are working across the globe to develop these types of products for effectively mitigating various life-threatening diseases.

From the commercial success of various complex injectables, it is very well understood that these drug products have the potential to improve the therapeutic outcomes along with significantly enhancing the patient compliance. Regulatory bodies are putting efforts to promote more generic product competition and emphasizing excessively on collaborating with the government, academia, and industry. In comparison to the pharmaceutical industries, academic research focuses more into the advanced drug delivery systems. In the future, such collaborative efforts by the regulatory bodies would help in translating the scientific knowledge from the academia to the industries resulting in the fruition of the products from the pipeline. Along with the product development, there is a requirement of rigorous research into the field of analytical, structural, and physicochemical characterization. Development of more reliable tools for characterization would help in better understanding of the quality attributes of the drug products, assisting the generic industries to match their drug product with the innovator. Further research in the field of *in silico* PK modeling and experimental *in vitro-in vivo* correlations is required which will help the researchers to predict the *in vivo* product performance resulting in designing more meaningful bioequivalence studies for the complex injectable formulations. Pharmaceutical industries have recognized the value of designing the drug products in the form of drug-device combinations such as pre-filled syringes because they provide the ease of administration along with the accurate dosage. However, this shift of trend towards drug-device combinations presents a challenge for the generic industries in designing the product. Therefore, there is a need for investments to build the facilities required for manufacturing the required tooling for drug-device combinations. Subsequently, the regulatory bodies will have to design additional guiding documents based on their requirements for ANDA approval.

In the coming decades, the USFDA may see a rise in the differentiated 505(b)(2) applications. 505(b)(2) products may serve the pharmaceutical industries with market exclusivity and intellectual property protection, but require investments to conduct clinical studies compared to the ANDA applications. ANDA and 505(b)(2) applications are highly encouraged as they help in increasing the generic competition, reduce costs of the treatment, and provide a better therapeutic option for the patients. Based on the current scenario, there lies a long way for the industries and regulators to harmonize the development, filing, and approval processes of these complex products. However, support from all stakeholders is equally needed in order to achieve the common goal of improving the overall quality of life and providing a holistic therapeutic approach to patients.

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