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RESEARCH ARTICLE
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A REVIEW ON PLGA-BASED NANOPARTICLE FOR ZICONOTIDE

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ABSTRACT: This article reviews the potential of poly (lactic-co-glycolic acid) (PLGA)-based nanoparticles for the delivery of ziconotide, a promising non-opioid peptide for neuropathic pain management. Ziconotide, derived from marine snail venom, is a selective N-type calcium channel blocker with significant analgesic potential but faces challenges like rapid degradation and the need for intrathecal administration due to poor blood-brain barrier permeability. PLGA offers a solution by enhancing drug stability, controlling release, and improving bioavailability. This review discusses formulation strategies, in vitro and in vivo studies, and future prospects for clinical application of PLGA-based ziconotide delivery systems.

KEYWORDS: Targeted drug delivery, Nanoparticle, Neuropathic pain, Ziconotide, Complication of ziconotide delivery, PLGA-peptide Drug delivery.

ABBREVIATIONS: NP: neuropathic pain, CaV: Voltage-gated calcium channel, PLGA: Poly (lactic-co-glycolic acid), DLX: Duloxetine, DCM: Dichloromethane, VASPI: Visual analogue scale of pain intensity, INDA: Investigational new drug application, NDA: New drug application, MA: Marketing authorization, GBP: Gabapentin, NCE: New chemical entity, PVA: Poly-vinyl alcohol.

1. Introduction:

Neuropathic pain is the pain caused by a lesion or a disease of the primary afferent neurons of the somatosensory nervous system. This definition of neuropathic pain was given by the neuropathic pain special interest group of the International Association for the Study of Pain [1]. It can also be said that NP (Neuropathic Pain) Is the pain triggered due to primary laceration or the dysfunction of the somatosensory system which is regulated for sensory information-like taste, touch, smell, etc. Neuropathic pain also known as neuralgia is a sudden pain or burst of pain sensed around the neuron affecting the quality of life and mental health [2]. Trigeminal neuralgia, painful polyneuropathy, postherpetic neuralgia, and pain following a stroke are a few instances of neuropathic pain. Clinically, neuropathic pain is characterized by increased pain responses in response to noxious or non-noxious stimuli, as well as spontaneous continuous or shooting pain [3]. Opioids topical anaesthetics antidepressants and antiepileptics are general medications given for NP and various techniques such are physical therapy spinal cord stimulation and surgeries. Neuropathic pain therapy is challenging but a combination approach world better than an individual regimen [4].

1.1 Overview of Ziconotide:

Currently, in the last phases of clinical testing, ziconotide is a neuroactive venom peptide being considered as a non-opioid therapy for severe chronic pain. It is the synthetic counterpart of ωconopeptide MVIIA, which is present in the venom of the marine piscivorous snail, Conus magus. Numerous studies and reviews have been conducted on ziconotide's chemical, biological, pharmacological, and therapeutic characteristics [5]. A synthetic 25-amino acid antagonist of the N-type calcium channel, ziconotide is administered intravenously and locally. Like other toxinderived peptide drugs (eptifibatide, lepirudin, bivalirudin, and ziconotide), its high molecular weight can be advantageous because it restricts the drug's distribution from the site of administration to other parts of the body [6].

1.2 Importance of N-type calcium channel blockers:

CaV channels play a major role in the transmission of pain. The N-type CaV channel (CaV2.2) is found in high concentrations in the central projections of primary sensory neurons that terminate in the dorsal horn of the spinal cord, where it is involved in the spinal processing of pain. Ziconotide selectively and reversibly binds to and blocks these channels without interacting with other ion channels or cholinergic, monoaminergic, or m- and d-opioid receptors. Ziconotide thus inhibits the spinal signalling of pain [7]. Transmission from primary nociceptive afferents by inhibiting the release of neurotransmitters and thus blocking signal transmission. Ziconotide also acts on calcium channels in the cerebral cortex, the neurohypophysis, and the spinal cord but not calcium channels in the neuromuscular junction. It shows no affinity for other ion channels or cholinergic, monoaminergic, mu, and kappa opioid receptors [8]. The classical calcium-blocking cardiovascular drugs, such as dihydropyridines, benzothiazepines, and phenyl alkylamines are potent blockers of L-type VSCCs but have essentially no effect on NVSCCs at therapeutic doses. By binding to N-type calcium channels, ziconotide appears to block nerve transmission from primary nociceptive afferents by inhibiting the release of neurotransmitters and thus blocking signal transmission. Ziconotide also acts on calcium channels in the cerebral cortex, the neurohypophysis, and the spinal cord [5].

1.3 Challenges in Ziconotide Delivery:

Since ziconotide has a limited capacity to cross the blood-brain barrier, patients must get intrathecally administered ziconotide to attain maximal analgesic efficaciousness with a decreased risk of major side effects. Ziconotide can quickly achieve its maximum local concentration when administered via the spinal route, which promotes a quick start of analgesia [9]. Ziconotide is a peptide, making it prone to degradation by proteolytic enzymes in the body, which can reduce its efficacy and require frequent dosing [8].

2. Poly (Lactic-co-glycolic acid) PLGA:

Novel Drug Delivery Systems, which seek to increase patient compliance, decrease side effects, and improve therapeutic efficacy, have caused a paradigm change in the pharmaceutical sciences. NDDs are revolutionizing the field of pharmacological therapy by providing previously unheardof possibilities for increased stability, targeted distribution, controlled release, and therapeutic efficacy [10]. Drug delivery is one of the most sophisticated uses of nanoparticles among their various applications. The success of drug delivery systems based on polymers and liposomes, many of which are currently being used in clinical settings, is largely to blame for this [12]. Polymerbased drug delivery and sustained drug-release systems are of high interest for both academic research and clinical applications. Many drug carriers based on polymer nanoparticles have been created over the past few decades, with an emphasis on the design and development of nanocarriers for therapeutic and diagnostic uses [11]. One of the challenges in creating successful treatments for neurodegenerative illnesses is getting drugs to the central nervous system. As a result, high doses must be administered, with increased risks of adverse side effects. Surmounting the innate inclination of the blood-brain barrier (BBB) to obstruct medication transport is a critical aspect of this undertaking [12].

2.1 Structure and Properties of PLGA:

Important physicochemical characteristics of biodegradable polymers include surface charge, hydrophobicity, molecular weight, crystallinity, biodegradability, co-polymer composition, and glass transition temperature [13]. The monomer ratio of glycolic acid and lactic acid mostly determines the physicochemical characteristics of PLGA [14]. Minimal systemic toxicity is linked to the use of PLGA for drug administration or biomaterial applications because these two monomers are endogenous and readily metabolized by the body through the Krebs cycle [15]. Lactic acid has less hydrophilicity compared to glycolic acid. Hence if the proportion of lactic acid increases the degradation rate of PLGA reduces and the reverse is the case, when the monomer units of glycolic acid increase the degradation of PLGA hastens. A 50:50 ratio of PLA and PGA can yield a polymer that has good biodegradability and the property of sustaining the drug release with good tensile strength, whereas high lactide content helps to sustain the re-release of the drug with bioerosion [14].

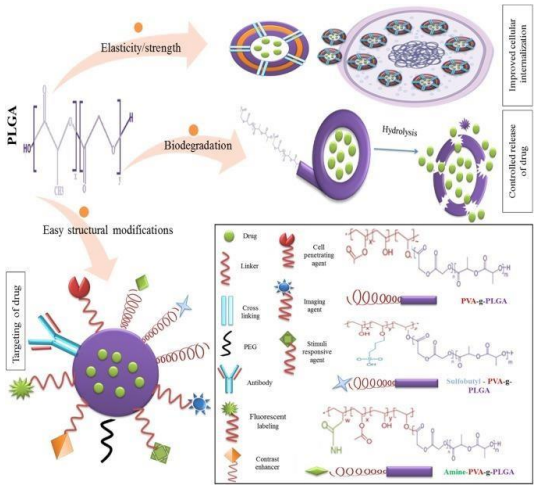

Figure 1. Show the role of the property of PLGA in Drug Delivery [13]

2.2 Biodegradability and Biocompatibility:

Lactic acid (LA) and glycolic acid (GA), which have strong biocompatibility and biodegradability, randomly polymerize to form PLGA [16]. The polymer breaks down most quickly when the ratio of the two monomers is 50:50. This is because PLGA50:50 facilitates water penetration into the polymer matrix due to its high hydrophilicity and lowest crystallinity. The biological milieu and the level of tolerability around particular drug-polymer-tissue interactions determine a material's biocompatibility, which is not an intrinsic quality of the material [17].

2.3 Application in Drug Delivery:

Because of their short half-lives in circulation, peptides and proteins may also require parenteral formulations for drug delivery to prevent degradation in the gastrointestinal tract and first-pass metabolism. This will also decrease the frequency of dosing. Injectable biodegradable and biocompatible PLGA particles could be used for controlled-release dose forms to circumvent the uncomfortable surgical insertion of big implants [13]. When compared to conventional medications, particle-based therapies have several advantages, including the ability to target certain regions, adjust release rates, and get past biological barriers while administering hydrophobic medications. To extend the encapsulated medications' half-life, polymeric particles protect the pharmaceuticals from enzymatic reactions [18]. With great affinity and specificity, PLGA nanoparticles or microspheres are utilized to target malignant tumours when coupled with bio-targeting ligands such as hormones, cytokines, vaccines, and chemotherapeutic drugs [19].

3. Ziconotide and its therapeutic potential:

It has been found by researchers that ω-MVIIA binds with great affinity and reversibility to a subset of voltage-sensitive calcium channels known as N-type channels. In contrast, ω-MVIIA has no effect

on other types of calcium channels that are sensitive to voltage [20]. Though expressed in many different parts of the brain, N-type calcium channels—which serve as binding sites for ziconotide or conopeptide—are mostly found in the spinal cord's superficial dorsal horn. This ziconotide binding zone is located on the Rexed laminae I and II, which are the areas on spinal pain transmission neurons where nociceptive primary afferents synapse [21]. N-type voltagesensitive calcium channels are selectively and reversibly blocked by ziconotide, as demonstrated in animal studies. This reduces the release of neurotransmitters from nociceptive afferents that terminate in the dorsal horn of the spinal cord [22], including glutamate and neuropeptides [20]. To determine the ultimate pain response and provide an affective and emotional context for nociception, millions of peripheral impulses arrive in the dorsal horn of the spinal cord, where they are filtered and then regulated [20]. Ziconotide blocks a wide range of neurotransmitters, such as glutamate, GABA, calcitonin, gene-related peptide (CGRP), substance P, and norepinephrine, that are released from brain slices and synaptosomes either electrically or by K+ depolarization. Not only does ziconotide impede the release of neurotransmitters through a presynaptic mechanism, but it also modifies the excitability of neurons by inhibiting calcium channels on cell bodies, dendritic shafts, and spines [23]. Based on a combination of these findings, the theory proposed that analgesia would result from the selective antagonistic action of N-type calcium channels in the spinal cord, which would prevent synaptic transmission between nociceptive sensory neurons and dorsal horn neurons in the cord [21].

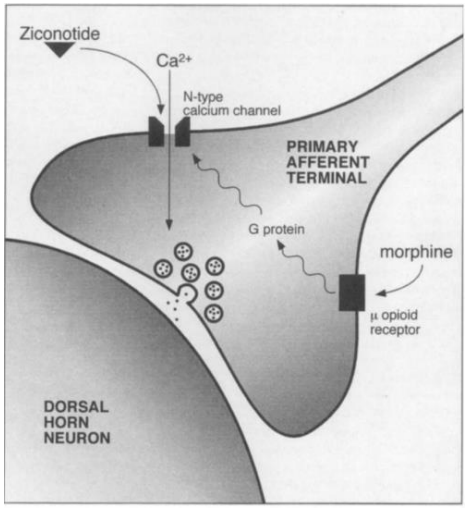

Figure 2 Shows the binding of ziconotide at the N-type Calcium Channel at the Primary afferent terminal [21].

3.1 Side effects of Ziconotide:

Since significantly smaller (mg) doses of the peptides are administered Intracranioventricularly (i.c.v.), it is highly likely that the trembling behaviour is caused by the peptide's central action [24]. Intrathecal ziconotide produced serious supraspinal and systemic adverse effects. Many symptoms were cerebellar in origin, including nystagmus and dysmetria, although the brain stem, cortical, and other systems were also affected (Table 1) [25]. The principal drug effect found was orthostatic hypotension [26], dizziness, nystagmus, nausea, postural hypotension, somnolence, confusion, fever, headache, and urinary retention [8].

Table 1: Adverse effects associated with Ziconotide [25]

System affected	Clinical findings
Cardiac	Bradycardia, orthostatic hypotension [27]
CNS-Brain stem	Nausea, vomiting, ataxia [28]
CNS-cerebellar	Nystagmus, dysmetria [29]
CNS-cortical	Agitations, hallucinations [30, 31]

Dermatologic	Rash **[31]**
Endocrine	Hypoglycaemia [25]
GI	Diarrhea [32]
Head and neck	Nasal congestion [33]
Hepatic	LFT elevation [25]
Urologic	Urinary retention [34]

4. PLGA as a delivery system for Ziconotide:

Protein and peptide medications frequently have brief half-lives in vivo, necessitating repeated injections to keep the drug at a therapeutic level [26]. Peptides and proteins that are placed into PLGA particles are shielded from proteolytic breakdown and given the appropriate plasma halflife **[35]**.

4.1 PLGA Nanoparticle and Microspheres:

PLGA-based Nanoparticle for the class of drugs: voltage-gated calcium channel blockers [Pregabalin and Gabapentin].

Preparation of Pregabalin NP using Nanoprecipitation method: Using the nanoprecipitation technique, pregabalin-containing nanoparticles with different drug-to-polymer ratios were created. To improve homogeneity, the drug was dissolved in water and combined with a cosolvent. Next, a polymer (Tween80, Poloxamer188, span20) dissolved in chloroform was added. Solvents were evaporated after mixing with ethanol, and nanoparticles were separated by centrifugation, washing, and drying [36].

Gabapentin NP by Spray Drying: GBP-loaded nanoparticles were produced by spray drying using the (BÜCHI Labortechnik) AG, Switzerland Nano Spray Dryer B-90. Using spray drying technology is one of the most fortunate ways to prepare NPs. This specifically created method is crucial for producing large quantities of powdered pharmaceutical formulations with excellent stability, including those that are thermosensitive [37].

Albumin Nanoparticle for Gabapentin: Through the process of pH coacervation, albumin was dissolved in a sodium chloride solution, ethanol was added, and glutaraldehyde was cross-linked to create albumin nanoparticles of gabapentin. The resultant nanoparticles were cryoprotectantfreeze-dried using glucose [38].

Other PLGA Formulations:

DLX encapsulated-PLGA by Double emulsion: Using a PLGA copolymer and dimethyl sulfoxide (DMSO), DLX nanoparticles (NPs) were created via a double emulsion process. Sonication and magnetic stirring were then used to form and harden the nanoparticles. For later usage, the NPs were then collected by centrifugation, cleaned, and freeze-dried [39].

4.2 Encapsulation techniques:

Single emulsion:

In this procedure, the drug is added to a single-phase solution made of the polymer dissolved in an organic solvent (such as DCM, acetone, or ethyl acetate) to produce a dispersion. This combination is emulsified at the proper temperature (o/w-25-40^{o}C, w/o- 40-60ºC) while being stirred in water that contains PVA as an emulsifier. After that, the organic solvent is either extracted by putting the emulsion in water or it is evaporated under low pressure [40].

Double emulsion:

Unlike the o/w approach, which works best for water-insoluble medications like steroids water-inoil-in-water (w/o/w) method is best suited to encapsulated water-soluble pharmaceuticals like peptides, proteins, and vaccines [41].

Phase separation:
In this procedure, a third component is added to the polymer solution in an organic solution, therefore lowering the solubility of the encapsulating polymer. The procedure eventually produces two liquid phases (phase separation)- the supernatant phase with reduced polymer content and the polymer-containing coacervate phase. mostly used to encapsulate medications that dissolve in water and substances like peptides, etc [40,41].

Spray Congealing and drying method:
After being scattered in a polymer solution, core particles are sprayed into a heated chamber. When the solvent evaporates, the shell material adheres to the core particles, forming polynuclear or matrix-type microcapsules [42].

Fluidized bed coating:
When applying fluidized bed coating, the coating material is sprayed into a heated fluidized bed containing a medication of interest. Controlling the size distribution and porosity is possible with this cost-effective method. This approach is unable to encapsulate thermally labile molecules [43].

Extrusion:
The process of encapsulation by extrusion entails dispersing the core material within a mass of molten carbohydrates. This combination is sent through a die into a liquid that is drying out, hardening the coating, and encasing the core substance [44].

Pan coating:
The coating solution is applied as an atomized spray to the solid core material in the coating pan. To remove the coating solvent warm air is passed over the coated material [42].

Freeze Drying:
Freeze-drying of an emulsion solution composed of the coating material and the drug of interest is carried out. An emulsion solution containing the target medication and the coating material is freeze-dried. This technique works well with medications that are unstable in water and those that are sensitive to temperature changes [43].

4.3 Release kinetics: Dissolution profiles
In vitro drug release: Using a drug-loaded nanosuspension in simulated tear fluid (pH 7.4) at 37°C and mechanical shaking, an in vitro drug release investigation was carried out. At predetermined intervals, samples were taken, fresh fluid was added, and UV spectrophotometry was used to examine the results at 290 nm. For release performance, the created nanosuspension was contrasted with a commercial formulation [45].

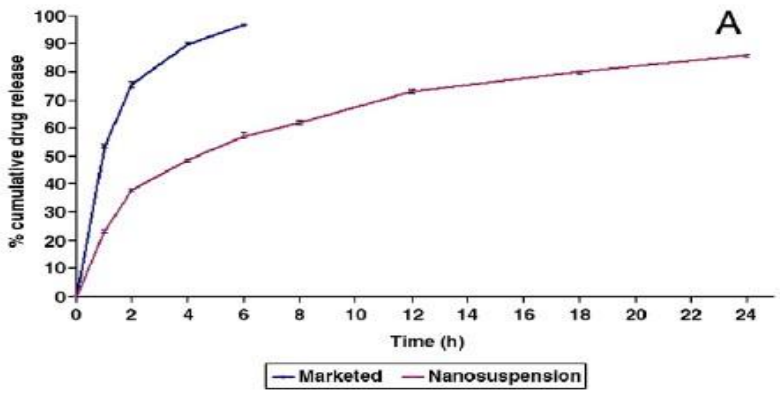

Figure 3 In vitro drug release profile of optimized sparfloxacin nanosuspension and marketed formulation [45].

Drug-release behaviour of microparticles (Effect of type of solvent): Drug dissolution characteristics at pH 6.8 are shown in Figs. 6 and 7, demonstrating bi-phasic release with a 72hour continuous release after an initial burst. Although pore formation, drug solubility, and polymer characteristics can cause this to change to a bi-phasic profile, PLGA microparticles normally show tri-phasic release. These variables have a major impact on the dynamics of drug release [46].

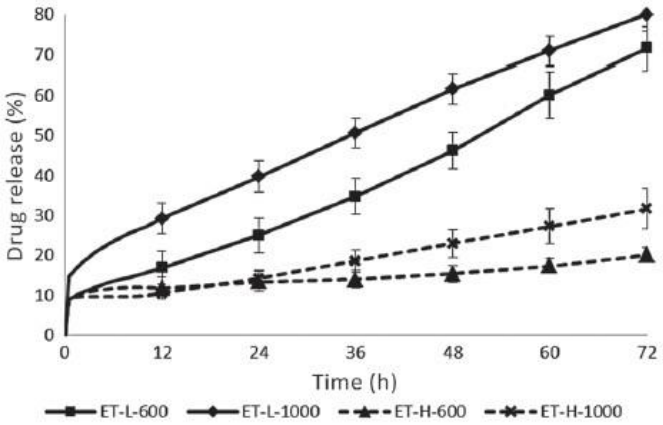

Figure 4 In vitro dissolution profiles of ibuprofen from PLGA samples prepared with dichloromethane as organic solvent [46].

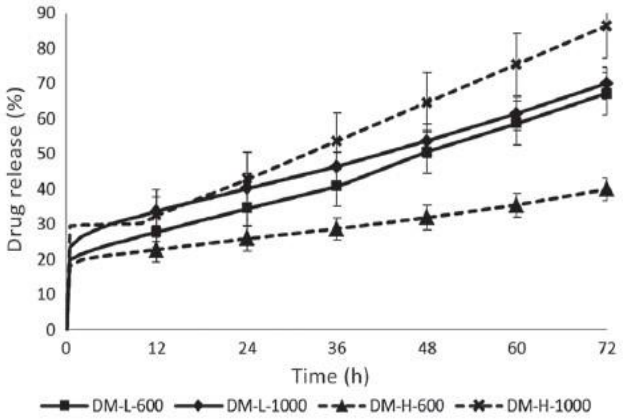

Figure 5 In vitro dissolution profiles of ibuprofen from PLGA samples prepared with ethyl acetate as organic solvent

[46].

Various Ratio of lactic acid to glycolic acid: The various ration of lactic acid to glycolic acid i.e. 50:50, 65:35, 75:25, and 85:15 polylactic-co-glycolic acid release patterns were modelled in vivo. PLGA with the notation 65:35 indicates that 35% of the copolymer is glycolic acid and 65% is lactic acid. It has been noted that the drug releases in two phases, the first having a zero-release duration and the second having a quick release. Additionally, the profiles indicate that the ratio of lactide to glycoside decreases as release rate increases [47].

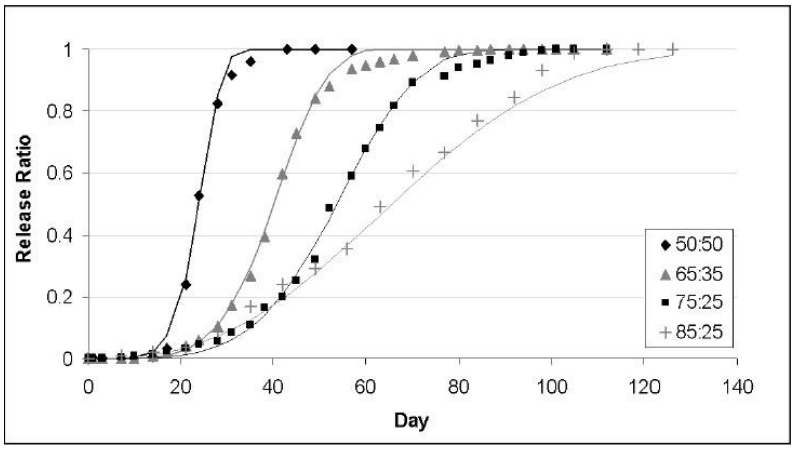

Figure 6 A biphasic curve for drug release as a result of PLGA biodegradation [47].

5. Formulation Stability and storage considerations:

Stability: The excellent performance of PLGA nanoparticles as a nano-carrier system is demonstrated by their wide range of degradation rates, which provide a desirable formulation opportunity, stability in long-term storage, and high encapsulation efficiency [48]. The C-PNPs in this study exhibit a negative zeta potential that is sufficiently high to guarantee that the nanoparticles will disperse readily in aqueous media and that the nanosuspensions will exhibit excellent stability and tolerance to aggregation [49]. After three months of storage in accelerated stability conditions, nanoparticles did not undergo any alterations in their characteristics [50].

Storage: It has been discovered that nanocapsules may become unstable during storage due to cryoprotectant crystallization as a result of a high residual water content [51]. After being stored for three months at 4 °C, the behaviour of the NPs in all formulations under investigation did not alter, according to the storage stability research [52]. The observed shelf life, under all temperature and humidity conditions, was approximately 12.5 months [53].

6. In vitro characterization:

6.1 Particle size and morphology:

Particle size: The particle size and morphology of Drug loaded nanoparticle using Scanning electron microscope (SEM). Using scanning electron microscopy, the appearance of the SLN dispersion loaded with pregabalin was investigated. Figure displayed the SEM picture. The particle size, as shown by the SLNdispersion, is 500 nm, and its surface is rather smooth [34]. The spray dried NPs variants were characterized for their sizes which ranged from 70 ± 9 nm to 555 ± 15 nm **[54].**

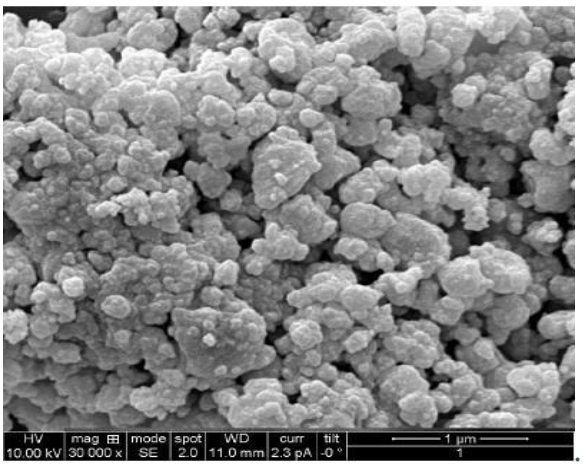

Figure 7Image of Gabapentin loaded Nanoparticle by SEM [38]

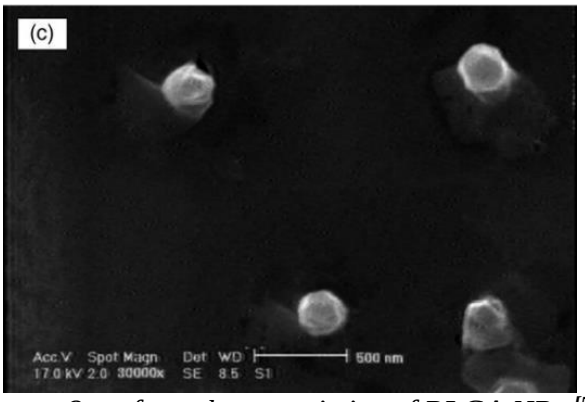

Figure 8 surface characteristics of PLGA NPs [55]

6.2 Drug loading and encapsulation efficiency:

Entrapment efficiency (%) = Total amount of drug added – amount of unentrapped drug ×100 **[54]**

Total amount of drug added

Drug loading (%) = Total amount of drug added – amount of unentrapped drug ×100 **[54]**

Total weight of nanoparticles

Uncoated PLGA NPs have EE %= 87.3% and drug loading % = 7.7% **[56]**. A high EE of over 95% (w/w) and up to 100% (w/w) was previously observed using single-step nano spray drying technique **[54]**.

The best entrapment efficiency for pregabalin surface-loaded nanoparticles was of formulation code F6 made of Poloxamer188, with an efficiency of 84.8±0.51 **[38]**.

6.3 Cytotoxicity studies:

Ziconotide is in the clinical trial phase and there isn't information determining the cytotoxicity of the drug. And PLGA is safe and has no cytotoxicity so it is termed as GRAS- Generally recognized as safe.

7. In vivo studies:

Pharmacokinetics: In the cerebral spinal fluid (CSF), Ziconotide is 100% bioavailable **[57]**. After eight hours, ziconotide plasma levels peaked and stayed relatively constant for the next eight to forty-eight hours of infusion **[58]**. Ziconotide's linear kinetics and 4.5-hour half-life have been clearly established in vivo experiments including both humans and animals **[59]**. Peptidases in serum and in organ tissues have been shown to degrade ziconotide **[60]**. The clearance median values, which ranged from 0.22 to 0.343 ml/minute, tended to be more constant **[61,62]**.

Pharmacodynamics and clinical studies: Because ziconotide binds directly to the calcium channel and not the G protein-coupled receptor, animal studies predicted that patients will not build a tolerance to the analgesic action of ziconotide during the human study **[63]**. The degree of pain relief was less than that reported in the two previous controlled trials of ziconotide when using the slow dose titration regimen, which started at 0.1 mg/hour (2.4 mg/day) and increased to a mean dose of 0.29 mg/hour (6.96 mg/day) over three weeks **[64]**. Wallace et al. studied ziconotide in a high-dose, fast-titration research. The beginning dose was 9.6 mcg/day, and the dosage was increased to 168 mcg/day before being lowered to a maximum of 57.6 mcg/day. the mean VASPI score improvement was 31.6% in 124 patients with neuropathic pain **[65]**.

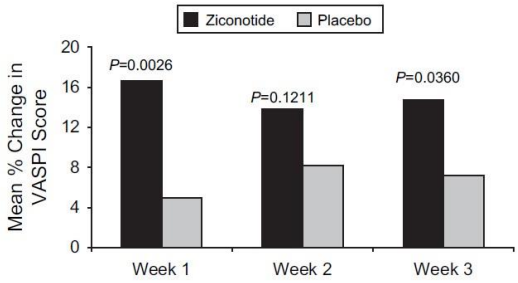

Figure 9 Ziconotide effectively reduced pain after 3 weeks of treatment with the onset of significant efficacy observed at Week 1

[64]

8. Regulatory considerations:

The novel product must abide by the rules established for biological pharmaceutical products and for new chemical entities (NCEs) when it comes to biological entities like proteins, peptides, or antibodies **[66,67]**. In actuality, NPs properties are easily changed by tiny adjustments made to manufacturing procedures and raw material composition which can influence biological prop and biodistribution patterns **[68]**. Nanomedicines have been found to interact with immune cells and adsorb plasma proteins, contingent on their size range and physicochemical features. Biocompatibility and immunotoxicity must therefore be taken into account during the preclinical evaluation **[69,70]**. The competitive nanomedicine-related drug market approval is influenced by the pharmaceutical regulatory environment, health care strategies, demographics, and broader economic conditions **[71]**. The increased attention being paid to nanosimilars, which blend generic medications with novel excipients like nanocarriers, is another problem in the ongoing regulatory Discussions **[72]**.

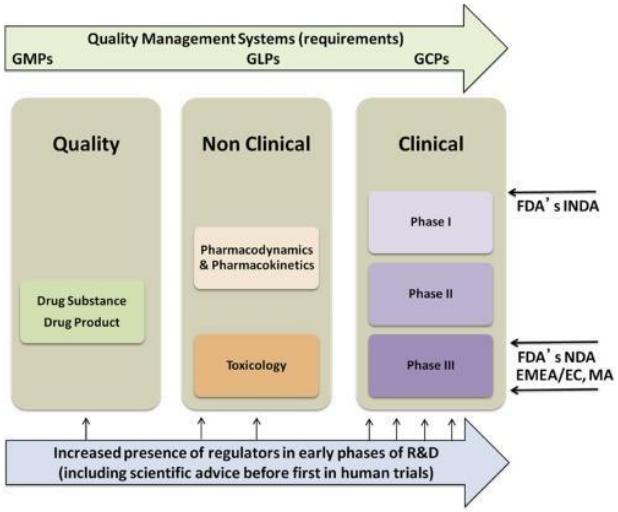

Figure 10 Overview of regulatory presence on the research and development life cycle of any medicinal product **[71]**

9. Future Prospects:

NDDS allows for targeted, controlled drug release with fewer side effects, they have a great deal of potential in medicine in the future. And precision in the treatment of diseases like cancer and neurodegenerative disorders. The use of nanomedicine will result in new treatment and diagnosis due to our expanding knowledge of molecular diseases or a similar identification for markers with a nanomaterial subcellular scale **[73]**. As drug carriers, nanoparticles increase bioavailability, regulate drug release, prolong the duration of circulation, and reduce harmful effects on cells other than cancerous ones. Because of their higher bioavailability, they may be able to be used at lower dosages, which could reduce adverse effects in patients and have positive economic effects **[74]**. Collaborative efforts could leverage the potential of nanoparticulate systems to enhance the therapeutic index of

non-toxic natural compounds (NCEs) [75]. A lot of research is being done to find new ways to deliver drugs to herbal medicines that would not be possible with the conventional molecular delivery system. These ways would include increased bioavailability, decreased toxicity, sustained release action, and GI safety [74].

10. Conclusion:

In conclusion, PLGA-based nanoparticle delivery systems for ziconotide represent a promising approach to overcoming challenges associated with conventional drug delivery. By improving bioavailability, stability, and targeted delivery, these systems could enhance therapeutic outcomes for treating chronic neuropathic pain, while minimizing side effects. Future research should view on refining these systems to ensure more efficient clinical translation and regulatory approval.

11. References:

1. Shinu P, Morsy MA, Nair AB, Mouslem AK, Venugopala KN, Goyal M, Bansal M, Jacob S, Deb PK. Novel therapies for the treatment of neuropathic pain: potential and pitfalls. Journal of Clinical Medicine. 2022 May 26;11(11):3002.
2. Choudhary S, Kaur R, Waziri A, Garg A, Kadian R, Alam MS. N-type calcium channel blockers: a new approach towards the treatment of chronic neuropathic pain. Exploration of Medicine. 2023 Feb 28;4(1):85-106.
3. Baron R, Binder A, Wasner G. Neuropathic pain: diagnosis, pathophysiological mechanisms, and treatment. The Lancet Neurology. 2010 Aug 1;9(8):807-19.
4. Barnes MP, Good DC. ERIC KERSTMAN, SANGMIN AHN, SREE BATTU, SHABREZ TARIQ, AND MARTIN GRABOIS. Neurological Rehabilitation. 2013 Jan 10:175.
5. Miljanich GP. Ziconotide: neuronal calcium channel blocker for treating severe chronic pain. Current medicinal chemistry. 2004 Dec 1;11(23):3029-40.
6. Stepensky D. Pharmacokinetics of toxin-derived peptide drugs. Toxins. 2018 Nov 20;10(11):483.
7. Werbitzky O, Giraud M. Manufacturing of venom-derived therapeutic peptides..
8. Williams JA, Day M, Heavner JE. Ziconotide: an update and review. Expert opinion on pharmacotherapy. 2008 Jun 1;9(9):1575-83.McGivern, J. G. (2007). Ziconotide: a review of its pharmacology and use in the treatment of pain. *Neuropsychiatric Disease and Treatment*, *3*(1), 69–85.
9. McGivern, J. G. (2007). Ziconotide: a review of its pharmacology and use in the treatment of pain. *Neuropsychiatric Disease and Treatment*, *3*(1), 69–85.
10. Khadam VK, Singh RP, Prajapati D, Yunus M, Prajapati B, Rai SK, Kumari P, Gogoi D. An Updated Comprehensive Review on Novel Drug Delivery Systems (NDDS) In the Pharmaceuticals. Asian Journal of Pharmaceutical Research and Development. 2024 Feb 15;12(1):55-64.
11. Kučuk N, Primožič M, Knez Ž, Leitgeb M. Sustainable biodegradable biopolymerbased nanoparticles for healthcare applications. International journal of molecular sciences. 2023 Feb 6;24(4):3188.
12. Murthy, S. K. (2007). Nanoparticles in modern medicine: State of the art and future challenges. *International Journal of Nanomedicine*, *2*(2), 129–141.
13. Mir M, Ahmed N, ur Rehman A. Recent applications of PLGA based nanostructures in drug delivery. Colloids and Surfaces B: Biointerfaces. 2017 Nov 1;159:217-31.
14. Surya N, Bhattacharyya S. PLGA–the smart polymer for drug delivery. Pharmacy & Pharmacology. 2021 Oct 15;9(5):334-45.
15. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. Journal of controlled release. 2012 Jul 20;161(2):505-22.
16. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers. 2011 Aug 26;3(3):1377-97.
17. Anderson JM, Shive MS. Biodegradation and biocompatibility of PLA and PLGA microspheres. Advanced drug delivery reviews. 1997 Oct 13;28(1):5-24.

18. Liu G, McEnnis K. Glass Transition Temperature of PLGA Particles and the Influence on Drug Delivery Applications. *Polymers*. 2022; 14(5):993
19. Kapoor, D. N., Bhatia, A., Kaur, R., Sharma, R., Kaur, G., & Dhawan, S. (2015). PLGA: a Unique Polymer for Drug Delivery. *Therapeutic Delivery*, *6*(1), 41–58.
20. Schmidtko A, Lötsch J, Freynhagen R, Geisslinger G. Ziconotide for treatment of severe chronic pain. The Lancet. 2010 May 1;375(9725):1569-77.
21. Mathur, V. S. (2000). *Ziconotide: A new pharmacological class of drug for the management of pain. Seminars in Anesthesia, Perioperative Medicine and Pain, 19(2), 67–75.*
22. Rauck, R. L., Wallace, M. S., Burton, A. W., Kapural, L., & North, J. M. (2009). *Intrathecal Ziconotide for Neuropathic Pain: A Review. Pain Practice, 9(5), 327–337.*
23. Wang, Y.-X., & Bowersox, S. S. (2006). *Analgesic Properties of Ziconotide, a Selective Blocker of N-Type Neuronal Calcium Channels. CNS Drug Reviews, 6(1), 1–20.*
24. Newcomb R, Abbruscato TJ, Singh T, Nadasdi L, Davis TP, Miljanich G. Bioavailability of Ziconotide in brain: influx from blood, stability, and diffusion. Peptides. 2000 Apr 1;21(4):491-501.
25. Penn RD, Paice JA. Adverse effects associated with the intrathecal administration of ziconotide. Pain. 2000 Mar 1;85(1):291-6.
26. Wu C, Mu H. Lipid and PLGA microparticles for sustained delivery of protein and peptide drugs. Pharmaceutical nanotechnology. 2020 Feb 1;8(1):22-32.
27. McGuire D, Bowersox S, Fellmann JD, Luther RR. Sympatholysis after neuronspecific, N-type, voltage-sensitive calcium channel blockade: first demonstration of Nchannel function in humans. Journal of cardiovascular pharmacology. 1997 Sep 1;30(3):400-3.
28. Bäckryd E. Do the potential benefits outweigh the risks? An update on the use of ziconotide in clinical practice. European Journal of Pain. 2018 Aug;22(7):1193-202.
29. Wallace MS, Rauck R, Fisher R, Charapata SG, Ellis D, Dissanayake S, Ziconotide 98022 Study Group. Intrathecal ziconotide for severe chronic pain: safety and tolerability results of an open-label, long-term trial. Anesthesia & Analgesia. 2008 Feb 1;106(2):628-37.
30. Nair A, Poornachand A, Kodisharapu P. Ziconotide: Indications, adverse effects, and limitations in managing refractory chronic pain. Indian journal of palliative care. 2018;24(1).
31. Jain KK. An evaluation of intrathecal ziconotide for the treatment of chronic pain. Expert Opinion on Investigational Drugs. 2000 Oct 1;9(10):2403-10.
32. Rauck RL, Wallace MS, Burton AW, Kapural L, North JM. Intrathecal ziconotide for neuropathic pain: a review. Pain Practice. 2009 Sep;9(5):327-37.
33. Wermeling DP. Ziconotide, an intrathecally administered N-type calcium channel antagonist for the treatment of chronic pain. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 2005 Aug;25(8):1084-94.
34. Brinzeu, A., Berthiller, J., Caillet, J.-B., Staquet, H., & Mertens, P. (2019). *Ziconotide for spinal cord injury related pain. European Journal of Pain.*
35. Allahyari M, Mohit E. Peptide/protein vaccine delivery system based on PLGA particles. Human vaccines & immunotherapeutics. 2016 Mar 3;12(3):806-28.
36. Ramchandar T, Rajkumar D. Design Prepare and characterization of Pregabalin loaded solid lipid Nanoparticles. Frontier Journal of Pharmaceutical Sciences and Research. 2023 Dec 27;6(3):37-41.
37. Salama AH, Salama AA, Elhabak M. Single step nanospray drying preparation technique of gabapentin-loaded nanoparticles-mediated brain delivery for effective treatment of PTZ-induced seizures. International Journal of Pharmaceutics. 2021 Jun 1;602:120604.
38. Wilson B, Lavanya Y, Priyadarshini SR, Ramasamy M, Jenita JL. Albumin nanoparticles for the delivery of gabapentin: preparation, characterization and pharmacodynamic studies. International journal of pharmaceutics. 2014 Oct 1;473(12):73-9.
39. Kim SI, Shin J, Tran Q, Park H, Kwon HH, Shin N, Hwang JA, Shin HJ, Lee J, Lee WH, Lee SY. Application of PLGA nanoparticles to enhance the action of duloxetine on microglia in neuropathic pain. Biomaterials Science. 2021;9(18):6295-307.

40. Khan I, Gothwal A, Sharma AK, Kesharwani P, Gupta L, Iyer AK, Gupta U. PLGA nanoparticles and their versatile role in anticancer drug delivery. Critical Reviews™ in Therapeutic Drug Carrier Systems. 2016;33(2).
41. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly (lactide-co-glycolide)(PLGA) devices. Biomaterials. 2000 Dec 1;21(23):2475-90.
42. Jyothi NV, Prasanna PM, Sakarkar SN, Prabha KS, Ramaiah PS, Srawan GY. Microencapsulation techniques, factors influencing encapsulation efficiency. Journal of microencapsulation. 2010 May 1;27(3):187-97.
43. Sasidharan S, Saudagar P. Encapsulation and delivery of antiparasitic drugs: a review. Encapsulation of Active Molecules and Their Delivery System. 2020 Jan 1:323-42.
44. Risch SJ. Encapsulation: overview of uses and techniques.
45. Gupta H, Aqil M, Khar RK, Ali A, Bhatnagar A, Mittal G. Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery. Nanomedicine: nanotechnology, biology and medicine. 2010 Apr 1;6(2):324-33.
46. Vysloužil J, Doležel P, Kejdušová M, Mašková E, Mašek J, Lukáč R, Košťál V, Vetchý D, Dvořáčková K. The influence of different formulations and process parameters during the preparation of drug-loaded PLGA microspheres evaluated by multivariate data analysis. Acta Pharmaceutica. 2014 Dec 31;64(4):403-17.
47. Makadia HK, Siegel SJ. Poly lactic-co-glycolic acid (PLGA) as biodegradable controlled drug delivery carrier. Polymers. 2011 Aug 26;3(3):1377-97.
48. Chatterjee M, Chanda N. Formulation of PLGA nano-carriers: specialized modification for cancer therapeutic applications. Materials Advances. 2022;3(2):837-58.
49. Akl MA, Kartal-Hodzic A, Oksanen T, Ismael HR, Afouna MM, Yliperttula M, Samy AM, Viitala T. Factorial design formulation optimization and in vitro characterization of curcumin-loaded PLGA nanoparticles for colon delivery. Journal of Drug Delivery Science and Technology. 2016 Apr 1;32:10-20.
50. Jain S, Mittal A, K Jain A, R Mahajan R, Singh D. Cyclosporin A loaded PLGA nanoparticle: preparation, optimization, in-vitro characterization and stability studies. Current Nanoscience. 2010 Aug 1;6(4):422-31.
51. Abdelwahed W, Degobert G, Stainmesse S, Fessi H. Freeze-drying of nanoparticles: formulation, process and storage considerations. Advanced drug delivery reviews. 2006 Dec 30;58(15):1688-713.
52. Holzer M, Vogel V, Mäntele W, Schwartz D, Haase W, Langer K. Physico-chemical characterisation of PLGA nanoparticles after freeze-drying and storage. European Journal of Pharmaceutics and Biopharmaceutics. 2009 Jun 1;72(2):428-37.
53. Mittal P, Vardhan H, Ajmal G, Bonde GV, Kapoor R, Mittal A, Mishra B. Formulation, optimization, hemocompatibility and pharmacokinetic evaluation of PLGA nanoparticles containing paclitaxel. Drug development and industrial pharmacy. 2019 Mar 4;45(3):365-78.
54. Rai G, Gauba P, Dang S. Surface modified biodegradable nanoparticles of Gabapentin. An approach to increase cell uptake. Materials Today: Proceedings. 2023 Apr 25.
55. Afshari M, Derakhshandeh K, Hosseinzadeh L. Characterisation, cytotoxicity and apoptosis studies of methotrexate-loaded PLGA and PLGA-PEG nanoparticles. Journal of microencapsulation. 2014 May 1;31(3):239-45.
56. Salama AH, Salama AA, Elhabak M. Single step nanospray drying preparation technique of gabapentin-loaded nanoparticles-mediated brain delivery for effective treatment of PTZ-induced seizures. International Journal of Pharmaceutics. 2021 Jun 1;602:120604.
57. Prommer EE. Ziconotide: can we use it in palliative care?. American Journal of Hospice and Palliative Medicine®. 2005 Sep;22(5):369-74.
58. Yaksh TL, de Kater A, Dean R, Best BM, Miljanich GP. Pharmacokinetic analysis of ziconotide (SNX-111), an intrathecal N-type calcium channel blocking analgesic, delivered by bolus and infusion in the dog. Neuromodulation: Technology at the Neural Interface. 2012 Dec 1;15(6):508-19.

59. Pope JE, Deer TR. Intrathecal pharmacology update: novel dosing strategy for intrathecal monotherapy ziconotide on efficacy and sustainability. Neuromodulation: Technology at the Neural Interface. 2015 Jul 1;18(5):414-20.
60. Newcomb R, Abbruscato TJ, Singh T, Nadasdi L, Davis TP, Miljanich G. Bioavailability of Ziconotide in brain: influx from blood, stability, and diffusion. Peptides. 2000 Apr 1;21(4):491-501.
61. Wermeling, D. P. (2005). *Ziconotide, an Intrathecally Administered N-Type Calcium Channel Antagonist for the Treatment of Chronic Pain. Pharmacotherapy, 25(8), 1084– 1094.*
62. Klotz U. Ziconotide-a novel neuron-specific calcium channel blocker for the intrathecal treatment of severe chronic pain-a short review. International Journal of Clinical Pharmacology & Therapeutics. 2006 Oct 1;44(10).
63. Staats PS, Yearwood T, Charapata SG, et al. Intrathecal Ziconotide in the Treatment of Refractory Pain in Patients With Cancer or AIDS: A Randomized Controlled Trial. *JAMA.* 2004;291(1):63–70.
64. Rauck RL, Wallace MS, Leong MS, MineHart M, Webster LR, Charapata SG, Abraham JE, Buffington DE, Ellis D, Kartzinel R, Ziconotide 301 Study Group. A randomized, double-blind, placebo-controlled study of intrathecal ziconotide in adults with severe chronic pain. Journal of pain and symptom management. 2006 May 1;31(5):393-406.
65. Rauck RL, Wallace MS, Burton AW, Kapural L, North JM. Intrathecal ziconotide for neuropathic pain: a review. Pain Practice. 2009 Sep;9(5):327-37.
66. G.A. Hodge, D.M. Bowman, A.D. Maynard (Eds.), International Handbook on Regulating Nanotechnologies, Edward Elgar Publishing Limited, Northampton, MA, USA (2010), pp. 291-320
67. Ehmann F, Sakai-Kato K, Duncan R. Hernan Perez de la Ossa D, Pita R, Vidal JM, et al. Next-generation nanomedicines and nanosimilars: EU regulators' initiatives relating to the development and evaluation of nanomedicines. Nanomedicine (Lond). 2013 May;8(5):849-56.
68. Duncan R, Gaspar R. Nanomedicine (s) under the microscope. Molecular pharmaceutics. 2011 Dec 5;8(6):2101-41.
69. Dobrovolskaia MA, McNeil SE. Understanding the correlation between in vitro and in vivo immunotoxicity tests for nanomedicines. Journal of controlled release. 2013 Dec 10;172(2):456-66.
70. Aggarwal P, Hall JB, McLeland CB, Dobrovolskaia MA, McNeil SE. Nanoparticle interaction with plasma proteins as it relates to particle biodistribution, biocompatibility and therapeutic efficacy. Advanced drug delivery reviews. 2009 Jun 21;61(6):428-37.
71. Hafner A, Lovrić J, Lakoš GP, Pepić I. Nanotherapeutics in the EU: an overview on current state and future directions. International journal of nanomedicine. 2014 Feb 19:1005-23.
72. Demetzos C, Pippa N. Fractal geometry as a new approach for proving nanosimilarity: a reflection note. International Journal of Pharmaceutics. 2015 Apr 10;483(1-2):1-5.
73. Rayate YT, Yadav AR, Mohite SK. Novel Drug Delivery systems and its future prospects. World J Appl Pharm. 2023 Jan;1(1):14-9.
74. Awasthi A, Paul P, Mir MS, Singh SK, Dua K. Novel Drug Delivery Systems in Colorectal Cancer: Advances and Future Prospects. Pathology-Research and Practice. 2024 Aug 21:155546.
75. Narang A, Chang RK, Hussain MA. Pharmaceutical development and regulatory considerations for nanoparticles and nanoparticulate drug delivery systems. Journal of pharmaceutical sciences. 2013 Nov 1;102(11):3867-82.