"DEVELOPMENT AND EVALUATION OF POLYSORBATE 80 COATED ALBUMIN NANOPARTICLES FOR BRAIN TARGETING OF PREGABALIN"

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Abstract

Epilepsy is a brain disease characterized by abnormal electrical activity causing seizures or unusual behavior, sensations and sometimes loss of awareness. An epileptic seizure is a transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain. Pregabalin is an antiepileptic drug belonging to BCS class 1 drugs i.e., having high solubility and high permeability. However, its delayed transport across BBB limits its use in emergency situations. The present work was performed to develop BSA nanoparticles of pregabalin coated with polysorbate 80. The selected formulation DP 1:2 was evaluated for particle size, zeta potential and surface morphology. The *in vitro* drug release study revealed that DP 1:2 showed maximum drug release as compared to other batches. The *in vivo* biodistribution study was performed in wistar rats, using two groups i.e pure drug and DP 1:2 formulation. It was observed from results that PS 80 coated BSA Nanoparticles showed maximum amount of drug reaches in brain in comparison to pure drug. Thus, from the results it was concluded that albumin nanoparticles coated with polysorbate 80 could efficiently target the drug pregabalin into brain.

Keywords: Epilepsy, nanoparticles, blood brain barrier, brain targeting, Bovine serum albumin, Polysorbate 80.

Chapter 1

INTRODUCTION

1.0 Introduction

1.1 Epilepsy:-

According to (David et al., 1978), "To emphasize that epilepsy is made up of many different diseases and conditions, it is generally referred to as a disorder or a family of disorders rather than a disease. While the term disease may (but is not always) imply a more persistent disruption of normal function, the term disorder suggests a functional disturbance that is not always enduring".

According to (Fisher et al.,2005), "a neurological condition where epileptic seizures are perpetually more likely to occur. Two unprovoked seizures that are more than 24 hours apart are typically how this criteria is used in practice. Such discharges can occur in various areas of the brain. The smallest muscular jerks or concentration lapses can be seizures, as can severe and protracted convulsions".

According to (Loscher et al., 2010), "There can be a wide range in the frequency of seizures, from fewer than one per year to several per day. Since up to 10% of people experience one seizure in their lifetime worldwide, it is not necessary to have epilepsy to experience seizures. Epileptic seizures are defined as two or more unannounced seizures. The first documented accounts of epilepsy date from 4000 BCE, making it one of the earliest recognized medical diseases in the world".

According to (Sisodiya et al., 2012), "For millennia, epilepsy has been enveloped in stigma, misinformation, prejudice, and societal humiliation. There are still many nations that hold this stigma. This Stigma persists today in many nations, which negatively affects the lives of those who have the condition and their families".

1.1.1 Signs and symptoms

Seizures can display a variety of traits based on where in the brain the disruption first appears and how far it progresses. According to (Glaeser et al., 2015), “Transient symptoms include loss of awareness or consciousness, as well as impairments of mobility, Sensation (such as sight, sound, and taste), emotion, or other cognitive processes. People with epilepsy are more likely to experience bodily problems (such as fractures and bruises from injuries brought on by seizures) as well as psychological problems (such as fear and despair)”.

According to (Perucca et al., 2016), “People with epilepsy are up to three times more likely to die prematurely than the general population, with low- and middle-income nations and rural areas having the highest rates of early mortality”.

According to (Szakacs et al., 2012), “Falls, burns, and prolonged seizures are only a few of the epilepsy-related causes of mortality that can be prevented, especially in low- and middle-income countries".

1.1.2 Epidemiology

According to (Kwan et al., 2014), “Worldwide, epilepsy affects people of all ages and genders. As a result of the higher frequency of stroke, neurological disorders, and tumors in this age group, males are slightly more likely than women to have epilepsy, and the condition tends to peak in the elderly. Both in children and adults, focal seizures are more frequent than generalized seizures.”

According to (Beghiet al., 2020), “The aetiology of epilepsy varies depending on the sociodemographic makeup of the affected populations and the depth of the diagnostic workup, although in roughly 50% of cases from high-income countries (HIC), there is currently no known cause. When epilepsy is evaluated by seizure freedom, the majority of patients have a favorable overall prognosis, reports from developing or middle-income nation’s reports from low- and middle-income nations (LMICs), where epilepsy patients are provided prevalence and remission rates that are similar to those of HICs and are largely treatable. Since epilepsy tends to be more common in most LMICs, the overlap in prevalence can be explained by misdiagnosis, sudden onset of symptoms, and early death. According to studies, almost half of the patients experience sustained seizure remission. Recent studies on the long-term prognosis of epilepsy, despite this, researchers have found a number of prognostic patterns, such as early and late remission, a relapsing-remitting course, and even a deteriorating course (characterised by remission followed by relapse and continuous seizures)”.

The lifetime prevalence of epilepsy is 7.6 per 1,000, and the prevalence of active epilepsy is 6.4 per 1,000. According to (Singh et al., 2016), "The prevalence tends to rise with age, peaking in the oldest age groups and among those from disadvantaged social backgrounds. Epilepsy occurs in 61.4 out of every 100,000 person-years. According to age, epilepsy has a bimodal distribution with peaks in both young and old people. The rise of age- and aging-related epileptogenic factors can be blamed for the higher incidence of seizures and epilepsy in the elderly can be attributed to the increase of age-related and aging-related epileptogenic conditions".

1.2 Causes of Epilepsy

According to (Arroyo et al., 2016), "Epilepsy cannot be spread, Although there are numerous underlying diseases that can cause epilepsy, only about 50% of cases around the world have a known cause". Epilepsy causes can be broken down into the following categories: structural, genetic, infectious, metabolic, immunological, and unidentified. Examples include:

- Brain injury from perinatal or prenatal causes, such as low birth weight, trauma duringbirth, or oxygen deprivation during birth;
- severe head injuries
- hereditary diseases that cause brain deformities, congenital abnormalities,
- strokes that reduce the quantity of oxygen reaching the brain,
- severe head injuries;
- a brain infection like meningitis, encephalitis, or neurocysticercosis, as well as several genetic disorders
- a brain tumor, (Thorne et al.,2012).

1.2.1 Types of Epilepsy:-

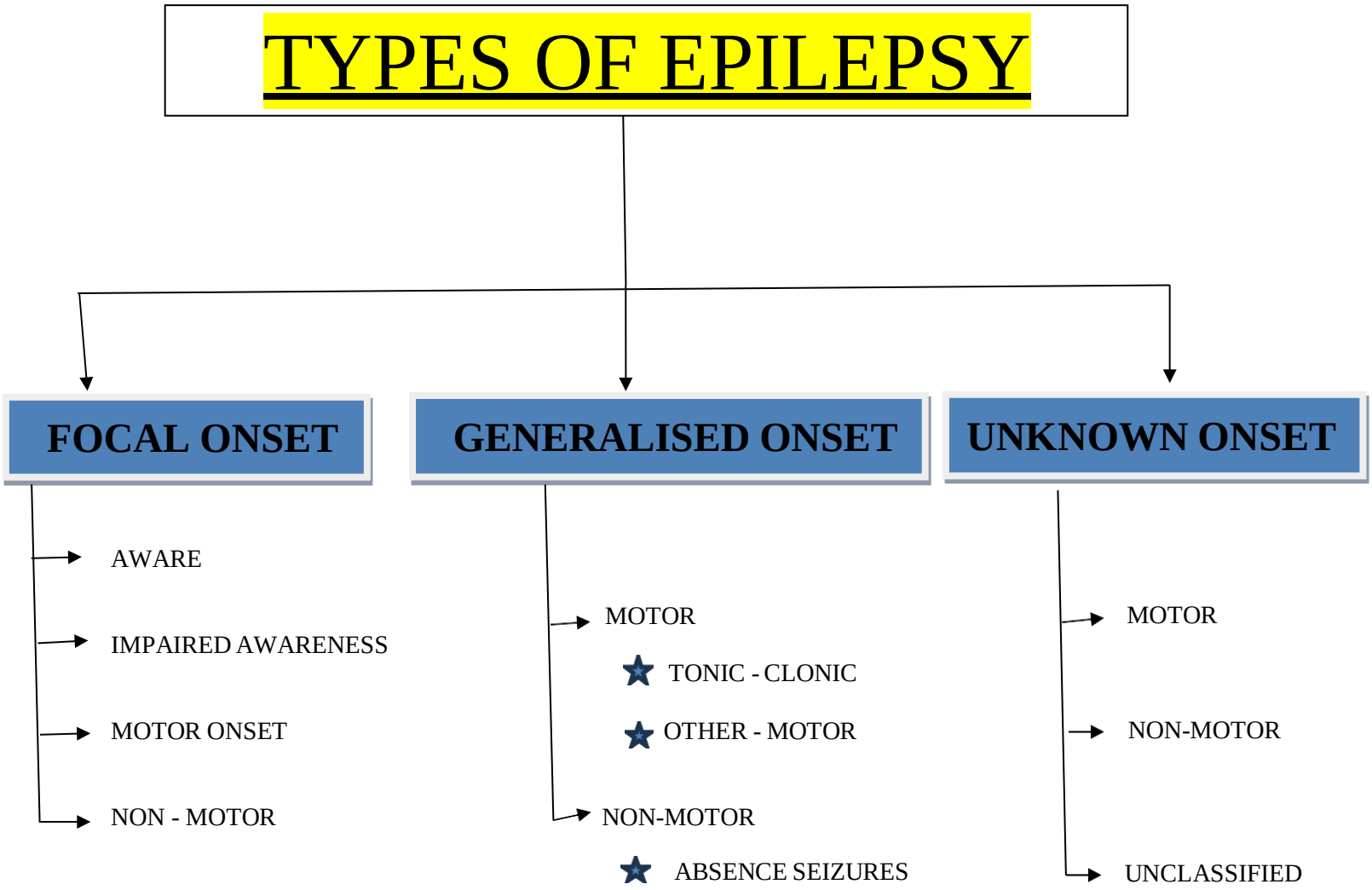

Fig 1 :- Types of epilepsy

1.2.2 Pathophysiology of Epilepsy:-

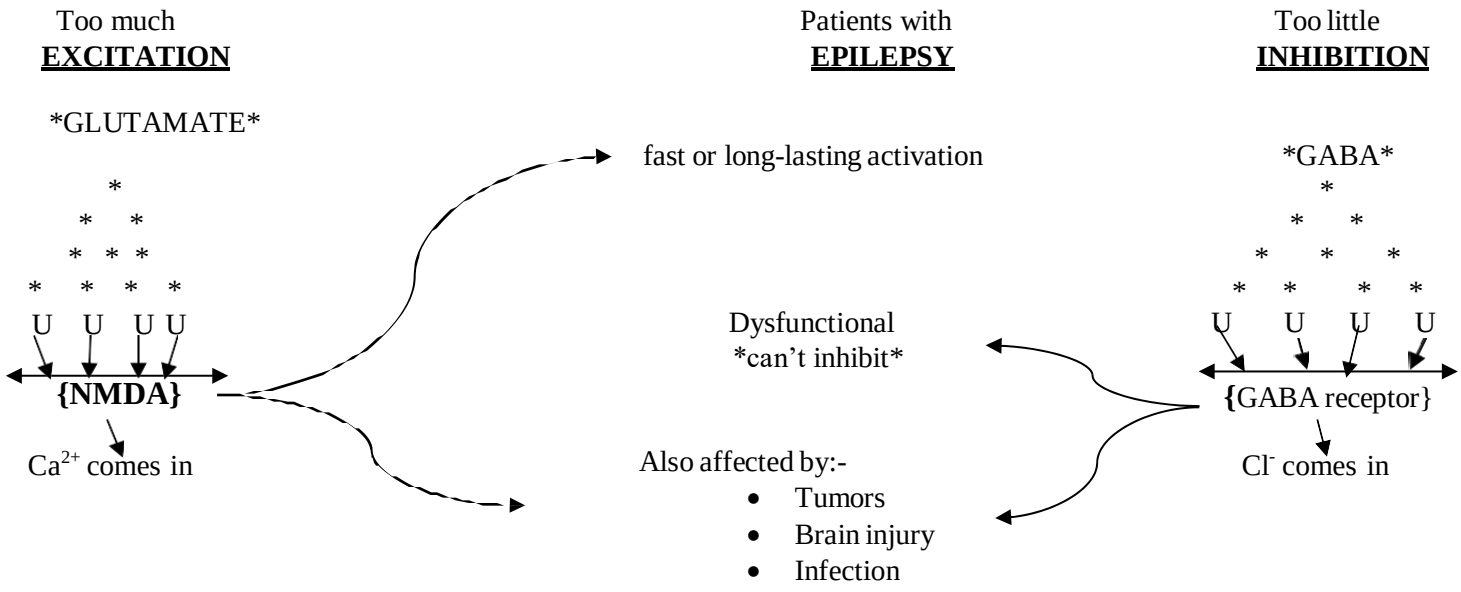

Fig 2 :- Pathophysiology of Epilepsy

Mechanism of seizure formation

- The stimulation of several nerves. Glutamate and aspartate, two excitatory neurotransmitters, have been affected by this.
- Epileptogenesis, hyperexcitability, and hyper synchronization of neurons promote spread. Inadequate inhibition.
- There must be improper synchronisation for a group of neurons to be able to discharge independently together.
- A hyperexcitable neuron cannot cause a seizure on its own (Jain et al., 2015).

➢ **Treatments:-**

Most epileptics can stop having seizures by taking just one anti-epileptic drug.

According to (Paasonen et al., 2014), "A combination of drugs may help some people reduce the frequency and severity of their seizures. "According to (Verdun et al., 2017), "Different anti-seizure drugs are on the market. Depending on the sort of seizures you experience, your doctor will select a medicine to treat your epilepsy, as well as other factors such as your age and other health conditions."

1.3 Anti-epileptic drugs

Table 1:- Antiepileptic drugs approved by FDA (FDA.org. 2019).

Antiepileptic drug	**Types of seizures**
Phenobarbital	Negative myoclonus, tonic ad absence
Benzodiazepines	Tonic-clonic seizures
Phenytoin	Tonic, clonic, tonic-clonic, and myoclonic Seizures
Carbamazepine	Tonic, atonic, absence, myoclonic
Oxcarbazepine	Absence and myoclonic
Valproate	Myoclonic and status epilepticus
Ethosuximide	Generalized nonconvulsive and atonic seizures
Lamotrigine	Myoclonic seizures
Gabapentin	Absence and myoclonic
Vigabatrin	Myoclonic

1.3.1 Nanoparticles:

According to (Dutt et al.,2018), "Nanoparticles are solid or liquid particulate dispersions with a size between 10 and 1000 nm. The medication was dissolved, trapped, encapsulated, or connected to the matrix of nanoparticles. Nanoparticles are in solid form and can be either amorphous or crystalline. They range in size from 10 to200 nm and include nanospheres and nanocapsules. The creation of nanoparticles has made considerable use of polymeric materials. Depending on the preparation technique, one can producenanoparticles, nanospheres, or nanocapsules".

According to (Alexus etal.,2018), "Nanospheres are matrix systems in which the drug is physically and uniformly spread, whereas nanocapsules are systems in which the drug is confined to a cavity and enclosed by a special polymer membrane. Due to their capacity to circulate for an extended period of time and their ability to target a specific organ, biodegradable polymeric nanoparticles—particularly those coated with a hydrophilic polymer like poly (ethylene glycol) (PEG)—have been used as potential drug delivery devices in recent years. They are also used as the DNA carrier in gene therapy".

1.3.2 Method of preparation of Nanoparticles:

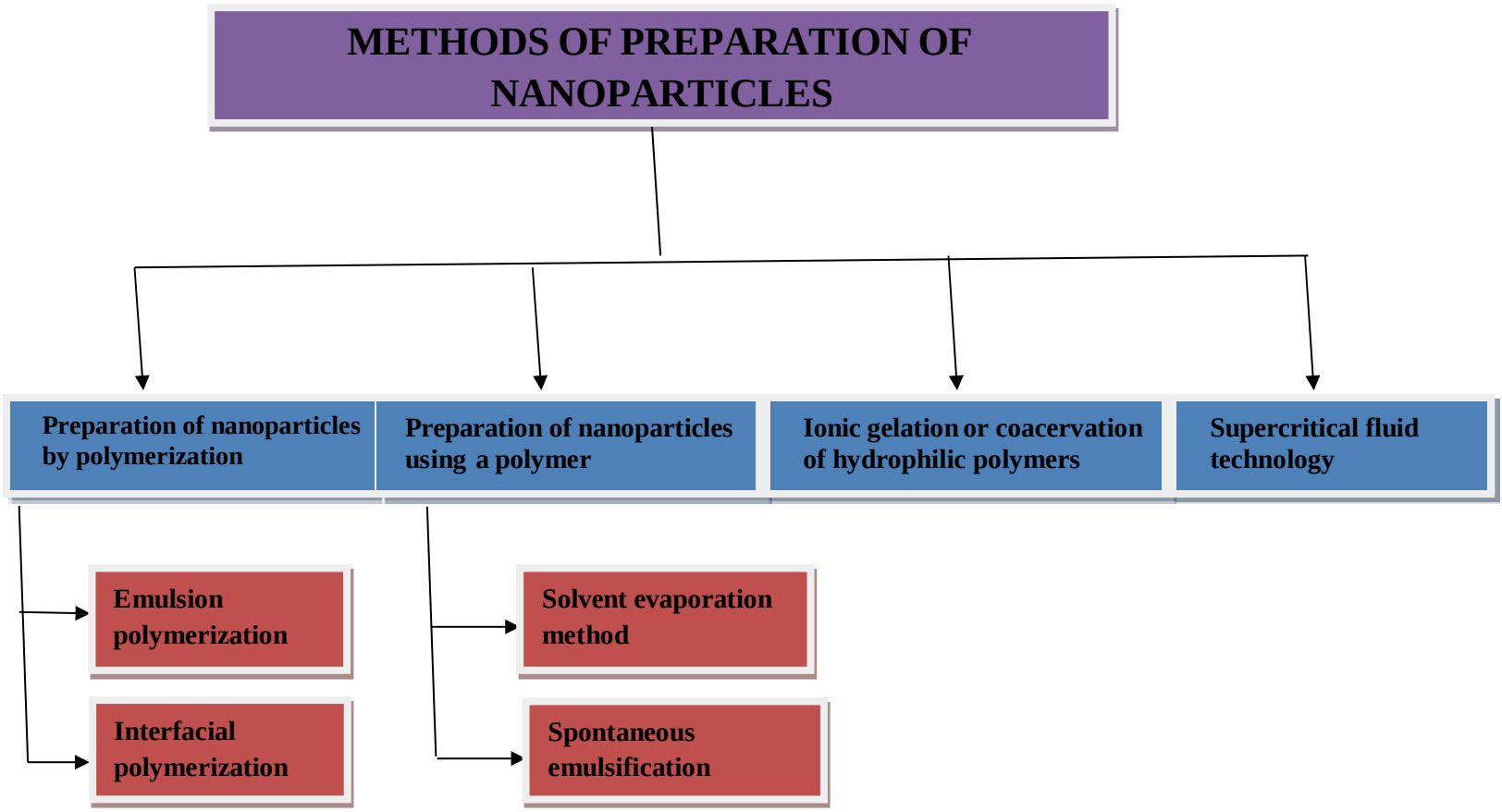

Fig 3:- Method of preparation of Nanoparticles

According to (Lockman et al.,2016), "Nanoparticles can be divided into many categories based on their size, morphology, physical characteristics, and chemical composition. They include lipid-based nanoparticles, metal nanoparticles, ceramic nanoparticles, semiconductor nanoparticles, polymeric nanoparticles, and nanoparticles based on carbon.

There are two primary categories of nanoparticles: organic and inorganic. Micelles, dendrimers, liposomes, and hybrid and compact polymeric nanoparticles are included in the first group. Fullerenes, quantum dots, silica, and metal nanoparticles are included in the second group.

1.3.3 Different Types of Nanoparticles:-

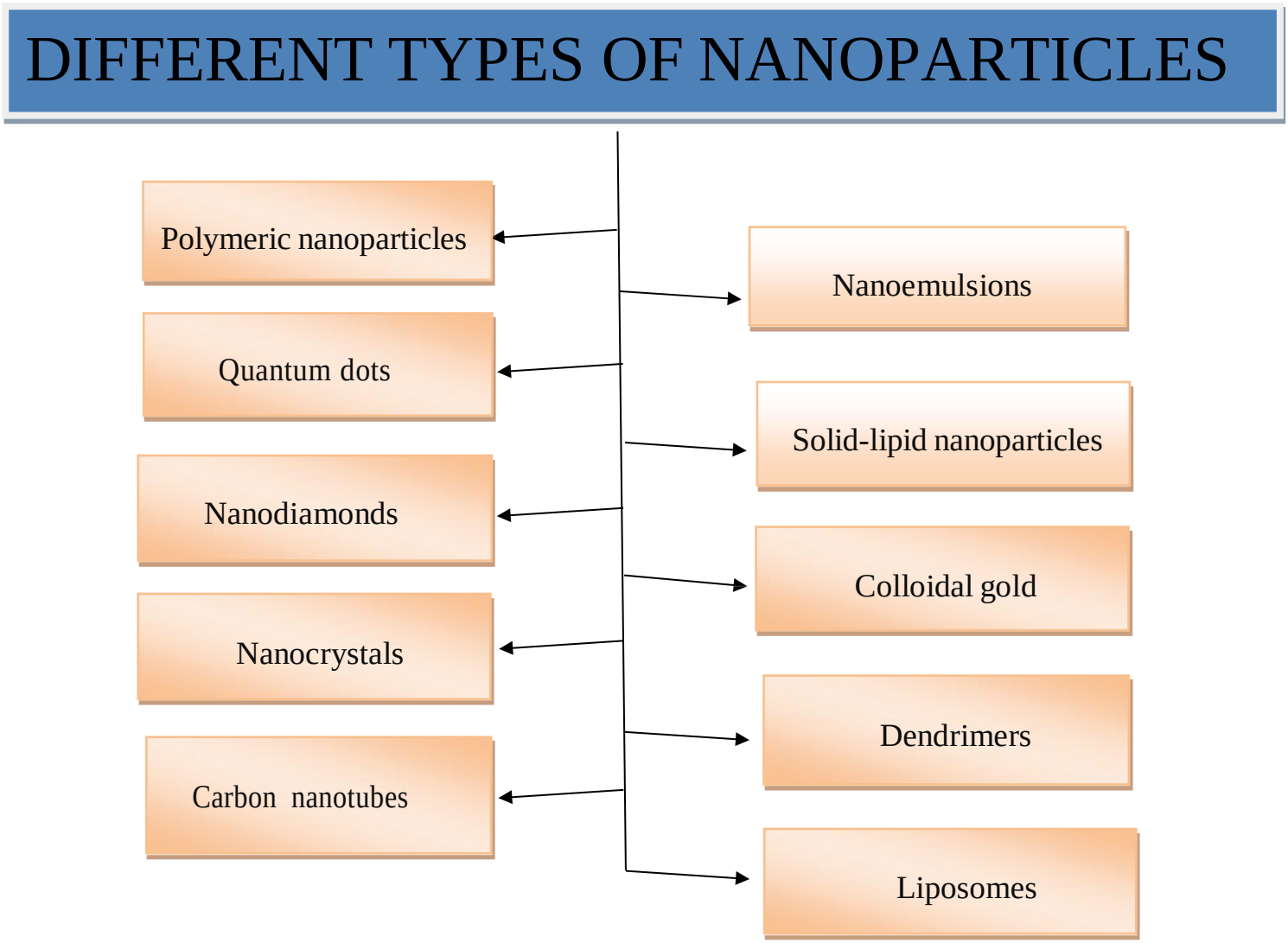

Fig 4:-Different types of nanoparticles

Chapter 2

AIM AND

OBJECTIVES

2.0 Rationale:-

Despite pregabalin high oral absorption and stronger intrinsic efficacy to inhibit seizure activity, it can only be used in emergency epileptic convulsions due to its delayed transit across the blood-brain barrier. Pregabalin is projected to become more permeable with the development of Polysorbate80-coated albumin nanoparticles, which would increase its effectiveness in the treatment of epilepsy and other disorders. Due to its sintering interaction with brain microchannel endothelial cells, Polysorbate80, a surface active chemical, has been demonstrated to increase the permeability of drug-loaded nanoparticles across the Blood Brain Barrier (BBB). The way that polysorbate80 penetrates BBB through p-gp in blood, to which it binds, and through receptors on the endothelial walls of the BBB.

Albumin is the most abundant plasma protein that is non-toxic and biodegradable, its water-soluble nature makes it suitable for injection.

2.1 Aim and Objectives:-

2.2 Aim:- Creation and assessment of polysorbate80-coated albumin nanoparticles for pregabalin brain targeting.

2.3 Objectives:-

1. Preformulation studies.

2. Development of polysorbate80 coated albumin nanoparticles of pregabalin.

3. *In vitro* evaluation of developed formulation.

4. *In vivo* biodistribution study

Chapter 3

LITERATURE REVIEW

3.0 Review of Literature

3.1 Review on Pregabalin Formulations

According to (Kanwar et al., 2016), "Wet granulation and compaction techniques were employed in the preparation of the non-effervescent floating and swelling tablets. The formulations demonstrated consistent release patterns and kept their buoyancy for more than 24 hours. It was discovered that crospovidone and hydroxypropyl methylcellulose concentrations had a significant impact on the produced tablets' in vitro dissolving and floating characteristics. Within three minutes, the pregabalin-containing optimized tablets began to float and grew in all dimensions—including length, width, and thickness—above 12.8 mm, the reported pyloric sphincter diameter during the fed condition. The optimized formulations are suitable as once-daily dosage forms, according to in vivo experiments in beagle dogs, and dose proportionality was seen at dosages between 75 and 300 mg. A gastro-retentive drug delivery method is required because the dogs given the formulation with poor in vitro gastro-retentive qualities displayed very variable and lowered levels of absorption. The created non-effervescent floating tablets are, in conclusion, promising candidates for once-daily pregabalin delivery."

According to (Mackay et al., 2017), "New three-layered (TL) tablet systems were compared to both monolithic matrix (MM) formulations and a commercial immediate-release (IR) capsule to make once-a-day (OAD) pregabalin tablets. In beagles and people, the TL tablet's pharmacokinetic parameters were compared to those of an IR capsule, as well as the TL tablet's physical properties, such as swelling and dissolving rates, to those of MM tablets. The findings showed that regardless of tablet geometry, formulations containing the same quantity of a hydrophilic polymer at 12 h had comparable dissolving patterns. Larger levels of polymer in the tablets showed a greater degree of swelling, but the extent of tablet swelling varied. Additionally, TL pills swelled quicker than MM tablets. In a pharmacokinetic investigation of the TL tablet, beagles showed absorption findings that were comparable to those of an IRcapsule, whereas humans showed low total absorption in comparison to an IR capsule. The findings of the beagle investigation were consistent with the highest plasma concentration of 6 hours in the fed condition of humans. In order to provide better formulations with greater continuous drug absorption for OAD administration, the novel TL tablet method of pregabalin may prove to be beneficial".

According to (Yasinet al., 2021), "The water-oil-oil double emulsion solvent evaporation process was used to make the microspheres. The particle size, encapsulation effectiveness, and in vitro drug release of microspheres were all characterized. It was investigated how the processing parameters affected the generated microspheres' properties. Utilizing differential scanning calorimetry, Fourier transform infrared spectroscopy, and scanning electronic microscopy, the solid-state of microspheres were characterized".

According to (Jadi et al., 2016), "Pregabalin Sellable Core Osmotic Pumps were created using the direct compression approach. Using an optimization technique, the number of polymers and osmogens that should be included in each formulation was determined. Using lactose, sodium carboxy methyl cellulose (sod. CMC), methylcellulose (MC), hydroxypropyl methylcellulose (HPMC), and sodium starch glycolate (SSG), the current experiment sought to produce matrix tablets. The made-up matrix tablets were identified. For physical evaluations to indicate tablet uniformity and mechanical integrity".

According to (Siddiqui et al., 2021), "Ketoprofen and Pregabalin Transdermal Drug Delivery Systems were created using the solvent casting technique. For the creation of transdermal patches, several ratios of hydrophilic (HPMC) and hydrophobic (Eudragit and Ethyl Cellulose) polymers were used. As a permeation enhancer, PG and oleic acid were used, while PEG-400 was used as a plasticizer. The homogeneous distribution of medicines throughout the matrix and the superb compatibility of the chosen constituents have been proven by surface morphology. The formulation was complete. More than 300 folds were completed, showing that all patches possess enough mechanical strength. The patches' excellent stability and compatibility with the chosen materials were further demonstrated by their complete flatness".

According to (Gabizon et al., 2018), "Pregabalin-containing polymeric nanoparticles were created, and the optimal polymer content was determined based on the 24-hour in vitro release profile. Using a solvent displacement approach and varyingquantities of Eudragit S100 (EPNP1-EPNP5), the nanoparticles were created. The produced nanoparticles were evaluated for their drug content, entrapment effectiveness, particle size and zeta potential as well as in vitro drug release profile. Preformulation research confirmed the compatibility between the medication and other excipients employed in the formulation. Based on its particle size, entrapment effectiveness, and in vitro drug release profile, the optimized formulation was chosen. As the formulation with the optimal concentration for the regulated release of Pregabalin, 300 mg of Eudragit S100 (EPNP5) was chosen as the optimized concentration for the controlled release of

Pregabalin for a period of 24hrs".

According to (Rapoport et al., 2018), "Pregabalin (PG)-entrapped niosomes were created utilizing span 60 and cholesterol in various combinations. The leftover PG from the hydrating solution was isolated from vesicles by freezing centrifugation. Molar ratios by the hydration procedure. Pregabalin (PG) nanotechnology optimization was completed. To get PG-loaded niosomes with the desired qualities, the Quality by Design technique was effectively used. The Minitab programme used a design of experiments (DOE), which forecast the best parameters by examining the combined effect of various elements concurrently to achieve the ideal particle size, drug release, and entrapment efficiency. Pareto charts were utilized in the screening stage to weed out variables that weren't important, and response surface methodology (RSM) was employed in the optimization stage to analyze the important variables. The most effective recipe was chosen to make topical niosomal PG into hydrogels utilizing HPMC and Carbopol 934. Mechanical and rheological tests were used to demonstrate that the addition of the vesicles to the gelmatrix had a substantial impact on the gel network. Ex vivo and in vitro, permeation tests were performed. The delivery of the PG molecules was Higuchi-based rather than Fickian-based. The pharmaceutical industry will be interested in the current work as a controlled transdermal substitute for the traditional oral route".

According to (Barnett et al., 2019), "Pregabalin is used to relieve pain brought on by seizures and neurologic conditions including neuralgias. In the current study, direct compression was used to create Pregabalin fast-dissolving tablets for the treatment of epilepsy with the goal of improving patient compliance. Fast-dissolving pills broke down in the mouth and disintegrated within a few seconds without the need for water. Fast-dissolving tablets (FDTs) were made using various super disintegrate concentrations and their pre-compression parameters were assessed. The produced pills underwent post-compressional testing. The wetting time of formulations containing crospovidone was found to be the shortest, and tablets demonstrated the quickest disintegration. Fast dissolving tablets (FDTs') medication release grew with increasing the formulations containing Crospovidone was found to have the highest concentration of super disintegrates".

Recently, various dosage forms have been designed and created using three-dimensional (3D) printing in order to facilitate on-demand production and personalized treatment. In this study, fused deposition modelling (FDM) was employed to create a floating sustained release system. Pregabalin, polyethylene glycol (PEG 400), and hypromellose acetate succinate (HPMCAS) were used to make the filaments. Using the FDM printer, cylindrical tablets with infill rates of 25%, 50%, and 75% were created.

With a slightly opened top layer and a closed bottom layer, an optimized formulation (F6) was created. Fourier- transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray powder diffraction and thermo gravimetric analysis (TGA) were used to characterize filaments and tablets. The findings demonstrate that the drug's stability was not significantly impacted by the processing conditions, and its crystallinity persisted even after printing. According to a dissolution study, open systems with low infill ratios release drugs more quickly than closed systems and open systems with high infill ratios. Zero-order drug release was demonstrated by the optimized formulation (F6) with a slightly opened top layer. According to the findings (Fischer et al., 2018), "FDM printing is appropriate for creating floating dosage forms with the desired drug release profile".

According to (Muller et al.2019), "For facilitating pregabalin administration to the brain, a hepatitis B core (HBc) protein nanocage (NC) with the insertion of brain-targeted TGN peptide was created. The outcomes showed that this nanocage can 2.4fold more effectively and specifically target brain tissue and 100 times the antiepileptic effectiveness of phenytoin in epilepsy models caused by pilocarpine. Human neural three-dimensional cortical organoids in vitro and in vivo both showed high penetrating ability. Rather than causing the brain-blood barrier to be disrupted, these tasks are accomplished by facilitating the brain target peptide TGN. In conclusion, we demonstrated an effective nanocage for the delivery of antiepileptic drugs for the treatment of refractory epilepsy. Additionally, this therapeutic modulation offers a viable method for treating various untreatable neurological conditions".

3.2 Nanoparticles formulations of other drugs used for the treatment of various diseases

According to (Prabhakar et al., 2013), "Indinavir lipid nanoemulsions were created utilizing an ultrasonic and heat homogenization method. 10% weight-to-volume soybean oil served as the oil core in the composition 1.2%w/v Tween -80 is used to emulsify phospholipids, oleic acid is used to create negative charges, tocopherol is used as an antioxidant, and glycerol is used to maintain isotonicity during intravenous delivery. Water-soluble chemicals were introduced to the aqueous phase while oil-soluble molecules were put into the soybean oil (oil phase). After bringing both phases to a temperature of 70 °C, the aqueous phase was introduced to the oil phase while stirring, and the mixture was then homogenized for approximately three minutes at a speed of 15,000 rpm".

According to (Kapoor et al.,2016), "Seratopeptidase nanoparticles were created utilizing the ionic gelation process. This approach involved the preparation of two phases, one of which was an acidic phase containing chitosan (0.1%) in aqueous acetic acid and the other of which was a basic phase containing Three Phase Partitioning (TPP) (0.1%). The formation of nanoparticles involved adding the basic phase (TPP) drop by drop into the acidic phase (chitosan) at room temperature while stirring continuously for 60 minutes at a speed of 1000 rpm. This resulted in the formation of intra molecular and intermolecular linkages between the phosphate groups of the TPP and the amino groups of the chitosan. The polymer and medication were combined in a 1:1 ratio. After adding the medication, the mixture was stirred for 1 hour at room temperature using a 4000 rpm speed. The drug that was not bound was then separated from the drug that was included in the produced nanoparticles by centrifugation for 30 minutes at 12000 rpm. Chitosan TPP polymer was produced in a variety of ratios ranging from 3:1 to 6:1, and 10 mg of medication was added to each".

According to (Klingeler et al., 2019), "Patients with primary brain tumors and brain metastases have an extremely dismal prognosis despite advancements in tumor therapy. The blood-brain barrier's resistance to cytotoxic drugs is the main cause of chemotherapy side effects. Gliomas and different types of brain metastases have both been demonstrated to be responsive to paclitaxel. However, due to low blood-brain barrier permeability and substantially adverse effects connected with the injection of the paclitaxel solvent, Cremophor EL, its application in the treatment of brain tumors is restricted. The p-glycoprotein (p-gp) efflux transporter is hypothesized to be connected to the lack of paclitaxel brain absorption. Despite reak through in cancer therapy, patients with primary brain tumors and brain metastases have

a very poor prognosis. The key contributing factor to chemotherapy side effects is the blood-brain barrier's resilience to cytotoxic medicines. Paclitaxel has been shown to have an effect on both gliomas and several types of brain metastases. Cremophor EL®, a paclitaxel solvent, can be injected; however, because of its low blood-brain barrier permeability and significant side effects, its use in the treatment of brain tumors is limited. The lack of paclitaxel brain absorption is thought to be related to the p-glycoprotein (p-gp) efflux transporter. In this study, new cetyl alcohol/polysorbate nanoparticles were used to entrap the drug paclitaxel (PX). The characteristics of paclitaxel nanoparticles (PX NPs) were size, short-term stability, drug entrapment effectiveness, and release profile. U-118 and HCT-15, two distinct cell lines, were used to monitor the PX NP cytotoxicity profile. An in situ rat brain perfusion model was used to assess the cerebral uptake of PX NPs. The findings imply that paclitaxel's entrapment in nanoparticles greatly improves both the drug's brain absorption and its toxicity towards tumor cells expressing p-glycoprotein. The idea was that PX NPs may disguise paclitaxel's properties and so restrict its ability to bind to p gp, which would then result in increased brain and tumor cell uptake of the otherwise effluxed medication".

According to (Saad et al., 2020), "In this case, Different antiepileptic medicines (AEDs) including valproic acid, tiagabine, phenytoin, carbamazepine, and lamotrigine were produced and characterized as functionalized titanium nanoparticles. To create a homogenous phase, AEDs were encapsulated by introducing them during the gelation process. By using N2 adsorption-desorption, FTIR spectroscopy, scanning electron microscopy, transmission electron microscopy, and thermal gravimetric measurement, the produced nanoparticles (empty and carrying the drug) were characterized. In order to assess the drug release over time, the delivery kinetics of the nano materials was also examined. It was shown that the AEDs put to the test may be contained in functionalized titanium nanoparticles. Because of the encapsulation procedure, infrared spectra demonstrate that the drug's structure has not undergone any chemical change. The SEM images of the drug-containing nanoparticles reveal a heterogeneous microstructure made up of amorphous, spherical aggregates. The evaluation of the in vitro release kinetics to the resulting nanomaterials revealed two distinct velocities: first, a quick release, and second, a steady and consistent release".

For the electrochemical determination of gabapentin (GB), a straightforward and sensitive platform based on glassy carbon electrode (GC) and gold nanoparticles (AuNPs) was built. The majority of traditional techniques for GB determination call for sample pre-treatment. Using a chronoamperometry approach, the AuNPs were electrodeposited on the surface of the GC electrode.

Investigations have been done on the electro deposition technique's applied potential and electro-deposition time. According to (Couvruer et al., 2018), "The electrochemical response for GB electro oxidation was significantly better than that of a standard GC electrode. For the electrochemical determination of gabapentin (GB), a straightforward and sensitive platform based on glassy carbon electrode (GC) and gold nano particles (AuNPs) was built. The majority of traditional techniques for GB determination call for sample pre-treatment. Using a chronoamperometry approach, the AuNPs were electrodeposited on the surface of the GC electrode. The electrochemical response for GB electrooxidation was significantly better than that of a standard GC electrode. The sensor response showed that the AuNPs/GC electrode had a 3.25m limit on the electrocatalytic ability to oxidize GB, which was a substantial electrocatalytic capability".

According to (Mora et al., 2019), "A promising method to improve anti-epileptic drug targeting in the brain is the use of nanoparticles (NPs) for intranasal (IN) drug delivery. Phenytoin (PHT)-loaded chitosan-lecithin NPs were created in the current study utilizing the nano-precipitation technique. While the average dynamic size and zeta potential were determined using dynamic light scattering, scanning and transmission electron microscopy was used to establish the NPs' spherical nature and their stability. All prepared NPs had a PHT encapsulation effectiveness of greater than 60%. According to release studies, the quantity of PHT released was inversely proportional to the amount of chitosan employed. PHT levels in the brain were assessed three times after the IN route was used to administer the best preparation, L10Ci+. Two experimental controls were administered intravenously (IN) and intraperitoneally (IP). The highest PHT concentration was found in L10Ci +, reaching 1.01 0.55%, which was related to a prolonged release of PHT. These first results indicate that controlling epilepsy with IN administration of PHT-loaded NPs is quite promising. The continuous release could boost patient compliance in a clinical context, while the direct nose-to-brain method could increase the safety margins of PHT."

According to (Kreuter et al., 2018), "Cubosomal-gel (cub-gel) was created to contain clonazepam (Cl) and be applied topically as a patch reservoir. A mixture of glycerol monooleate (GMO) and pluronic F127 (P F127) was used to create cubosomes. The impact of various stabilizing agents (Ethanol and PVA) and surfactant concentration on cubosomes' particle size and entrapping efficiency was examined using an actual-statistical approach. The stability, in vitro drug release, and particle morphology of the chosen formulations were tested for evaluation. In order to make cubgel, specific cubosome formulae were used.

When PVA or Et are used as stabilizers with PF127, the average cubosome's PS is greatly reduced (352 2.8 and 264 2.16 nm, respectively), and the EE is increased (58.97 4.57% and 54.21 3.89%). Cubosomes speed up the first hour's release of Cl to ensure a quick therapeutic impact (37.39% and 46.04% in the first hour), which is then followed by a prolonged release over the next four hours. When compared to Cl suspension, PVA demonstrated much greater Cl-transdermal permeability. The ideal cub-gel was tested for in vitro dissolution, ex vivo permeability, and skin deposition before its pharmacological activity was investigated. Additionally, it lengthens the retention period (89.57% at 48 hours) and multiplies skin deposition by six. Additionally, it lessens epileptic seizures and modifies pilocarpine-induced behavioural characteristics. Therefore, Cubosomal-gel could be viewed as a cutting-edge transdermal dosage form for Cl."

According to (Aronica et al., 2018), Antiepileptic medication carbamazepine (CBZ) has weak water solubility and is hence poorly bioavailability. The evaporation assisted solvent-antisolvent interaction (EASAI) approach was successful in producing spherical nanoparticles of CBZ with a particle size less than 50 nm. The same technique was used to create nanoparticles that are stabilised by polyvinylpyrrolidone (PVP). CBZ nanoparticles and CBZ-PVP nanoparticles were more soluble than raw CBZ by 11.9 and 21.5 times, respectively. Almost all of the drug was released from CBZ nanoparticles and CBZ-PVP nanoparticles in less than 60 minutes, according to in vitro dissolution experiments, whereas just 34% of the drug was released from raw-CBZ even after 180 minutes. The impact of various experimental variables, including drug concentration and PVP presence, on CBZ particle size, shape, solubility, and in vitro drug release rate was carefully examined. Transmission electron microscopy (TEM) and field emission scanning electron microscopy (FESEM) were used to confirm the nanoparticles' spherical form. The hydrogen bonding between PVP and CBZ molecules in the CBZ- PVP nanoparticles was discovered by FTIR spectroscopy research. The CBZ-PVP nanoparticles' X-ray diffraction (XRD) pattern showed that the raw-CBZ crystal structure had undergone a slight modification. According to investigations using differential scanning calorimetry (DSC), the nanoparticles were comparatively less crystalline than the raw CBZ".

3.3 Pregabalin Profile

According to (Frampton et al., 2005), "a gamma-amino butyric acid (GABA) 3-isobutyl derivative with anticonvulsant, antiepileptic, anxiolytic, and analgesic properties. Pregabalin selectively binds to alpha2delta (A2D) subunits of presynaptic voltage dependent calcium channels (VDCCs) in the central nervous system (CNS), while the precise mechanism of action is uncertain. Pregabalin blocks calcium influx and the subsequent calcium-dependent release of many neurotransmitters via binding to VDCC A2D subunits.

According to (Ben-Menachem et al.,2004) Pregabalin has a similar pharmacological profile to that of its developmental ancestor gabapentin, but it has shown stronger analgesic effectiveness in treating partial seizures in rodent models and neuropathic pain. Pregabalin is the pharmacologically active S-enantiomer of 3- aminomethyl-5-methyl-hexanoic acid.

According to (Dworkin et al.,2005) Although the exact mechanism of pregabalin's effect is uncertain, it is believed to prevent the release of excitatory neurotransmitters by interacting with the protein subunit 2 of voltage-gated calcium channels.

According to (Sills et al., 2006) In three randomised, double-blind, placebo-controlled, multicenter studies with a total of 776 evaluable patients with post herpetic neuralgia over the course of 8–13 weeks, oral pregabalin 150–600 mg/day, given twice or three times per day, was more effective than a placebo in reducing pain and enhancing pain-related sleep interference.

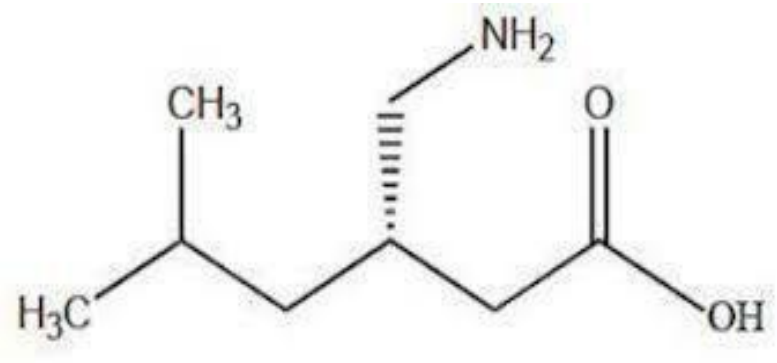

Fig 5:- Structure of Pregabalin

Molecular weight	159.23
Color form	White off to white crystalline
Solubility	freely soluble in both basic and acidic solutions as well as in water
Log P log Kow	-1.78 (est)
Boiling point	144 – 147 ºC
Melting point	176 – 178ºC

3.4 Pharmacology

According to (Williamson et al., 2008), “Despite having a structure that resembles gamma-aminobutyric acid (GABA), pregabalin does not interact with GABA receptors. Instead, it binds to the central nervous system's presynaptic voltage-gated calcium channels' alpha2-delta subunit”.

Chapter 4

MATERIAL AND METHODS

4.0 Material and Methods

4.1 Materials

Pregabalin was received as a gift sample from Gracure Pharmaceuticals Ltd, Bhiwadi, Rajasthan. Bovine serum Albumin was purchased from International Scientific and Surgicals, Solan, Acetone, 8% v/v glutaraldehyde solution and Tween 80 and distilled water were purchased from Qualikems Laboratory Reagents, New Delhi. All other chemicals and reagents used were of AR grade.

4.2 Methods

4.2.1 Preparation of Calibration Curve

The stock solution was prepared by dissolving 10mg of Pregabalin drug in 10 ml of solvent to get 1000µg/ml concentration. The different concentrations of dilutions were prepared from 10- 100µg/ml. The lambda max was determined by UV spectrophotometer (Thermo Scientific) in phosphate buffer pH 7.4. At 210 nm, absorbance was measured in comparison to a reagent blank. Plotting the absorbance vs the pregabalin final concentration resulted in calibration curves.

4.2.2 Drug Excipient Compatibility Study Using FT-IR

The compatibility of excipients was checked by performing the FTIR of the drug, excipients and a mixture of drug and excipients.

4.2.3 Preparation of Albumin Nanoparticles Coated with Polysorbate80

According to an earlier research by Marty et al., nanoparticles were created using the desolution approach. As indicated in Table 2, three batches of nanoparticles were created using various BSA and PGB concentrations. Here, the drug and polymer masses varied, but the volume of the resulting solution stayed the same across all formulations. 5 mL of BSA solution were first filtered through a Millipore membrane filter with a pore size of 0.45 mm to create the nanoparticles. To obtain nanoparticles with smaller particle sizes, the filtrate was collected and its pH was raised to 9 using a 0.5 mol/litre NaOH solution. PGB was combined with BSA solution using a magnetic stirrer, and then 20 mL of ethanol were continuously added dropwise at a rate of 1 ml per minute from a syringe until turbidity was seen in the mixture.

In order to decrease BSA's solubility in water and promote the precipitation of nanoparticles, ethanol functions as an antisolvent. By adding 500 ul of 8% (V/V) glutaraldehyde and stirring continuously for 4 hours at 500 rpm at room temperature, the nanoparticles created were cross-linked. The drug-loaded nanoparticles were then filtered through a Millipore filter with a 1-mm size after the cross-linking stage. A high-speed centrifuge was used to centrifuge the filtrate for 20 min. at 20,000 rpm. To remove the adsorbed glutaraldehyde and PGB from the nanoparticles surface, the supernatant was decanted, and the solution was washed three times with distilled water/ethanol (1:1). In an ultrasonication bath, each redispersion step was carried out. Before analysis, the cleaned samples were added to glass and quickly redispersed in an aqueous buffer solution by shaking. Pregabalin nanoparticle dispersion was mixed with P80 1% (m/v) for 30 minutes to create P80-coated nanoparticles.

4.3 In Vitro Evaluation

4.3.1 Particle Size distribution and Zeta Potential

Dynamic light scattering technique (Beckman Coulter) was employed to determine particle size and size distribution. A small amount from all the prepared batches of 1 ml was taken for particle size distribution and as a result, the particle size and PDI of samples were obtained and the zeta potential of selected formulation DP 1:2 was determined by zeta sizer (Beckman Coulter).

4.3.2 Surface morphology

The selected formulation DP 1:2 was selected for surface morphology, The surface morphology of the particles was determined by FESEM (Hitachi), SAIF, Panjab University, Chd.

4.3.3 Entrapment Efficiency Determination

The mass of dried nanoparticles recovered from each batch relative to the total starting material was used to calculate nanoparticle recovery. By mixing 5 mg of sample from each batch with 10 mL of a pH 7.0 phosphate buffer/ethanol (1:1) solution containing 1 mL of 50% (m/V) trichloroacetic acid, the amount of pregabalin in each batch was calculated. The samples were centrifuged after 24 hours, and the amount of pregabalin in the supernatant was calculated using UV spectroscopy.

Drug entrapment efficiency was calculated as the ratio of actual drug content to theoretical drug content.

$$\% \text{ Entrapment efficiency} = \frac{\text{Amount of drug present}}{\text{Amount of drug added}} \times 100$$

4.4 *In Vitro* drug release study

Pregabalin release from the pregabalin nanoparticles was assessed using a dialysis technique in pH 7.4 phosphate buffer. In order to do this, the necessary quantity of nanoparticles containing 5 mg of pregabalin were dissolved in 2 mL of dissolving medium, placed into a dialysis tube, knotted at both ends, and fully dissolved in 100 mL of pH 7.4 phosphate buffer. The buffer was kept at a temperature of 37 °C while being stirred at a speed of 100 rpm. To maintain sink conditions, 5 mL of buffer was taken at various intervals and replenished with a similar volume of fresh buffer. By using UV spectroscopy, the Pregabalin concentration was evaluated.

4.5 *In Vivo* Bio distribution Study

The male Wistar rats used for the bio distribution investigation were healthy and weighed 180–220 g. The ratswere kept in climate-controlled environments with a 12:12 h light/dark cycle. The rats were randomly and evenly separated into the pregabalin and pregabalin-nanoparticles-polysorbate80 groups after being acclimated for a week prior to the studies. The tail vein was used to administer the mixtures. Blood was drawn 1 hour after injection (1 ml), and the animals were promptly put to death. The brain, heart, lungs, liver, and kidneys were then removed, homogenised in normal saline for 10 ml, and centrifuged for 2 ml. The content of pregabalin in the homogenate will then be measured by HPLC.

Chapter 5

RESULTS AND DISCUSSION

5.0 Results

5.1 Preparation of Calibration Curve

From the stock solution (10 mg/10 ml i.e. 1000 ug/ml), the dilutions were prepared in the range of 10-100 µg/ml. The maximum absorbance was found to be 224nm in phosphate buffer pH 7.4.

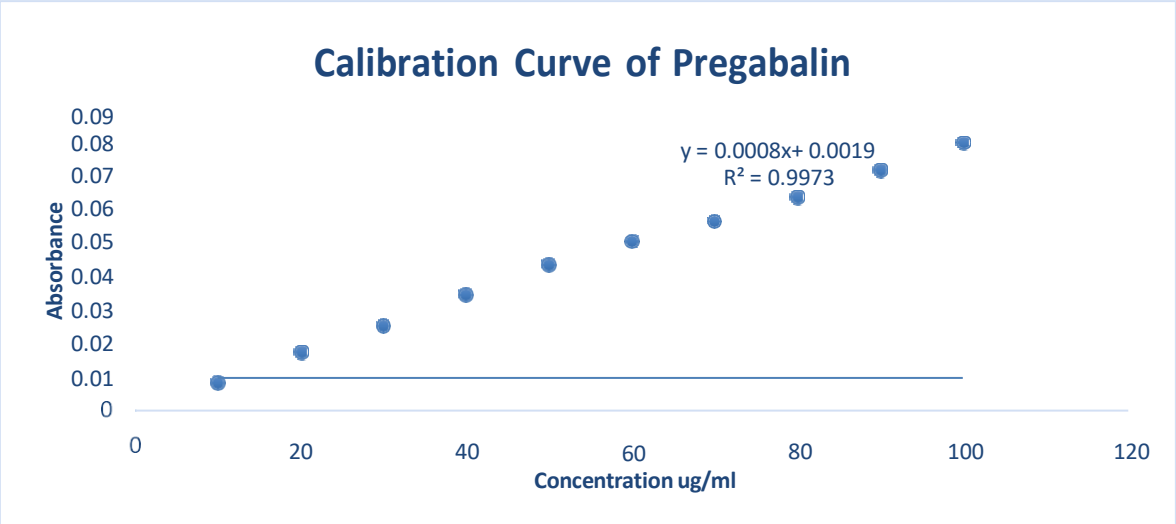

Fig 6:- Standard calibration curve using UV Spectroscopy

5.2 Drug Excipient Compatibility Study by FT-IR Spectroscopy

The FTIR spectra of Pregabalin (pure drug) showed absorption peaks at 2958cm $^{-1}$(C-H stretching), acidic O-H group peak at 2898cm^{-1}, -OCH_2 group at 1469cm^{-1}, C=N stretching at 1700cm^{-1}, N-H bending of 2°amine group at 1625cm^{-1}. The C-H stretching and acidic O-H group peak showed peaks in excipients at 2928cm^{-1} and 2862cm^{-1} respectively Major peaks of the drug were found in the FT-IR spectra of drug – excipient mixture indicating no chemical interaction between the drug and excipients as shown in fig. 7.

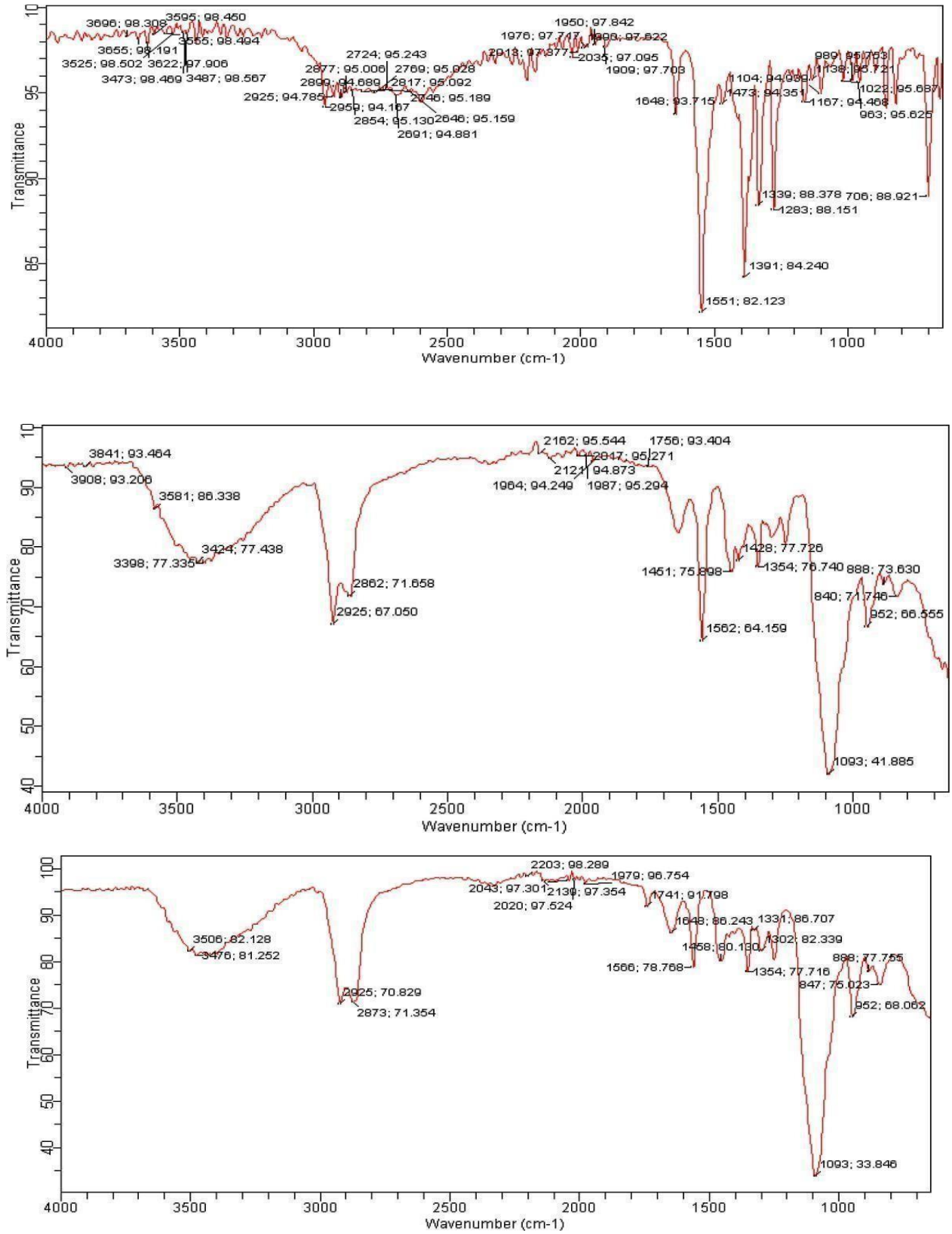

Fig 7:-FTIR spectra of A) Pure drug B) Excipients mixture C) Drug + Excipients mixture

Table 2:- Absorption bands of FTIR spectra of the pure drug, excipient and drug + excipients mixture.

Functional Groups	Absorption peak (cm^{-1}) Pure drug	Excepients	Dr + Exc
-OCH2	1469	1921	2929
Acidic O-H peak	2898	2962	2852
C=O stretching	1700	1728	1737
N-H bending of 2°amine group	1625	1648	
C-F group	1342	1352	
OH group	2898	3491	

5.3 Preparation of Albumin Nanoparticles of Pregabalin

The nanoparticles were prepared by desolution method and 3 batches in different concentrations of drug and polymer, i.e., DP 1:1, DP 1:2, DP 1:3 were prepared as shown in Table 3 indicating nanoparticles coated with polysorbate80.

Table 3:- Different compositions of BSA nanoparticles of pregabalin coated with Polysorbate80.

Ingredients	**Drug: polymer 1:1**	**Drug: polymer 1:2**	**Drug: polymer 1:3**
Pregabalin	100 mg	100 mg	100 mg
Albumin	100 mg	200 mg	300 mg
10 Mm NaCl solution	8 ml	8 ml	8 ml
4% v/v glutaraldehyde Solution	200 ul	200 ul	200 ul
Acetone	5 ml	5 ml	5 ml
Polysorbate80	1% v/v	1% v/v	1% v/v

5.4 In Vitro Evaluation

5.4.1 Particle Size and Zeta Potential Determination of Selected Formulation

The particle size plays an important role in the Nanoparticles formulation as the smaller the particle size, the better will be the uptake of the particles. The formulations DP 1:1, DP 1:2, DP 1:3 showed particle size of 211 nm, 192 nm and 213nm respectively. The smallest particle size showed by DP 1:2 formulation was selected for zeta potential that was found to be -0.10 mV as shown in fig 8. The lower value was due to the coating of particles with a non-ionic surfactant i.e., Polysorbate80. Table 4 shows the particle size and PDI of prepared formulations.

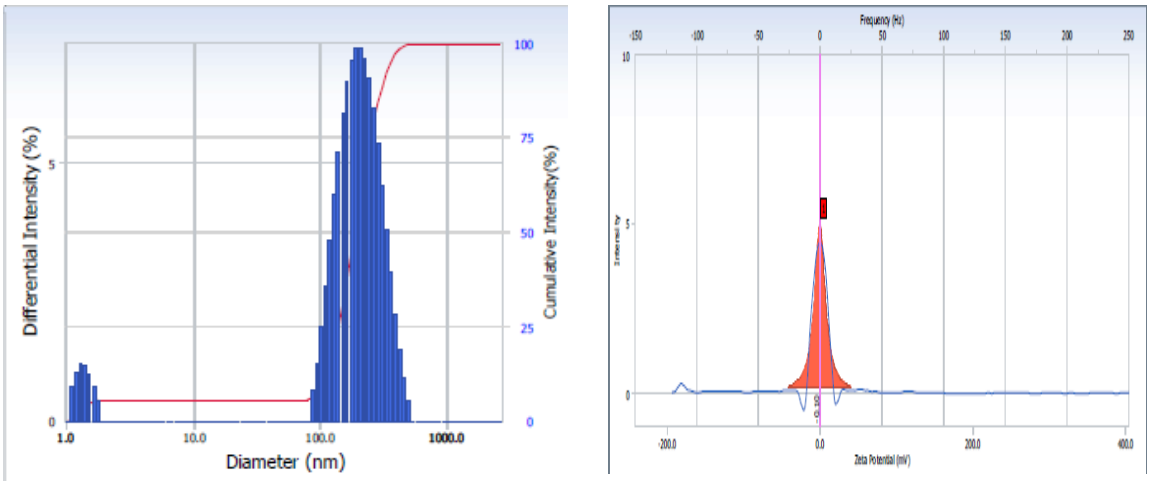

Fig 8, 9:- Representative particle size and zeta potential graphs of selected formulation (DP 1:2)

Table 4: Particle size and PDI of all prepared formulations:-

Formulation	Particle size	PDI
DP1:1	211 nm	0.18
DP1:2	192 nm	0.15
DP1:3	213 nm	0.21

5.4.2 Surface Morphology:-

The surface morphology of the prepared nanoparticles was evaluated by FE-SEM. Fig. 11 shows a photomicrograph of nanoparticle formulation (DP 1:2). The particles were found to be non-aggregated and spherical in shape with size in the nm range. The particles were coated with PS 80.

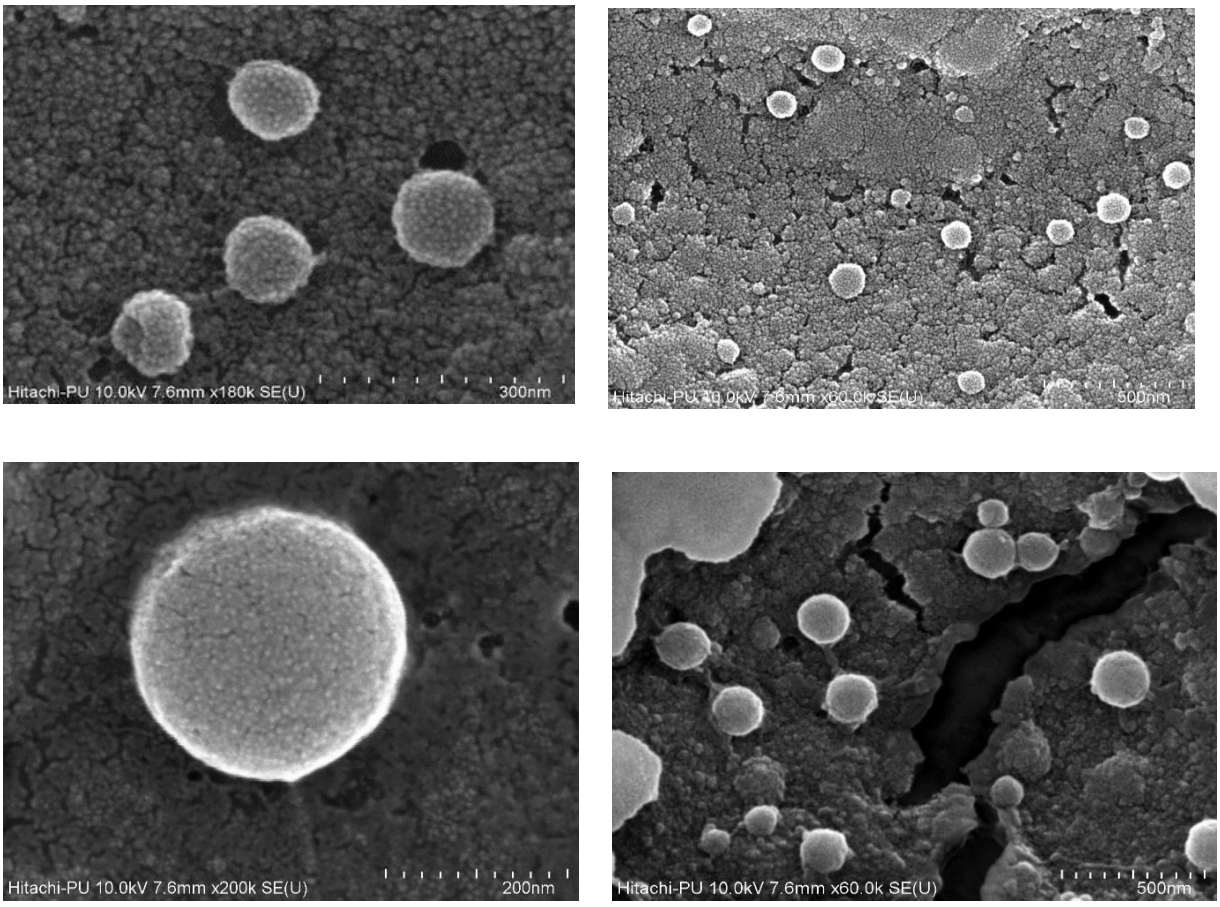

Fig 10, 11:- Surface Morphology of Selected Formulation (1:2) Drug: Polymer

5.4.4 Entrapment Efficiency:-

From the entrapment efficiency results, it may be concluded that the optimal EE (81%) was found for the formulation with drug: polymer 1:2. Further increasing the drug: polymer does not increase the EE.

Table 5: Entrapment efficiency of different drug-polymer ratios of pregabalin nanoparticles

Formulations	Entrapment Efficiency
Drug: polymer 1:1	79%
Drug: polymer 1:2	81%
Drug: polymer 1:3	75%

5.5 *In Vitro* Drug Release Study

The percentage of drug release from nanoparticle formulation (DP 1:2) was found to be higher as compared to DP 1:1 and DP 1:3, as shown in figure 12. The release was studied for up to 12 hours continuously and samples were withdrawn at different time intervals and sink conditions were maintained during the whole study process. At the end of 12 hours, the drug release from DP 1:1, DP 1:2 and DP 1:3 was found to be 90%, 93%, and 91% respectively as shown in fig 12. The percentage of drug release was nearly the same in formulations DP 1:1, DP 1:2 and DP 1:3. The maximum amount ofrelease was found in DP 1:2, i.e., 93%.

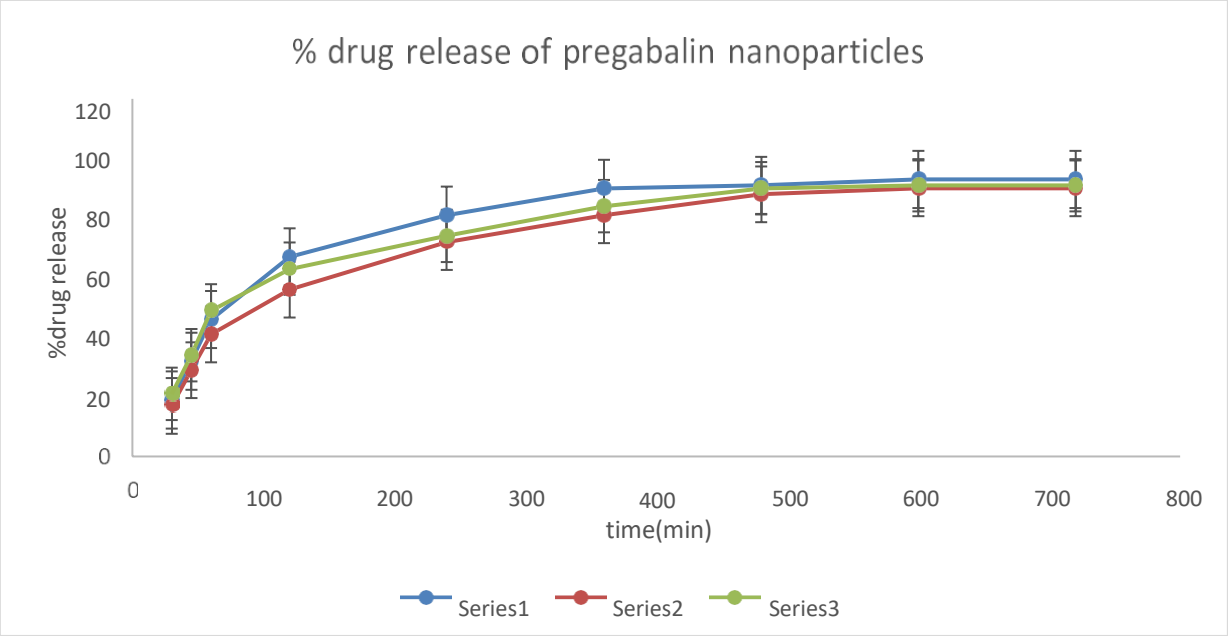

Fig 12:- in vitro drug release of prepared nanoparticle formulations DP 1:1,DP 1:2 and DP 1:3

5.6 In Vivo Bio distribution Study:

Preparation of Standard Calibration Curve using HPLC Method

The standard calibration curve of pregabalin was prepared by preparing a stock solution of pregabalin by adding 1 mg of pregabalin into 10 ml methanol. Then dilution was prepared by the addition of 100 ulof the above solution in 1900 ml of methanol and the sample was kept in the HPLC vial and then the process was repeated in triplicate and the standard curve was plotted as shown in fig 13.

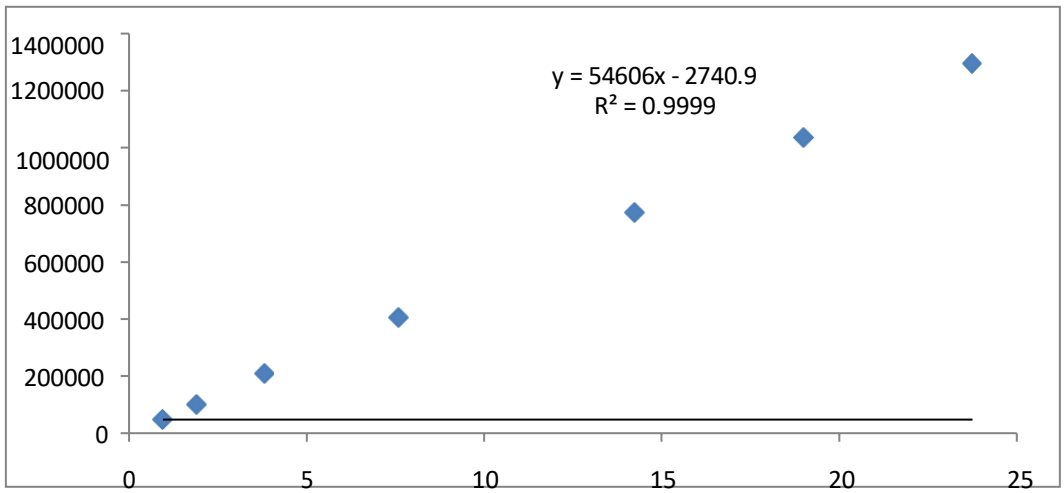

Fig 13:- Calibration curve of pregabalin by HPLC method.

The pure drug concentration achieved in the blood, heart, lungs, liver, and kidneys was 0.94 ± 0.022, 1.106 ± 0.002, 0.85 ± 0.23,0.241 ± 0.002,0.55 ± 0.01,0.375 ± 0.013 µg/g respectively, after 1 h of IV injection by tail vein. The NPs concentration achieved in the brain, blood, heart, lungs, liver and kidneys was 1.724 ± 0.002,1.39 ± 0.29,1.365 ± 0.364,0.45 ± 0.14,0.87 ±0.22,0.67 ±0.20 respectively as shown in fig 14. It was observed that t h e maximum concentration of the drug was found in the liver when delivered in the form of polysorbate80 nanoparticles. This could be due to the passive targeting of the nanoparticles. This occurs due to the uptake of particles by RES. However, in comparison to pure drug higher concentration of the drug was found in the brain when delivered in the form of polysorbate80 nanoparticles.

Table 6:- Concentration of pure drug and nanoparticles in various organs

Organs	Pure drug	DP 1:2 Nanoparticles
Liver	1.106	1.724
Blood	0.94	1.39
Brain	0.85	1.365
Lungs	0.55	0.87
Kidney	0.371	0.67
Heart	0.241	0.45

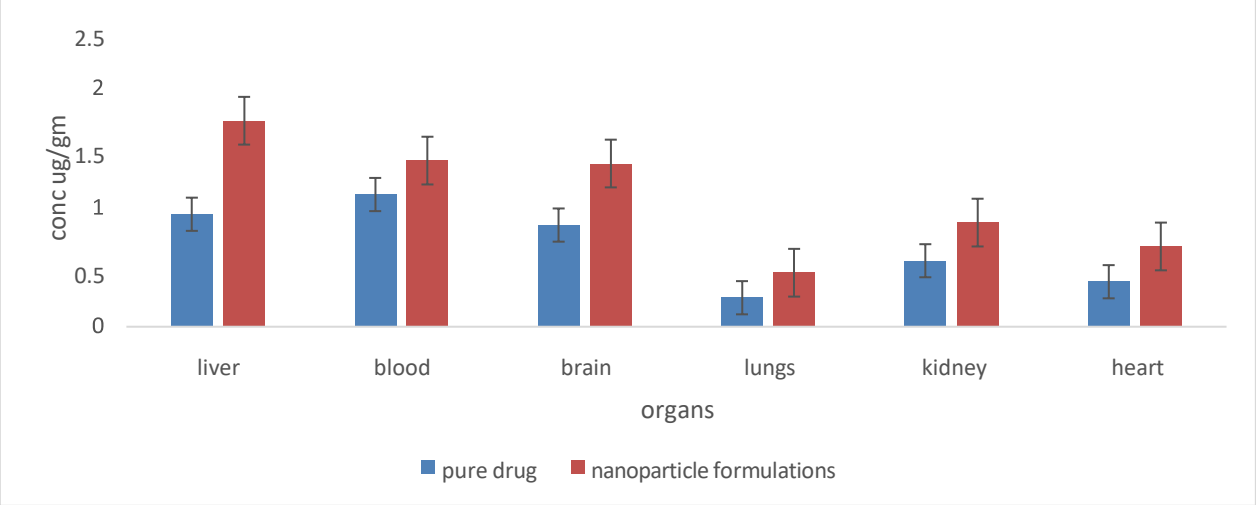

Fig 14:- PGB concs (µg/gm) in different organs after IV inj. of free drug and nanoparticles

CHAPTER 6

CONCLUSION

6. Conclusion

In this study, albumin nanoparticles of pregabalin coated with polysorbate 80 were formulated. It was concluded that nanoparticles showed particle size in the nanometric rangeless than 200 nm. *In-vitro* dissolution studies showed that faster drug release showed nearly similar drug release from the three formulations. However, the maximum release was found from DP 1:2 which was selected for *in vivo* studies. It was observed that in comparison to pure drug higher concentration of the drug was found in the brain when delivered in the form of polysorbate80 coated nanoparticles. In conclusion, pregabalin-loaded BSA nanoparticles. Nanoparticles showed a promising approach for improving the penetration of drug across BBB.

CHAPTER 7

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