ISSN No: 2348-4845
International Journal & Magazine of Engineering, Technology, Management and Research
A Peer Reviewed Open Access International Journal

Preparation and Optimization of ORO-Dispersible Films Containing Clonazepam Nanoparticles - A Novel Approach for the Better management of Epilepsy

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ABSTRACT

The aim of present study was formulation and evaluation of fast dissolving oro-dispersible film containing nanoparticles of Clonazepam poorly water soluble drug to improve solubility and onset of action in treatment of epilepsy. To improve the solubility of Clonazepam, nanosuspension was prepared by using high speed homogenizer. HPMC E15 and SDS were used to stabilize the nanosuspension. HPMC E15 is a key ingredient in formulation of film which rapidly disintegrates in presence of water or saliva. Formulations were prepared by varying the concentration of polymer and homogenization time. The formulations were achieved through the preparation of nanosuspension by homogenization and films by solvent casting method. The prepared nanoparticle loaded films were characterized by particle size, scanning electron microscopy, Fourier transfer infrared spectroscopy, film thickness, weight variation, tensile strength, surface pH and %CDR *in-vitro* drug release. The results showed that the average particle size of nanoparticle formulations were 259.3nm. The in-vitro drug release study revealed that optimized formulation the drug release was found to be 62.62%. SEM revealed nanoparticle loaded films were irregular in shape. Upon fitting of *in-vitro* drug release data into various kinetic models, the release was found to follow karsmayers-peppa's model.

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KEY WORDS:

Clonazepam, Sodium Dodecyl Sulphate, HPMC E15, Homogenization, solvent casting method.

INTRODUCTION

EPILEPSY

Epilepsy is a chronic mental disorder or condition, usually resulting in unpredictable, unprovoked recurrent seizures that affect a variety of mental and physical functions. It is one of the most common neurological diseases, affecting more than 3 million people in the U.S.[1] Epilepsy is a chronic non-communicable disease of the brain that affects people of all ages. More than 50 million people worldwide have epilepsy, making it one of the most common neurological diseases globally.[2]

As per WHO, epilepsy is one of the most common serious brain disorder that affects not only the individual, but also disturbs the family and the society in general.

Cite this article as: L.Geetha, Vijaya G Joshi, Geetha.M & Swapna.B "Preparation and Optimization of ORO-Dispersible Films Containing Clonazepam Nanoparticles - A Novel Approach for the Better management of Epilepsy", International Journal & Magazine of Engineering, Technology, Management and Research (IJMETMR), ISSN 2348-4845, Volume 7 Issue 11, November 2020, Page 1-17.

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WHO estimates that 8 per 1000 population worldwide have epilepsy, with higher prevalence in developing countries as compared to developed countries. Further, there are approximately 10 million people estimated to be with epilepsy in India accounting for 1/5th of the global burden.

Epilepsy occurs in men and women and can begin at any age, but is most frequently diagnosed in early life or in old age. Up to 5% of the world's population may have a single seizure at some time in their lives, but a diagnosis of epilepsy is reserved for those who have recurring seizures, i.e. at least two unprovoked seizures.[3]

Classification of seizures:

- **Focal seizures:** Also called partial seizures, these start in an area or network of cells on one side of the brain.
- **Generalized seizures:** Also called primary generalized, these engage or involve networks on both sides of the brain at the onset.
- **Unknown onset:** If the onset of a seizure is not known, the seizure falls into the unknown onset category.
- **Focal to bilateral seizure:** A seizure that starts in one side or part of the brain and spreads to both sides has been called a secondary generalized seizures.[4]

Symptoms:

As the epilepsy is caused by abnormal activity in the brain, seizures can affect any process of your brain coordinates. Seizure signs and symptoms include:

- Temporary confusion
- A staring spell.
- Uncontrollable jerking movements of the arms and legs
- Loss of consciousness or awareness
- Psychic symptoms such as fear, anxiety. Symptoms vary depending on the type of seizure. In most cases, a person with epilepsy will tend to have the same type of seizure each time, so the symptoms will be similar from episode to episode.[5]

Principles of management of epilepsy:

1. Any causative factors of epilepsy must be treated, e.g. cerebral neoplasm.
2. The patients should be educated about the disease, duration of treatment and need for compliance.
3. Precipitating factors should be avoided, e.g. alcohol, sleep deprivation, emotional stress.
4. Natural variation should be anticipated, e.g. fits may occur particularly or exclusively around periods in women.
5. Antiepileptic drug should be given only if seizure type and frequency require it, i.e. more than one fit every 6-12 months.[6]

CLONAZEPAM:

Clonazepam is one of the 1,4-benzodiazepine and belongs to BCS class II drugs which has low solubility and high permeability. And clonazepam is commonly used in epilepsy management, and recommended for second-line adjunctive treatment for various types of seizures (NICE 2012;Riss 2008 Shorvon 2009). It is superior to diazepam due to its lower dosage, almost complete absence of side effects, and more favourable efficacy (Shorvon 2009).[7]

Clonazepam, like other benzodiazepines, while being a first-line treatment for acute seizures, is not suitable for the long-term treatment of seizures due to the development of tolerance to the anticonvulsant effects.

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Clonazepam has been found effective in treating epilepsy in children, and the inhibition of seizure activity seemed to be achieved at low plasma levels of clonazepam.[18] As a result, clonazepam is sometimes used for certain rare childhood epilepsies; however, it has been found to be ineffective in the control of infantile spasms.[19] Clonazepam is mainly prescribed for the acute management of epilepsies. Clonazepam has been found to be effective in the acute control of non-convulsive status epilepticus. However, the benefits tended to be transient in many people, and the addition of phenytoin for lasting control was required in these patients.[20]

It is also approved for treatment of typical and atypical absences, infantile myoclonic, myoclonic and akinetic seizures. A subgroup of people with treatment resistant epilepsy may benefit from long-term use of clonazepam; the benzodiazepine clorazepate may be an alternative due to its slow onset of tolerance.[8]

- Clonazepam has also been found effective in treating other anxiety disorders, such as social phobia, but this is an off-label use.[9]

The effectiveness of clonazepam in the short-term treatment of panic disorder has been demonstrated in controlled clinical trials. Some long-term trials have suggested a benefit of clonazepam for up to three years without the development of tolerance but these trials were not placebo-controlled. Clonazepam is also effective in the management of acute mania.[10]

Restless legs syndrome can be treated using clonazepam as a third-line treatment option as the use of clonazepam is still investigational. Bruxism also responds to clonazepam in the short-term. Rapid eye movement behavior disorder responds well to low doses of clonazepam.[11]

Mechanism of action of CLONAZEPAM:

Clonazepam's primary mechanism of action is the modulation of GABA function in the brain, by the benzodiazepine receptor, located on GABA receptors, which in turn, leads to enhanced GABAergic inhibition of neuronal firing. Benzodiazepines do not replace GABA, but instead enhance the effect of GABA at the $GABA_A$ receptor by increasing the opening frequency of chloride ion channels, which leads to an increase in GABA's inhibitory effects and resultant central nervous system depression. In addition, clonazepam decreases the utilization of 5-HT (serotonin) by neurons[14] and has been shown to bind tightly to central-type benzodiazepine receptors.[15] Because clonazepam is effective in low milligram doses (0.5 mg clonazepam = 10 mg diazepam), it is said to be among the class of "highly potent" benzodiazepines.[16] The anticonvulsant properties of benzodiazepines are due to the enhancement of synaptic GABA responses, and the inhibition of sustained, high-frequency repetitive firing.[17]

Pharmacokinetics:

Clonazepam is lipid-soluble, rapidly crosses the blood–brain barrier, and penetrates the placenta. It is extensively metabolized into pharmacologically inactive metabolites, with only 2% of the unchanged drug excreted in the urine. Clonazepam is metabolized extensively via nitro-reduction by cytochromeP450 enzymesincluding CYP3A4. Erythromycin, clarithromycin, ritonavir, itraconazole, ketoconazole, nefazodone, cimetidine, and

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grapefruit juice are inhibitors of CYP3A4 and can affect the metabolism of benzodiazepines.[120] It has an elimination half-life of 19–60 hours.[8] Peak blood concentrations of 6.5–13.5 ng/mL were usually reached within 1–2 hours following a single 2 mg oral dose of micronized clonazepam in healthy adults. In some individuals, however, peak blood concentrations were reached at 4–8 hours.[18]

Oral-dispersible films:

Mouth dissolving film is the most advanced oral solid dosage form due to its flexibility and comfort in use. Mouth dissolving films are oral solid dosage form that disintegrate and dissolve within a minute when placed in mouth without taking water or chewing. This dosage form allows the medication to bypass the first pass metabolism so bioavailability of medication may be improved. [22] ODFs are also applicable when local action in the mouth is desirable such as local anesthetic for toothaches, oral ulcers, cold sores, or teething and also used where quick onset of action of drug is required. ODFs are prepared using hydrophilic polymer that rapidly dissolve /disintegrated in the mouth within seconds without water and reduces fear of chocking. Mainly the fast dissolving film can be considered as an ultra thin strip of postage stamp size with an active pharmaceutical ingredient and other excipients. Most fast dissolving films are having taste masked active ingredients. These masked active ingredients are swallowed by the saliva of patients along with the soluble and insoluble excipients.

COMPOSITION OF FILMS FORMULATION:

Composition	Concentration
Drug	1-25%
Water soluble-polymer	40-50%
Plasticizers	0-20%
Fillers, colors, flavors	0-40%.[23]

Table: composition of films formulation

Manufacturing Methods of films:

There are five methods which are used alone or in a combination with the following process for the manufacture of the fast dissolving oral films.

i) Solvent casting
ii) Semisolid casting
iii) Hot melt extrusion
iv) Solid dispersion extrusion and v) Rolling

NANOTECHNOLOGY :

The development of nanoparticle-based drug formulations has yielded the opportunities to address and treat challenging diseases. Nanoparticles vary in size but are generally ranging from 100 to 500 nm. Though the manipulation of size, surface characteristics and material used, the nanoparticles can be developed into smart systems, encasing therapeutic and imaging agents as well as bearing stealth property.[30]Nanoparticles (NPs) are defined as particulate dispersions or solid particles drug carrier that may or may not be biodegradable. The drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. The term nanoparticle is a combined name for both nanospheres and nanocapsules. Drug is confined to a cavity surrounded by a unique polymer membrane called nanocapsules, while nanospheres are matrix systems in which the drug is physically and uniformly dispersed.[31]

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OBJECTIVES

The objective of the present study:

- To prepare mouth dissolving films containing Clonazepam in the form of nanoparticles.
- To carry out evaluation parameters for nanoparticles and films.
- To perform compatibility studies on drug and polymer using Fourier Transform-Infrared Spectroscopy (FT-IR) and Differential Scanning Calorimetry (DSC).
- To carry out preliminary investigation studies with different material variables and process variables to arrive an optimum formulation.
- The current work will contribute significantly to an important research effort aimed at improving the quality of life of patient suffering from Epilepsy.

REVIEW OF LITERATURE

Deepthi A, Reddy BV, Navaneetha K. their study was aimed to formulate and evaluate fast dissolving oral films of Zolmitriptan using sodium alginate, xanthan gum and sodium starch glycolate, guar gum. They selected suitable plasticizer and its concentration on the basis of flexibility, tensile strength and stickiness of the film. The films were prepared by solvent casting method and characterized by UV, FTIR studies. The films were evaluated for disintegration time, Folding endurance, Tensile Strength, Mouth dissolving time, Thickness, content uniformity and In-vitro dissolution studies. The F5 formulation has given 98.5% drug release within 6 minutes and has a tensile strength of 1.80 MPa[33].

Bala R, Pawar P, Khanna S, Arora S designed films to dissolve upon contact with a wet surface, such as the tongue, within a few seconds, meaning the consumer can take the product without need for additional liquid. Their convenience provided both a marketing advantage and increased patient compliance. As the drug is directly absorbed into systemic circulation, degradation in gastrointestinal tract and first pass effect can be avoided. These points made this formulation most popular and acceptable among pediatric and geriatric patients and patients with fear of choking[34].

Pawar SV, Junagade MS they formulated Risperidone fast dissolving films by solvent-casting method containing HPMC E5 as polymer and Propylene glycol as plasticizer. All films prepared were smooth and elegant in appearance and showed no visible cracks and were uniform in thickness, weight and drug content. Formulation A2 is considered as the optimized formulation as it showed good % elongation (120%), good folding endurance (185), faster disintegration rate (13 sec.) and maximum in vitro drug release (93.57%) within 10 mins. No significant changes were observed during stability studies for the optimized formulation. It was concluded that Risperidone fast dissolving oral films can be formulated as a potentially useful tool for an effective treatment of Schizophrenia with improved bioavailability[35].

Patel D, Patel M, Upadhyay P, Shah N, Shah S this review gives an idea about formulation techniques, evaluation parameters, overview on packaging and some available marketed products of mouth dissolving films. Mouth dissolving film is the most advanced oral solid dosage form due to its flexibility and comfort in use. This dosage form allows the medication to bypass the first pass metabolism

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so bioavailability of medication may be improved. Mouth dissolving film has potential to improve onset of action lower the dosing and eliminate the fear of chocking. Formulation of mouth dissolving films involves both the visual and performance characteristics as plasticized hydrocolloids[36].

Ekambaram P, Sathali AA formulated and evaluated solid lipid nanoparticles of Ramipril. This drug has many side effects such as postural hypotension, hyperkalemia, and angioedema, when given as an immediate dosage form. To overcome the side effects and to increase the bioavailability of Ramipril, solid lipid nanoparticles of Ramipril are prepared by using lipids (glyceryl monostearate and glyceryl monooleate) with stabilizers (tween 80, poloxamer 188, and span 20). The prepared formulations have been evaluated for entrapment efficiency, drug content, *in-vitro* drug release, particle size analysis, scanning electron spectroscopy, Fourier transform-infrared studies, and stability. A formulation containing glyceryl monoleate, stabilized with span 20 as surfactant showed prolonged drug release, smaller particle size, and narrow particle size distribution, as compared to other formulations with different surfactants and lipids[37].

Karthikeyan D, Srinivas M, Santhosh Kumar C prepared and characterized surface modified stavudine entrapped low molecular weight chitosan (CS) nanoparticles as potential drug delivery system for anti-HIV chemotherapy. The particle size and the surface morphology results revealed that stavudine nanoparticles (SNPs) were smooth spheroidal with a size ranging from 260 nm-632 nm. The drug entrapment efficiency was found to be near 83%. In vitro release studies revealed that the rate of drug release was 93% in 24 hours. Release of drug followed zero order and show sustained release behavior. Koresmeyer peppas models showed that the drug followed non-Fickian transport with value of n>0.5. The results suggested that chitosan polymer based Nano particulate formulations were potential means to achieve release of stavudine for the prolonged period of time for effective therapy[38].

Shirsand SB, Suresh S, Swamy PV, Kumar DN, Rampure MV they designed fast dissolving tablets of clonazepam by direct compression method with a view to enhance patient compliance. Three superdisintegrants viz crospovidone, croscarmellose sodium and sodium starch glycolate in different ratios with microcrystalline cellulose (Avicel PH-102) along with directly compressible mannitol (Pearlitol SD 200) to enhance mouth feel. The prepared batches of tablets were evaluated for hardness, friability, drug content uniformity, wetting time, water absorption ratio and *in vitro* dispersion time. Based on *in vitro* dispersion time, three formulations were tested for the *in vitro* drug release pattern short-term stability and drug-excipient interaction (IR spectroscopy). Among the three promising formulations, the formulation prepared by using 10% w/w of crospovidone and 35% w/w of microcrystalline cellulose emerged as the overall best formulation ($t_{50\%}$ 1.8 min) based on the *in vitro* drug release[39].

DRUG PROFILE
CLONAZEPAM
Molecular structure

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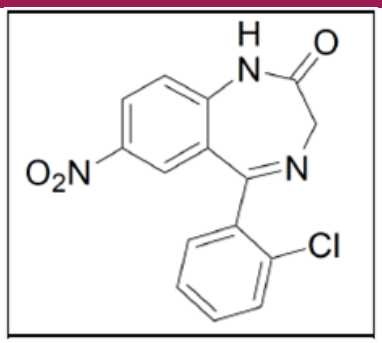

Fig. Structure of CLONAZEPAM

Molecular formula: $C_{15}H_{10}ClN_3O_3$

Molecular weight: 315.715 g/mol

Description: Clonazepam(CLO; 5-(o-chlorophenyl) -1,3-dihydro-7-nitro-2H-1,4-benzodiazepin-2-one), a benzodiazepine with prominent anticonvulsant, anxiolytic properties. It has been most effective in the treatment of typical and atypical absence, myoclonic and akinetic seizures, and infantile spasms

Solubility: It is insoluble in water and soluble in organic solvent like methanol.

Storage: Stored in tightly closed container.

EXCIPIENTS PROFILE:

SODIUM LAURYL SULPHATE:

Molecular formula: $CH_3(CH_2)_{11}SO_4$ Na

Molecular structure:

Fig. structure of SDS

Molecular weight: 288.372g/mol

Description: SLS is a colorless powder**.**

Category : SLS is a surfactant and used as a stabilizer in the formulation of nanosuspension.

Storage : stored in air tight container**.**

HYDROXY PROPYL METHYL CELLULOSE :

Molecular structure:

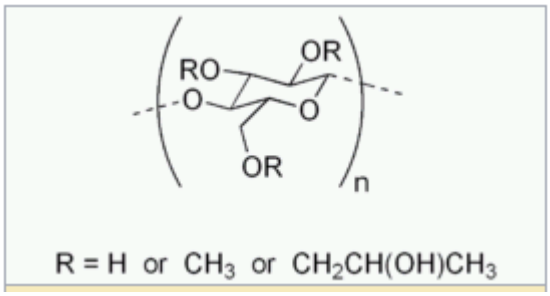

Fig. structure of HPMC

Description:

Hydroxyl propyl methylcellulose (HPMC), is a semisynthetic, inert, viscoelastic polymer used as eye drops, as well as an excipient and controlled-delivery component in oral medicaments, found in a variety of commercial products. HPMC E15 is hydrophilic and a good film forming polymer.

As a food additive, HPMC is an emulsifier, thickening and suspending agent.

Poly ethylene glycol (PEG 400):

Molecular weight: 380-420 g/mol

Molecular formula:

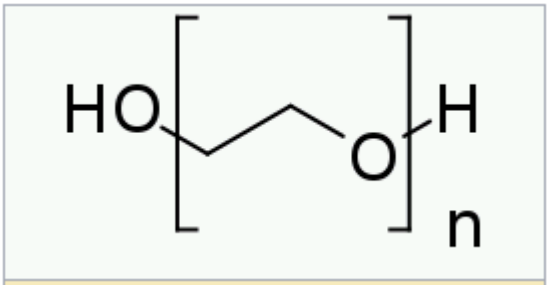

Fig.structure of PEG

Description :

PEG 400 is a low-molecular-weight grade of polyethylene glycol. It is a clear, colorless, viscous liquid.

PEG 400 is widely used in a variety of pharmaceutical formulations.

PEG 400 is strongly hydrophilic.

PEG 400 is soluble in water, acetone, alcohols, benzene, glycerin, glycols, and aromatic

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hydrocarbons, and is slightly soluble in aliphatic hydrocarbons.

METHANOL :
Molecular formula: CH_3OH
Molecular weight: 32.04g/mol
Description :
Methanol is also known as **methyl alcohol**, Methanol acquired the name **wood alcohol** because it was once produced chiefly by the destructive distillation of wood.

Methanol is the simplest alcohol, consisting of a methyl group linked to a hydroxyl group. It is a light, volatile, colorless, flammable liquid with a distinctive odor similar to that of ethanol.

Storage: stored in amber colored bottle in tightly closed container in a cool place.

5. METHODOLOGY

5.1 Drug analysis

Determination of λ_{max}:
Clonazepam was analyzed using ultraviolet (UV) spectrophotometric method. Stock solution (100µg/ml) of clonazepam was prepared in methanol. The solution was further diluted to get a concentration of 4-28µg/ml. The resultant solution was scanned for λ_{max} with in a wavelength range of 200-400nm using Shimadzu UV-Visible spectrophotometer. The λ_{max} of the drug was found to be 309nm.

PREPARATION OF STANDARD CALIBRATION CURVE OF CLONAZEPAM IN BUFFER :
Preparation of stock solution:
Accurately weighed 10mg quantity of clonazepam was transferred to 100ml volumetric flask and dissolved in phosphate of pH 6.8 using ultra sonication to give a stock solution having concentration of 100µg/ml.

Preparation of calibration curve: aliquots of 0.5-3.0ml of stock solutions were transferred to series of 10ml volumetric flasks, and volume made up to mark using methanol to give concentration range(4-28µg/ml). The absorbance was measured at 309nm against buffer as blank and calibration curve was plotted.

5.2 COMPATIBILTY STUDIES:

The formulation of a dosage form requires considerations of physical, chemical and biological characteristics of both drug and excipients used in the formulation. Compatibility must be established between the active ingredient and the excipient to produce a stable, efficacious, attractive and safe product.

5.3 Formulation design:

A central composite design is a 2^k full factorial to which the central point and the star points are added. The star points are the sample points in which all the parameters but one are set at the mean level "m". The value of the remaining parameter is given in terms of distance from the central point. If the distance between the central point and each full factorial sample is normalized to 1, the distance of the star points from the central point can be chosen in different ways:

- If it is set to 1, all the samples are placed on a hyper sphere centered in the central point (central composite circumscribed CCC).
- If it is set to $\sqrt{k}/k$, the value of the parameter remains on the same levels of the 2^k full factorial (central composite faced, or CCF).

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- This design analyzes the main effects and also helps to identify the significant factors. The lower and higher levels of independent factors are selected .The independent variables selected were polymer concentration and stirring time.

5.4 Method of preparation of nanoparticle loaded oral films:

The preparation of nanoparticle loaded oral films involves mainly 2steps:

1. Preparation of Nano suspension.
2. Preparation of oro-dispersible films.

5.5 Characterisation Of Nanoparticles Loaded Films:

PARTICLE SIZE ANALYSIS:

The particle size of all the formulations was measured using Horiba Zeta size analyzer, the nanosuspension was diluted in the ratio of 1:10 with Millipore water, vortexed for a minute and subjected for analysis. Measurements were made in triplicate, standard deviation and standard error mean was calculated.

Drug Entrapment Efficiency:

A film of 4cm^2(2×2) equivalent to 0.5mg of drug is dissolved in 10ml of buffer solution of pH 6.8 sonicated and analyzed by UV-spectrometer at 309nm.

Weight variation

Film was cut in to five different strips from casted petridish. Weight of each film was taken and variation was calculated.

Film thickness

The thickness of 3 film was measured by screw gauge micrometer at different position of film and average thickness was calculated.

Folding endurance

A film of 2 x 2 cm2 was repeatedly folded and unfolded at the same place till it breaks. The number of times, the film could be folded at same place, without breaking was recorded as the value of folding endurance. This gives an indication of brittleness of the film.

Surface pH

The film to be tested was placed in a petridish 1 ml of distilled water was added and kept for 30seconds. The pH was noted after by electrode of the pH meter allowing contact time of 1 min. the average of three measurements for each formulation was carried out.

5.6 Scanning Electron Microscopy

The shape and surface characteristics of the prepared film were evaluated by using scanning electron microscope (SEM) TESCAN-VEGA3 LMU. The samples for SEM were prepared by on both sided adhesive tape stuck to a stub. Gold palladium coating was done on the prepared stub by using sputter coater. The coated substances were then randomly scanned and photomicrographs were taken with a SEM.

5.7 In-vitro drug release studies of the films:

The drug release from film was determined by using activated cellophane membrane. A film of 4cm^2 (2×2) equivalent to 1mg transferred to a glass cylinder having a length of 7 cm and diameter of 2.5 cm (Dis-integration tubes) where one side of the tube is completely covered with pre activated cellophane membrane. This cylinder was fitted to the stand and was suspended in the beaker of the which contains 5ml of buffer solution of pH6.8. And the samples of 1ml were collected

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at 5, 10, 15, 20, 25,30mins replaced with fresh buffer and assayed using UV spectroscopy for drug content at 309 nm.

RESULTS

ANALYTICAL METHODS:

UV SPECTROPHOTOMETRIC METHOD FOR ESTIMATION OF CLONAZEAPAM

Determination of λ_{max} for clonazepam in buffer of pH 6.8 :

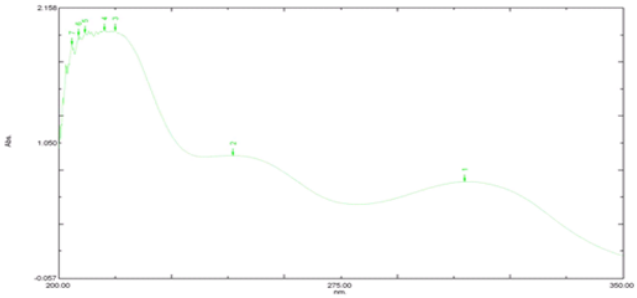

Fig. wavelength estimation of clonazepam

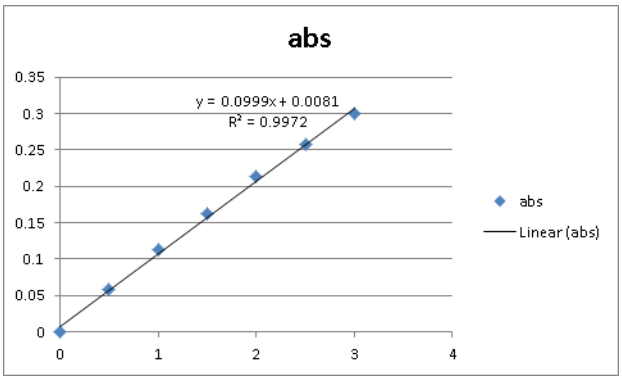

Fig: Standard calibration curve of clonazepam

COMPATIBILTY STUDIES:

FOURIER TRANSFORM INFRARED SPECTROSCOPY:

Bonds	Expected range (cm^{-1})	OBSERVED PEAKS	
		Pure drug	MIXTURE
N=O	1550-1475	1539.8	1468.0
C=O	1730-1540	1600	1695.9
C=C	1680-1600	1580	1581.7
C-Cl	600-800	750	750.9
C-H	3110-3090	3056	2968.5
N-H	3450-3310	3104	3361

Table: Functional groups present in IR spectrum of Clonazepam

FTIR SPECTRA:

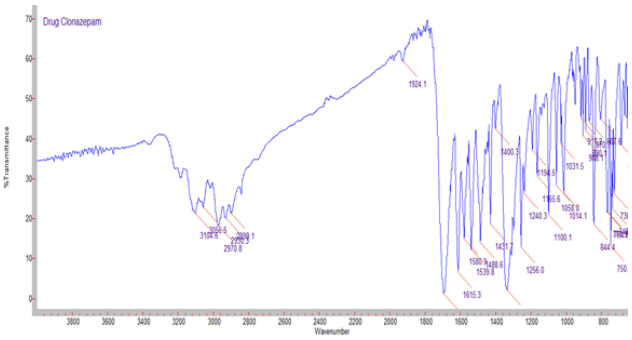

Fig: FTIR spectrum of pure drug

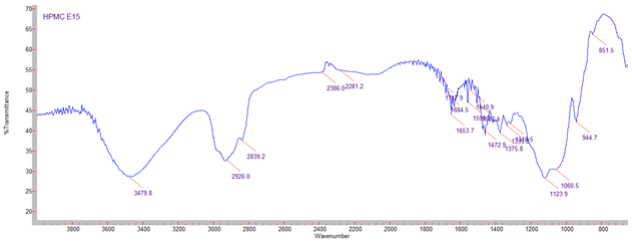

Fig: FTIR spectrum of polymer HPMCE15

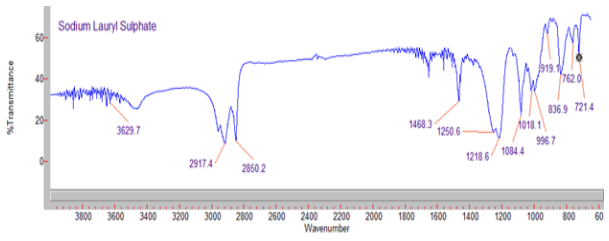

Fig: FTIR spectrum of SDS

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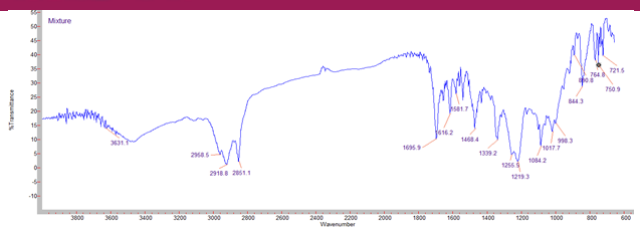

Fig: FTIR spectrum of MIXTURE

DIFFERENTIAL SCANNING CALORIMETRY:

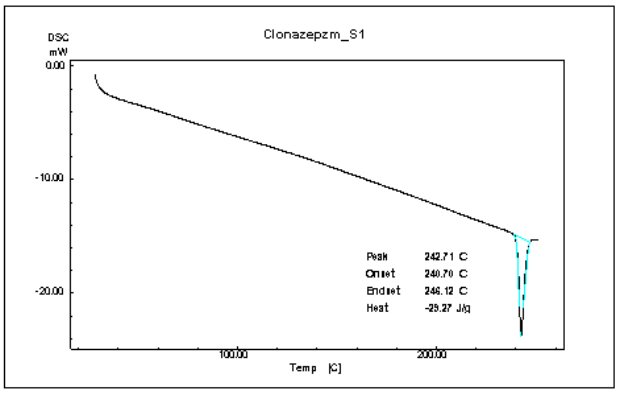

Fig: DSC of Pure drug

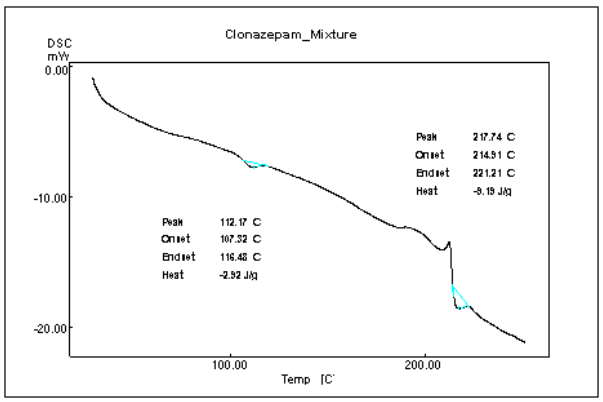

Fig: DSC of optimized formulation

***IN VITRO* DRUG RELEASE STUDIES :**

Specifications of release study:

- **Apparatus:** attached with disintegration tube
- **Dissolution medium:** Phosphate buffer pH 6.8
- **Volume of dissolution medium:** 5ml
- **RPM:** 100rpm
- **Volume of sample withdrawn:** 1ml
- **λ_{max}:** 309nm in Phosphate buffer 6.8 pH
- **Slope:** 0.0005 in Phosphate buffer 6.8 pH

***IN VITRO* DRUG RELEASE**:

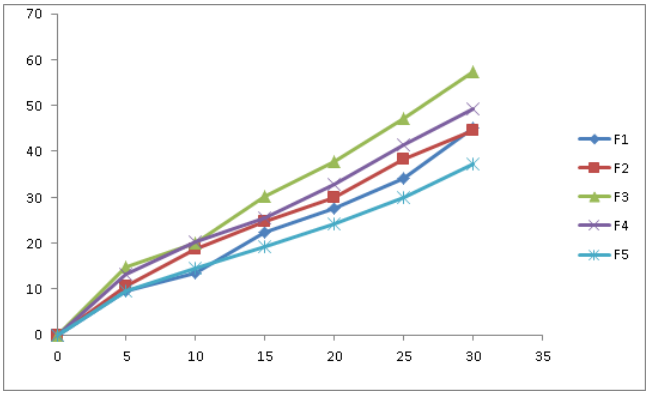

Fig No: Graphical representation for % CDR of formulations (F_{1-5})

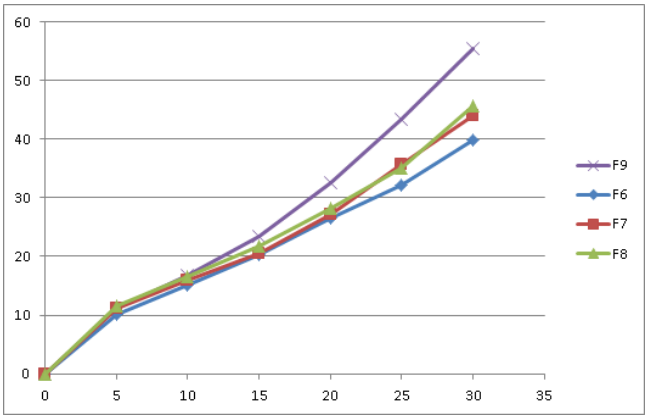

Fig No.: Graphical representation for % CDR of formulations (F_{6-9})

6.5 OPTIMIZATION

ANOVA for Quadratic model

Response 1: particle size

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Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1.120E+05	5	22399.68	1.89	0.3184
A-polymer conc	9664.11	1	9664.11	0.8153	0.4331
B-homogenization time	43401.01	1	43401.01	3.66	0.1516
AB	219.04	1	219.04	0.0185	0.9005
A^2	5195.20	1	5195.20	0.4383	0.5553
B^2	53519.01	1	53519.01	4.52	0.1236
Residual	35558.50	3	11852.83		
Cor Total	1.476E+05	8			

Table: Factorial model for response Particle size.

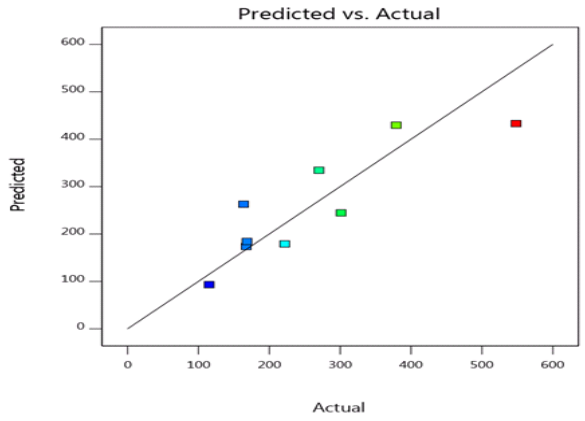

Fig No: Predicted v/s actual correlation of Particle size

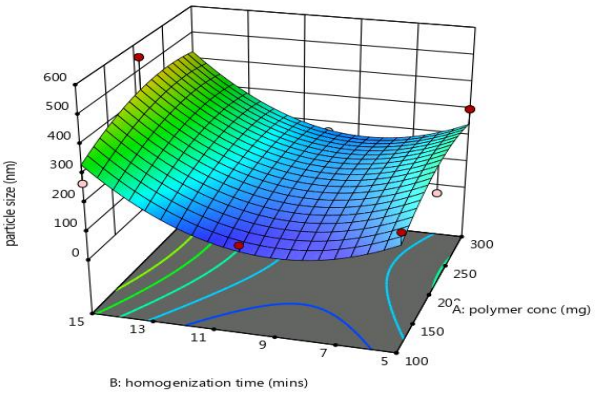

Fig No: 3D response graph of Particle size

ANOVA for Linear model

Response 2: %CDR

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	541.98	2	270.99	12.68	0.0070	significant
A-polymer conc	466.58	1	466.58	21.82	0.0034	
B-homogenization time	75.40	1	75.40	3.53	0.1095	
Residual	128.28	6	21.38			
Cor Total	670.26	8				

Table: Factorial model for response DEE.

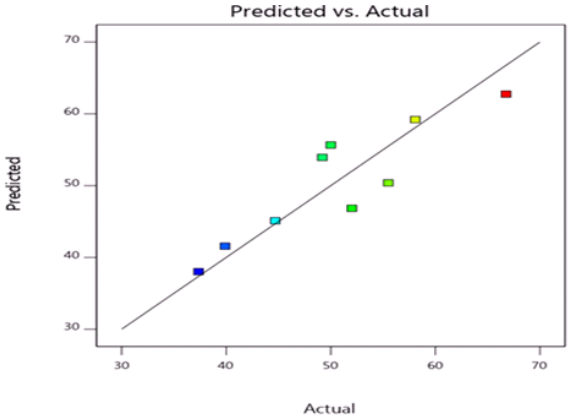

Fig No: Predicted v/s actual correlation of DEE

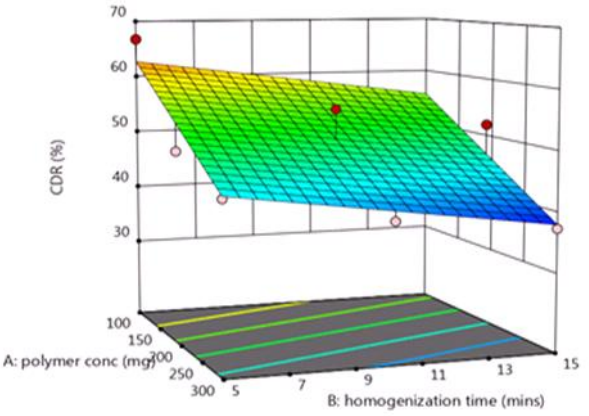

Fig No: 3D response graph of DEE.

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CHARACTERIZATION OF OPTIMIZED FORMULA:

EVALUATION PARAMETERS	RESULTS
Particle size (nm)	159
% CDR	62.6262
Thickness (mm)	0.0799
Weight (mg)	0.1921
Folding endurance	199
Surface pH	7.101
Disintegration time	56

Table: Results of optimized formulation

In vitro drug release of optimized formula:

Time	Log Time	SQRT Time	% Cumula Release	Log % Release	% Drug remaining	Log % Dru remaining
5	0.698970004	2.236067977	11.6161	1.065060342	88.3839	1.94637316
10	1.000	3.162	17.9798	1.255	82.0202	1.91392082
15	1.176	3.873	26.6666	1.426	73.3334	1.86530182
20	1.301	4.472	35.4545	1.550	64.5455	1.80986596
25	1.398	5.000	46.6666	1.669	53.3334	1.72699927
30	1.477	5.477	62.6262	1.797	37.3738	1.57256725

TABLE: *in-vitro* drug release study of optimized formula

GRAPHICAL REPRESENTATION OF OPTIMIZED FORMULA DRUG RELEASE

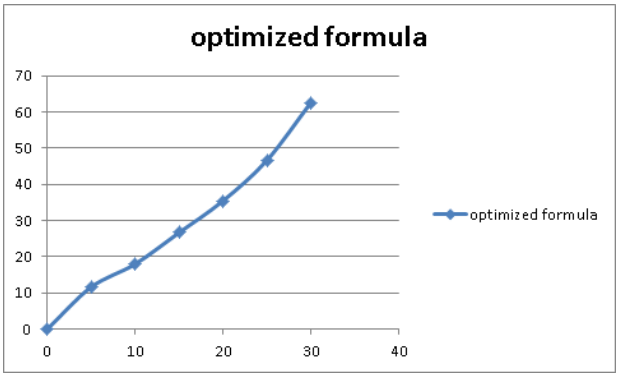

Fig no.19 Graphical representation of optimized formulation drug release.

SCANNING ELECTRON MICROSCOPY

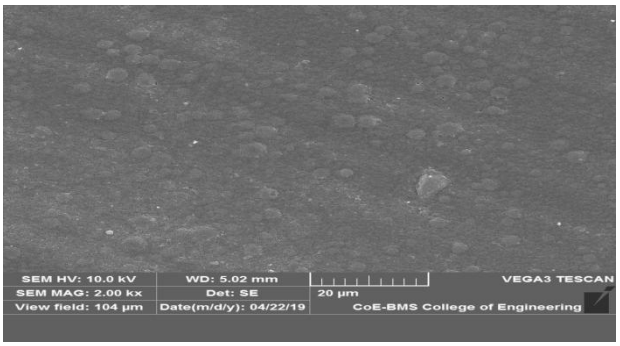

Fig No.20 SEM image of optimized formulation

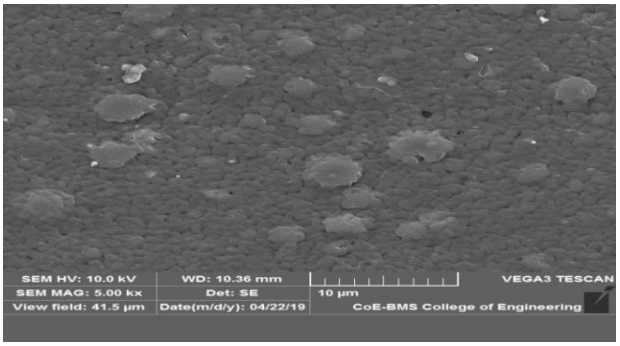

Fig no.21 SEM images of optimized formulation

DRUG RELEASE KINETICS

Reports:

	Zero order	Korsmeyer-Peppas	Higuchi	First Order
$K_{0(Slope)}$	1.999422	1.1110	4.337590128	-0.014
R^2	0.9760	0.9898	0.9274	0.9228

Table: Values of various kinetics models

Graphical representation of drug release kinetic models:

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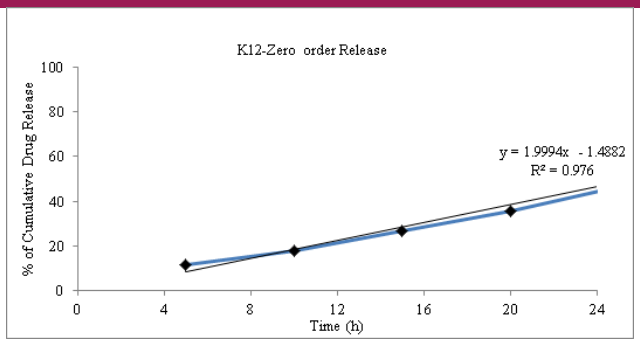

Fig No. 22 Graphical representation of zero order release from optimized Formulation.

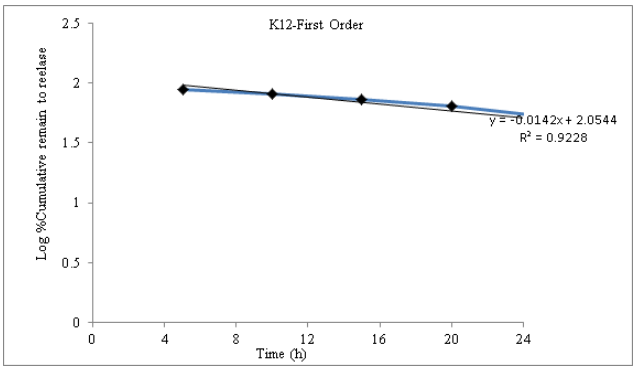

Fig No.23 Graphical representation of first order release of optimized formulation

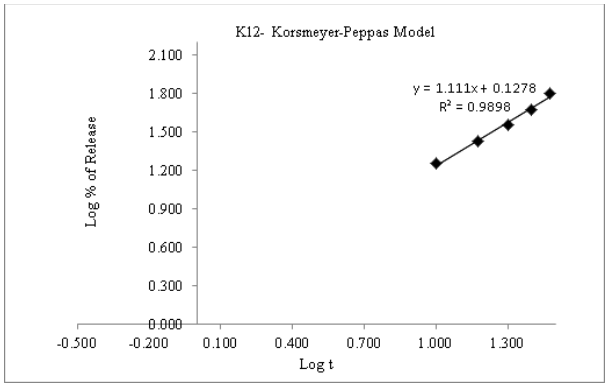

Fig No.24 Graphical representation of Peppas model from optimized formulation

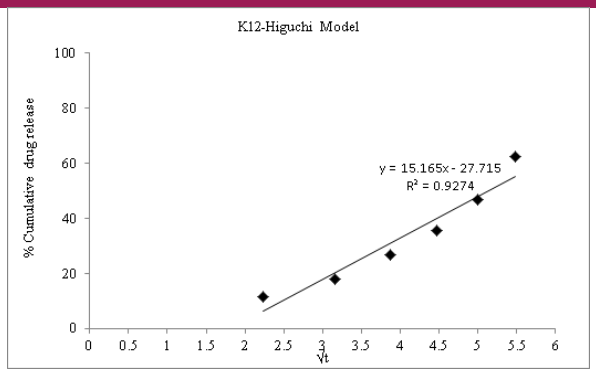

Fig No.25 Graphical representation of Higuchi from optimized formulation.

7. CONCLUSION

- In the present work, Clonazepam nanoparticle loaded films were prepared using homogenization and film casting method. Details regarding the preparation and evaluation of formulated Nanoparticle loaded films have been discussed in the previous chapters. From the study following conclusions were drawn:
- The results of this investigation indicated that homogenization method can be employed successfully to formulate nanoparticles.
- FT-IR spectra and DSC thermograms of the pure drug, physical mixture and formulations revealed that the drug is compatible with the polymer used.
- SEM analysis of the nanoparticle loaded films revealed that all the prepared nanoparticles were irregular in shape and had smooth surface morphology.
- The particle size and %CDR were depend on homogenization time and polymer concentration.
- The *in-vitro* drug release was found to decrease with increase in polymer concentration. Further the release was controlled by it. From the drug release kinetics study, it was found that the drug release followed Korsmeyer-Peppas model.

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International Journal & Magazine of Engineering, Technology, Management and Research
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8. SUMMARY

The aim of the present work was to formulate and evaluate nanoparticle loaded films containing
Clonazepam for immediate release for better management of epilepsy.

The scheme of work has been divided into various parts. The collection of theoretical and technical data by extensive literature survey, review of literature and drug profile is presented in chapter 3 and 4 respectively. This was followed by procurement and screening of all materials used in the formulation of nanoparticle loaded films. The nanoparticle loaded films were prepared by homogenization and solvent casting method using HPMC E15as polymer.

The nanoparticle loaded films were prepared where drug concentration was maintained constant. Polymer concentration and homogenization time were two independent variables. Evaluation responses namely particle size and %CDR were considered. The optimized formulation was evaluated for SEM, DSC, *In -vitro* release kinetics. The results obtained have been discussed in chapter 6. Based on the desirability the optimized formulation was selected. Results of FT-IR and DSC revealed that there was no chemical interaction between the drug and the polymer used. The obtained nanoparticle were irregular in shape. The kinetic model fitting data showed that the release of drug from nanoparticle loaded films followed Karsmayer-peppa's model.

It is evident from study that Epilepsy can be managed by Films containing nanoparticles of Clonazopam by fast releasing the drug in the mouth and thereby better absorption of Drug. However Kinetic studies need to be carried out for further confirmation.

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