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ENHANCING THE ORAL BIOAVAILABILITY OF CARBAMAZEPINE THROUGH NANOSUSPENSION TECHNOLOGY

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ABSTRACT

Carbamazepine is an antiepileptic drug with poor aqueous solubility, which can limit its dissolution and oral absorption. The present study was aimed at developing carbamazepine nanosuspension by the anti-solvent precipitation technique to reduce particle size and improve dissolution characteristics. Carbamazepine nanosuspensions were prepared using ethanol as the solvent and PVP K90, sodium lauryl sulfate (SLS), and Tween 80 as stabilisers. Four formulations (F1–F4) were prepared with different concentrations of stabilisers and evaluated for drug content, viscosity, particle size, polydispersity index (PDI), zeta potential, and *in-vitro* drug release. Pre-formulation studies including organoleptic properties, melting point, solubility, FTIR spectroscopy, λ max determination, and calibration curve were also performed. Among the formulations, F2 was selected as the optimized formulation. F2 showed 97.20% drug content, 1.77cP viscosity, 153.45nm particle size, and 0.335 PDI. The zeta potential was found to be −32.3mV. *Invitro* drug release using the egg shell membrane diffusion method showed 89.15% cumulative drug release at 360 minutes. Release kinetics showed the highest R^2 value for the Higuchi model (0.987). The study indicates that anti-solvent precipitation is a suitable approach for developing carbamazepine nanosuspension with improved dissolution characteristics and potential for enhanced oral delivery.

KEYWORDS: Carbamazepine, Nanosuspension, Anti-solvent precipitation, Bioavailability enhancement, Particle size, Zeta potential, *Invitro* drug release, Release kinetics.

INTRODUCTION

NANOTECHNOLOGY[1]

Nanotechnology is the field of science and engineering concerned with the design, fabrication, characterisation, and application of materials and devices that have at least one dimension on the nanometer scale (1–100 nm), where one nanometer represents one-billionth of a meter. This technology enables the development of materials with unique physical, chemical, and biological properties due to their extremely small size. Nano-science and nanotechnology have the potential to significantly improve human healthcare by offering innovative solutions in drug development, water purification, and the production of stronger and lighter materials. Their application in healthcare research is expected to provide substantial medical benefits and enhance the prevention, diagnosis, and treatment of diseases. Nanotechnology-based drug delivery systems can protect drugs from degradation, thereby improving their stability and therapeutic effectiveness. These systems can also reduce the required dosing frequency, enhance patient compliance, and lower the overall cost of treatment.

NANOSUSPENSION[2]

Nano-suspensions are submicron-sized colloidal dispersions composed of nanometer-sized drug particles that are stabilised using surfactants or other suitable stabilising agents. They contain poorly water-soluble drug particles dispersed in a liquid medium without the use of a carrier or matrix material. It is a pharmaceutical

dosage form in which poorly water-soluble drug particles are uniformly dispersed in an aqueous medium for administration through oral, topical, parenteral, or pulmonary routes. Nanosuspensions are an effective strategy for improving the solubility of drugs that exhibit poor solubility in both aqueous and lipid media. The particle size of drug crystals in nanosuspensions is generally below 1 µm, with an average size ranging from 200 to 600 nm**.** By enhancing drug solubility and dissolution rate, nanosuspensions increase the rate of drug absorption, allowing the drug to reach its maximum plasma concentration more rapidly and improving its bioavailability.

ADVANTAGES[3]

- Improves the solubility and bioavailability of drugs.
- Suitable for delivering poorly water-soluble drugs.
- Allows higher drug loading compared to many conventional dosage forms.
- Enables dose reduction by improving therapeutic efficiency.
- Enhances the physical and chemical stability of drug molecules.
- Facilitates passive targeting of drugs to the desired site of action.

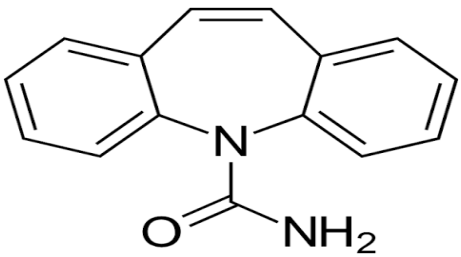
DISADVANTAGES[3]

- Nano-suspensions may undergo physical instability, including sedimentation, aggregation, and particle compaction during storage.
- Inaccurate dosing may occur if the suspension is not properly dispersed before administration.
- Achieving a uniform and precise dose can be challenging due to particle settling.
- Careful handling, storage, and transportation are required to maintain the stability and quality of the formulation.
- Uniform and accurate dosing can only be ensured when the suspension is thoroughly redispersed before use.

Drug Profile

Drug: Carbamazepine
IUPAC name: 5H-dibenzo[b,f]azepine-5-carboxamide
Molecular formula: $C_{15}H_{12}N_2O$

Structure:



Molecular weight: 236.27g/mol
Melting point: $189^0C - 193^0C$
Solubility: Pratically insoluble in water and soluble in ethanol
Half Life: 12-17hrs
Side Effects: Dizziness, Nausea, Dry mouth, Blurred or Double Vision.
Uses: Epilepsy, Trigeminal Epilepsy

MATERIALS AND METHODS

Materials

The carbamazepine was purchased from Dhamtec Pharma Pvt. Ltd. Polyvinylpyrrolidone K90 and Sodium Lauryl Sulphate were purchased from Narvon Pharma Pvt. Ltd. Tween 80 was purchased from Mohini Organics Pvt. Ltd.

Methods

Organoleptic Properties[4]

The colour, odour and taste of the drug were recorded using descriptive terminology.

Determination of Melting Point[7]

A small amount of drug was loaded into a capillary tube, where one end of the capillary tube was closed, and kept in the melting point apparatus; the temperature was noted when the drug melted.

Solubility Profile

The solubility of Carbamazepine was determined by10mg of the drug to the solvent. The drug was added in increments of 10mg. The quantity after which there is no further solubilisation of the drug was taken as the endpoint of drug solubility.

Determination of λ max

Carbamazepine (1000 µg/ml) was prepared by dissolving 10mg of carbamazepine in 10ml of methanol with vigorous shaking. 1mLof this standard stock solution was transferred to a 10 mL volumetric flask and diluted to volume with methanol to obtain a working concentration of 100µg/ml. Subsequently, 1 mL of this solution was diluted to 10 mL with methanol to achieve a final concentration of 10µg/ml. This solution was then scanned at 284nm in a UV-visible spectrophotometer to attain the absorption maximum (λ max).

Calibration Curve[5]

10mg of Carbamazepine was dissolved in 0.1N HCl (10mL) to get a 1mL/mg concentration, which can also be said to be 1000 (µg/ml) and is referred to as a stock solution. From the above stock solution 1ml was pipetted out and diluted with 0.1N HCl and make upto 10mL (100 µg/ml). From the stock, again pipette out 1ml and make up to 10 mL with 0.1N HCl (10µg/ml) and the concentration was referred to as the primary standard solution. From the above primary standard solution, the following working standard solutions are prepared0.2ml, 0.4ml, 0.6ml, 0.8ml, 1.0ml is referred to as 2,4,6,8,10(µg/ml) by diluting the primary standard solution with appropriate amount of 0.1N HCl. The working standard solutions were then measured at

284nm using a UV-visible spectrophotometer. The plot of absorbance vs concentration was plotted.

Fourier Transform Infrared Spectroscopy[6]

FTIR spectroscopy is one of the important analytical techniques for chemical identification. The drug-polymer interaction was studied by using FTIR spectroscopy. The spectra were recorded for the pure drug using Shimadzu. The scanning range was 4000 – 400 Cm^{-1}.

METHOD OF PREPARATING NANOSUSPENSION[7]

Nanosuspension was prepared by the anti-solvent precipitation method. First, the drug was mixed in an organic solution, such as ethanol, which is used to dissolve the drug completely; this is the solvent phase. Secondly, the anti-solvent phase was prepared in which the polymers and surfactant were dissolved in distilled water. Then the solvent phase was mixed with the anti-solvent phase dropwise under constant stirring using a magnetic stirrer at 100RPM for 15 min. And at last, distilled water was added to make the volume 100 mL.

Batches	Drug(mg)	Ethanol(ml)	PVP k90(mg)	SLS(mg)	Tween 80(mg)	Distilled water(ml)
F1	100	5	125	25	0.05	q.s to 100ml
F2	100	5	250	50	0.10	q.s to 100ml
F3	100	5	375	75	0.15	q.s to 100ml
F4	100	5	500	100	0.20	q.s to 100ml

EVALUATION OF NANOSUSPENISON

1) Drug Content[8]

The dispersed system was assayed spectrophotometrically for the drug content at a wavelength of 284nm with proper dilution of the formulation taking 0.1N HCl.

2) Viscosity Determination[9]

The prepared nanosuspension viscosity was determined using an Ostwald viscometer. The viscosity for nanosuspension range is between from 1- 5 centipoise

3) Particle size and Polydispersity Index[9]

Particle size and Polydispersity index analysis studies are carried out to find out the diameter of the particles present in the nanosuspension. Particle size and Polydispersity index analysis were done using a Malvern instrument.

4) Zeta Potential[10]

Zeta potential studies are used to know the overall stability of the Nanosuspension. Zeta potential analysis was done using a Zeta sizer(Malvern instrument).

5) Scanning Electron Microscopy[11]

The surface morphology of carbamazepine nanosuspension was visualised with a field-emission scanning electron microscope. Before observation, the samples were fixed onto metal stubs using double-sided sticky tape previously secured onto aluminium stubs and then coated with gold under a vacuum.

***6) In vitro* drug release[12]**

An *in vitro* dissolution test was carried out using an eggshell membrane. Carbamazepine nanosuspension was loaded in egg shell membrane and cover it tightly, and this membrane was kept in a 250ml in a medium of 0.1N HCl and maintained at 37± 0.5^0C with 100rpm by using a magnetic stirrer under constant stirring. At specified intervals, an aliquot of 5ml sample was withdrawn from the medium and replaced with fresh medium containing 0.1N HCl. After proper dilution, the samples were analysed for the drug using a UV-visible spectrophotometer at 284nm for 0.1N HCl.

***7) In vitro* drug release kinetics[13]**

The release data obtained from the in vitro drug release study were fitted to different mathematical kinetic models to determine the mechanism and kinetics of Carbamazepine release. The data were found by using zero-order, first-order, Higuchi and Korsmeyer–Peppas models.

Zero-order model

$Q = Q_0 + K_0t$

where Q is the cumulative amount of drug released at time t, and K_0 is the zero-order release constant. A plot of cumulative percentage drug release versus time was prepared.

First-order model

$\log (100 - Q) = \log 100 - K_1t/2.303$

where Q represents the cumulative percentage of drug released at time t, and K_1 is the first-order release constant. A plot of log percentage drug remaining versus time was prepared.

Higuchi model

$Q = K_H\sqrt{t}$

where Q is the cumulative amount of drug released, and K_H is the Higuchi release constant. A plot of cumulative percentage drug release versus square root of time was prepared.

Korsmeyer–Peppas model

$Mt/M\infty = Kt^n$

or

$\log(Mt/M\infty) = \log K + n \log t$

where Mt/M∞ is the fraction of drug released at time t, K is the release rate constant, and n is the release exponent. A plot of log cumulative fraction of drug released versus log time was prepared.

The coefficient of determination (R^2) obtained from each kinetic model was compared. The model showing the highest R^2 value was considered the best-fitting kinetic model. The release exponent (n) obtained from the Korsmeyer–Peppas model was used to indicate the possible drug-release mechanism.

RESULT AND DISCUSSION

1) ORGANOLEPTIC PROPERTIES

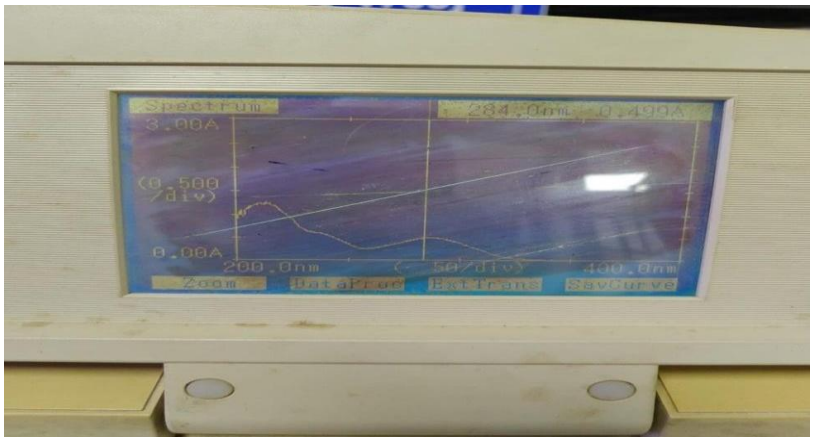
S.NO	PARAMETER	OBSERVATION
1.	Colour	White to off-White
2.	Odour	Odourless
3.	Appearance	White Crystalline Powder

2) MELTING POINT

S.NO	MELTING POINT	AVERAGE
1.	190^0C	190^0C
2.	191^0C	
3.	190^0C	

3) SOLUBILITY PROFILE

S.NO	SOLVENT	SOLUBILITY
1.	Acetone	Sparingly Soluble
2.	Dimethyl Chloride	Freely Soluble
3.	Ethanol	Freely Soluble
4.	Water	Insoluble
5.	0.1N HCl	Soluble

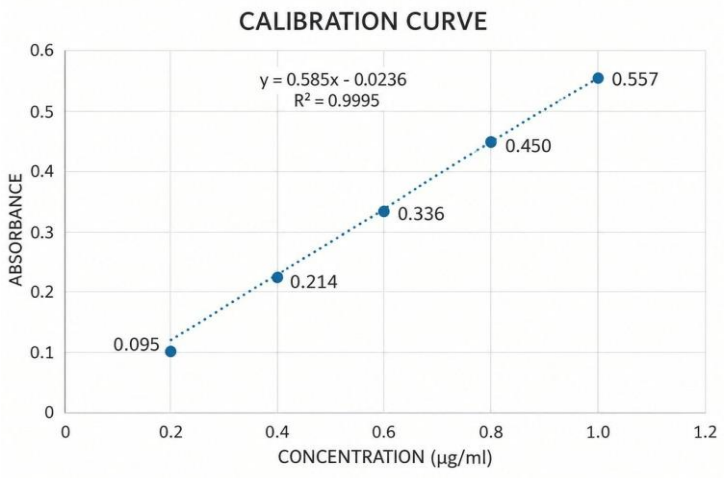


Calibration Curve

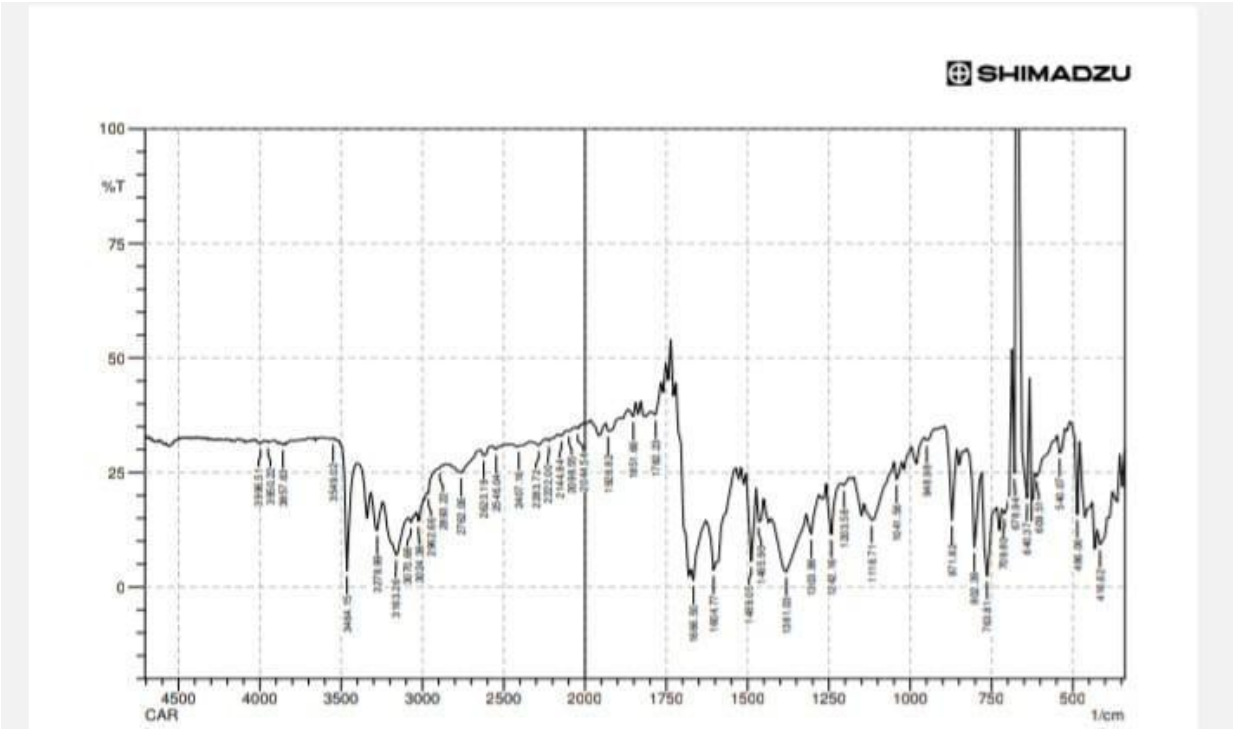
The wavelength of maximum absorbance (λ max) of carbamazepine was obtained at 284nm with 0.1N HCl. The calibration curve was found to be linear in the range of 2-10µg/ml. and straight line equation was obtained, and having regression coefficient value of 0.9995.

Data calibration curve of carbamazepine with 0.1N HCl

S.NO	Concentration(µg/ml)	Absorbance
1	2	0.095
2	4	0.214
3	6	0.336
4	8	0.450
5	10	0.557



5) FTIR STUDY FOR CARBAMAZEPINE



Interpretation	Wave Number(cm^{-1})
N-H Stretching	3465
N-H Stretching	3161
C-H Stretching	3050
C=O Stretching	1680
C=C Stretching	1600
C=C Bending	1488

6) DETERMINATION OF DRUG CONTENT

FORMULATION CODE	%DRUG CONTENT
F1	88.64%
F2	**97.20%**
F3	92.74%
F4	91.38%

Inference

The percentage drug content was found to be in the range from 88.64% to 97.20%.

7) VISCOSITY DETERMINATION

FORMULATION	FLOWTIME(sec)	Viscosity(cP)
F1	65	1.84 cP
F2	**59**	**1.77 cP**
F3	63	1.88 cP
F4	67	1.89 cP

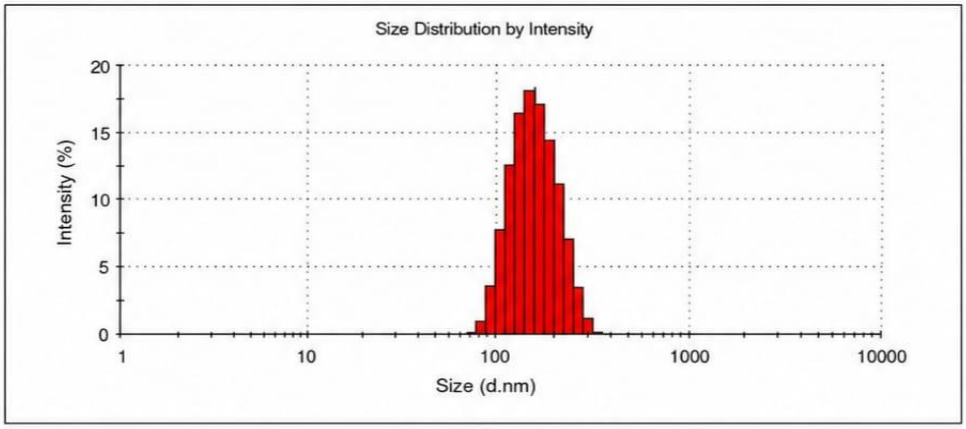
Inference

The Viscosity of given formulation found in the range between 1.77cP to 1.89cP.

8) DETERMINATION OF PARTICLE SIZE AND POLYDISPERSITY INDEX

Results

			Size (d.nm):	% Intensity	Width (d.n.):
Z-Average (d.nm):	153.42	Peak 1:	152.3	100.0	68.72
PdI:	0.335	Peak 2:	0.000	0.0	0.000
Intercept:	0.356	Peak 3:	0.000	0.0	0.000
Result quality :	Good				



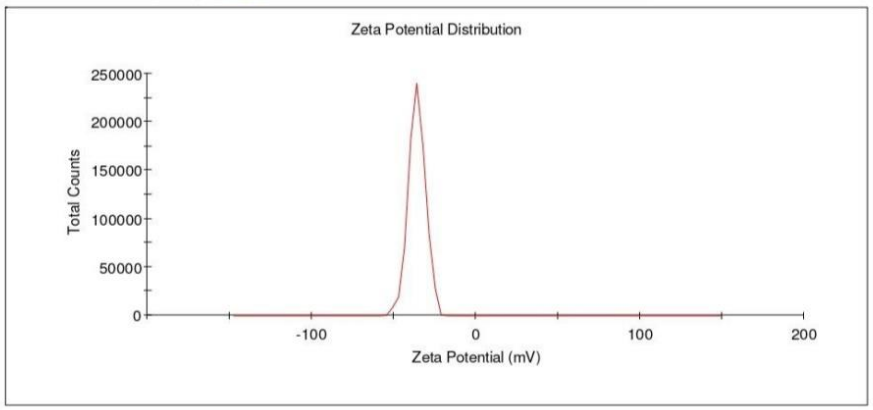
Inference

The particle size and poly dispersity of the formulation F2 was found to be 153.42 nm and 0.335.

9) DETERMINATION OF ZETA POTENTIAL

Results

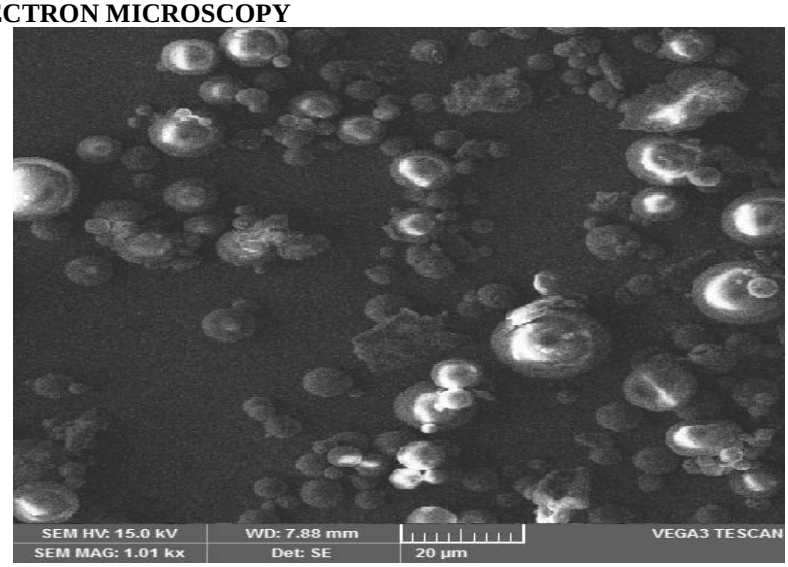
			Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV):	-32.3	Peak 1:	-32.3	100.0	6.14
Zeta Deviation (mV):	4.73	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm):	1.016				
Result quality :	Good				



Inference

The Zeta Potential of the formulation F2 was found to be -32.3 mv and shows the formulation is stable.

10) SCANNING ELECTRON MICROSCOPY

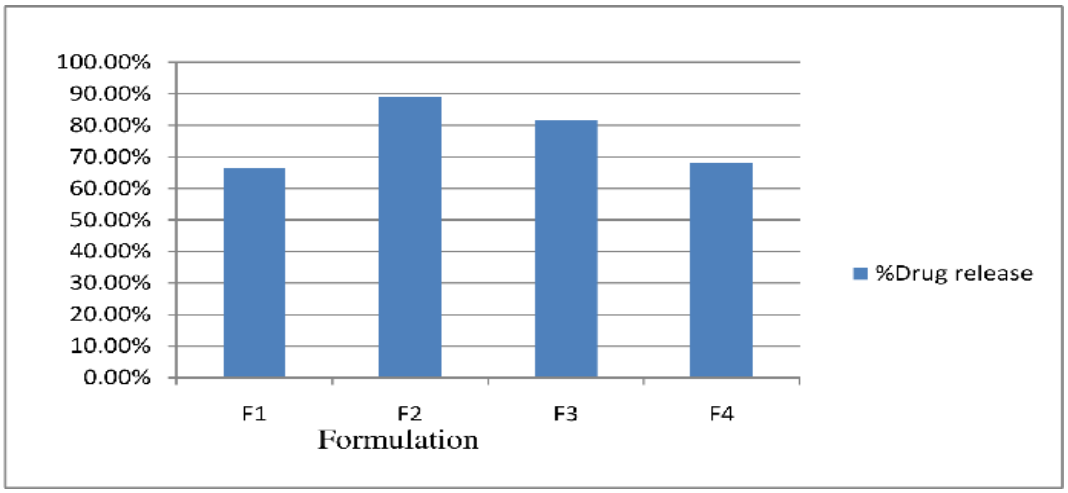


Inference

The SEM micrograph of prepared nanosuspension showed that the nanoparticles was synthesized in spherical shape.

***11) IN-VITRO* DRUG RELEASE**

Time(hrs)	Cumulative Percentage Drug Release			
	F1	F2	F3	F4
0	0	**0**	0	0
0.25	8.43%	**11.48%**	9.03%	9.11%
0.50	10.65%	**17.03%**	17.15%	10.32%
0.75	13.93%	**26.05%**	23.90%	13.17%
1	18.65%	**30.09%**	30.10%	14.54%
1.5	22.17%	**41.32%**	38.80%	18.92%
2	29.01%	**46.86%**	45.02%	22.27%
2.5	32.46%	**51.32%**	50.91%	28.25%
3	38.71%	**54.52%**	57.25%	30.91%
3.5	41.91%	**59.75%**	59.60%	36.10%
4	48.75%	**62.82%**	65.21%	40.96%
4.5	53.58%	**70.22%**	68.79%	47.95%
5	59.08%	**77.20%**	72.58%	52.84%
5.5	62.02%	**82.16%**	77.36%	60.37%
6	66.46%	**89.15%**	81.78%	68.04%

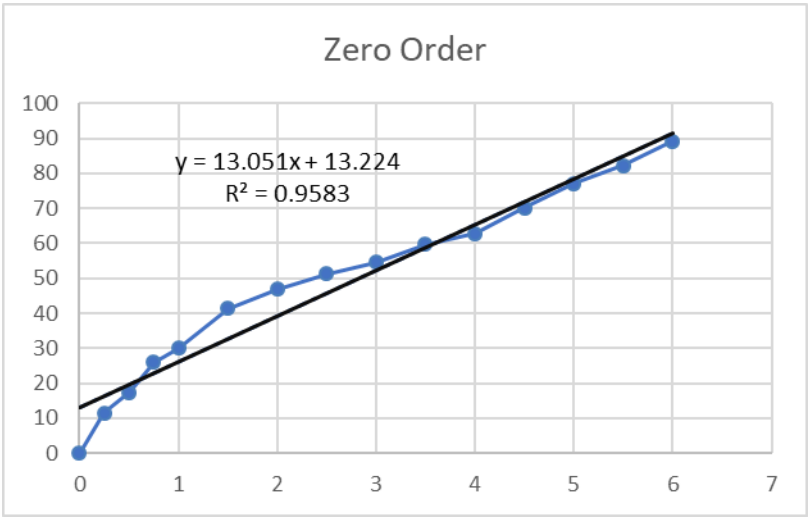


Cumulative Percentage Drug Release

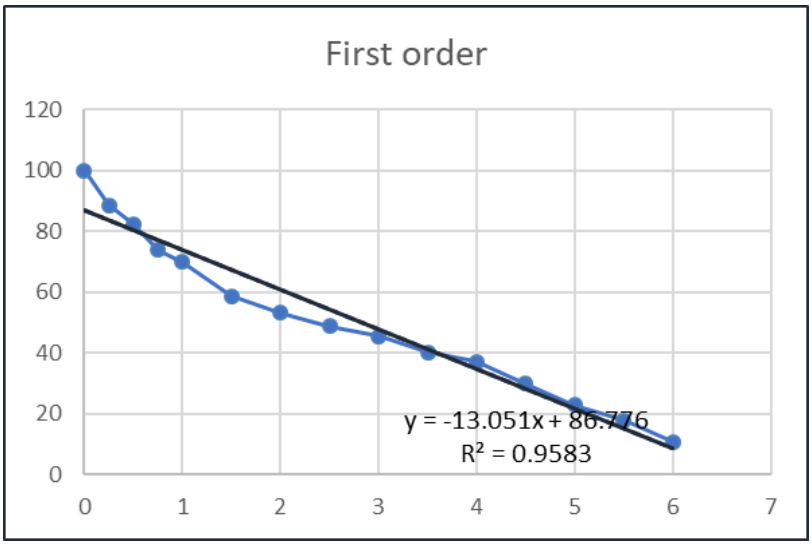
12) RELEASE KINETICS OF CARBAMAZEPINE NANOSUSPENSION

Time (hours)	Log time	Square root of time	Cumulative %drug release	Cumulative % drug remaining	Log cumulative %drug release	Log cumulative % drug release remaining	Cube root of cumulative %drug remaining
0	∞	0	0	100	∞	2	4..642
0.25	-0.602	0.5	11.48	88.52	1.0591	1.947	4.458
0.5	-0.301	0.707	17.03	82.97	1.231	1.918	4.361
0.75	-0.124	0.866	26.05	73.95	1.415	1.868	4.198
1	0	1	30.09	69.91	1.478	1.844	4.119
1.5	0.176	1.224	41.32	58.68	1.616	1.768	3.891
2	0.301	1.414	46.86	53.14	1.670	1.725	3.759
2.5	0.397	1.581	51.32	48.68	1.710	1.687	3.652
3	0.477	1.732	54.52	45.48	1.736	1.657	3.568
3.5	0.544	1.870	59.75	40.25	1.776	1.604	3.429
4	0.602	2	62.82	37.18	1.798	1.570	3.337
4.5	0.653	2.121	70.22	29.78	1.846	1.473	3.100
5	0.698	2.236	77.20	22.8	1.887	1.357	2.835
5.5	0.740	2.345	82.16	17.84	1.914	1.251	2.613
6	0.778	2.449	89.15	10.85	1.950	1.035	2.212

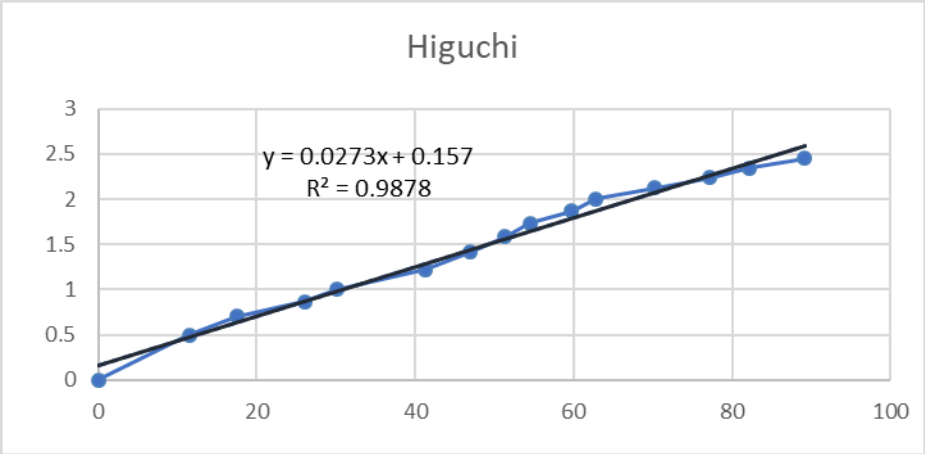
ZERO ORDER KINETICS



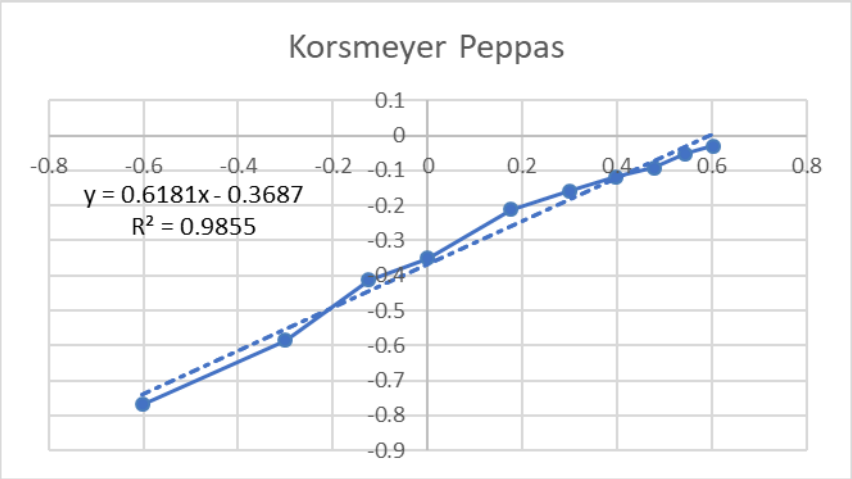
FIRST ORDER KINETICS



HIGUCHI RELEASE KINETICS



KORSMEYER PEPPAS PLOT KINETICS



The co efficient of determination (R^2) was taken as standard for choosing the most appropriate model. The R^2 values of different models are given in Table.

Table No. 8.3: Kinetics Model and its R^2 value.

S.NO	KINETICS MODEL	Co efficient of determination (R^2) of formulation F2
1.	Zero order	0.958
2.	First order	0.958
3.	Higuchi	0.987
4.	Korsmeyer and peppas	0.985

The data from *invitro* release of optimized formulation was fit into various kinetic models. Good linearity was observed with the Higuchi Release Kinetics.(R^2 = 0.987).

Hence the result indicating that the drug release was based on their change in surface area and diameter of the particles.

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