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A RESEARCH ON FORMULATION AND EVALUATION OF FLOATING MICROSPHERES OF TOLPERISONE HYDROCHLORIDE

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ABSTRACT

Tolperisone HCl, a centrally acting muscle relaxant. Tolperisone HCl with its shorter half life, of 1.5- 2.5 hours, high first pass metabolism with frequent dosing characteristics makes it suitable candidate for development of oral controlled release dosage form. Nine formulations of drug loaded microspheres using three different polymeric combinations and ratio were prepared by emulsion solvent evaporation technique. The various polymer used in the formulations were Ethyl cellulose, Eudragit RS100 & Eudragit S100 (all hydrophobic). Average particle size ranged from 56.68 µm to 87.50 µm. Percentage yield ranged from 77.28 to 90.22% and drug entrapment efficiency ranges from 69.12 to 82.64%. Percentage drug loading was found to be satisfactory. Kinetics study showed that drug release follows first order kinetics. Batch F4 shows best dissolution profile because of highest similarity factor f_2 (f_2=75.62) among all the batches (which ranged from 51.74 to 75.62). FTIR study confirmed the stable character of Tolperisone HCl in formulations. Scanning electron microscopy revealed that the microspheres were spherical. Nine different formulations (F1 to F9) of Tolperisone HCl loaded microspheres were prepared using various polymeric combinations with a view for the sustained delivery of the drug over a time period of 12 hours.

KEYWORDS: Floating microspheres, Gastro retentive drug delivery system, Emulsion solvent evaporation technique, Muscle relaxant.

INTRODUCTION

For the delivery of therapeutic agents oral route has being used because of the low cost of therapy and ease of administration which lead to high level of patient compliance.[1] Controlled release drug delivery system (CRDDS) provide drug release at predetermined, predictable and controlled rate. Many benefits can be achieved by controlled release drug delivery system like maintenance of optimum therapeutic drug concentration in blood with predictable and reproducible release rates for extended time period; enhancement of activity of duration for shorter half life drugs; elimination of side effects; reducing frequency of dosing and wastage of drugs; optimized therapy and better patient compliances.[2,3]

Three aspects are required for the successful development of oral controlled drug delivery system, namely,

1. The physiochemical characteristic of the drug.
2. Anatomy and physiology of GIT and
3. Characteristics dosage forms.[4]

However, shorter residence of time of the dosage forms and incomplete release of drugs in the upper gastro intestinal tract, a prominent site for the absorption of many drugs will leads to lower bioavailability. Efforts to improve oral bioavailability have grown parallel with the pharmaceutical industry. As the number and chemical diversity of drugs has increased, new strategies are required to develop the orally active therapeutics. Thus gastro retentive dosage forms which prolong the residence time of the drug in the stomach and improve their bioavailability, have been developed. One of the most feasible approaches for achieving a prolonged and predictable drug delivery profile in the GIT tract is to control the gastric residence time i.e. Gastro retentive dosage forms [$GRDS_s$].[5]

Gastro retentive floating drug delivery system[GRFDDS] have a bulk density lower than that of gastric fluids thus remains buoyant in the stomach without effecting gastric emptying rate for a prolonged period of time.[6]

Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers having a particle size ranging from 1-1000 μm.[7]

Floating microspheres are gastro retentive drug delivery system based upon non-effervescent approach. The floating microspheres have been utilized to obtain prolonged and uniform release in the stomach for development of a once daily formulation. When microspheres come in contact with gastric fluid the gel formers polysaccharides, and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release.[8]

Tolperisone, a centrally acting muscle relaxant agent. It has been used for the treatment of Myasthenia, Pregnancy and Breastfeeding. It has also been used in treatment of conditions which includes dymenorrhoea, climacteric complaints, lock jaw and neurolatyrism. The usually recommended dose of Tolperisone HCl in adults is 50mg tablet given three times a day (i.e. a daily dose of 150mg) that can be increased to a maximum daily dose of 600mg. if required in children. The drug is administered in a daily dose of 5-10 mg/kg/day, given in three divided doses. The dose of the drug should be reduced in the elderly and in patients with hepatic or renal insufficiency. The dosage of the drug should be maintained until the therapeutic effect is reached. Afterwards, the dosage of the drug should be reduced gradually.[9-13]

In the present investigation floating sustained release microspheres of Tolperisone HCl were prepared by emulsion solvent evaporation technique using three different polymers Eudragit RS100, Eudragit S100 and Ethyl cellulose. The aim of the work was to evaluate microspheres for size, in-vitro release, buoyancy and incorpraton efficiency. The effect of various formulation variables on the size and drug release was also investigated.

MATERIALS

Tolperisone HCl was obtained as a gift sample from Alkem Labs Ltd, Mumbai(India). Eudragit RS 100 and Eudragit S100 were obtained from Alkem Labs Ltd, Mumbai, Ethyl Cellulose, Dicloromethane and Tween 80 were obtained from Central Drug House Ltd, Mumbai. All other chemical/reagents used were of analytical grade, available commercially and used as such without further processing. FT-IR ALPHA-E (Bruker USA), Double beam UV-Visible spectrophotometer (UV-2700, Thermoscientific USA) and USP Eight Stage Dissolution Test Apparatus (DS 8000) were the instruments employed in the current study.

METHOD

Following studies were performed to formulate and evaluate Tolperisone HCl floating microspheres.

Preformulatuion Studies

The first step in the rational development of dosage forms of a drug is the preformulation testing. It can be defined as an investigation of Physical and chemical properties of drug substance, alone and when combined with excipients. The overall objective of preformulation testing is to generate information useful to the formulation developing stable and bioavailable dosage forms, which can be produced at large scale.[14]

Identification of Pure Drug[15]

The selected drug Tolperisone HCl was subjected for investigation of physical characterization parameters such as colour, state, odour, IR spectroscopy, solubility analysis, melting point Determination.

Compatibility Studies of Drug and Polymers

It is essential to confirm that drug is not interacting with the polymer under certain experimental studies before formulating a dosage form. Interacting among drug and polymer may affect the efficacy of final dosage form.

FTIR spectrum of pure drug, polymers and physical mixture of drug with polymers as per different formulation combinations were taken over. The wave number range of 4000-400 cm^{-1}. Also spectrums of drug in different types of formulations were also taken. FTIR helps to confirm the identity of the drug and to detect the interaction of the drug with the carriers.

SCANNING OF TOLPERISONE HYDROCHLORIDE IN S.G.F. (STANDARD CALIBRATION CURVE)

Preparation of 10 µg/ml Solution

A stock solution of Tolperisone Hydrochloride containing 100mg in 100ml was prepared using simulated gastric fluid (SGF) without enzymes of pH 1.2. From this solution one ml was diluted upto 100 ml in a volumetric flask with simulated gastric fluid (SGF).

Scanning

This resulting 10 µg/ml solution was scanned in UV/Visible double beam spectrophotometer (Thermo scientific, India) in the range 200-400 nm. Tolperisone Hydrochloride shows

maximum ultraviolet absorbance at 260 nm. Based on this information, a standard graph was constructed using simulated gastric fluid (pH 1.2) without enzymes as detailed below to estimate its amount either in dissolution fluids or matrix tablets.

Preparation of Stock Solution: A stock solution of Tolperisone Hydrochloride containing 100mg in 100ml was prepared using simulated gastric fluid (SGF) without enzymes of pH 1.2.

Standard Dilutions: From the stock solution different concentrations of Tolperisone Hydrochloride viz, 2, 4, 6, 8, 10, 12, 14 16, 18, and 20 µg/ml were prepared by diluting with SGF without enzymes of pH 1.2 and their absorbance were measured at 260 nm using UV/Visible double beam spectrophotometer (Thermoscientific, India). The absorbance of the above solutions was tabulated in the following table (Table.). A graph was plotted by taking concentration of Tolperisone Hydrochloride (µg/ml) on X-axis and absorbance on the Y- axis (Fig.5).

Preparation of Microspheres of Tolperisone Hcl

Various batches of floating microspheres of Tolperisone HCl were prepared by the emulsion solvent evaporation method. Calculated quantities of polymers were dissolved into a mixture of dichloromethane and ethanol (7:3) in which the calculated quantity of drug is previously dissolved. This viscous solution was then added drop wise to a 50 ml beaker containing liquid paraffin having 0.4% Tween 80 as emulsifying agent. The resulting mixture was agitated at 40^0C for 3-4 hrs at 200-300 rpm. After the complete removal of the solvent, the prepared microspheres were filtered, washed repeatedly with n-hexane and dried in hot air oven at 50^0C.

Table No.1: Composition of Formulation.

Formulations	Tolperisone HCl (mg)	Eudragit RS100 (mg)	Ethyl Cellulose (mg)	Eudragit S100 (mg)
F 1	500	500	-	-
F 2	500	1000	-	-
F 3	500	1500	-	-
F 4	500	750	750	-
F 5	500	1000	500	-
F 6	500	500	1000	-
F 7	500	750	-	750
F 8	500	1000	-	500
F 9	500	500	-	1000

EVALUATION OF THE FORMULATED FLOATING MICROSPHERES

1. Micromeritic Studies

The prepared microspheres are characterized by their micromeritic properties[16,17] such as microsphere size, tapped density, Carr's compressibility index, Hausner's ratio and angle of repose.

Bulk Density

The bulk density is defined as the mass of powder divided by bulk volume. The bulk density was calculated by dividing the weight of the samples in grams by the final volume in cm^3.

Bulk density = Mass of microspheres/Volume of microspheres before tapping

Tapped Density

Tapped density is the volume of powder determined by tapping by using a measuring cylinder containing weighed amount of sample. The cylinder containing known amount of microspheres was tapped for about 1 minute on a tapped density apparatus until it gives constant volume.

Tapped density = Mass of microspheres/ Volume of microspheres after tapping

Carr's Compressibility Index

This is an important property in maintaining uniform weight. It is calculated using following equation**,**

% Compressibility Index = [(Tapped density-Bulk Density)/ (Tapped Density)] X 100

Lower the compressibility values indicate better flow.

Hausners ratio

A similar index like percentage compressibility index has been defined by Hausner. Values less than 1.25 indicate good flow, where as greater than 1.25 indicates poor flow. Added glidant normally improve flow of the material under study. Hausner's ratio can be calculated by formula,

Hausner's ratio = (Tapped density/ Bulk density)

Angle of Repose (θ)

Good flow properties are critical for the development of any pharmaceutical tablet, capsules or powder formulation. Interparticle forces between particles as well as flow characteristics

of powders are evaluated by angle of repose. Angle of repose is defined as the maximum angle possible between the surface and the horizontal plane.

The angle of repose of each powder blend was determined by glass funnel method. Powders were weighed accurately and passed freely through the funnel so as to form a heap. The height of funnel was so adjusted that the tip of the funnel just touched the apex of the heap. The diameter of the powder cone so formed was measured and the angle of repose was calculated using the following equation,

$$\mathbf{Tan\ \Theta = h/r}$$

$\Theta = Tan^{-1}\ (h/r)$

Where,

θ = Angle of repose

h = Height of the pile

r = Radius of the powder cone

Angle of repose affects particle size distribution, as larger the particle size, it will flow freely and vice-versa. It is a helpful parameter to monitor quality of powdered or granular pharmaceutical formulations. For good flowing materials, the angle of repose should be less than 30°.

2. Particle Size & Surface Morphology[18]

Particle Size Determination

Microsphere size was determined by using an optical microscope under regular polarized light, and the mean microsphere size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer.

Morphological Study using SEM

The morphological study was carried out by Scanning Electron Microscope (SEM). Microspheres were scanned and examined under Electron Microscope HITACHI SU 1500, Japan. The sample was loaded on copper sample holder and sputter coated with carbon followed by Gold.

3. Percentage Yield[19]

The prepared microspheres of all batches were accurately weighed. The measured weight of prepared microspheres was divided by the total amount of all the excipients and drug used in

the preparation of the microspheres, which give the total percentage yield of floating microspheres. It was calculated by using following equation,

$$\% \text{ Yield } = \frac{\text{Actual weight of product}}{\text{Total weight of excipients and drug}} \times 100$$

4. Drug Loading and Drug Entrapment[20,19]

Microspheres equivalent to 50 mg of the drug were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1M HCl (pH-1.2) repeatedly. The extract was transferred to a 100ml volumetric flask and the volume was made up using 0.1M HCl (pH-1.2). The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically (UV 2700, Shimadzu, Japan) at 260 nm against appropriate blank. The amount of drug loaded and entrapped in the microspheres was calculated by the following formulas:

$$\% \text{ Drug Loading } = \frac{\text{Weight of the drug loaded in the microspheres}}{\text{Total weight of microspheres}} \times 100$$

$$\% \text{ Drug entrapment Efficiency } = \frac{\text{Amount of drug actual present}}{\text{Theoretical drug load expected}} \times 100$$

5. Floating behaviour (buoyancy %)

50 mg of the microspheres were placed in 100 ml of stimulating gastric fluid (pH 1.2). The mixture was stirred at 100 rpm and separated by filtration particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in desiccators. Both the fractions of microspheres were weighed and buoyancy percentage was calculated as the ratio of the mass of the microspheres that remained floating and the total mass of the microsphere.[21]

$$\text{Buoyancy (\%)} = \frac{\text{Weight of floating microspheres after time t}}{\text{Initial weight of microspheres}} \times 100$$

5. *In-vitro* Release Study[22,19]

The drug release study was performed for microsphere containing quantity equivalent to 100mg of Tolperisone HCl by using USP type-II dissolution test apparatus (USP TDT 08L) in 900 ml of dissolution media (pH-1.2) at 50 rpm and 37±0.1^{0}C temperature. 5 ml of sample was withdrawn at predetermined time interval for 1 hour and same volume of fresh medium was replaced to maintained sink condition. Withdrawn samples were assayed

spectrophotometrically at 260 nm. Drug release was also performed for pure drug. The cumulative % drug release was calculated using standard calibration curve method.

Details of dissolution testing

- Apparatus: USP TDT 08L
- Dissolution media: 0.1 M HCl
- Speed: 50 rpm
- Volume of medium: 900 ml
- Temperature: 37±0.1°C
- Wavelength: 260 nm**.**

6. Release Kinetics

The matrix systems were reported to follow the Peppas release rate and the diffusion mechanism for the release of the drug.[23] To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to, Zero order, First order, Higuchi matrix, Peppas and Hixson Crowell model. In this by comparing the R^2-values obtained, the best-fit model was selected.

Zero Order Kinetics

Drug dissolution from Pharmaceutical dosage forms that do not disaggregate and releases the drug slowly, assuming that the area does not change and no equilibrium conditions are obtained can be represented by the following equation

$$Q_t = Q_0 + K_0 t$$

Where,

Q_t = Amount of drug dissolved in time t,

Q_0 = Initial amount of drug in the solution and

K_0 = Zero order release constant.

First Order Kinetics

To study the first order release rate kinetics the release rate data were fitted to the following equation.

$$\log Q_t = \log Q_0 + K_1 X t / 2.303$$

Where,

Q_t = Amount of drug released in time t,

Q_0 = Initial amount of drug in the solution and

K_1 = First order release constant.

Higuchi Model

Higuchi developed several theoretical models to study the release of water soluble and low-soluble drugs incorporated in semisolids and or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The Higuchi equation is

$$Q_t = K_H \times t^{1/2}$$

Where,

Q_t = Amount of drug released in time t and,

K_H or (D) = Higuchi diffusion coefficient.

Korsmeyer-Peppas Release Model

To study this model the release rate data is fitted to the following equation

$$M_t / M_\infty = K.t^n$$

Where,

M_t / M_∞ = Fraction of drug release,

K= Release constant,

t = Drug release time and

n = Diffusional exponent for the drug release that is dependent on the shape of the matrix dosage form.

Hixson-Crowell Model

To study the Hixson–Crowell model the release rate data are fitted to the following equation:

$$W_0^{1/3} - Wt^{1/3} = Ks\ t$$

Where,

W_0= Amount of drug in the pharmaceutical dosage form,

Wt = Remaining amount of drug in the pharmaceutical dosage form,

Ks = Constant incorporating the surface-volume relation.

7. Stability Studies

Stability of a drug has been defined as the ability of a particular formulation[14,24], in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of

environmental factors such as temperature, humidity, light, and enables recommended storage conditions.

As per ICH guidelines the lengths of study and storage conditions are:
Accelerated testing - 37°C/75% RH for 6 months.

Procedure

In the present study, stability study was carried out by keeping samples for a period up to the 3 months at 37°C/75% RH for optimized formulation F4. After three months, samples were analyzed for the physical appearance, drug entrapment efficiency, *in vitro* release study and possible drug-excipient interactions using Infrared (FTIR) spectrophotometry.

RESULTS AND DISCUSSION

Preformulation studies

Description of drug

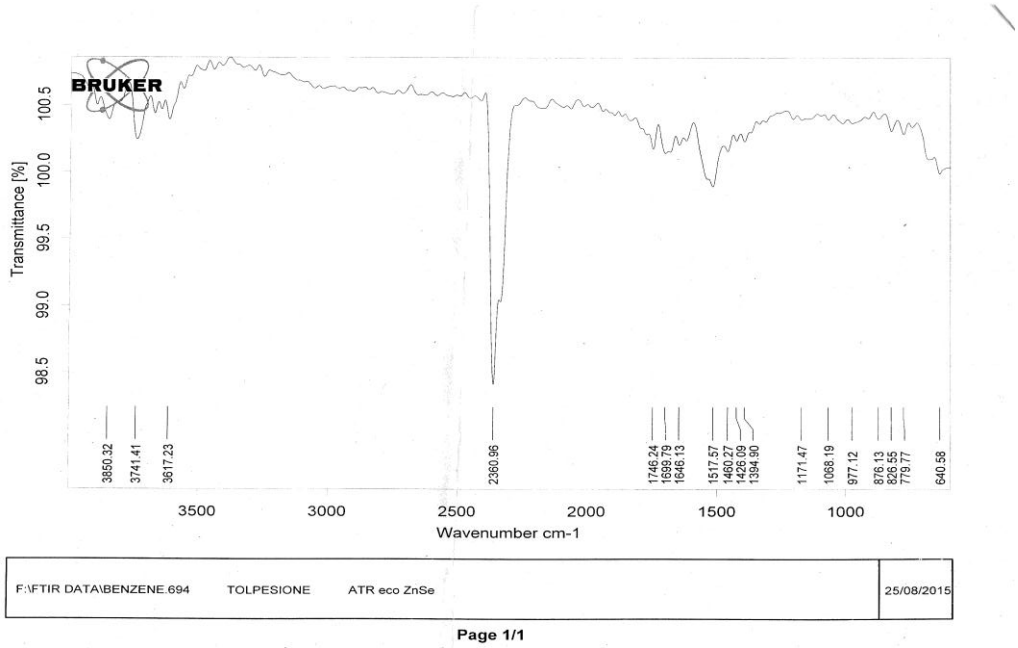
Various properties of drug related with physical appearance, state and solubility given in Table no. 2.

Table No.2: Description on Drug.

S.NO	PROPERTIES	INFERENCE
1.	Colour	White colour
2.	Solubility	Freely soluble in Methanol, Water and Dichrolomethane
3.	Odour	Odourless

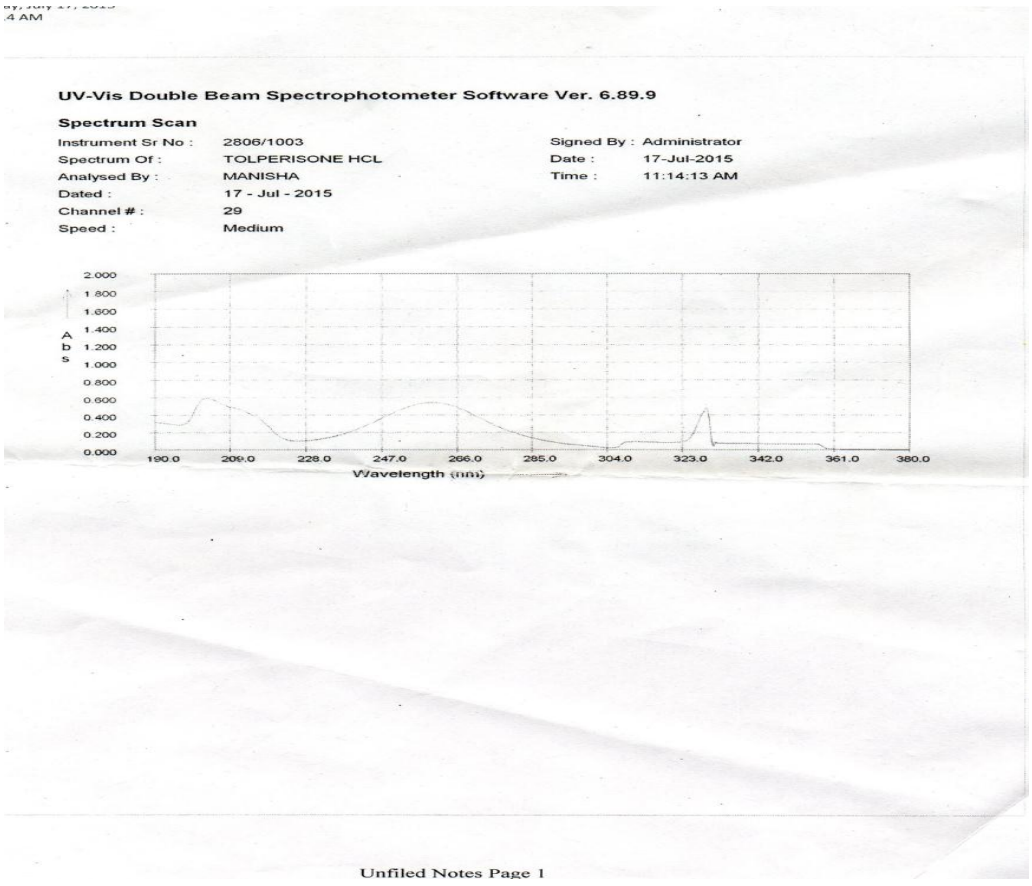
FTIR Spectroscopy

The FTIR spectrum of the Tolperisone HCl pure drug was found to be similar to the standard spectrum of Tolperisone HCl. The spectrum of Tolperisone HCl showed the characteristic peaks at the wave number: 3850cm^{-1}, 3741cm^{-1}, 3617cm^{-1}, 2360cm^{-1}, 1746cm^{-1}, 1699cm^{-1}, 1646cm^{-1},1517cm^{-1},1460cm^{-1},1394cm^{-1}, 1171cm^{-1}, 1068cm^{-1},977cm^{-1}, 876cm^{-1} and 685cm^{-1}.



UV Spectroscopy

The absorption spectrum of Tolperisone HCl pure drug was scanned between 400-200 nm with concentration of 10 µg/ml prepared in 0.1 M HCl (pH-1.2) (Fig. 4). The absorption maxima λ max was noted at 260 nm.



Melting Point Determination

The melting point of the obtained drug sample was found to be 170^{o}C which was within the reported range of 167^{0}C-174^{o}C. It complies with the purity of the drug sample.

DRUG EXCIPIENT COMPATIBILITY STUDIES

FTIR Study

The FTIR spectra of the pure drug and pure polymers were taken initially. The combination spectra of drug were also taken with the polymers in physical mixture as well as in formulations of different polymer combinations (F1, F4, F7) (Fig.28, Fig.29, Fig.30) which were taken after keeping samples for 3 months at 37^{0}C/75% RH. It was observed that all the characteristic peaks of were Tolperisone HCl present in the combination spectra as well thus indicating the compatibility of the drug with the polymers used in various formulations (Fig. 24, Fig. 25, Fig. 26, & Fig.27).

In comparison with pure drug, the absorption peak of the spectra for Tolperisone HCl in different formulations showed no significant shift and no disappearance of characteristic peaks suggesting that there was no interaction between drug and polymer matrices or no degradation in Tolperisone HCl molecule. The differences in transmittance may be due to concentration of drug present in formulations.

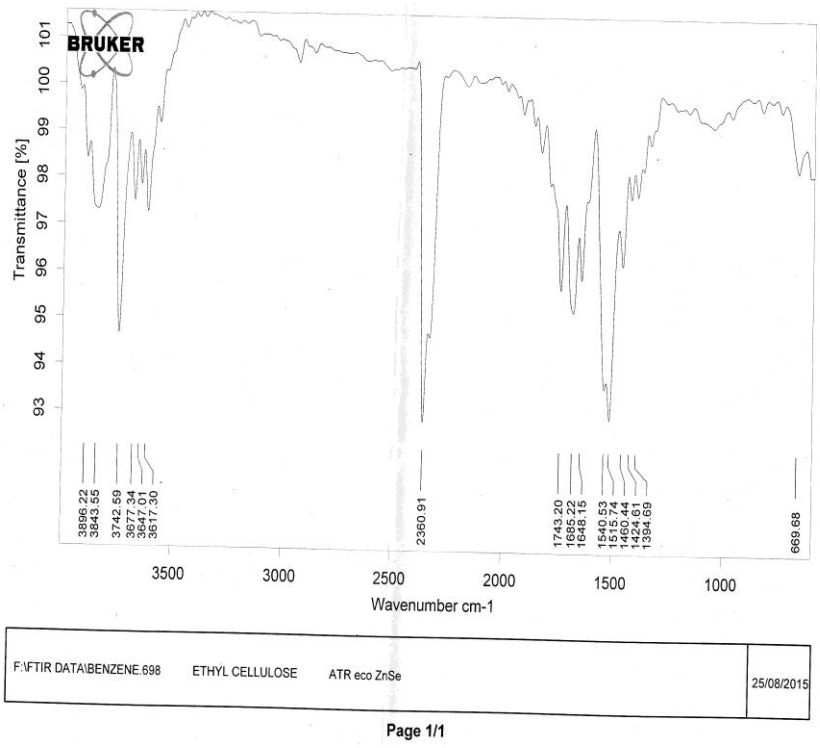


Fig. 25: IR Spectra of Ethyl Cellulose.

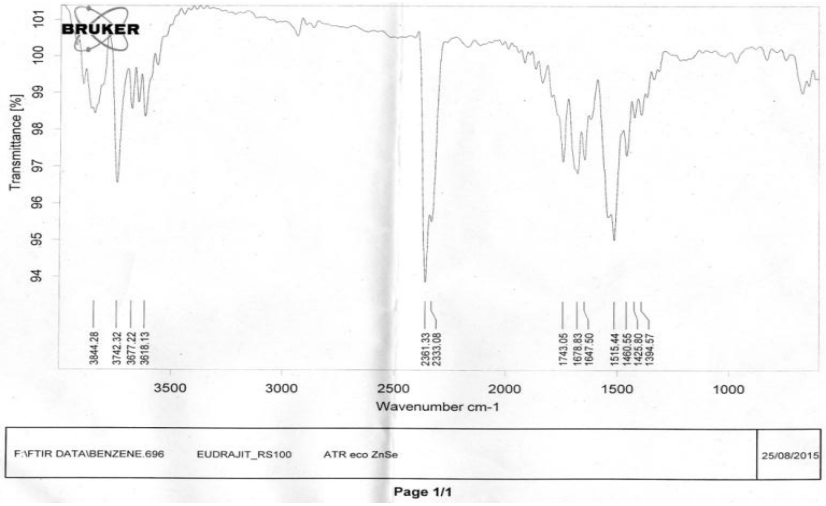


Fig. 26: IR Spectra of Eudragit RS100.

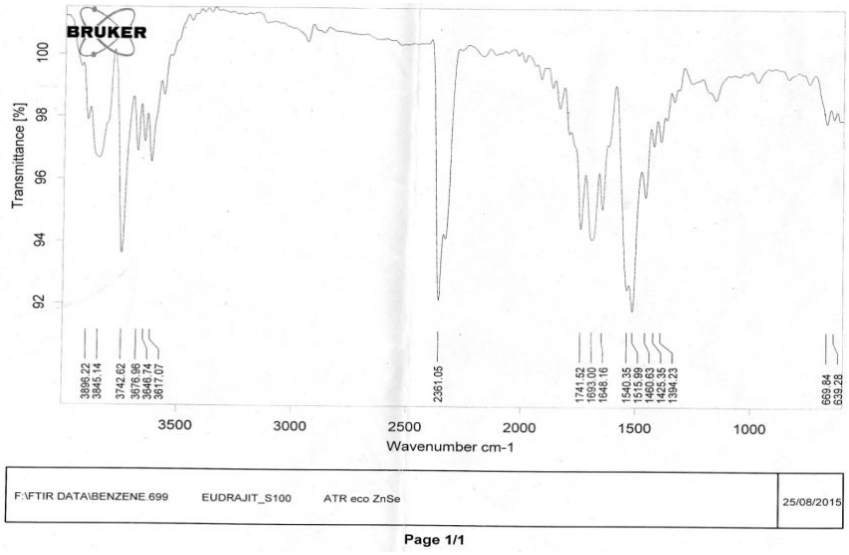


Fig. 27: IR Spectra of Eudragit S100.

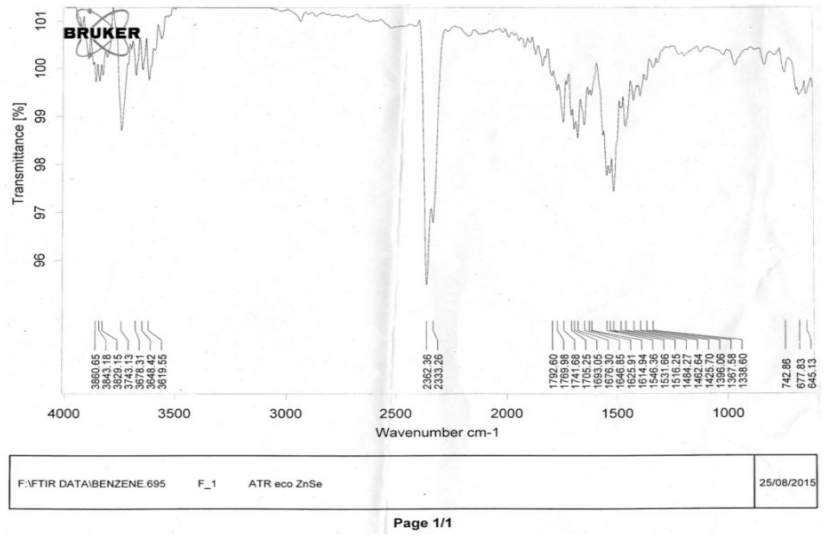


Fig. 28: IR Spectra of Drug + Eudragit (RS100) F1 (after 3 months).

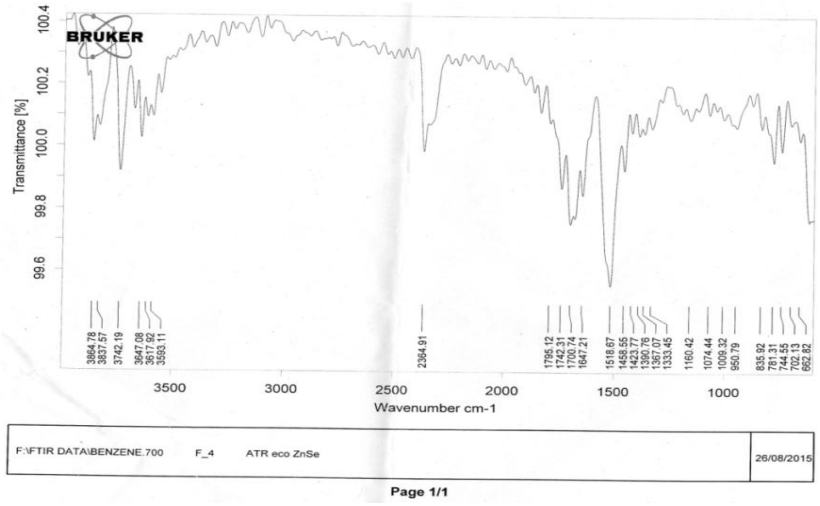


Fig. 29: IR Spectra of Drug + Eudragit (RS100) + Ethyl cellulose F4 (after 3 months).

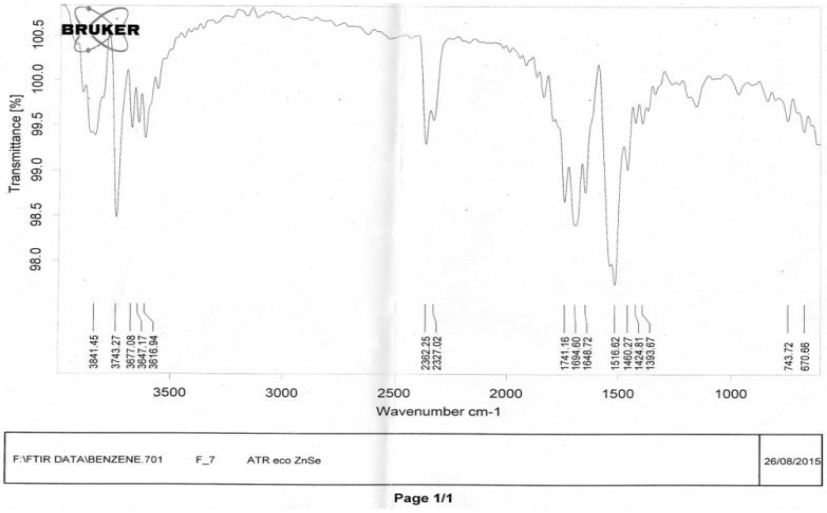


Fig. 30: IR Spectra of Drug + Eudragit (RS100) + Ethyl cellulose F7 (after 3 months).

STANDARD CURVE OF TOLPERISONE HCl IN 0.1M HCl (pH-1.2)

Scanning of Tolperisone HCl in 0.1 M HCl (pH-1.2)

The absorption spectrum of pure drug was scanned between 400-200 nm with concentration of 10 µg/ml prepared in 0.1 M HCl (pH-1.2) (Fig. 4). The absorption maxima λmax was noted at 260 nm.

Preparation of Standard Curve

Table No. 4 shows the absorbance of standard solutions of Tolperisone HCl ranging from 2-20 µg/ml in 0.1M HCl (pH-1.2). Figure 5 shows the standard calibration curve of Tolperisone HCl. The curve was found to be linear in the range of 2-20 µg/ml at λmax 260nm. The regression value was found to be 0.995.

The calculations of drug content, *in vitro* release and stability studies are based on this calibration curve.

Table 4: Standard Curve of Tolperisone Hydrochloride in S.G.F.

Conc.(µg/ml)	Absorbance at 260 nm
0	0
2	0.153
4	0.267
6	0.357
8	0.493
10	0.617
12	0.795
14	0.901
16	1.022
18	1.166
20	1.352

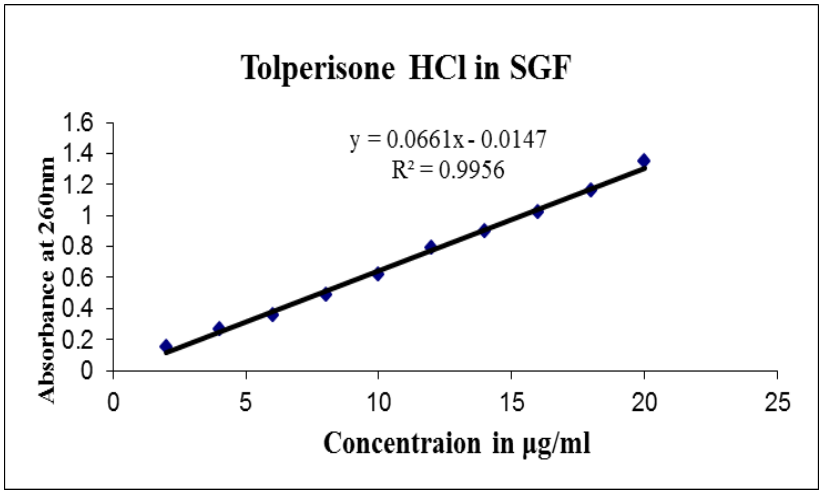


Fig. 5: Standard Curve of Tolperisone HCl.

EVALUATION TEST

Micromeritic Properties

The Micromeritic properties of all formulations F1 to F9 of microspheres were shown in Table No. 9, which were evaluated for variable parameters such as bulk density, tapped density, % Compressibility index, Hausner's ratio and angle of repose. The % Compressibility index was in the range of 12-18 for all the formulations F1 to F9 indicating good flow property. The value of Hausner's ratio for the all formulation F1 to F9 was below 1.140 which indicates good flow property. The values of angle of repose for formulations F1 to F9 was found to be in the range of 20^{o}-25^{o} which indicates good flow property of microspheres**.**

Table No. 9: Micromeritic Properties of Tolperisone HCl Floating Microspheres.

Formulation Code	Bulk Density (g/cm^3)	Tapped Density (g/cm^3)	Compressibility Index (%)	Hausner's Ratio	Angle of Repose (θ)
F1	0.158	0.181	10.30	1.147	$23^0$24'
F2	0.149	0.179	14.3	1.170	$22^0$80'
F3	0.143	0.169	13.6	1.158	$21^0$10'
F4	0.519	0.622	17.8	1.200	$20^0$20'
F5	0.570	0.682	14.9	1.189	$23^0$20'
F6	0.589	0.678	14.1	1.16	$22^0$40'
F7	0.420	0.495	14.3	1.163	$21^0$70'
F8	0.449	0.514	13.9	1.158	$23^0$60'
F9	0.480	0.557	12.3	1.141	$24^0$50'

Where, n

Particle size

Average particle size of microspheres as determined by optical microscopy by using stage micrometer and ocular micrometer are shown in Table No. 10. The particle size of different formulations F1 to F9 were found to be in between 56.68 to 87.50 µm.

Table No. 10: Average Particle Size of Tolperisone HCl Microspheres.

Formulation Code	Average Particle Size(µm)
F1	67.31
F2	62.54
F3	55.68
F4	63.46
F5	66.30
F6	78.81
F7	86.33
F8	81.25
F9	76.17

n = 100

Percentage Yield

Percentage yield of different formulation F1 to F9 were shown in Table No. 11 and the yield was found in between 77.28% to 90.22%. (Table No. 11).

Results showed that as the concentration of Eudragit RS 100 increased from formulation F1 to F3, the % yield decreased. Among formulation F4 to F6 as the concentration of Eudragit RS 100 increases and ethyl cellulose decreases % yield decreases in the following manner F5 > F4 > F6. Among formulation F7 to F9 as the concentration of Eudragit RS 100 increases and Eudragit S100 decreases % yield decreases on the following manner F8 >F7 > F9.

Drug Loading And Drug Entrapment Efficiency

The values of % drug loading and % drug entrapment efficiency of different formulations were shown in Table No.11.

Results showed that among formulation F1, F2 and F3, as the concentration of Eudragit RS100 increases, there is a decrease in the % drug loading and % drug entrapment efficiency. Among formulation F4 to F6 as the concentration of Eudragit RS 100 increases and ethyl cellulose decreases % drug loading and % drug entrapment efficiency decreases in the following manner F5 > F4 > F6. Among formulation F7 to F9 as the concentration of Eudragit RS 100 increases and Eudragit S100 decreases % drug loading and % drug entrapment efficiency decreases on the following manner F8 >F7 > F9.

Table No. 11: % Yield, % Drug Loading and % Drug Entrapment Efficiency.

Formulation Code	% Yield	% Drug Loading	% Drug Entrapment Efficiency
F1	90.22	35.21	82.64
F2	88.71	30.44	77.25
F3	82.49	29.33	69.12
F4	80.63	33.20	74.83
F5	84.71	35.72	76.83
F6	79.42	30.67	72.73
F7	83.34	29.25	76.65
F8	87.87	31.93	80.34
F9	77.28	28.20	71.65

Buoyancy Studies

From the study of floating properties (Table no 27.)**,** it was observed that the % floating ranges from 81.2 - 91.2% of different formulation. Maximum % floating was found in F4 i.e. 90.12%

Table 27: Floating Properties in SGF.

Formulation	% Floatation
F 1	85.16
F 2	84.45
F 3	83.67
F 4	90.12
F 5	88.56
F 6	87.12
F 7	84.82
F 8	85.56
F 9	83.12

n=3

***IN VITRO* DRUG RELEASE STUDIES**

Dissolution studies on all the nine formulations (F1 to F9) of Tolperisone HCl microspheres were carried out using by USP dissolution apparatus Type II. 0.1M HCl (pH 1.2) was used as the dissolution medium. The dissolution test was performed at 37±0.1^{0}C at 50 rpm.

From the *in vitro* drug dissolution profiles of all batches (Table No. 12-20), it was observed that batch F4 gave best dissolution profile from the rest of the formulations. It was evident from the similarity factor (f_2) of 75.62 as compared to theoretical dissolution profile of modified release Tolperisone HCl (Table No.24). The similarity factor (f_2) as proposed by Moore and Flanner of different formulations (Table No.24) were found be similar as compared to theoretical dissolution profile.

In order to find the effect of change in the content of polymers, formulations F1, F2 & F3 have been designed with gradual increase of Eudragit RS 100. Results showed that drug release decreases as the content of Eudragit RS 100 increases (Fig. 6). Similarly, among formulation F4, F5 & F6, as the concentration of Eudragit RS100 & Ethyl cellulose increases, the drug release was increased (Fig. 11). Also, from formulations F7, F8 & F9 as the concentration of Eudragit S100 increased, the drug release was decreased (Fig. 16).

Table No. 12: *In vitro* Drug Release of Formulation F1

Time(T) in Hrs	Sq.Rt.T	Log T	Cum. % Drug Released	Cum. %Drug Remaining	Log % Drug Remaining	Log % Drug Released	Cube Rt. % Drug Remaining
0.0	0.0	----	0.00	100.00	2.00		4.64
1.0	1.0	0.0	27.21	72.79	1.86	1.43	4.18
2.0	1.4	0.2	37.59	62.41	1.80	1.58	3.97
3.0	1.7	0.2	44.21	55.79	1.75	1.65	3.82
4.0	2.0	0.3	51.32	48.68	1.69	1.71	3.65
5.0	2.2	0.3	57.92	42.08	1.62	1.76	3.48
6.0	2.4	0.4	66.59	33.41	1.52	1.82	3.22
7.0	2.6	0.4	71.90	28.10	1.45	1.86	3.04
8.0	2.8	0.5	78.77	21.23	1.33	1.90	2.77
9.0	3.0	0.5	82.13	17.87	1.25	1.91	2.61
10.0	3.2	0.5	87.52	12.48	1.10	1.94	2.32
11.0	3.3	0.5	89.52	10.48	1.02	1.95	2.19
12.0	3.5	0.5	92.77	7.23	0.86	1.97	1.93

Table No. 13: *In vitro* Drug Release Formulation of F2.

Time(T) in Hrs.	Sq. Rt.T	Log T	Cum. %Drug Released	Cum. %Drug Remaining	Log % Drug Remaining	Log % Drug Released	Cube Rt. % Drug Remaining
0.0	0.0		0.00	100.00	2.00		4.64
1.0	1.0	0.0	25.32	74.68	1.87	1.40	4.21
2.0	1.4	0.2	33.39	66.61	1.82	1.52	4.05
3.0	1.7	0.2	40.11	59.89	1.78	1.60	3.91
4.0	2.0	0.3	48.31	51.69	1.71	1.68	3.73
5.0	2.2	0.3	55.32	44.68	1.65	1.74	3.55
6.0	2.4	0.4	61.89	38.11	1.58	1.79	3.37
7.0	2.6	0.4	67.22	32.78	1.52	1.83	3.20
8.0	2.8	0.5	70.31	29.69	1.47	1.85	3.10
9.0	3.0	0.5	75.62	24.38	1.39	1.88	2.90
10.0	3.2	0.5	78.52	21.48	1.33	1.89	2.78
11.0	3.3	0.5	85.13	14.87	1.17	1.93	2.46
12.0	3.5	0.5	90.99	9.01	0.95	1.96	2.08

Table No. 14: *In vitro* Drug Release Formulation of F3.

Time(T) in Hrs	Sq. Rt. T	Log T	Cum. %Drug Released	Cum. %Drug Remaining	Log % Drug Remaining	Log % Drug Released	Cube Rt. % Drug Remaining
0.0	0.0		0.00	100.00	2.00		4.64
1.0	1.0	0.0	22.11	77.89	1.89	1.34	4.27
2.0	1.4	0.2	29.31	70.69	1.85	1.47	4.13
3.0	1.7	0.2	36.39	63.61	1.80	1.56	3.99
4.0	2.0	0.3	41.24	58.76	1.77	1.62	3.89
5.0	2.2	0.3	52.19	47.81	1.68	1.72	3.63
6.0	2.4	0.4	57.31	42.69	1.63	1.76	3.49
7.0	2.6	0.4	62.31	37.69	1.58	1.79	3.35
8.0	2.8	0.5	65.21	34.79	1.54	1.81	3.26
9.0	3.0	0.5	71.91	28.09	1.45	1.86	3.04
10.0	3.2	0.5	73.99	26.01	1.42	1.87	2.96
11.0	3.3	0.5	81.17	18.83	1.27	1.91	2.66
12.0	3.5	0.5	88.91	11.09	1.04	1.95	2.23

Table No. 15: *In vitro* Drug Release Formulation of F4.

Time(T) in Hrs	Sq. Rt. T	Log T	Cum. %Drug Released	Cum. %Drug Remaining	Log % Drug Remaining	Log % Drug Released	Cube Rt. % Drug Remaining
0.0	0.0		0.00	100.00	2.00		4.64
1.0	1.0	0.0	30.21	69.79	1.84	1.48	4.12
2.0	1.4	0.2	35.21	64.79	1.81	1.55	4.02
3.0	1.7	0.2	40.23	59.77	1.78	1.60	3.91
4.0	2.0	0.3	49.59	50.41	1.70	1.70	3.69
5.0	2.2	0.3	56.38	43.62	1.64	1.75	3.52
6.0	2.4	0.4	64.42	35.58	1.55	1.81	3.29

7.0	2.6	0.4	69.17	30.83	1.49	1.84	3.14
8.0	2.8	0.5	76.33	23.67	1.37	1.88	2.87
9.0	3.0	0.5	82.11	17.89	1.25	1.91	2.62
10.0	3.2	0.5	85.31	14.69	1.17	1.93	2.45
11.0	3.3	0.5	89.32	10.68	1.03	1.95	2.20
12.0	3.5	0.5	92.20	7.80	0.89	1.96	1.98

Table No. 16: *In vitro* Drug Release Formulation of F5.

Time(T) in Hrs	Sq. Rt. T	Log T	Cum. %Drug Released	Cum. %Drug Remaining	Log % Drug Remaining	Log % Drug Released	Cube Rt. % Drug Remaining
0.0	0.0		0.00	100.00	2.00		4.64
1.0	1.0	0.0	27.39	72.61	1.86	1.44	4.17
2.0	1.4	0.2	31.91	68.09	1.83	1.50	4.08
3.0	1.7	0.2	38.31	61.69	1.79	1.58	3.95
4.0	2.0	0.3	46.37	53.63	1.73	1.67	3.77
5.0	2.2	0.3	53.17	46.83	1.67	1.73	3.60
6.0	2.4	0.4	60.24	39.76	1.60	1.78	3.41
7.0	2.6	0.4	65.72	34.28	1.54	1.82	3.25
8.0	2.8	0.5	71.82	28.18	1.45	1.86	3.04
9.0	3.0	0.5	78.03	21.97	1.34	1.89	2.80
10.0	3.2	0.5	83.37	16.63	1.22	1.92	2.55
11.0	3.3	0.5	87.32	12.68	1.10	1.94	2.33
12.0	3.5	0.5	89.32	10.68	1.03	1.95	2.20

Table No. 17: *In vitro* Drug Release Formulation of F6

Time(T)in Hrs	Sq. Rt. T	Log T	Cum. %Drug Released	Cum. %Drug Remaining	Log % Drug Remaining	Log % Drug Released	Cube Rt. % Drug Remaining
0.0	0.0		0.00	100.00	2.00		4.64
1.0	1.0	0.0	28.14	71.86	1.86	1.45	4.16
2.0	1.4	0.2	33.32	66.68	1.82	1.52	4.06
3.0	1.7	0.2	39.17	60.83	1.78	1.59	3.93
4.0	2.0	0.3	47.11	52.89	1.72	1.67	3.75
5.0	2.2	0.3	54.17	45.83	1.66	1.73	3.58
6.0	2.4	0.4	62.23	37.77	1.58	1.79	3.36
7.0	2.6	0.4	68.23	31.77	1.50	1.83	3.17
8.0	2.8	0.5	74.39	25.61	1.41	1.87	2.95
9.0	3.0	0.5	80.77	19.23	1.28	1.91	2.68
10.0	3.2	0.5	84.39	15.61	1.19	1.93	2.50
11.0	3.3	0.5	88.81	11.19	1.05	1.95	2.24
12.0	3.5	0.5	91.47	8.53	0.93	1.96	2.04

Table No. 18: *In vitro* Drug Release Formulation of F7.

Time(T) in Hrs	SQ. Rt. T	Log T	Cum. %Drug Released	Cum. %Drug Remaining	Log % Drug Remaining	Log % Drug Released	Cube Rt. % Drug Remaining
0.0	0.0		0.00	100.00	2.00		4.64
1.0	1.0	0.0	27.32	72.68	1.86	1.44	4.17
2.0	1.4	0.2	33.42	66.58	1.82	1.52	4.05
3.0	1.7	0.2	39.32	60.68	1.78	1.59	3.93
4.0	2.0	0.3	46.31	53.69	1.73	1.67	3.77
5.0	2.2	0.3	53.17	46.83	1.67	1.73	3.60
6.0	2.4	0.4	61.07	38.93	1.59	1.79	3.39
7.0	2.6	0.4	67.82	32.18	1.51	1.83	3.18
8.0	2.8	0.5	73.77	26.23	1.42	1.87	2.97
9.0	3.0	0.5	77.82	22.18	1.35	1.89	2.81
10.0	3.2	0.5	81.02	18.98	1.28	1.91	2.67
11.0	3.3	0.5	85.32	14.68	1.17	1.93	2.45
12.0	3.5	0.5	88.01	11.99	1.08	1.94	2.29

Table No. 19: *In vitro* Drug Release Formulation of F8.

Time(T)in Hrs	Sq. Rt. T	Log T	Cum. %Drug Released	Cum. % Drug Remaining	Log % Drug Remaining	Log % Drug Released	Cube Rt. % Drug Remaining
0.0	0.0		0.00	100.00	2.00		4.64
1.0	1.0	0.0	24.70	75.30	1.88	1.39	4.22
2.0	1.4	0.2	31.11	68.89	1.84	1.49	4.10
3.0	1.7	0.2	36.42	63.58	1.80	1.56	3.99
4.0	2.0	0.3	41.82	58.18	1.76	1.62	3.87
5.0	2.2	0.3	50.77	49.23	1.69	1.71	3.67
6.0	2.4	0.4	58.18	41.82	1.62	1.76	3.47
7.0	2.6	0.4	64.07	35.93	1.56	1.81	3.30
8.0	2.8	0.5	70.88	29.12	1.46	1.85	3.08
9.0	3.0	0.5	74.08	25.92	1.41	1.87	2.96
10.0	3.2	0.5	79.22	20.78	1.32	1.90	2.75
11.0	3.3	0.5	83.87	16.13	1.21	1.92	2.53
12.0	3.5	0.5	86.32	13.68	1.14	1.94	2.39

Table No. 20: *In vitro* Drug Release Formulation of F9.

Time(T)in Hrs	Sq. Rt. T	Log T	Cum. %Drug Released	Cum. %Drug Remaining	Log % Drug Remaining	Log % Drug Released	Cube Rt. % Drug Remaining
0.0	0.0		0.00	100.00	2.00		4.64
1.0	1.0	0.0	25.19	74.81	1.87	1.40	4.21
2.0	1.4	0.2	31.93	68.07	1.83	1.50	4.08
3.0	1.7	0.2	37.25	62.75	1.80	1.57	3.97
4.0	2.0	0.3	44.17	55.83	1.75	1.65	3.82
5.0	2.2	0.3	57.99	42.01	1.62	1.76	3.48
6.0	2.4	0.4	60.07	39.93	1.60	1.78	3.42
7.0	2.6	0.4	65.19	34.81	1.54	1.81	3.27
8.0	2.8	0.5	71.22	28.78	1.46	1.85	3.06
9.0	3.0	0.5	75.33	24.67	1.39	1.88	2.91
10.0	3.2	0.5	80.31	19.69	1.29	1.90	2.70
11.0	3.3	0.5	84.07	15.93	1.20	1.92	2.52
12.0	3.5	0.5	87.88	12.12	1.08	1.94	2.30

Table No. 24: Similarity Factors (f_2) of Formulations Compared to Theoretical Dissolution Profile (Th.).

Formulation	Similarity Factor
F1	72.01
F2	64.27
F3	51.74
F4	75.62
F5	66.95
F6	73.76
F7	63.85
F8	56.52
F9	60.18

Release Kinetics

The results obtained from *in vitro* drug release studies were plotted adopting five different mathematical models of data treatment as follows:

- % Cum. Drug Release vs. Time (Zero order rate kinetics).
- Log % Cum. Drug Retained vs. Time (First order rate kinetics).
- % Cum. Drug release was plotted against √T (Square root time). (Higuchi model)
- Log % Cum. Drug Release vs. Log Time (Korsmeyer & Peppas exponential equation).
- Hixson-Crowell's erosion equation, (% Cum. Drug Retained)$^{1/3}$ Vs. Time.

The curve fitting results of the release rate profiles of the designed formulation were shown in the Figures 6-20, which gave an idea on the release rate and the mechanism of drug release from matrices.

In accordance with the results of Alderman[114], the quick formation of gelatinous viscous layer resulting from hydration is considered to be the first essential step for delivery/release of drug.

The experimental data was fitted to different kinetic models like zero order and first order etc in order to establish the release pattern of the drug from the microspheres. The experimental data was also fitted to Higuchi's model, Korsmeyer model and Hixson Crowell to ascertain the mechanism of drug release from the matrix system.

The correlation coefficient of the slopes of these matrices showed an adequate fit to the first order kinetics. This was confirmed by the linearity of the plots obtained when log percent drug remaining to be released was plotted as a function of time (Fig.7, Fig.12 & Fig.17).

All the formulations followed Higuchi's equation proving that the drug release was by diffusion mechanism. The 'n' values obtained for microspheres after fitting into Korsmeyer and Peppas equation were found between 0.5 - 1 (Table No. 22), indicating Anomalous transport (Higuchi Matrix).

Fig. 6: Combined Zero Order Plot Of Drug Released (F1,F2 & F3).

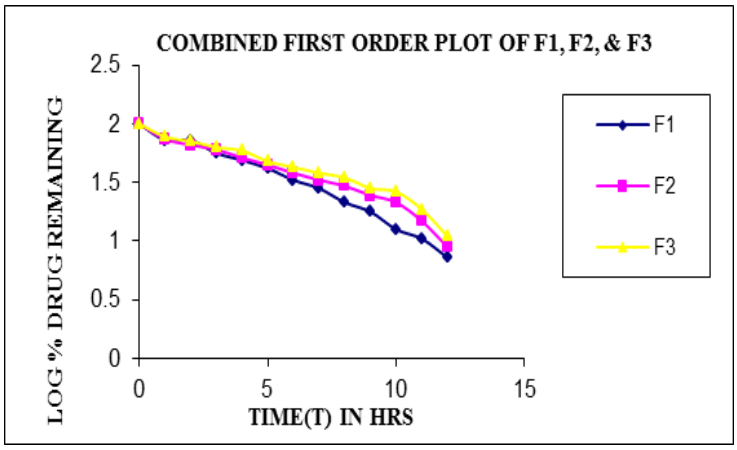


Fig. 7: Combined First Order Plot Of Drug Released (F1,F2 & F3).

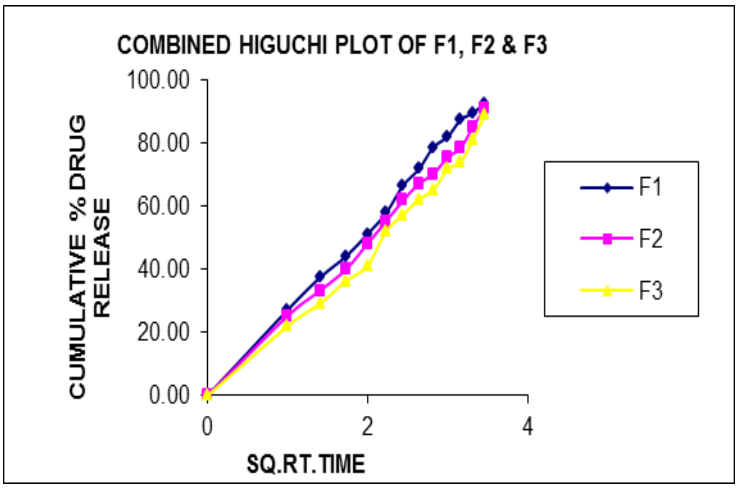


Fig. 8: Combined Higuchi Plot Of Drug Released (F1,F2 & F3).

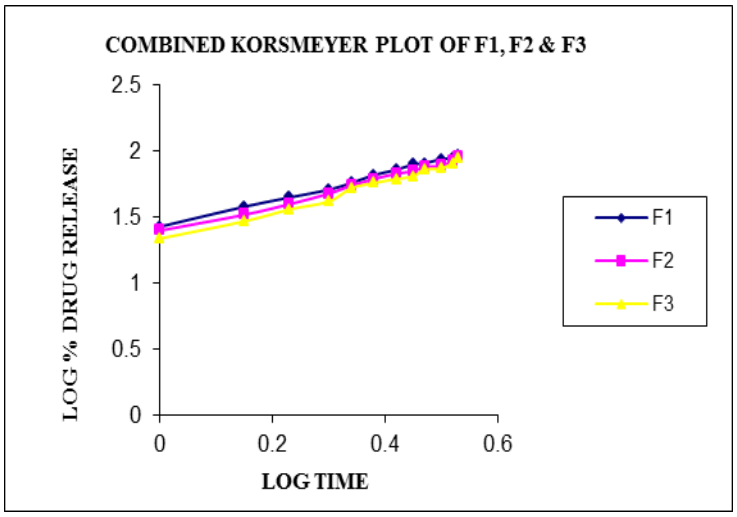


Fig. 9: Combined Korsmeyer Plot of Drug Released (F1, F2 & F3).

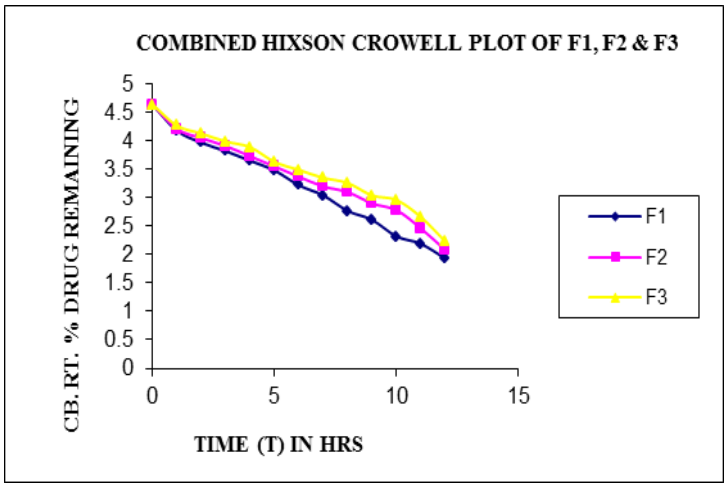


Fig. 10: Combined Hixson Crowell Plot of Drug Released (F1,F2 & F3).

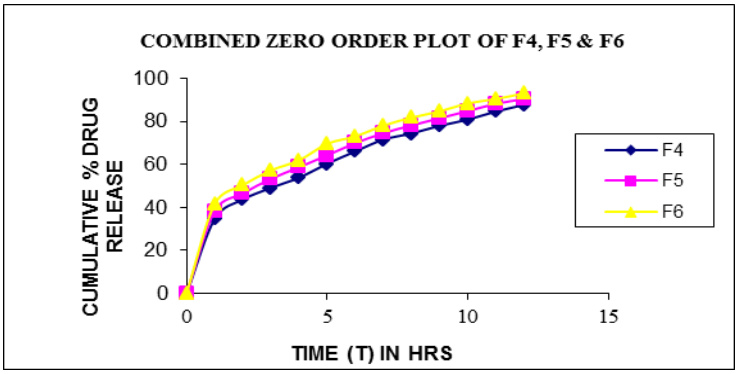


Fig. 11: Combined Zero Order Plot Of Drug Released (F4, F5 & F6).

Fig. 12: Combined First Order Plot Of Drug Released (F4, F5 & F6).

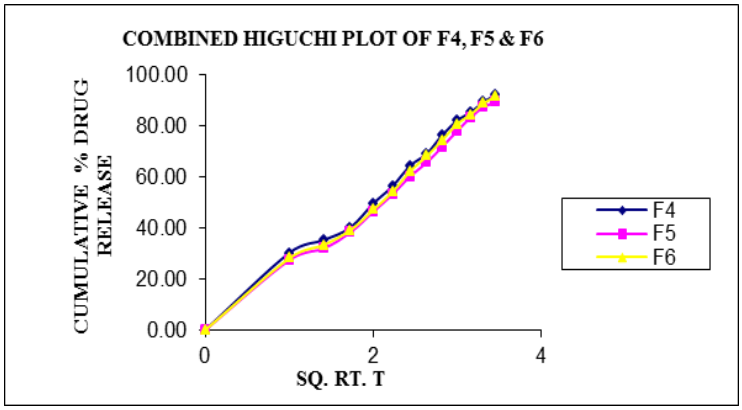


Fig. 13: Combined Higuchi Plot of Drug Released (F4, F5 & F6).

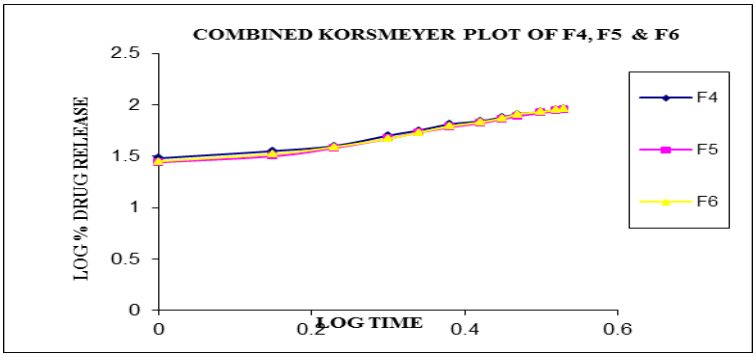


Fig. 14: Combined Korsmeyer Plot Of Drug Released (F4,F5 & F6).

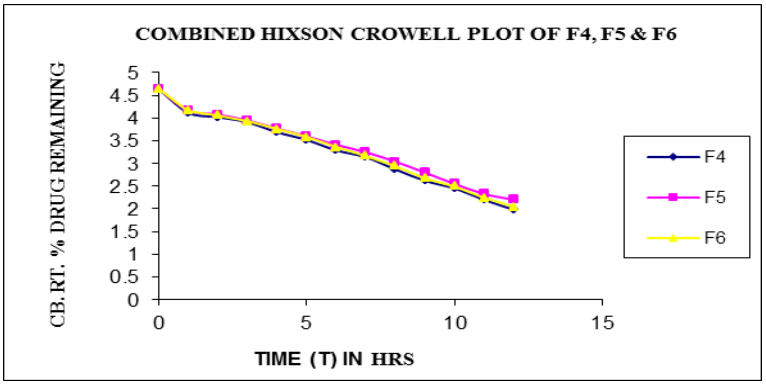


Fig.15: Combined Hixson Crowell Plot Of Drug Released (F4, F5 & F6).

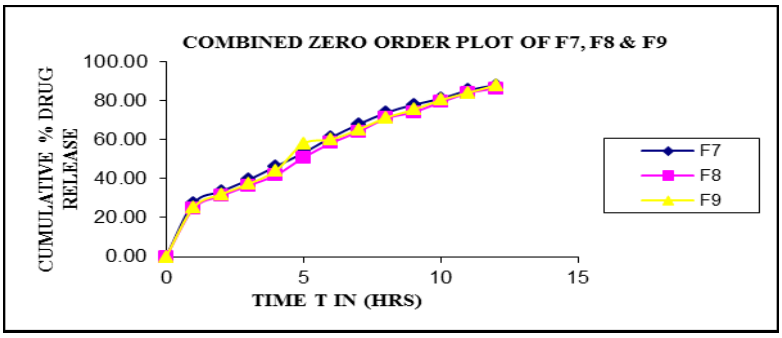


Fig. 16: Combined Zero Order Plot Of Drug Released (F7, F8 & F9).

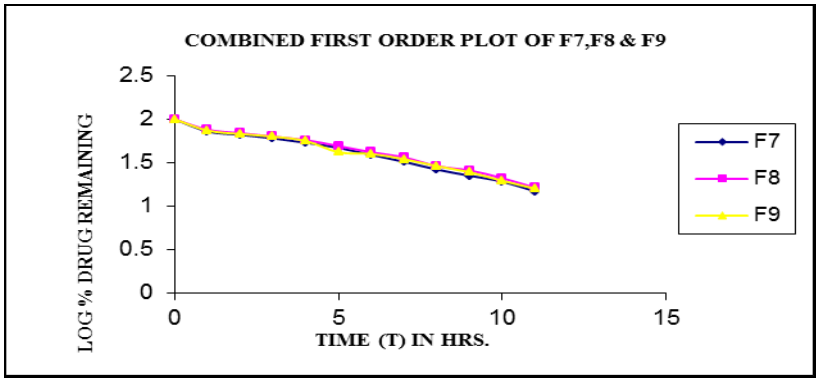


Fig. 17: Combined First Order Plot Of Drug Released (F7, F8 & F9).

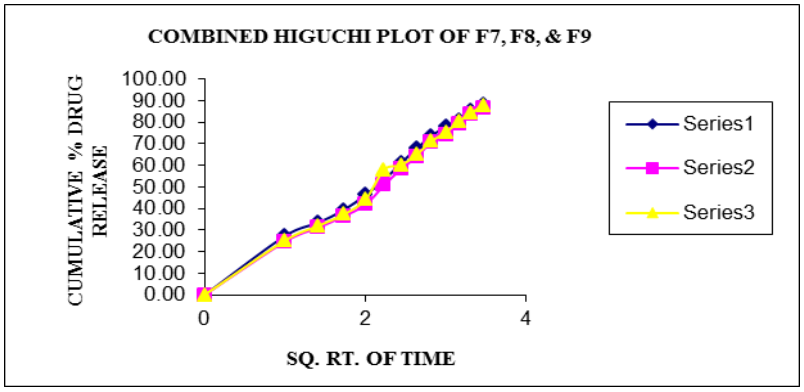


Fig. 18: Combined Higuchi Plot Of Drug Released (F7, F8 & F9).

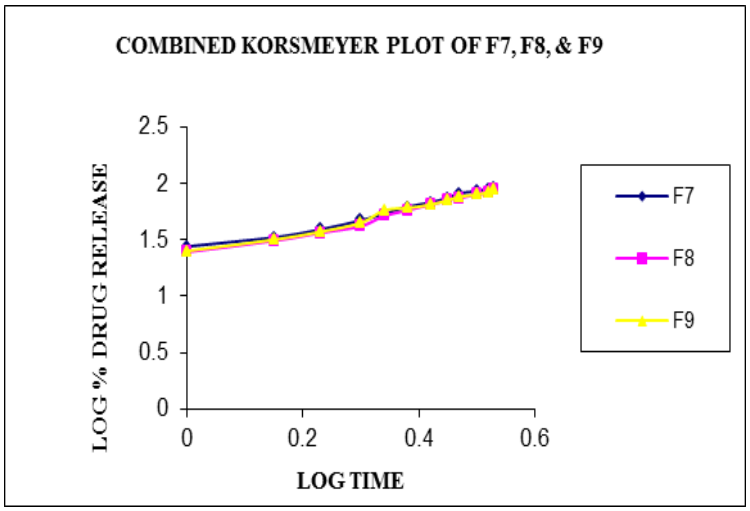


Fig. 19: Combined Korsmeyer Plot Of Drug Released (F7, F8 & F9).

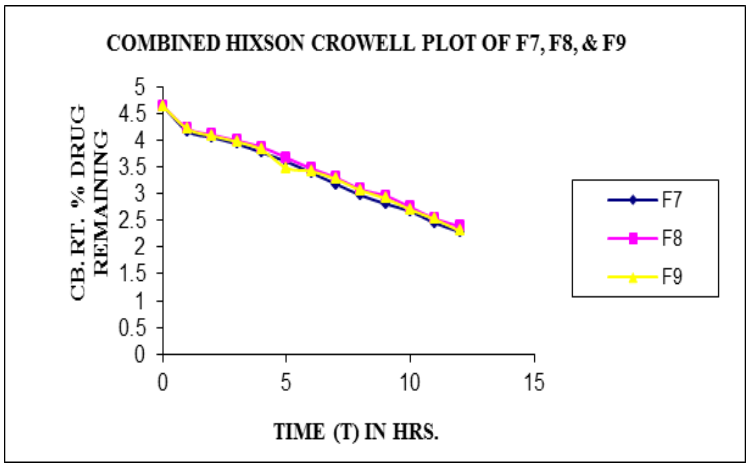


Fig. 20: Combined Hixson Crowell Plot Of Drug Released (F7, F8 & F9).

Table No. 21: Release Profiles of Tolperisone HCl from Different Formulations (R^2 values).

Formulation	Zero Order	1st Order	Higuchi	Korsmeyer	Hixson Crowell
F 1	0.930	0.983	0.996	0.996	0.993
F 2	0.942	0.957	0.995	0.992	0.983
F 3	0.960	0.946	0.987	0.988	0.978
F 4	0.939	0.975	0.991	0.973	0.991
F 5	0.953	0.975	0.989	0.975	0.990
F 6	0.950	0.974	0.989	0.973	0.991
F 7	0.941	0.988	0.992	0.980	0.991
F 8	0.956	0.986	0.987	0.980	0.992
F 9	0.952	0.984	0.990	0.982	0.991

Table No. 22: Dissolution Kinetics of Tolperisone HCl from Different Formulations

Formulation	Zero Order(K_O)	1st Order(K_1)	Higuchi Drug Diffusion Coefficient (D)	Values of Korsmeyer Release Exponent (n)	Hixson Crowell
F 1	6.871	0.204	27.51	1.032	0.212
F 2	6.527	0.170	25.96	1.063	0.185
F 3	6.462	0.154	25.28	1.144	0.173
F 4	6.819	0.198	27.09	0.995	0.207
F 5	6.725	0.177	26.51	1.058	0.193
F 6	6.867	0.191	27.11	1.057	0.204
F 7	6.557	0.165	26.05	1.029	0.184
F 8	6.541	0.156	25.72	1.104	0.178
F 9	6.575	0.163	25.49	1.077	0.180

Table 23: Theoretical (Expected) Dissolution Profile of Tolperisone Hydrochloride (12 Hours Modified Release).

Time in Hrs.	Percentage Drug Release (%)
0	0
1	29.52
2	35.92
3	42.32
4	48.72
5	55.12
6	61.52
7	67.92
8	74.32
9	80.12
10	87.12
11	93.52
12	99.92

Table No. 24: Similarity Factors (f_2) of Formulations Compared to Theoretical Dissolution Profile (Th.).

Formulation	Similarity Factor
F1	72.01
F2	64.27
F3	51.74
F4	75.62
F5	66.95
F6	73.76
F7	63.85
F8	56.52
F9	60.18

Table No. 25: % Drug Entrapment of Formulation F4 before & after 3 Months

Formulation	Initial	After 3 Months
F4	74.83	73.56

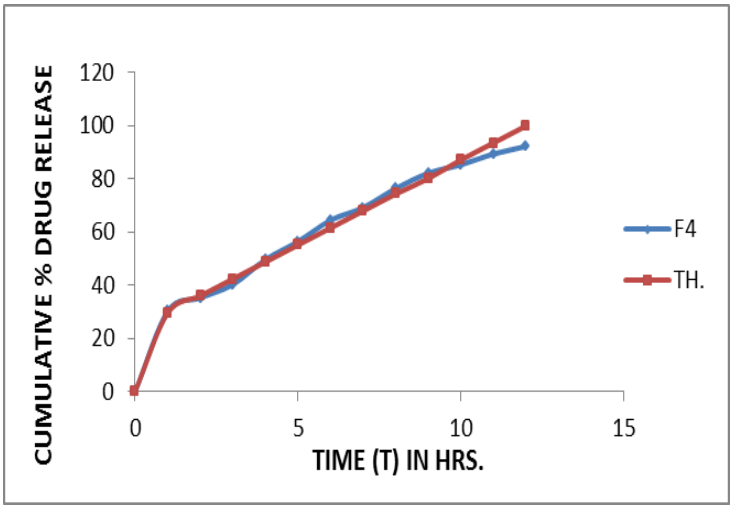


Fig. 21: Comparison of *in vitro* dissolution profiles of formulation F4 & Theoretical dissolution profile (Th.).

STABILITY STUDIES

Microspheres from optimized batch F4 were put on short-term stability study at 37^{o}C/75% RH condition for the period of three months. After three months, the microspheres were evaluated for physical appearance, drug entrapment efficiency, *in vitro* release study and possible drug-excipients interactions using Infrared (FTIR) spectrophotometry. Results showed that the microspheres did not show any significant changes in physical appearance, drug content (Table No.25) and cumulative % drug release (Table No.26). FTIR study (Fig.28) also revealed that there was no evidence of incompatibility between the pure drug Tolperisone HCl and various polymers that were used in the formulations. Hence we can conclude that the formulations should be kept at a temperature not above 30^{o}C and in a dry place.

Table-26: Cumulative % Drug Released from F4 initially and after 3 months (kept at 37^0C/75%RH)

Time (T) in Hours	Initially	After 3 months
0	0.00	0.00
1	30.21	29.45
2	35.21	34.56
3	40.23	39.43
4	49.59	48.74
5	56.38	56.35
6	64.42	63.63
7	69.17	67.25
8	76.33	725.97
9	82.11	81.37
10	85.31	83.84
11	89.32	86.27
12	92.20	91.47

Scanning Electron Microscopy

The determination of shape and surface morphology was done by scanning electron microscope HITACHI SU 1500, Japan.SEM analysis of the samples revealed that all microspheres were smooth, spherical and slightly aggregated. The surface topography reveals that the drug is well dispersed on the surface suggesting the prepared microspheres were matrix based wherein the drug is evenly distributed in the entire polymeric matrix. Also they were porous in nature due to the rapid escape of the volatile solvents molecules during formulation. Inward dents were seen on the surface probably due to collapse of the walls of the microspheres during the in situ drying process. (Fig. 31).

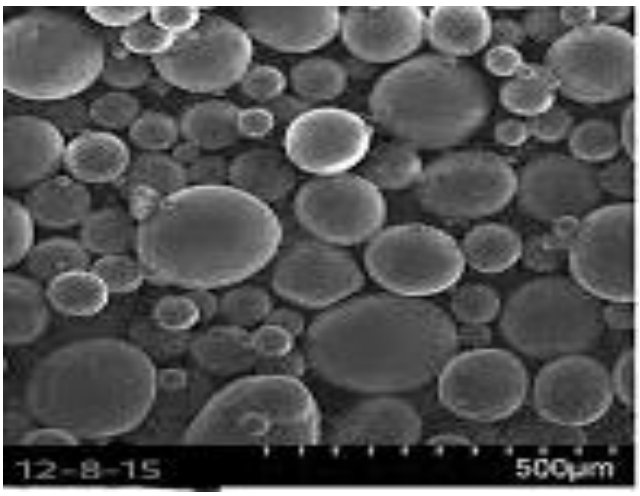


Fig. 31: Scanning Electron Microscopic View of Microsphere.

CONCLUSION

1. In the present study, nine different formulations (F1 to F9) of Tolperisone HCl loaded microspheres were prepared using various polymeric combinations with a view for the sustained delivery of the drug over a time period of 12 hours.
2. All the polymers and the drug used were of pharmaceutical grade.
3. Biocompatible polymers such as Eudragit (RS100 & S100) & Ethyl Cellulose (EC) all hydrophobic can be used to formulate sustained release microspheres especially in case of a highly water soluble drug like Tolperisone HCl.
4. Good percentage yield was obtained with all the formulations particularly microspheres prepared with a Eudragit RS 100.
5. Good loading efficiency and entrapment efficiency were obtained with all the batches.
6. All the formulations showed very good flow properties and were within acceptable range and therefore they can easily be filled into capsules.
7. Particle size analysis revealed that the particle size of all different formulations were in acceptable range.
8. From the *in vitro* drug dissolution profiles of all batches of all batches, it was observed that batch F4 gave best dissolution profile from the rest of the formulations. It was evident from the Similarity Factor (f_2) of 75.62 as compared to theoretical dissolution profile of modified release Tolperisone HCl and hence batch F4 was optimized out as the best formulation among all the formulations.
9. % Floating studies revealed that microspheres were able to float in the dissolution medium for the entire drug release period
10. Dissolution results showed that drug release decreases as the content of Eudragit RS 100 increases among formulations F1 to F3. Among formulation F4, F5 & F6, as the concentration of Eudragit RS100 & Ethyl cellulose increases, the drug release was increased. Also, among formulations F7, F8 & F9 as the concentration of Eudragit S100 increases, the drug release was decreased.
11. Analysis of dissolution profiles (R^2values) showed that drug release from matrix followed first order kinetics.
12. Analysis of dissolution profiles on the basis of Higuchi's model and that of Korsmeyer model suggested that drug release was basically Fickian diffusion controlled (Higuchi Diffusion).
13. FTIR study revealed that there was no evidence of interaction between pure drug Tolperisone HCl and the polymers used in the study.

14. SEM analysis of the samples revealed that all microspheres were smooth, spherical and slightly aggregated. The surface topography reveals that the drug was well dispersed on the surface suggesting the prepared microspheres were matrix based.
15. Stability studies revealed that the formulation F4 was stable after keeping them at 37^0C/75% RH for three months.
16. All these results show that the prepared microspheres seem to be a potential candidate for oral sustained release of the drug.

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