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PREPARATION AND CHARACTERISATION OF TORSEMIDE MICROCAPSULES

Mitta Chaitanya*and Dr. P. Narayana Raju
Department Of Pharmaceutical Science,
ShriJagdish Prasad Jhabarmal Tibrewala University, Vidyanagari, Jhunjhunu, Rajasthan – 333001

Abstract:
Hypertension (HTN or HT), also known as high blood pressure (HBP), is a long term medical condition in which the blood pressure in the arteries is persistently elevated. Microencapsulation is a process of applying relatively thin coatings to small particles of solids or droplets of liquids and dispersions. It provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and of controlling the release characteristics or availability of coated materials. Batches of microcapsules were prepared by ionotropic gelation method which involved reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate and the mucoadhesive polymer were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. Torsemide microcapsules were prepared with Eudragit RL 100 and Eudragit RS 100 by using solvent evaporation method.Preformulation and evaluation studies were performed for all the microcapsules. The in vitro dissolution studies were performed using USP type I dissolution apparatus at 100 rpm. Invivo clinical studies were conducted. Microcapsules prepared with ion tropic gelation method T-F7 formulation shown maximum drug release. DSC and FTIR studies were also performed.

Keywords: *Hypertension, Torsemide microcapsules, ionotropic gelation method.*

Corresponding authour:
Mitta Chaitanya,
Department Of Pharmaceutical Science,
ShriJagdish Prasad Jhabarmal Tibrewala University,
Vidyanagari, Jhunjhunu,
Rajasthan – 333001

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INTRODUCTION:

Hypertension

Hypertension (HTN or HT), also known as high blood pressure (HBP), is a long term medical condition in which the blood pressure in the arteries is persistently elevated. High blood pressure usually does not cause symptoms. Long term high blood pressure, however, is a major risk factor for coronary artery disease, stroke, heart failure, peripheral vascular disease, vision loss, and chronic kidney disease. High blood pressure is classified as either primary (essential) high blood pressure or secondary high blood pressure. About 90–95% of cases are primary, defined as high blood pressure due to nonspecific lifestyle and genetic factors. Lifestyle factors that increase the risk include excess salt, excess body weight, smoking, and alcohol. The remaining 5–10% of cases are categorized as secondary high blood pressure, defined as high blood pressure due to an identifiable cause, such as chronic kidney disease, narrowing of the kidney arteries, an endocrine disorder, or the use of birth control pills.

Blood pressure is expressed by two measurements, the systolic and diastolic pressures, which are the maximum and minimum pressures, respectively. Normal blood pressure at rest is within the range of 100–140 millimeters mercury (mmHg) systolic and 60–90 mmHg diastolic. High blood pressure is present if the resting blood pressure is persistently at or above 140/90 mmHg for most adults. Different numbers apply to children.[8] Ambulatory blood pressure monitoring over a 24-hour period appears more accurate than office best blood pressure measurement

Microencapsulation

Microencapsulation is a process of applying relatively thin coatings to small particles of solids or droplets of liquids and dispersions. It provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and of controlling the release characteristics or availability of coated materials. [1]

Size for microcapsules range from 5-500-µm and may be isolated as free flowing powders called as aggregates, or suspended directly in a vehicle for administration.

Microcapsules assume various shapes such as globular, spherical, bean like, rice grain like, flocculated masses. The thickness exceeds 10 µm with the walls having single layered or multilayered structures. Further microcapsules may contain 1 to thousands of core substances.

The capsule wall should be inert to the substance it contains, possess enough strength to allow for normal handling without rupture. The contents of capsule are contained within the wall until related by some means that serve to break, crush, melt, dissolve, rupture or remove the capsule shell or until the internal phase is caused to diffuse out through the capsule wall.

Fundamental considerations[2]

For the microencapsulation to be successful, due attention must be given to the physical and chemical characteristics of the core material, nature and properties of the wall material (prior to and after encapsulation) and methods available for the encapsulation. The intended physical characters of the encapsulated product and the intended use of the final product must also be considered.

a) Characteristics of the core material/drug

The specific material to be coated is defined as the core material, can be either liquid or solid in nature. The composition of the core material can be varied as the liquid core can include dispersed and /or dissolved material. The solid core can be a mixture of active constituents, stabilizers, diluents, excipients and release rate retardants or accelerators.

b) Characteristics of the wall material

The coating material should be capable of forming a film that is cohesive with the core material, be chemically compatible and non reactive with the core material and provide the desired coating properties such as strength, flexibility, impermeability, optical properties and stability. The total thickness of the coatings achieved with microencapsulation techniques is microscopic in size.

c) Physical character of the final product

Microcapsule should have desirable physical properties like ability to flow, to be compacted or to be suspended and the capsule wall must be capable of resisting the pressure during compression etc.

d***) Intended route of administration of the drug***

Microcapsules intended for oral use may dissolve in the environment of the stomach or may be enteric coated. They may be designed to burst while being chewed or to release their ingredients on contact with saliva.

It can be seen that when the decision is made to microencapsulate particular material, it is imperative to have the necessary knowledge of the core material, the available coat material, the nature of the final wall, and the available methods for microencapsulation.

MATERIALS AND METHODS:

USED: Toresamide, Cellulose Acetate Phthalate, Cellulose acetate butyrate, Eudragit RL-100, Eudragit RS-100, Sodium alginate, Ethyl cellulose.

Torsemide Microcapsules Prepared With Ion Tropic Gelation Method

Batches of microcapsules were prepared by ionotropic gelation method which involved reaction between sodium alginate and polycationic ions like calcium to produce a hydrogel network of calcium alginate. Sodium alginate and the mucoadhesive polymer were dispersed in purified water (10 ml) to form a homogeneous polymer mixture. The API,torsemide (100mg) were added to the polymer premix and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added through a 22G needle into calcium chloride (4% w/v) solution. The addition was done with continuous stirring at 200rpm. The added droplets were retained in the calcium chloride solution for 30 minutes to complete the curing reaction and to produce rigid spherical microcapsules. The microcapsules were collected by decantation, and the product thus separated was washed repeatedly with purified water to remove excess calcium impurity deposited on the surface of microcapsules and then air-dried.

Formulation of Torsemide microcapsules

The Torsemide microcapsules were prepared by Ionotropic gelation technique, the composition of various formulations was mentioned in Table 3.4. Torsemideand polymers were individually passed through sieve ≠ 60. The required quantities of polymers were dissolved in purified water to form a homogenous polymer solution. Torsemide was added to the polymer solution and mixed thoroughly with stirrer at 400 rpm to form a homogeneous dispersion. The resulting homogeneous dispersion was sonicated for 30 min to remove any air bubbles .For the formation of microcapsules the dispersion was then extruded manually drop wise into aluminum sulphate solution (10%) using polyethylene syringe (needle size 24 G). The extruded droplets were retained in the aluminiumsulphate solution for 30 minutes to complete the curing reaction and to produce spherical rigid Torsemide microcapsules. The obtained microcapsules were collected by decantation, washed repeatedly with distilled water to remove excess aluminum impurity and dried at 45°C for12 hour.

***In Vitro* Dissolution**

The in vitro dissolution studies were performed using USP type I dissolution apparatus (LABINDIA, DISSO-2000, Mumbai, India) at 100 rpm. The microcapsules equivalent to 20 mg based on the assay were weighed and filled in the empty hard gelatin capsule shells size "2" and placed in the basket. The dissolution medium consisted of 900 ml of 0.1 N HCl maintained at 37 ±0.5 °C. An aliquot (5 mL) was withdrawn at specific time intervals and drug content was determined by UV visible spectrophotometer (Schimadzu, UV-1700 E 23) at 263 nm. The release studies were conducted in triplicate.

RESULTS:

Table 1: Sieve analysis, Drug content and entrapment efficiency of Torsemide microcapsules

	TORSEMIDE :EUD RL 100				TORSEMIDE :EUD RS 100			
Size	**1:0.5**	**1:1**	**1:1.5**	**1:2**	**1:0.5**	**1:1**	**1:1.5**	**1:2**
10/20 (1242 µ)	9 ±0.15	2 ±0.15	5 ±0.15	9 ±0.85	6 ±0.87	4 ±0.87	9 ±0.56	10 ±0.56
20/30 (666.5 µ)	10 ±1.01	18 ±1.01	12 ±0.26	13 ±1.21	3 ±0.78	5 ±0.78	8 ±0.74	10 ±0.21
30/40 (445 µ)	70 ±0.56	69 ±0.56	76 ±0.69	71 ±0.88	79 ±0.56	83 ±0.56	73 ±0.87	72 ±0.32
60/80 (225 µ)	10 ±0.87	11 ±0.87	7 ± 0.99	7 ±0.78	12 ±0.56	8 ±0.56	10 ±0.47	9 ±0.87
Drug content (%)								
Theoretical (%)	66.66	50	33.3	25	66.65	50	33.3	25
Estimated (%)	41.54±1.5	38.51± 2.2	26.31±1.1	19.9±1.6	43.15 ±1.1	33.11±1.1	25.32±0.2	21.17±1.1
Entrapment efficiency (%)	62.31	77.02	78.93	79.64	64.73	66.22	75.03	84.68

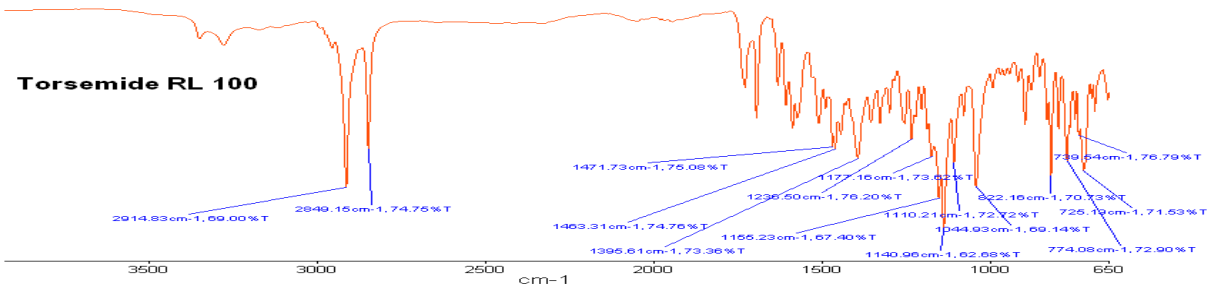

Fig.1: FTIR spectra of Torsemidemicrocapsules prepared with Eudragit RL 100

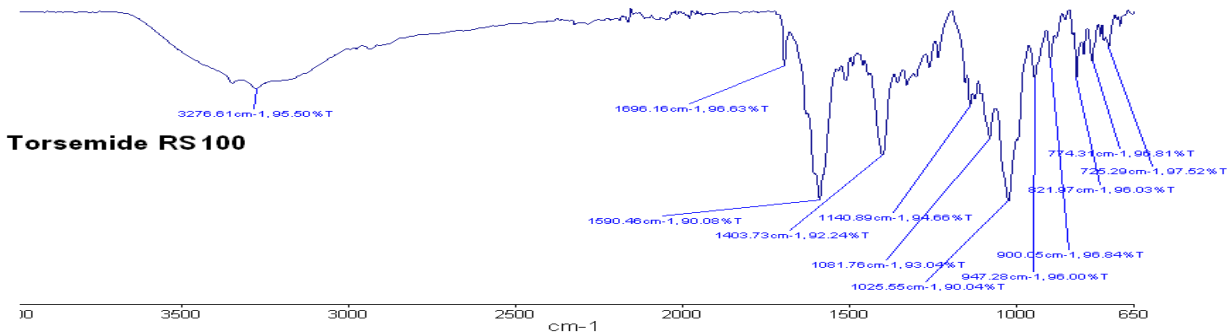

Fig.2: FTIR spectra of Torsemide microcapsules prepared with Eudragit RS 100

Fourier Transforms Infrared Radiation measurement (FT-IR)

FTIR studies were conducted on the prepared microcapsules. Absorption peaks of C=N stretch at 1695 cm^{-1} , C-N stretch at 1280cm^{-1} and 3280 cm^{-1} for presence of hetero atom for Torsemide pure drug. Similar absorption peaks were observed for the torsemide microcapsules prepared with Eudragit RL 100 and RS 100 clearly indicated the stable nature of microcapsules prepared with these polymers

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Differential scanning calorimetry (DSC) study

Differential scanning calorimetric (DSC) study of drug loaded microcapsules was performed using a Diamond DSC (Mettler Star SW 8.10) to determine the drug excipients compatibility. DSC thermograms of pure Torsemide show sharp endothermic peaks at 167.8°C. DSC thermograms of Torsemide microcapsules prepared with Eudragit RS 100 show endothermic peaks at 168.0°C and Torsemide microcapsules prepared with Eudragit RL 100 show endothermic peaks at 165.2°C clearly indicated no drug polymer interaction.

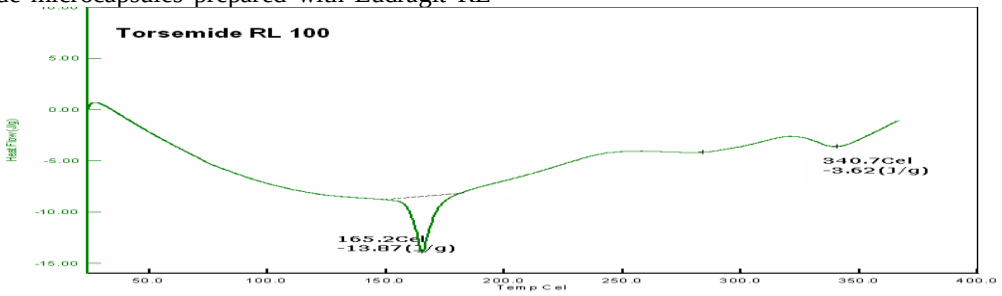

Fig. 3: DSC thermogram of Torsemide microcapsules prepared with Eudragit RL 100

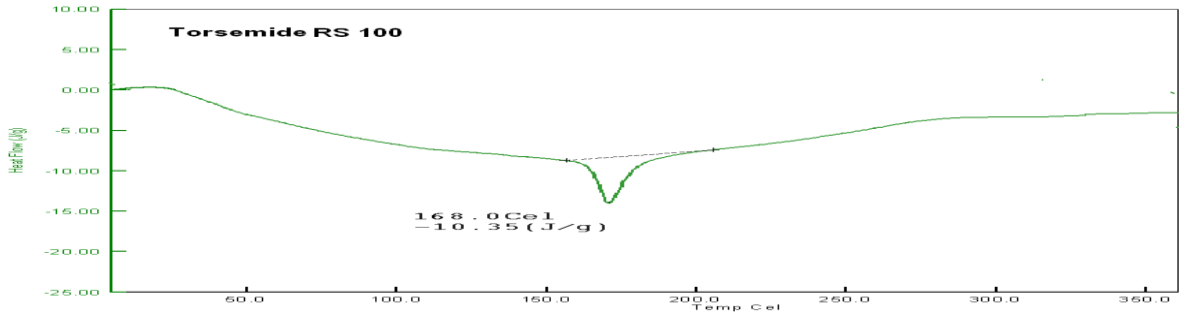

Fig.4: DSC thermogram of Torsemide microcapsules prepared with Eudragit RS 100

Differential scanning calorimetric (DSC) study of drug loaded microcapsules was performed using a Diamond DSC (Mettler Star SW 8.10) to determine the drug excipients compatibility. DSC thermograms of pure Torsemide show sharp endothermic peaks at 167.8°C. DSC thermograms of Torsemide microcapsules prepared with Eudragit RS 100 show endothermic peaks at 168.0°C andTorsemide microcapsules prepared with Eudragit RL 100 show endothermic peaks at 165.2°C clearly indicated no drug polymer interaction.

Table 2: Percent drug released of Torsemide microcapsules with Eudragit RL 100 and Eudragit RS 100

Formulation	**DRUG :RL 100**				**DRUG :RS 100**			
Time (hr)	**1:0.5**	**1:1**	**1:1.5**	**1:2**	**1:0.5**	**1:1**	**1:1.5**	**1:2**
0	0	0	0	0	0	0	0	0
1	39	28	21	13	33	16	11	8
2	56	42	34	25	48	29	22	17
3	67	53	44	35	59	40	32	25
4	77	63	54	42	69	48	40	32
5	87	70	61	51	78	56	48	42
6	94	78	69	59	85	64	56	49
7	99	83	76	65	92	70	62	56
8		89	83	72	95	77	69	61
9		94	88	78	99	84	77	68
10		99	92	84		88	84	73
11			96	88		93	89	79
12 13 14			99	92 97		95	93 97 99	83 87 91

Table 3: Release kinetics of Torsemide microcapsules with Eudragit RL 100 and Eudragit RS 100

Formu Lation	**DRUG :RL 100**				**DRUG :RS 100**			
Kinetics	**1:0.5**	**1:1**	**1:1.5**	**1:2**	**1:0.5**	**1:1**	**1:1.5**	**1:2**
Zero order	0.9682	0.9673	0.9665	0.9782	0.9331	0.9638	0.9788	0.9858
First order	0.8876	0.8566	0.8867	0.9733	0.8692	0.9543	0.9395	0.9845
Higuchi	0.9961	0.9984	0.9976	0.9982	0.9934	0.9983	0.9957	0.9941
Peppas	0.9972	0.9978	0.9967	0.9953	0.9962	0.9939	0.9962	0.9968
Peppas (n)	0.482	0.5471	0.6199	0.7896	0.5091	0.7123	0.8503	0.9333

Torsemide Microcapsules Prepared With Iontropic Gelation Method

Table 4: In vitro dissolution data and release kinetic data of Torsemide microcapsules prepared with Sodium alginate and Pectin.

Time (Hrs)	TF-1	TF-2	TF-3	TF-4	TF-5	TF-6	TF-7	TF-8	TF-9	TF-10	TF-11	TF-12
0	0	0	0	0	0	0	0	0	0	0	0	0
1	28.2	21.3	17.6	14.3	20.6	14.3	11.6	8.96	15.3	12.6	7.87	4.57
2	34.2	26.2	22.6	19.6	26.9	21.6	17.6	14.3	22.3	17.9	13.3	8.54
3	46.8	38.5	33.2	29.6	37.2	29.6	24.6	19.3	30.2	24.6	17.6	12.81
4	64.8	50.5	45.8	41.5	48.2	39.2	34.6	28	40.5	33.6	25.2	19.3
5	76.7	67.1	60.5	56	60	48	42.9	35.2	50.2	41.5	32.47	24
6	89.7	79.4	73.4	69.1	68	60	55.1	48	59.64	51.5	41.88	32.2
7	96.7	89.7	82.7	76.7	79.4	70.1	64.8	58.1	70.8	62.5	51.6	43.68
8	99	94.4	90.4	85	90.4	81.4	74.88	67.4	80	71.4	63.36	53.2
9		99	96	91.32	95.7	89.7	81.7	75.48	89.4	81.4	74.64	63.8
10			99	95.3	99	95.7	87	83.1	93.7	88	83.1	75
11				97.7		99	91	87.4	97	91.4	87	82.1
12				99			94	90	99	96	92	85.7
							97	93		99	93.7	89.7
							99	95			95	91.4
Zero order	0.9724	0.9776	0.9768	0.9530	0.9896	0.9918	0.9638	0.9711	0.9802	0.9843	0.9751	0.9803
First order	0.8934	0.8651	0.8756	0.9278	0.8473	0.8320	0.9414	0.9551	0.9096	0.9116	0.9269	0.9180
Higuchi	0.9241	0.9728	0.9769	0.9788	0.9807	0.9749	0.9801	0.9704	0.9740	0.9711	0.9569	0.9378
Peppas	0.9638	0.9673	0.9738	0.9766	0.9821	0.9889	0.9870	0.9844	0.9869	0.9835	0.9845	0.9877
Peppas(n)	0.6780	0.7848	0.8432	0.8752	0.7449	0.8672	0.9078	1.0064	0.8370	0.8955	1.0585	1.2531

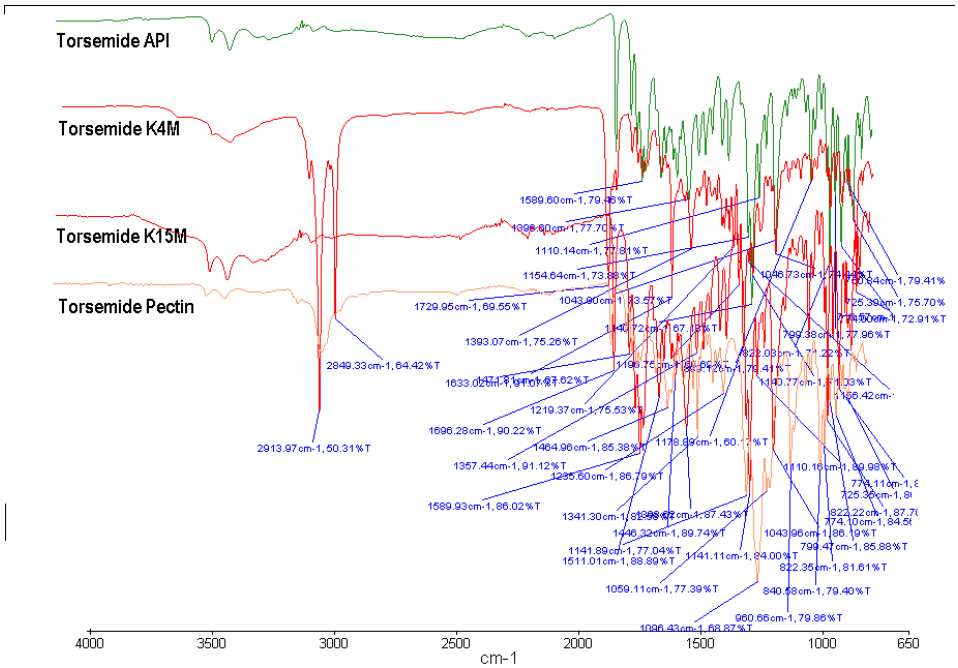

Fig.5: FTIR Spectrum of Pure Torsemide and Torsemide microspheres with Sodium alginate-Pectin, Sodium alginate –HPMCK4M and Sodium alginate HPMC k 15 M.

FTIR studies were conducted on the prepared microcapsules. Absorption peaks of C=N stretch at 1695 cm^{-1} , C-N stretch at 1280 cm^{-1} and 3280 cm^{-1} for presence of hetero atom for Torsemide pure drug. Similar absorption peaks were observed for the torsemide microcapsules prepared with Sodium alginate Pectin, Sodium alginate HPMC K 4 M and Sodium alginate HPMC K 15M clearly indicated the stable nature of microcapsules prepared with these polymers. Figshows the spectrum peaks points of Torsemide pure drug.

Differential scanning calorimetry (DSC) study

Differential scanning calorimetric (DSC) study of drug loaded microcapsules was performed using a Diamond DSC (Mettler Star SW 8.10) to determine the drug excipients compatibility. DSC thermograms of pure Torsemide show sharp endothermic peaks at 167.8°C. DSC thermograms of Torsemide microspheres prepared with sodium alginate pectin show endothermic peaks at 165.8°C, Torsemide microspheres prepared with sodium alginate HPMCK4M show endothermic peaks at 167.9°C and Torsemide microspheres prepared with sodium alginate HPMCK15M show endothermic peaks at 165.9°C clearly indicated no drug polymer interaction. Fig shows the endothermic peaks of Torsemide microcapsules during melting process.

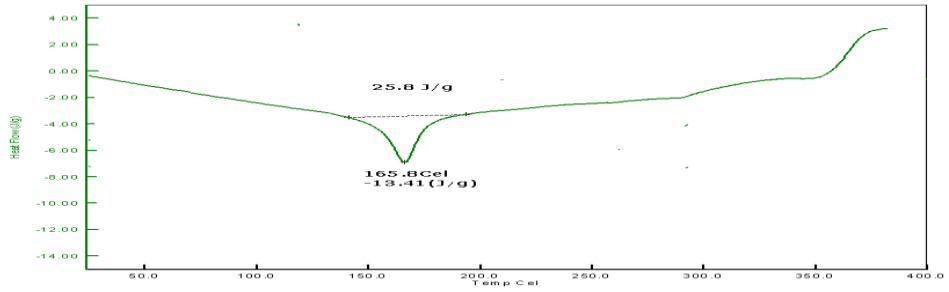

Fig. 6:DSC thermogram of Torsemide microspheres prepared with sodium alginate -pectin

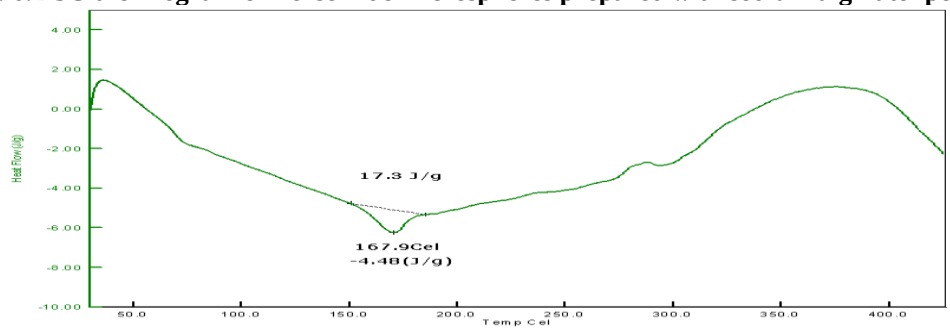

Fig.7: DSC thermogram of Torsemide microspheres prepared with sodium alginate –HPMCK4M

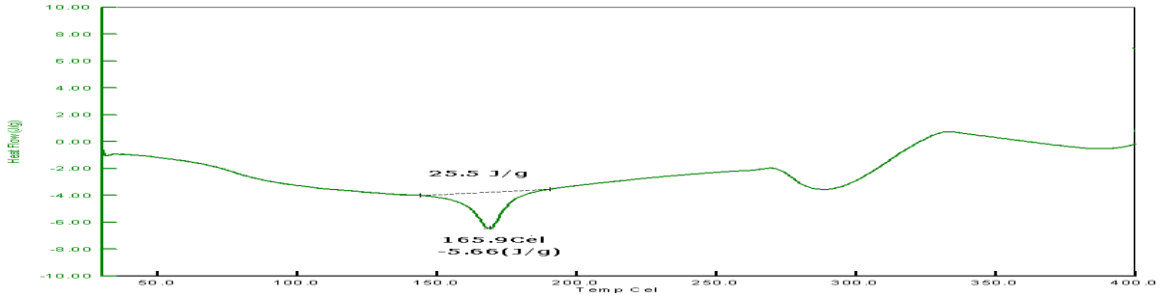

Fig.8: DSC thermogram of Torsemide microspheres prepared with sodium alginate –HPMCK15M

Differential scanning calorimetric (DSC) study of drug loaded microcapsules was performed using a Diamond DSC (Mettler Star SW 8.10) to determine the drug excipients compatibility. DSC thermograms of pure Torsemide show sharp endothermic peaks at 167.8°C. DSC thermograms of Torsemide microcapsules prepared with Eudragit RS 100 show endothermic peaks at 168.0°C and Torsemide microcapsules prepared with Eudragit RL 100 show endothermic peaks at 165.2°C clearly indicated no drug polymer interaction. Fig shows the endothermic peaks of Torsemide microcapsules during melting process.

***In Vivo* Clinical Study of Torsemide Sodium Alginate Microbeads**

Table 5: Pharmacokinetic data of TORSEMIDE 20 mg microcapsules (R)

Treatment	Subjects	T_{max}	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$	K_{el}	$T_{½}$
Reference (R)-IR	6	1.08	3390	6456.3	7137.3	0.31	2.23
Test (T)-ER	6	3.1	1360	14951.5	15073.1	0.14	4.56

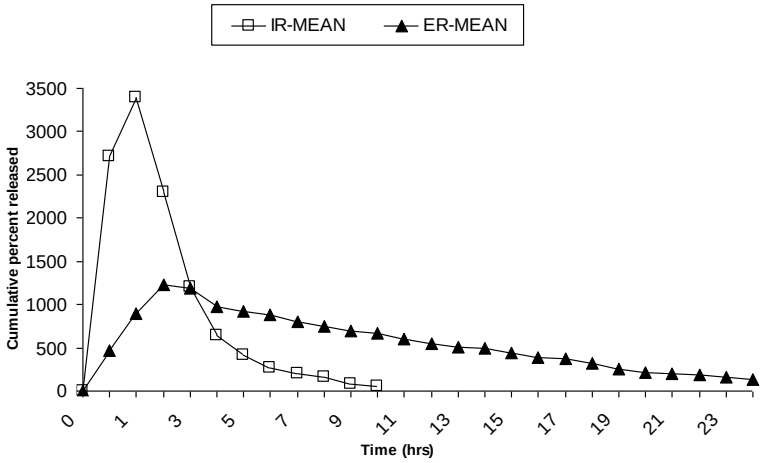

Fig.9: Comparative plasma profiles of TORSEMIDE 20 mg conventional formulation (R) with TORSEMIDE 20 mg extended release beads formulation (T)

Pharmacokinetic assessment

The plasma kinetic data was assessed with KINETIKA 5.0 software. Fig show the mean comparative data plot of the of the mean plasma concentration of the TORSEMIDE in both test (ER formulation) and reference (conventional formulation). The mean peak plasma concentration of test (T) formulation C_{max} 1360 ng/ml was gradually reached in 3.1 hr. In case of conventional reference formulation (R) the C_{max} was 3390 ng/ml which was reached in 1.08 hr. The Concentrationmaximum of the Test formulation (T)was lower when compared with Reference (R) formulation. The increased Tmax was clearly indicates the drug's availability for longer duration. Tables and show the kinetic data of TORSEMIDE conventional formulation (R) and extended release formulations (T) respectively.

The reference (R) formulation was absorbed rapidly, the T_{max} reached in about 1.08 hr. After reaching the T_{max} the drug starts rapid elimination and the concentration gradually reduced. In case of test (T) formulation the T_{max} achieved gradually and the drug availability was long time.

The AUC_{0-t}, of the reference (R) was found to be 6456.3 ng.min/ml. The increase in $AUC_{0-t}$of around 14951.5 ng.min/ml was observed in the test (T) formulation, this clearly indicates the drug availability for long period of time.

Decrease in elimination rate constant (K_{el}) from 0.31 hr^{-1} (Reference) (R) to 0.14 hr^{-1} (Test) indicates the lower release rate of drug in the body.

The half life ($T_{1/2}$) of the reference (R) and test (T) formulations were 2.33 hr and 4.56 hours, respectively, which were significantly different. Thus the prolonged $T_{1/2}$ [is] another indication on the in vivo performance of the TORSEMIDE extended release beads.

The overall C_{max}, T_{max}, AUC_{0-t}, K_{el} and $T_{1/2}$ were completely different between both test and reference formulation. Therefore the prepared formulation was releasing the drug for a prolonged period of time.

CONCLUSION:

This research study will divided into different major parts to support the primary goal of this research. Preformulation Studies, Drug Recovery from Microcapsules, Investigation of Formulation Parameters on Drug Release, Formulation, Drug excipient interaction study The prepared microcapsules will characterized for Drug entrapment efficiency, Particle size distribution, Assay, Invitro

dissolution, Differential scanning calorimetry, FTIR ,Scanning Electron microscopy, In vivo studies ,To analyze the pharmacokinetic parameters of the prepared microcapsule formulations.Torsemide microcapsules were prepared with Eudragit RL 100 and Eudragit RS 100 by using solvent evaporation method.Preformulation and evaluation studies were performed for all the microcapsules. The mobile phase was prepared using phosphate buffer of pH 7.4 and Acetonitrile at a ratio of 40:60 (Buffer: Acetonitrile).The retention time was found to be 2.971. Torsemide microcapsules were prepared with eudragit RL 100 and eudragit RS 100 of all the ratios the formulation with ratio 1:2 shown maximum drug release,in Torsemide Microcapsules prepared with ion tropic gelation method T-F7 formulation shown maximum drug release.

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