DEVELOPMENT AND CHARACTERIZATION OF TORSEMIDE MICROSPHERES

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ABSTRACT
The development of oral sustained release dosage form of Torsemide microspheres. Such drug is difficult to be delivered orally in a sustained or controlled release manner and, Due to its effectiveness and intensive use as a drug of choice in the treatment of fluid retention (Edema) sustained release formulations of Torsemide have been made and reported. Torsemide microsphere were prepared with a coat consisting of alginate and polymer such as HPMC and Sodium alginate by Ionic cross-linking technique using $CaCl_2$. The microspheres were evaluated with respect to the yield, particle size, incorporation efficiency, in vitro drug release and stability. Microspheres were characterized by FTIR studies. It was found that the particle size and incorporation efficiency of microspheres increases with increasing drug-to-polymer ratio.
Keywords: Torsemide, Ionotropic gelation technique, Sodium alginate, HPMC, FTIR studies, In vitro drug release studies.

I. INTRODUCTION

Microspheres are small spherical particles with diameters from 1 to 1000 µm. In some cases, microspheres are also known as micro particles. Microspheres can be produced from several natural and synthetic polymeric materials or even from inorganic materials For example, microspheres can be produced from commercially available polymers or ceramics.[1] Depending on the method, solid or porous microspheres can be obtained for specific intended applications.[2] Microspheres improve the drugs therapeutic efficacy and bioavailability, reduces toxicity and minimizes side effects. Microspheres can be prepared by various materials such as natural and synthetic materials. It plays a crucial role in enhancing the absorption of traditional medicines and microencapsulation is a alter method to delay the release of medicine. Because of smaller particle size it broadly dispersed throughout the GIT and improves drug absorption.[3]Torasemide inhibits the Na^+ /K^+ /$2Cl^-$ symporter in the thick ascending limb of the loop of Henle, reducing reabsorption of sodium, chloride, and water. This leads to increased urine output and decreased blood volume, helping to lower blood pressure and reduce edema. Used to treat edema (due to heart failure, liver or kidney disease) and hypertension.[4]

II. MATERIALS

Torsemide procured from Hetero Labs, HYD. HPMCand sodium alginate was obtained from Synpharma Research Labs, Hyderabad. Other chemicalsand the reagents used were of analytical grade.

METHODOLOGY

Fourier transform infrared spectroscopy studies

Drug and drug-polymer compatibility research identification procedure: The FTIR spectra of the pure drug, excipient and physical mixture of drug and excipient were recorded in between 400-4000 wave number (cm-1). No peaks are observed which interfere with the main drug peaks. The following spectrum and table show IR spectrum for drug and polymer and the wave number of characteristic bands for the same. [5]

Formulation table

Table-1: Formulation development of Torsemide microspheres

F. no	Torsemide	HPMC	Sodium alginate	$Cacl_2$
F1	10	100	-	1%
F2	10	200	-	1%
F3	10	300	-	1%
F4	10	400	-	1%
F5	10	-	100	1%

F6	10	-	200	1%
F7	10	-	300	1%
F8	10	-	400	1%

Ionotropic Gelation Technique

In this method, polymers in different concentrations was dispersed in suitable solvent solution and homogenized for 1hr. Drug polymer solution was prepared by dispersing the drug (10 mg) slowly into previously prepared polymers slurry in different ratios with continuous and uniform stirring for 3 hr. A gelation medium was prepared separately by dissolving different percentages of calcium chloride in distilled water. Bubble free dispersion medium was extruded through glass syringe (20 Guaze) into the gently agitated calcium chloride solution. The agitation was carried out by mechanical stirrer at different rpm. Microspheres were separated by filtration from the solution, washed with water and dried.[6]

Evaluation of microspheres

Percentage Yield

To prepared oral microsphere of all batches accurately weight. The measured weight of prepared microspheres was divided by total amount of all excipient and drug used in preparation of oral microspheres, which give the total percentage yield of total microspheres.It was calculated by following equation[7]

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

Drug Entrapment Efficiency

Entrapment efficiency of oral microspores was evaluated by deriving percent drug entrapment. the drug content of drug loaded oral microsphere was determine by dispersing 10 mg of oral microspheres in 10 ml ethanol followed by agitation with of magnetic stirrer for about 30 min to extract the drug and dissolved completely. After filtration though paper the 1 ml of filtrate is pipette out and diluted up to 10 ml volumetric flask. Drug concentration in ethanol phase was recorded by taking absorbance of this solution. The drug concentration was calculated. Thus, the total drug entrapped in total yield of microspheres from the procedure was calculated. It is express in percentage it is called as % drug entrapment. The amount of drug loaded and entrapped in oral microsphere was calculated by following formula. [8]

% Drug Entrapment = Amount of drug actually present / Theoretical drug loaded expected × 100

Particle size analysis

Particle size was determined by using an optical microscope under regular polarized light and the mean particle size was calculated by measuring 50-100 particles with the help of a calibrated ocular micrometer.[9]

Morphological characterization using SEM

The prepared microspheres were coated with a thin layer of gold by sputtering (EmitechK450X, England) and then the microstructure were observed in a scanning electron microscope (SEM; AIS-2100 780, Seron, South Korea) that operated at an acceleration voltage of 20 kV.[10]

In-Vitro Dissolution Study

The release studies were carried out in 10 ml Franz diffusion cell containing 10 ml Phosphate buffer. Phosphate buffer pH 7.4 (10ml) was placed in a 10 ml beaker. The beaker was assembled on a magnetic stirrer and the medium was equilibrated at 37±50C. Dialysis membrane was taken and one end of the membrane was sealed. After separation of non-entrapped BisoprololMicrospheres was filled in the dialysis membrane and other end was closed. The dialysis membrane containing the sample was suspended in the medium. 1ml of aliquots were withdrawn at specific intervals, filtered after withdrawal and the apparatus was immediately replenished with same quantity of fresh buffer medium.[11]

Kinetics of drug release studies[12]

Zero order release kinetics

It refers to the process of constant drug release from a drug delivery device independent of the concentration. In its simplest form, zero order release can be represented as

$Q = Q0 + K0\ t$

Where Q is the amount of drug released or dissolved,

Q0 is the initial amount of drug in solution (it is usually zero), and K0 is the zero order release constant. The plot made: cumulative drug release vs. time. Graphical representation of fraction of drug dissolved verses time will be linear. The slope of the curve gives the value of K in zero order release kinetics. This is ideal behaviour for a dosage form and leads to minimum fluctuations in drug plasma levels. This is expressed mainly by osmotic pump systems and also transdermal systems, matrix tablets with low soluble drugs and coated forms.

First order release kinetics

The first order Equation describes the release from system where release rate is concentration dependent, expressed by the equation:

$dC / dt = - Kt$

Where

K is first order rate constant expressed in units of time-1 .

This equation can be expressed as: $Log\ Ct = Log\ C0 – k\ t / 2.303$

Where,

C0 is the initial concentration of drug and

Ct is the concentration of drug in solution at time t.

The equation predicts a first order dependence on the concentration gradient (Co - Ct) between the static liquid layer next to the solid surface and the bulk liquid. The plot made: log cumulative of % drug remaining vs. time which would yield a straight line with a slope of –K/2.303.

Higuchi Model

The first example of a mathematical model aimed to describe drug release from a matrix system was proposed by Higuchi in 1963 this model is applicable to study the release of water soluble and low soluble drugs incorporated in semisolid and solid matrices Model expression is given by the equation:

$Q = A\ [D\ (2C - Cs)\ Cs\ t]\ 1/2$

Where

Q is the amount of drug released in time t per unit area A,

C is the drug initial concentration,

Cs is the drug solubility in the media and D is the diffusivity of the drug molecules (diffusion coefficient) in the matrix.

Simplified Higuchi model describes the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion Equation.

$Q = KH\ t1/ 2$

The data obtained were plotted as cumulative percentage drug release versus square root of time. The slope of the plot gives the Higuchi dissolution constant KH.

Korsmeyer - Peppas Model

To find out the mechanism of drug release, first 60% drug release data was fitted in Korsmeyer – Peppas model

$Mt / M\alpha = K\ tn$

Where

$Mt/M\alpha$ is fraction of drug released at time t, k is the rate constant (having units of tn) incorporating structural and geometric characteristics of the delivery system. n is the release exponent indicative of the mechanism of transport of drug through the polymer. The n value is used to characterize different release mechanisms.

Stability studies [13]

Once the delivery system was developed, the practical utility of the formulation depends on the maintenance of the therapeutic efficacy throughout the shelf-life under different storage conditions. Various Invitro characterization parameters (physical appearance, entrapment efficacy, and drug release) of the microspheres were assessed after storage of the best formulations for 3 and 6 months at $40±2^{o}C$/ 75±5% RH according to ICH guidelines, and results were compared with those obtained before storage.

III. RESULTS AND DISCUSSION

Drug - excipient compatibility studies (FT-IR)

The compatibility between the drug and the selected lipid and other excipients was evaluated using FTIR peak matching method. There was no appearance or disappearance of peaks in the drug-lipid mixture, which confirmed the absence of any chemical interaction between the drug, polymers and other chemicals.

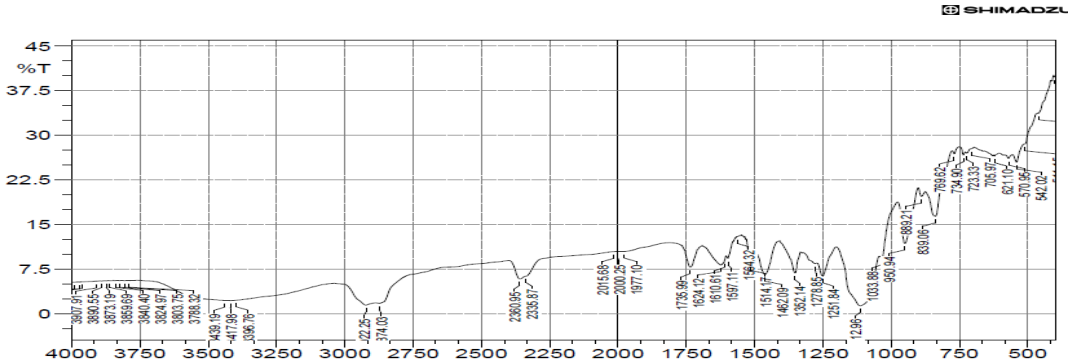

Fig-1: FTIR Studies of Torsemide

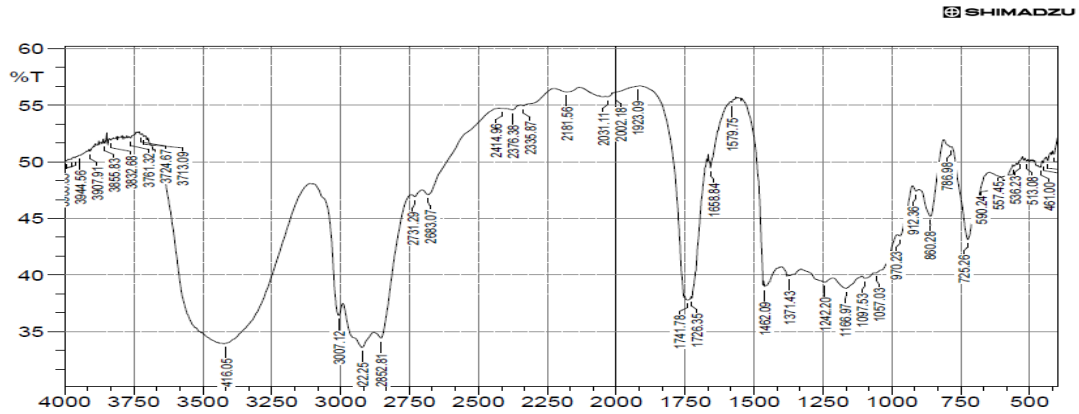

Fig-2: FTIR Studies of physical mixture of drug and excipients

Characterization of microspheres

Surface topography by scanning electron microscopy (SEM)

SEM photographs showed discrete, spherical microspheres. SEM photographs also showed the presence of drug crystal on the surface of microspheres revealing that the microspheres were having some rough surface. The drug crystals on microspheres were may be due to the presence of unentrapped drug in dispersion medium.

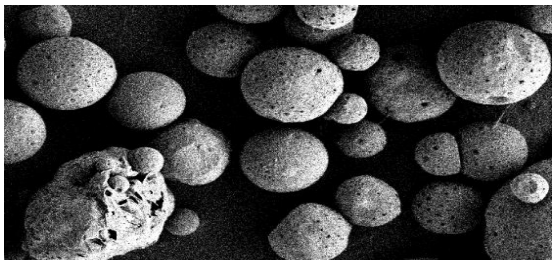

Fig-3: SEM photograph

Effect of formulation and process variables on Yield of sustained-release microspheres, Particle size,

Drug entrapment efficiency.

Table-2: Effect of drug polymer ratio on Yield of microspheres, Particle size, Drug entrapment efficiency

Formulation code	%yield	Particle size (µm)	Drug Entrapment Efficiency
F1	69.86±1.25	269±1.76	78.82±1.30
F2	70.22±1.36	290±1.85	80.20±1.25
F3	70.10±1.42	246±1.98	79.86±1.27
F4	71.22±1.76	273±1.92	82.30±1.26
F5	72.30±1.80	292±1.78	84.25±1.37
F6	75.68±1.36	286±1.85	85.21±1.35
F7	74.60±1.43	265±1.89	86.98±1.32
F8	73.12±1.38	236±1.91	84.50±1.28

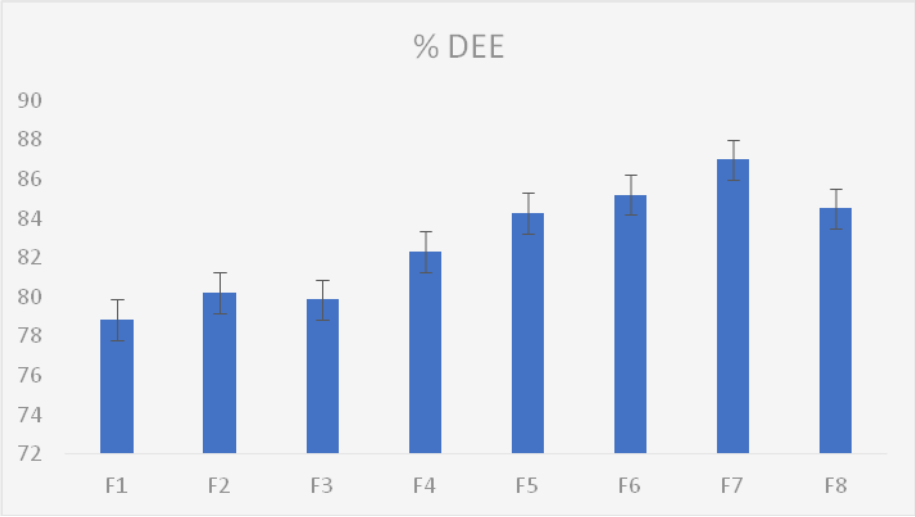

Fig-4: Drug Entrapment Efficiency of all formulations

Drug release studies

Table-3: *In vitro* release data of microspheres (F_1 to F_8)

Time (hrs.)	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8
0	0	0	0	0	0	0	0	0
1	16.82±0.29	17.50±0.47	18.10±0.63	15.30±0.45	16.30±0.43	14.50±0.50	15.10±0.42	14.69±0.54
2	27.50±0.20	26.54±0.53	27.19±0.56	28.12±0.43	27.36±0.43	28.12±0.49	29.68±0.51	27.48±0.52

3	33.69±0.23	32.22±0.48	35.93±0.50	36.89±0.50	35.90±0.50	36.98±0.43	38.12±0.39	37.16±0.40
4	47.41±0.26	46.39±0.54	47.50±0.49	48.96±0.49	47.64±0.57	46.89±0.55	49.66±0.46	48.55±0.48
5	53.50±0.37	55.24±0.63	58.93±0.36	57.10±0.53	52.99±0.46	55.52±0.50	57.45±0.54	55.42±0.56
6	65.19±0.26	66.89±0.24	68.90±0.48	66.52±0.37	68.10±0.43	67.29±0.41	68.55±0.42	69.86±0.40
7	77.20±0.56	78.10±0.46	76.51±0.43	78.12±0.29	77.85±0.50	76.85±0.36	79.82±0.57	82.56±0.37
8	91.25±0.48	93.36±0.51	94.89±0.37	95.40±0.50	94.59±0.46	95.33±0.51	97.58±0.36	94.53±0.45

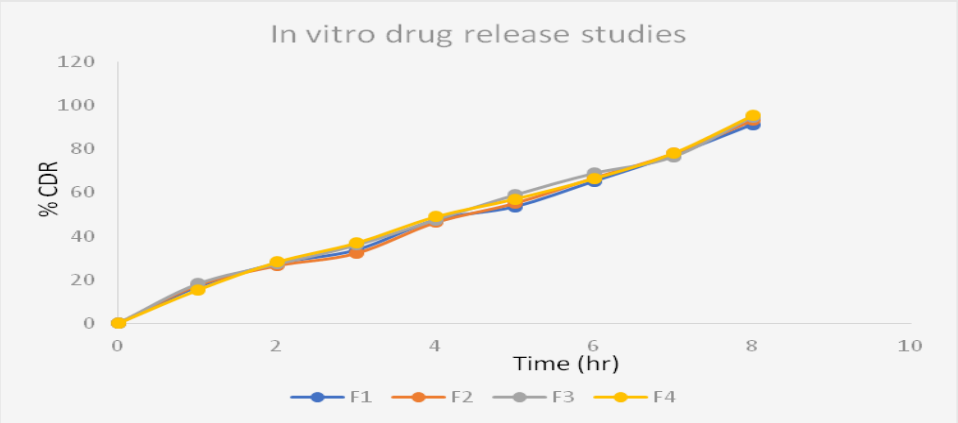

Fig-5: In vitro drug release of (F1- F4) formulation

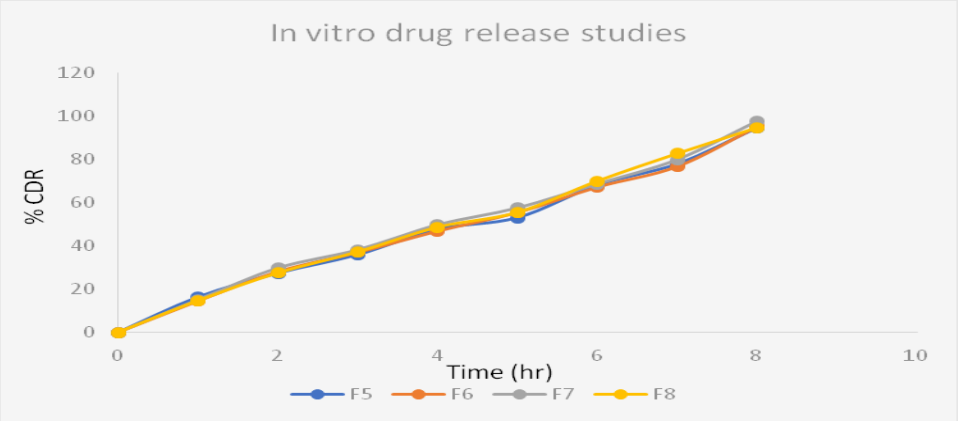

Fig-6: In vitro drug release of (F5- F8) formulation

Drug release kinetics:

The results obtaining in vitro release studies were plotted in different model of data treatment as follows:

- Cumulative percent drug released vs. time (zero order rate kinetics)
- Log cumulative percent drug retained vs. time (First Order rate Kinetics)

- Cumulative percent drug released vs. square root of time (Higuchi's
- Classical Diffusion Equation)
- Log of cumulative % release Vs log time (Peppas Exponential Equation)

Zero order kinetics

Fig-7: Zero order kinetics of optimized formulation

First order kinetics

Fig-8: First order kinetics optimized formulation

Higuchi model

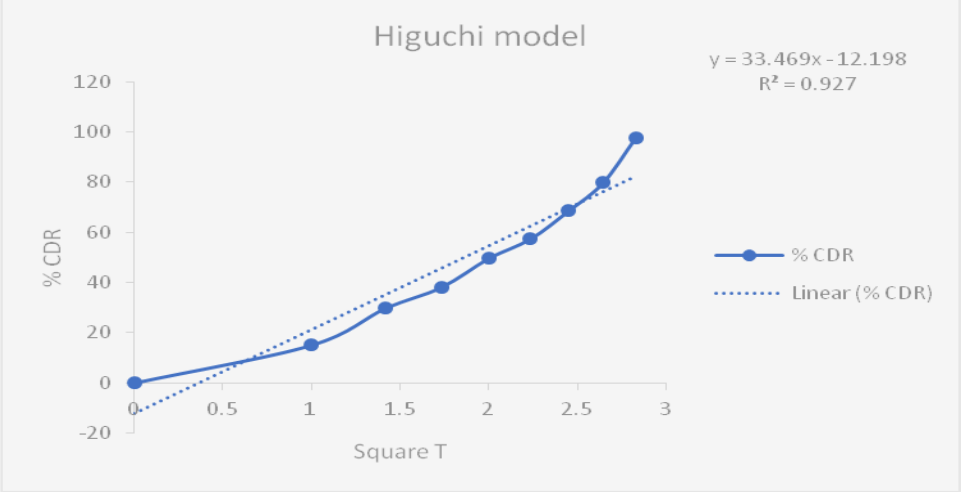

Fig-9: Higuchi modeloptimized formulation

Korsmeyer peppas

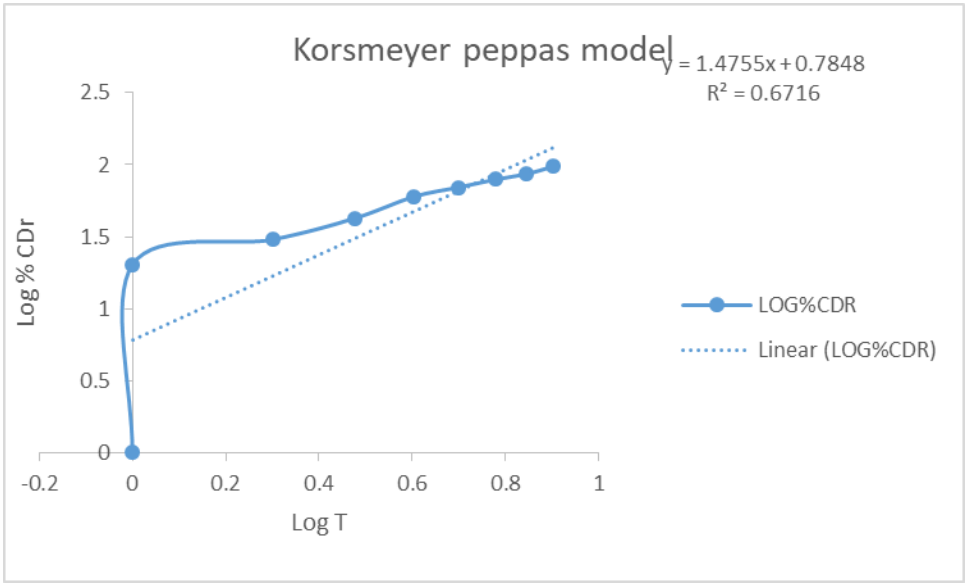

Fig-10: Korsmeyer peppasoptimized formulation

The values of in vitro release were attempted to fit into various mathematical models. Plots of zero order, first order, Higuchi matrix, Peppas were respectively.

Regression values are higher with Zero order release kinetics. Therefore, all the Torsemide microspheres Zero order release kinetics.

Stability studies

There was no significant change in physical and chemical properties of the Microspheres optimized formulation after 90 days. Parameters quantified at various time intervals were shown

Table-4: Results of stability studies of optimized formulation

F. Code	Parameters	Initial	1st Month	2nd Month	3rd Month	Limits as per Specifications
F-7	25^0C/60%RH % Release	97.58±0.36	96.59±0.34	95.15±0.27	94.14±0.31	Not less than 85 %
F-7	30^0C/75% RH % Release	97.58±0.36	96.43±0.30	95.59±0.32	94.42±0.33	Not less than 85 %
F-7	40^0C/75% RH % Release	97.58±0.36	96.32±0.29	95.13±0.29	94.50±0.27	Not less than 85 %

CONCLUSION

The yield and entrapment efficiency was high for Sodium alginate microspheres were Particle size, entrapment efficiency and production yield were influenced by the type of polymer, polymer concentration, stirring speed and combination of polymers. *In vitro* dissolution of optimized formulations of various Polymer in pH 7.4 formulations are releasing the drug up to 8 hrs. Hence, it is concluded that Torsemidepolymeric microspheres can be selected for the development of sustained drug delivery system for potential therapeutic uses

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