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Formulation and In-Vitro Assessment of Torsemide-Loaded Microspheres for Controlled Drug Delivery

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The present work aimed at formulation development and evaluation of Torsemide (TOR) microspheres. The loop diuretic TOR is used to treat congestive heart failure and edema. Due to its short half-life of two to three hours, weak basicity, and high solubility, TOR is released rapidly and does not provide sustained drug release. To achieve controlled drug release at a predetermined rate, TOR was prepared into microspheres. A total of 12 formulations were prepared by combining Sodium alginate with varying proportions of the polymers Eudragit RL100 and Hydroxy propyl methyl cellulose K15 (HPMC K15) by the ionotropic gelation technique and evaluated for micromeritic properties, percentage yield, drug entrapment efficiencies and in- vitro dissolution studies. Stability tests were performed out on the optimized formulation. Particle size of formulations was within acceptable limits, with percentage yields ranging from 80.5±0.012 to 95.3±0.028 and entrapment efficiencies from 72.5±0.024 to 86.8±0.020. Formulation F6 exhibited the highest drug release of 100% in a controlled manner, thus it was considered the optimized formulation and no stability issues were found.

Keywords: Eudragit RL100; HPMC; Ionotropic Gelation Technique; Torsemide; Sodium alginate.

A promising area of study and development is controlled drug delivery, which has the potential to significantly enhance the efficacy and safety of medication therapy. A method of administering drugs or therapeutic agents to a specific site of the body at predetermined rates and for predetermined amounts of time is known as controlled drug delivery. It entails the use of drug delivery systems or tools that control medication release, enabling the best possible therapeutic results while reducing adverse effects and enhancing patient compliance[1].

Traditional medication administration techniques, including oral tablets or injections, can cause rapid drug release and changes in drug levels in the body. This may result in less effective treatment, ineffective drug use, and possible negative effects. These restrictions are intended to be overcome by controlled drug delivery systems, which offer a more accurate and regulated release of drugs. In controlled drug delivery systems, a variety of techniques and technologies are employed based on the particular application and goals[2,3].

Microspheres are small, spherical particles and size range usually between 1µm to 1000 µm [4]. They are useful for targeted drug delivery and sustained release applications as they can encapsulate and release active components. The biocompatible polymers can be used to formulate microspheres[5].

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Torsemide (TOR) is a loop diuretic. It is generally used to treat ailments such as congestive heart failure, liver disease, and kidney problems that are characterized by fluid retention. TOR works on the kidneys to help boost urine production and decrease extra fluid in the body. TOR acts by preventing sodium and chloride ions from being reabsorbed in the kidneys' ascending loop of Henle. By preventing the re absorption of these ions, it promotes diuresis, which increases the outflow of water and electrolytes[6,7]. The traditional formulations of TOR like tablets and capsules exhibits quick absorption, resulting in high plasma concentrations, more availability of drug and rapid elimination, that require more frequent administration. As a result, the controlled release formulation is essential. TOR-controlled release microspheres have a similar systemic exposure, and they are far more tolerable due to their large reduction in absorption rate and variations in plasma concentrations.

These considerations led to present study aiming to develop TOR-controlled-release microspheres by Ionotropic gelation method using Sodium alginate along with Hydroxy propyl methyl cellulose K15 and Eudragit RL100.

MATERIALS AND METHODS

Materials

TOR was a gift sample from Yarrow Chemicals, Mumbai. Sodium alginate (CDH, New Delhi), Calcium Chloride ($CaCl_2$) (Qualigens, Mumbai), Eudragit RL100 (S.D. Fine Chemicals, Mumbai.) Hydroxyl propyl methyl cellulose (HPMC K15), Hexane (Spectro chem Pvt. Ltd, Mumbai) was commercially obtained. All other reagents used in experiment were of analytical grade and purchased from their commercial sources.

Preparation of TOR Microspheres

Ionotropic gelation method was employed to formulate microspheres containing TOR. Initially, a 1% Sodium alginate solution (1% solution of Sodium alginate was prepared in 100ml of distilled water) was prepared using a magnetic stirrer. After achieving a homogeneous mixture, HPMC K15 and Eudragit RL100 were added in combination to formulations F1-F6, HPMC alone to F7-F9, and Eudragit alone to F10-F12, as detailed in Table 1. The second step was preparation of a drug solution. For this drug was dissolved in 0.1N HCl with magnetic stirring and was slowly poured into the polymer solution on a magnetic stirrer. In the final step, a 5% $CaCl_2$ solution was prepared and maintained at 600 RPM on a magnetic stirrer. The drug-polymer mixture was gradually added drop by drop to the 5% $CaCl_2$ crosslinking solution using syringe with 20-gauge needle. The resulting microspheres were filtered, rinsed with hexane, and dried[8,9].

Evaluation of TOR microspheres

Micromeritic characteristics

The micromeritic characteristics of the microspheres characterized by assessing their bulk density, tapped density, compressibility index, Hausner's ratio and particle size.

Bulk density

The bulk density of a microsphere is defined as the ratio of its entire mass to its bulk volume. A measuring cylinder was filled with one gram of weighted microspheres, and the bulk volume was noted.

Bulk density = Microspheres total weight / Bulk volume

Tapped density

Microsphere's tapped density is determined by dividing its entire mass by its tapped volume. A measuring cylinder was filled with one gram of weighted microspheres and were tapped 100 times to obtain the tapped volume.

Tapped density = Microspheres total weight / Tapped volume

Hausner's ratio

Hausner's ratio is the relationship between the bulk density and the tapped density of microspheres.

Hausner's ratio = Tapped density/ Bulk density

Carr's index (% Compressibility)

The % compressibility of the bulk medication was calculated using the bulk density and the Tapped density.

Carr's Index = Tapped density " Bulk density/ Tapped density X 100

Particle size determination

Microspheres were divided into various fractions of sizes, passing through a 10-minute screening process in a mechanical shaker using standard sieves with pore diameters that conformed to IP standards[10]. Calculated the particle size by using formula:

Mean particle size = (Average particle size of fraction X weight fraction) / weight fraction

Particle surface morphology

Surface morphology of optimized formulation particles was assessed with Scanning Electron Microscope (SEM model LEO 430), which provides information about the microspheres' surface and form.

Practical yield of microspheres

Practical yield of the microspheres was calculated based on weight of the prepared microspheres obtained from each batch relative to the total initial weight of the drug and polymer[11]. The practical yield was determined using following formula;

% Yield = Weight of microsphere obtained / Total weight of drug and polymer X 100

Drug entrapment efficiency

Accurately weighed 25 mg samples of drug-loaded microspheres were combined with 25 ml of 0.1 N HCl and stirred using a magnetic stirrer for 24 hours. After this, 1 ml of the drug mixture was extracted, filtered, and appropriately diluted. The drug concentration was then measured using spectrophotometer at 290 nm. The drug entrapment efficacy (EE) of the microspheres determined using the formula provided[12].

Drug entrapment efficiency = (Actual drug content /Theoretical drug content) X100

***In -vitro* drug release study**

In- vitro studies of TOR microspheres determined using a USP basket type dissolution equipment (LAB INDIA DISSO 2000) with a paddle mesh size of #22. The study was conducted at 37°C for up to 12 hours. A 50 mg of prepared microspheres of each formulation was accurately weighed and mixed in 900 ml of 0.1 N HCl dissolution media for the first 2 hours, followed by a pH 6.8 phosphate buffer for the remaining time at 50 rpm. Samples were taken at regular intervals, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12hrs, immediately restored with the same amount of medium. Collected samples were properly diluted and spectrophotometrically analyzed at 290 nm[13].

Drug release kinetics

The mechanism of drug release from the microspheres was examined by fitting the *in-vitro* dissolution data into zero order, first order, Higuchi's release model, and Korsmeyer-Peppas model[14].

Stability studies

A stability analysis of the optimized formulation was carried out under various conditions in compliance with the standards of the ICH. For stability investigations, optimized microspheres were kept in stability equipment (REMI manufacture). For six months, accelerated stability investigations were conducted for the optimum formulations at room temperature 25 ± 2° C, refrigerated temperature 4 ± 2° C. During the stability study period, the microspheres were evaluated for their practical yield, EE, and cumulative percentage of drug released[15].

RESULTS AND DISCUSSION

Micromeritic characteristics

The bulk density and tapped density values of formulations found to be between 0.8168±0.0060 to 0.8640± 0.0080 and 0.9456±0.006 to 0.9755±0.0060 g/cm^3, respectively. This demonstrated the TOS microsphere compositions' exceptional flow characteristics. The compressibility index and Hausner's ratio ranged from 11.028±0.006 to 13.627±0.005 and from 1.123±0.0061 to 1.157±0.006, respectively, suggesting satisfactory formulation flow characteristics. The outcome values are shown in Table 2.

Particle size determination

The size of particles of all formulations F1- F12 ranged between 161.2 ± 0.96 to 187.3 ± 1.37ìm and were within the acceptable limits (Table 3). The formulation F3 containing highest polymer

concentration had the particle size 187.3 ± 1.37, and F7, F10 containing lowest polymer concentration exhibited lowest particle size. The polymer concentration has an impact on the microspheres' particle size. When polymer ratio increased particle size increased. Increasing polymer concentration mostly leads to larger particle sizes, because higher concentrations result in more polymer available to encapsulate the drug, thereby increasing the size of the particles formed[13,14,17,18]. The particle size acceptable limit range in between 150 to 200ìm (90 to 110%). The outcome values are included in Table 3.

Particle Surface Morphology

The shape of microspheres particles was spherical, smooth surface and nonporous. The microspheres were appeared in white to pale yellow in colour. The results are shown in Fig.1.

Percentage yield of microspheres

The percentage yield of all formulations ranged between 83.5% ± 0.012 to 89.2% ± 0.032. Formulation F3 had the highest polymer concentration, resulting in a more viscous solution as a consequence, it had a lower practical yield compared to other formulations. As the polymer ratio increased the practical yields of

Table 1. Composition of TOR microspheres formulations

Formulation code	TOR (mg)	Sodium alginate (mg)	HPMC K15 (mg)	Eudragit RL 100 (mg)	Calcium chloride (%)
F1	20	1000	40	40	5
F2	20	1000	80	80	5
F3	20	1000	120	120	5
F4	20	1000	40	80	5
F5	20	1000	80	120	5
F6	20	1000	120	40	5
F7	20	1000	40	-	5
F8	20	1000	80	-	5
F9	20	1000	120	-	5
F10	20	1000	-	40	5
F11	20	1000	-	80	5
F12	20	1000	-	120	5

Table 2. Micromeritic characteristics of TOR microsphere formulations (F1-F12)

Formulation code	Bulk density (g/cc)	Tapped density (g/cc)	Compressibility index (%)	Hausner's ratio
F1	0.8194±0.001	0.9487±0.009	13.627±0.005	1.157±0.005
F2	0.8168±0.006	0.9456±0.006	13.620±0.006	1.157±0.006
F3	0.8515±0.004	0.9661±0.002	11.862±0.003	1.134±0.003
F4	0.8519±0.008	0.9673±0.004	11.557±0.006	1.135±0.006
F5	0.8488±0.006	0.9598±0.008	11.564±0.007	1.130±0.007
F6	0.8499±0.004	0.9659±0.004	12.009±0.004	1.136±0.004
F7	0.8640± 0.008	0.9711±0.004	11.028±0.006	1.123±0.006
F8	0.8566±0.003	0.9655±0.007	11.279±0.005	1.127±0.005
F9	0.8583±0.002	0.9668±0.004	11.429±0.003	1.126±0.003
F10	0.8536±0.005	0.9662±0.007	11.653±0.006	1.131±0.006
F11	0.8642±0.002	0.9755±0.006	11.409±0.004	1.128±0.004
F12	0.8534±0.005	0.9712±0.007	12.129±0.005	1.138±0.005

Values are given as average mean ± S.D (n=3).

all formulations slightly decreased. Increased polymer concentration led to higher viscosity of the solution, which can hinder the formation or recovery of microspheres. The viscus polymer solution adhered to the walls of the beaker and magnetic bead during the process might led to the decreased practical yield of microspheres[11,16,19,20]. The results are included in Table 3.

Drug entrapment efficiency

The EE of TOR microspheres ranged from 73.8% to 86.9%. The EE of microspheres varied across different formulations. Optimized formulation among the formulations tested F1 to F12, F6 showed the highest EE when an optimized ratio of polymers HPMC K15 and Eudragit RL 100 was used. This suggests that the combination of these two polymers in specific proportions resulted in better drug entrapment. Formulations F1 to F5, which likely had higher combined polymer concentrations of HPMC and Eudragit, showed higher EE compared to F7 to F12. This suggests that the combination of both polymers generally led to better EE than formulations containing only one polymer type. Formulations containing only HPMC F7 to F9 showed higher EE compared to formulations containing only Eudragit F10 to F12[12,16,17,19,21]. This indicates that HPMC might be more effective in entrapping TOR compared to Eudragit RL 100.The outcome values are included in Table 3.

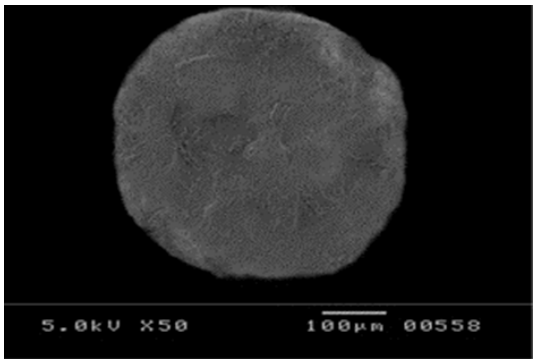

Fig. 1. SEM images of microspheres of optimized formulation F6

***In- vitro* studies**

In-vitro studies behaviour was examined over a 12-hour period under conditions simulating physiological environments, gastric fluid (0.1N HCl, pH 1.2) for initial 2 hrs and synthetic intestinal fluid (pH 6.8) for later by *in-vitro* dissolution method. After 11 hrs, F1 released 99.6% of the medication, whereas F2 released 98.1%. Formulation F3 showed 88.9% drug release rate throughout 12-hour period indicating extended drug release profile. In a 12-hour period, formulations F4, F5, and F6 demonstrated 100%, 99.1%, and 100% drug release, respectively. F4 and F6 demonstrated themselves to be suitable for controlled drug release. The formulations F7 to F9 prepared using HPMC K15 were unsuccessful in achieving the desired 12-hour drug release period even with increasing concentration of HPMC K15. Subsequently, formulations F10 to F12, which used

Table 3. Characterization of TOR microspheres

Formulation code	Practical yield (%)	Entrapment efficiency (%)	Drug content (%)	Particle size (µm)
F1	88.9±0.032	82.5±0.024	88.28 ± 0.73	174.5 ± 1.21
F2	87.9±0.023	84.3±0.026	90.75 ± 0.89	176.1 ± 1.19
F3	83.5±0.012	86.8±0.020	96.35 ± 0.82	187.3 ± 1.37
F4	86.9±0.025	83.2±0.017	100.61 ± 1.04	175.5 ± 1.25
F5	86.2±0.025	85.6±0.013	10l.53 ± 0.95	182.8 ± 1.02
F6	89.2±0.032	86.9±0.021	101.49 ± 0. 87	162.9 ± 0.96
F7	88.5±0.043	76.9±0.031	85.37 ± 1.18	163.2 ± 0.96
F8	88.1±0.014	78.6±0.056	87.75 ± 0.93	171.2 ± 1.34
F9	87.2±0.034	79.5±0.032	89.81 ± 1.05	179.1 ± 1.06
F10	88.5±0.028	73.8±0.014	83.74 ± 1.14	161.2 ± 0.96
F11	87.9±0.026	75.3±0.028	89.04 ± 1.09	175.7 ± 1.27
F12	86.9±0.024	77.8±0.012	87.65 ± 1.21	178.4 ± 1.02

Values are given as average mean ± S.D (n=3).

Eudragit RL 100 instead, also did not achieve the 12-hour drug release target. This suggests that neither the increase in concentration of HPMC K15 nor the use of Eudragit RL 100 was effective in extending the drug release period to 12 hours as intended[16,18,20,21,22]. The results are shown in Fig.2.

Drug release kinetics

The R^2 values of zero order kinetics for all formulations found to be in between the range of 0.953 to 0.989. The R^2values of first order kinetics for all the formulations were found to be in between the range of 0.700 to 0.909. The R^2values of Higuchi model for all formulations were found to be in between the ranges of 0.842 to 0.933. The R^2values of Korsmeyer peppas model for all the formulations were found to be in between the range of 0.760 to 0.866.

The regression coefficient for the zero-order plot was 0.982, which was close to unity (1.0). In kinetic studies, a regression coefficient close to 1 indicates good linearity and suggests that the data points fit the zero-order kinetics model well. The plot according to the first-order equation shows less linearity compared to the

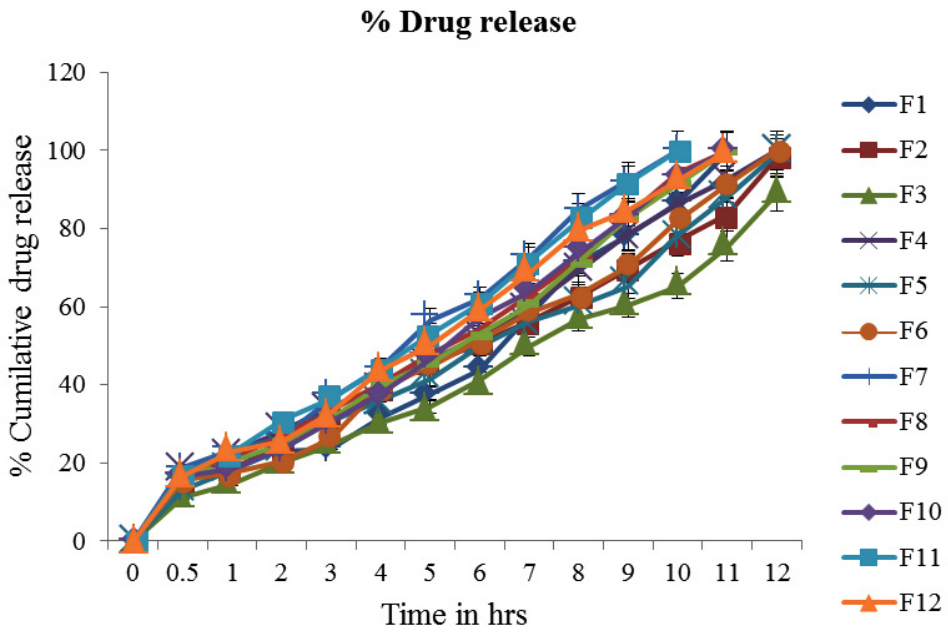

Fig. 2. *In- vitro* studies of TOR microspheres F1- F12

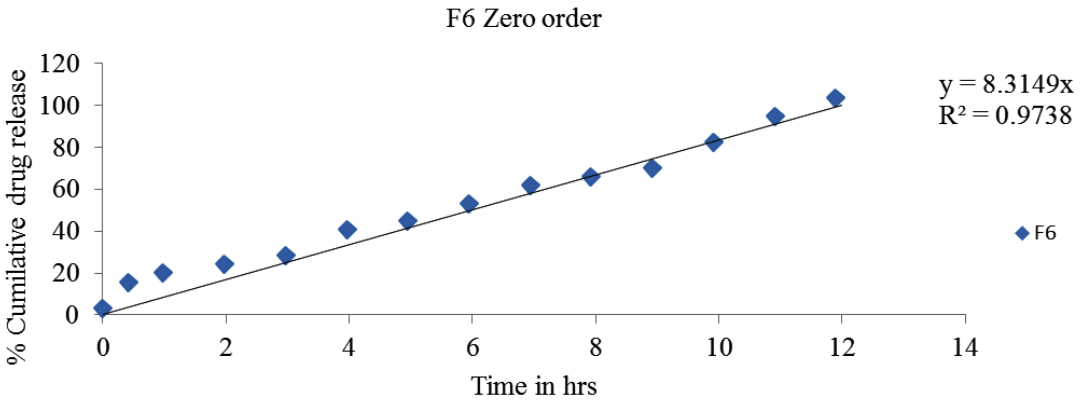

Fig. 3. Drug release kinetics of optimized formulation F6 Zero order

zero-order plot. Therefore, based on the higher regression coefficient (0.982) for the zero-order plot and the better fit of the data to the zero-order kinetics compared to the first-order kinetics, it was reasonable to conclude primary mechanism of drug release was zero-order kinetics. The R^2 values for Higuchi model ranged in between 0.842 to 0.933, suggesting a diffusion-controlled release mechanism. The R^2 values for the Korsmeyer-Peppas model ranged from 0.760 to 0.866, indicating a decent fit, but potentially suggesting additional mechanisms or complexities in the release process, and it was non- Fickian diffusion [14]. The kinetic values are included in Table 4 and the kinetic graphs of optimized formulation F6 are shown in Fig. 3, 4, 5, 6.

Stability studies

Optimized formulation F6 was chosen for stability analysis because of its high entrapment efficiency, cumulative percentage of drug releases and R^2 value of zero order kinetics. In accordance with ICH norms, stability investigations were carried out for 6 months. Based on the findings, it was indicated that the optimized formulation was stable and has mostly preserved its original qualities[15]. Color of microspheres was not changed after stability studies. However particle size, EE and drug content were slightly warried because of temperature effect. The stability study outcome values are shown in Table 5 and release shown in Fig. 7.

Table 4. *In- vitro* release kinetics of all formulations (F1- F12)

Formulation code	Zero order R^2	First order R^2	Higuchi model R^2	Korsmeyer peppas model R^2
F1	0.987	0.852	0.842	0.760
F2	0.988	0.700	0.930	0.859
F3	0.992	0.852	0.893	0.831
F4	0.987	0.885	0.933	0.842
F5	0.991	0.639	0.906	0.832
F6	0.973	0.867	0.903	0.858
F7	0.989	0.825	0.930	0.859
F8	0.990	0.864	0.916	0.835
F9	0.991	0.864	0.904	0.833
F10	0.993	0.852	0.903	0.861
F11	0.996	0.885	0.933	0.844
F12	0.990	0.909	0.933	0.866

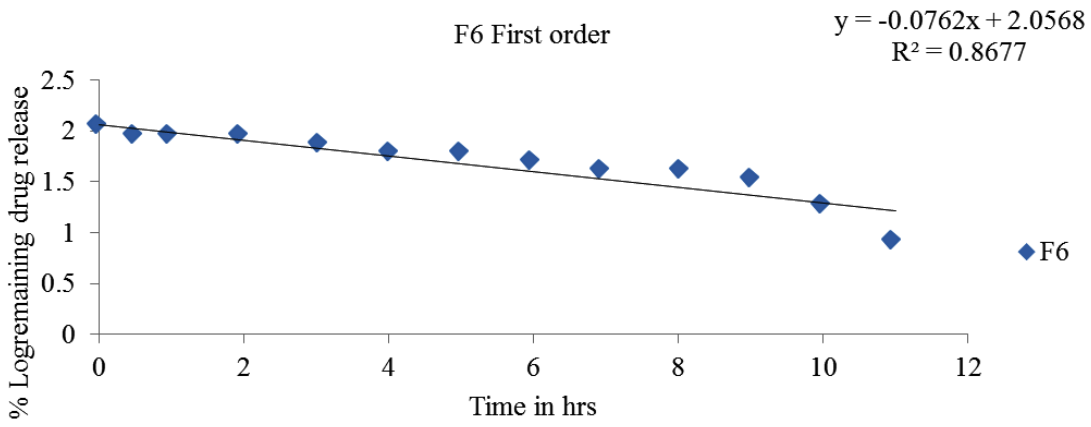

Fig. 4. Drug release kinetics of optimized formulation F6 First order

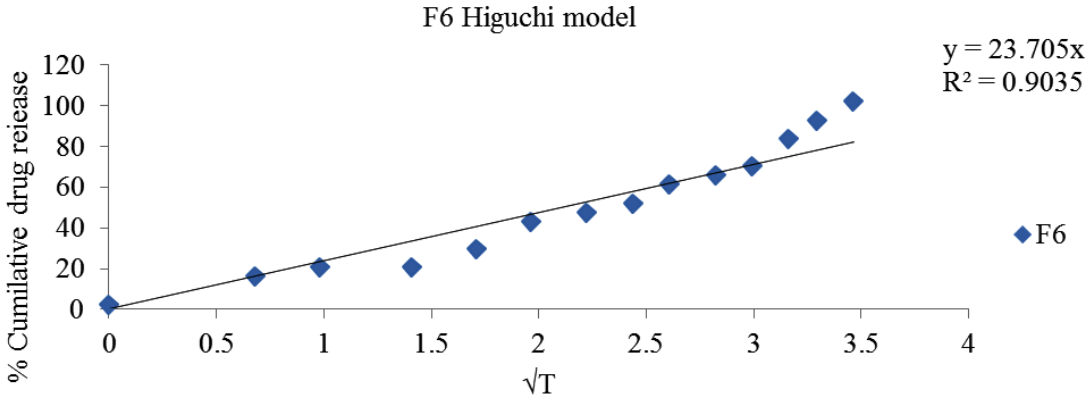

Fig. 5. Drug release kinetics of optimized formulation F6 Higuchi model

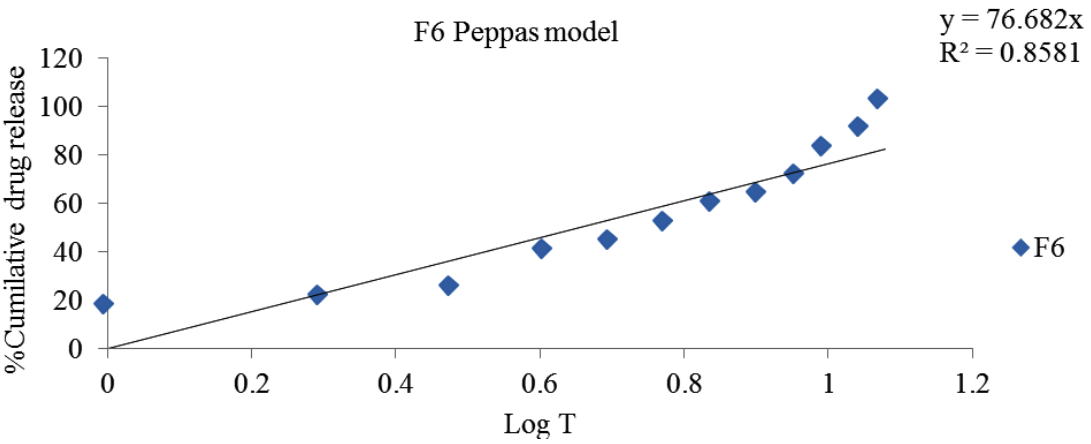

Fig. 6. Drug release kinetics of optimized formulation F6 - Peppas model

Table 5. Stability studies of optimized formulation F6

Storage condition	Time intervals (Months)	Particle size (µm)	Entrapment efficiency (%)	Drug content (%)
Room temperature (25 ± 2°C)	Initial	162.9 ± 0.96	86.9 ±0.021	101.49 ± 0.87
	1	162.9 ± 0.95	86.9 ± 0.021	101.39 ± 1.02
	2	162.1 ± 0.19	86.8 ± 0.022	101.35 ± 1.32
	3	161.5 ± 0.19	86.8± 0.52	101.34± 1.15
	6	161.2 ± 1.01	86.7 ± 0.32	101.27 ± 1.12
Refrigerated temperature (4 ± 2°C)	Initial	162.9 ± 0.96	86.9 ± 0.021	101.49 ± 0.87
	1	162.9 ± 1.35	86.9 ± 0.022	101.43 ± 1.03
	2	162.9 ± 1.14	86.9 ± 0.026	101.39 ± 1.12
	3	163.1± 1.15	86.9± 0.034	101.37± 1.13
	6	163.2 ± 1.27	86.9 ± 0.036	101.35 ± 1.25

All the values were expressed in average mean ± SD (n=3)

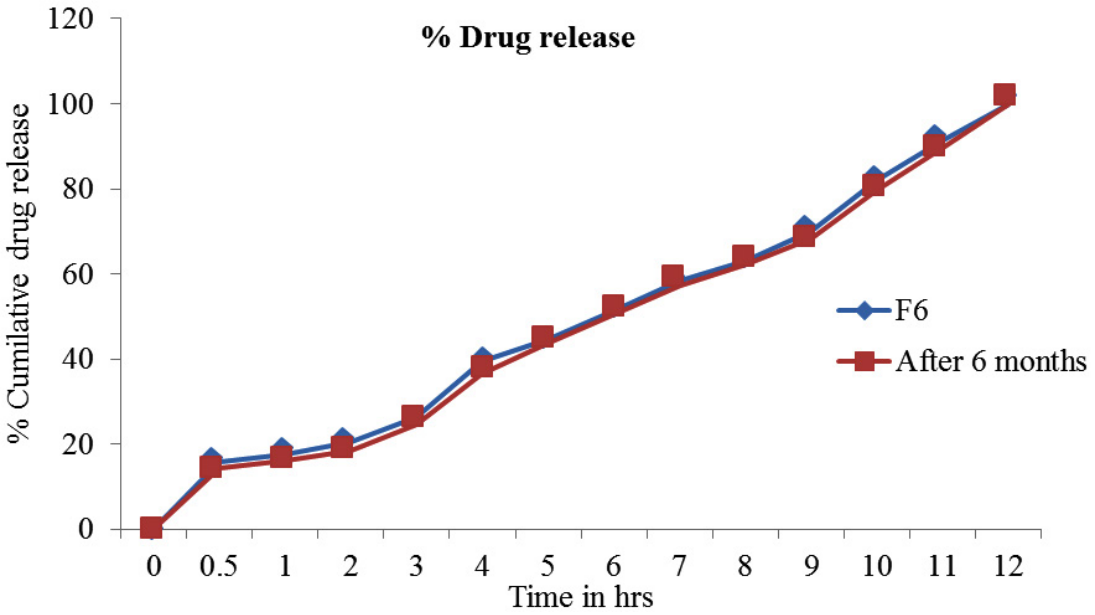

Fig. 7. % Cumulative drug release of optimized formulation before and after stability studies

CONCLUSION

TOR microspheres were formulated by inotropic gelation method using sodium alginate in combination with Eudragit RL100 and HPMC K15 in different concentrations. The prepared microspheres were assessed for particle size, practical yield, drug entrapment efficiency, and *in- vitro* studies. Formulation F6 was selected as the optimal formulation, as it exhibited acceptable results with respect to various evaluation parameters. The *in- vitro* release data of formulation F6 showed 100% regulated release up to 12 hours following zero order kinetics. Additionally, no considerable change in drug content was observed in optimized formulation during a six-month period of stability testing. Therefore, it can be assumed that the TOR microspheres are promising pharmaceutical dosage forms as they provide controlled-release drug delivery system.

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