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Evaluation and optimization of preparative variables for controlled-release floating microspheres of levodopa/carbidopa

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Levodopa, a prodrug of dopamine, is the first line drug in the treatment of Parkinson's disease. All current levodopa products are formulated in combination with aromatic amino acid decarboxylase inhibitors such as carbidopa or benserazide to prevent the peripheral metabolism of levodopa. The objective of the present investigation was to produce floating microspheres of carbidopa (CD)/levodopa (LD) to enhance their efficacy by increasing their gastric residence time, which is major technique to improve efficacy of narrow absorption window drugs. The microspheres were prepared by the o/w emulsion-solvent diffusion method using polymers hydroxypropylmethyl cellulose K15 M (HPMC K15 M) and ethyl cellulose (EC). The effects of various formulation and process variables on the particle size, *in vitro* floating behavior, percent drug entrapment, and *in vitro* drug release were studied. The size and surface morphology of prepared microspheres were characterized by optical and scanning electron microscopy, respectively. *In vitro* drug release studies were performed and drug release kinetics was evaluated using the linear regression analysis. The prepared microspheres exhibited prolonged drug release (approximately 10 h) and remained buoyant for >12 h. Spherical and smooth-surfaced microspheres with encapsulation efficiency ranging from 43% to 80% were obtained. *In vitro* studies demonstrated diffusion-controlled drug release from the microspheres.

1. Introduction

Gastric retention has received attraction of various researchers in the past few decades to eliminate the limitations connected with gastric emptying time. In fact, variable and too rapid gastrointestinal transit can result in incomplete drug release and less time span for drugs to get completely absorbed. This leads to lesser efficacy of the administered dose, especially for drugs that are absorbed to the greatest extent in the upper part of the small intestine (Iannucelli et al. 1998). Various attempts have been made to increase the retention of an oral dosage form (Talwar et al. 2001; Klausner et al. 2003; Sato et al. 2004), but floating drug delivery systems offer the most effective and rational protection against early gastric emptying. These systems remain buoyant on the gastric content for extended periods of time because of their low densities compared to that of the gastric fluid (Goole et al. 2007). Floating dosage forms can be classified as single- and multiple-unit systems (Fell et al. 2000). The single-unit floating systems are more popular but have the disadvantage of high variability of the gastrointestinal transit time (Whitehead et al. 1998; Talukder et al. 2004). Still, the multiple-unit dosage forms may be better than single-unit because they are claimed to reduce the intersubject variability in absorption and lower the probability of dose dumping (Shrivastava et al. 2005). Various natural and synthetic polymers have been reported to prepare floating microspheres in many research works like hollow microspheres of ibuprofen using acrylic polymers (Kawashima et al. 1992), cimetidine-loaded floating

microspheres using hydroxypropyl methylcellulose and ethyl cellulose (Srivastava et al. 2005), hollow microspheres or microballoons (MB) of riboflavin, aspirin, salicylic acid, ethoxybenzamide, and indomethacin using Eudragit S100 as enteric polymer (Sato et al. 2003a).

Levodopa is the primary drug used in the treatment of Parkinson's disease. Levodopa is generally administered orally or intravenously in combination with a decarboxylase inhibitor (Kao et al. 2000). Orally administered levodopa causes variable and untrustworthy clinical responses because of its inconsistent oral absorption and first-pass metabolism. The selection of the drug for preparing microspheres was based on the facts that it is a narrow absorption window (NAW) drug having relatively short elimination plasma half-time ($t_{1/2} = 1$ h) (Goole et al. 2008), and oral bioavailability of levodopa alone is estimated at about 5%, and less than 1% of the orally administered dose reaches the brain (Klausner et al. 2003), hence development of a slow release formulation could reduce fluctuations in the therapeutic effect and so improve its clinical efficacy. Carbidopa was used in combination with levodopa as a peripheral decarboxylase inhibitor, which results in effective level of dopamine and reduction of side effects (Safavi et al. 2007).

The objective of the present investigation was to produce floating microspheres of (LD)/(CD) using the polymers HPMC K15 M and EC and investigate the effects of various formulation and process variables on the microsphere size, *in vitro* floating behavior and percentage drug entrapment, in order to design formulation with better efficacy.

Table 1: Effect of preparative variables on various parameters on LD microspheres

Batch	Polymer ratio (HPMC K15M/EC)	Mean particle size (μm)	Buoyancy (%)	Drug entrapment efficiency (%)
LDM1	1:1	436.33 $\pm$ 8.64	70.07 $\pm$ 3.45	73.66 $\pm$ 3.88
LDM2	1:2	575.21 $\pm$ 6.22	80.40 $\pm$ 1.75	87.43 $\pm$ 1.61
LDM3	2:1	421.25 $\pm$ 6.83	66.22 $\pm$ 3.12	46.34 $\pm$ 2.95
LDM4	1:3	594.79 $\pm$ 7.46	76.03 $\pm$ 3.45	84.25 $\pm$ 3.20
LDM5	3:1	486.14 $\pm$ 4.61	44.16 $\pm$ 4.32	34.75 $\pm$ 4.62
LDMS1	1:2	732.62 $\pm$ 8.57	72.73 $\pm$ 3.62	82.54 $\pm$ 3.89
LDMS2	1:2	189.74 $\pm$ 5.57	70.04 $\pm$ 5.02	74.43 $\pm$ 3.46
LDMT1	1:2	428.72 $\pm$ 3.65	72.73 $\pm$ 3.62	42.64 $\pm$ 1.89
LDMT2	1:2	589.32 $\pm$ 8.37	74.24 $\pm$ 4.23	74.43 $\pm$ 3.46
LDMV1	1:2	752.16 $\pm$ 7.35	76.22 $\pm$ 2.94	84.14 $\pm$ 3.51
LDMV2	1:2	412.89 $\pm$ 4.87	67.21 $\pm$ 4.12	78.12 $\pm$ 2.91

2. Investigations, results and discussion

2.1. Effect of preparative variables on particle size

The effect of various preparative variables is shown in Tables 1 and 2 for LD and CD respectively. The mean particle size of the microspheres significantly increased with increasing EC ratio and was in the range of 421.25 ± 6.83 to $594.79 \pm 7.46 \mu\text{m}$ for LD and 375.44 ± 4.81 to $554.62 \pm 2.96 \mu\text{m}$ for CD, respectively. The particle size increased with increase in polymer concentration ratio, which is due to increased viscosity of the medium. Due to increased viscosity large emulsion droplets are formed which were difficult to break and precipitated as such leading to an increase in mean particle size (Sahoo et al. 2007).

An increase in average particle size was observed with decreasing agitation speed from 750 to 250 rpm. This may be attributed to the inability of the stirrer at low speed to break up the bulk of the polymer into finer droplets. An increase in particle size was observed at increasing temperature conditions, which may be attributed to different rate of dichloromethane evaporation in the emulsion solvent diffusion method. A decrease in particle size was observed at increasing volume of medium which may be attributed to availability of large free space for movement, results in reduced aggregation.

2.2. Buoyancy

The effect of preparative variables on buoyancy is shown in Tables 1 and 2. At increasing concentration of HPMC K15 M a decrease in buoyancy was observed, while in case of temperature and stirring speed, 40°C and 500 rpm showed optimized results, respectively. Microspheres prepared at 30°C were highly porous and showed less buoyancy due to easy penetration of solution through the porous surface, at 40°C showed formation of shell due to diffusion of ethanol into the aqueous solution and

simultaneous evaporation of dichloromethane, while at 50°C a single large depression at the surface was observed, which was a consequence of rapid evaporation of dichloromethane, which results in high apparent particle density and low buoyancy due to the absence of a cavity (Sato et al. 2003b). The effect of phase volume on buoyancy was measured in terms of best results with 200 ml of processing medium which may be attributed to free movement of emulsion droplets resulting in reduced collision induced aggregation as compared to 150 ml processing medium.

2.3. Drug entrapment efficiency

Drug entrapment was found to be 80.40 ± 1.75 to $44.16 \pm 4.32\%$ for LD microspheres and 78.24 ± 2.64 to $38.22 \pm 5.36\%$ for CD microspheres. It was observed that the entrapment efficiencies of both LD and CD microspheres were different depending on the polymers. The data are shown in Tables 1 and 2.

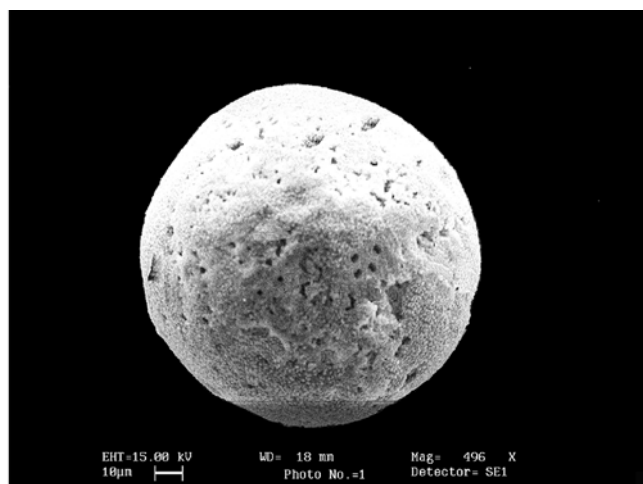
A decreasing entrapment efficiency was observed with increasing HPMC K15 M concentration which may be the result of a compact internal matrix of the resultant microsphere which may prevent drug entrapment. Highest entrapment was obtained at 500 rpm. Low stirring may cause improper mixing while higher, breaking of bonds formed during entrapment.

2.4. Morphological characterization of the microspheres

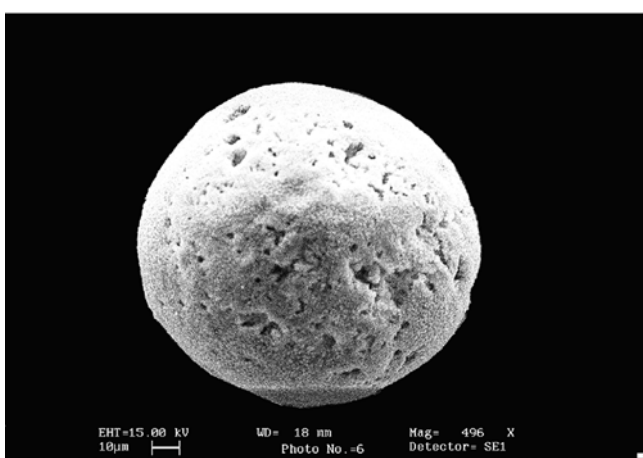
Shape and surface morphology of the microspheres were investigated by SEM as shown in Fig. 1a and b, both LDM2 and CDM2 which indicates that the drug-loaded microspheres are spherical in shape and have a smooth surface, distinct pores and cavities are evident on the surface of microspheres, which will be responsible for the release and made them float. The pores and cavities in the microspheres were formed by the rapid evaporation of dichloromethane.

Table 2: Effect of preparative variables on various parameters on CD microspheres

Batch	Polymer ratio (HPMC K15M/EC)	Mean particle size (μm)	Buoyancy (%)	Drug entrapment efficiency (%)
CDM1	1:1	396.14 $\pm$ 3.64	80.16 $\pm$ 4.32	68.11 $\pm$ 4.32
CDM2	1:2	479.14 $\pm$ 2.45	90.64 $\pm$ 2.16	78.24 $\pm$ 2.64
CDM3	2:1	375.44 $\pm$ 4.81	47.54 $\pm$ 1.75	62.34 $\pm$ 3.32
CDM4	1:3	554.62 $\pm$ 2.96	85.23 $\pm$ 3.45	56.04 $\pm$ 3.48
CDM5	3:1	442.14 $\pm$ 2.41	38.40 $\pm$ 1.72	38.22 $\pm$ 5.36
CDMS1	1:2	712.46 $\pm$ 4.43	84.62 $\pm$ 4.47	67.65 $\pm$ 3.12
CDMS2	1:2	160.32 $\pm$ 5.32	72.12 $\pm$ 3.18	64.08 $\pm$ 4.12
CDMT1	1:2	392.42 $\pm$ 2.48	46.28 $\pm$ 4.23	70.63 $\pm$ 2.68
CDMT2	1:2	516.43 $\pm$ 3.46	76.72 $\pm$ 3.62	72.56 $\pm$ 2.13
CDMV1	1:2	746.23 $\pm$ 4.15	83.54 $\pm$ 1.31	75.16 $\pm$ 3.35
CDMV2	1:2	426.76 $\pm$ 3.81	74.61 $\pm$ 4.16	67.21 $\pm$ 4.12



(a)



(b)

Fig. 1: SEM micrographs of CD and LD floating microspheres for formulations coded LD-M2 (a) CD-M2 (b); showing surface morphology

Fig. 2a and b, both LDM2 and CDM2 exhibited a range of sizes within each batch.

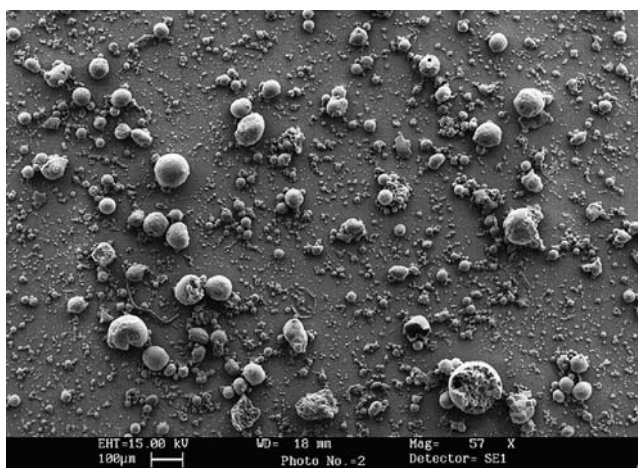
2.5. *In vitro* release studies

In vitro release studies of both LD and CD microspheres were performed in 0.1 N HCl containing 0.02 w/v% Tween 20 (pH 1.2) for 10 h. Both LD and CD loaded microspheres displayed controlled release properties *in vitro* as shown in Figs. 3 and 4, respectively.

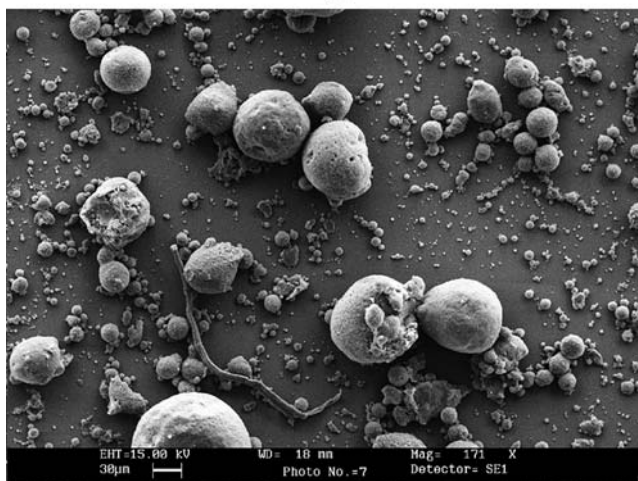
The cumulative release of drug decreased with increasing EC concentration from 1:1 to 1:2, while increasing again from 1:2 to 1:3 for both LD and CD microspheres (Figs. 3 and 4).

The observed results may be attributed to smaller microspheres which are formed at a lower polymer concentration (1:1) and have a larger surface area exposed to dissolution medium, giving rise to faster drug release. In case of higher concentrations (1:3) the average particle size of microspheres was similar to that of ratio 1:2, but due to greater total mass of microspheres there may be a higher number of microspheres units formed as compared to that obtained with ratio 1:2 which resulted in increased surface area, subsequently more drug release per unit time.

The amount of both LD and CD released from microspheres increased on increasing the HPMC K15 M ratio as shown in Figs. 3 and 4, respectively. This observation could be attributed to the increased contact area with the solution due to the poor buoyancy associated with the increased HPMC ratio. The *in vitro* release profile was biphasic with an initial burst release in



(a)



(b)

Fig. 2: SEM micrographs of CD and LD floating microspheres for formulations coded LD-M2 (a) CD-M2 (b); showing Different Size

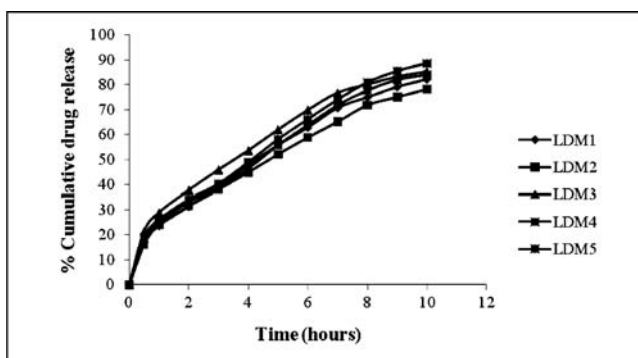


Fig. 3: *In vitro* release profiles of LD microspheres

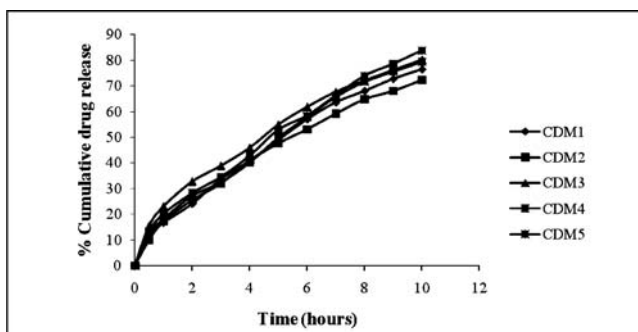


Fig. 4: *In vitro* release profiles of CD microspheres

Table 3: Kinetic equation parameters of selected formulations

B. No.	Zero order		First order		Higuchi		Korsmeyer Peppas	
	K_0	r^2	K_1	r^2	K_h	r^2	n	r^2
LDM1	7.0207	0.9835	0.166	0.9922	27.988	0.9912	0.4419	0.9910
LDM2	6.5328	0.9858	0.1427	0.9924	26.02	0.9917	0.4312	0.9935
CDM1	7.0489	0.9852	0.1423	0.9957	28.073	0.9909	0.4326	0.9953
CDM2	6.4812	0.9815	0.1229	0.9984	25.927	0.9960	0.4426	0.9981

Table 4: Batch specifications of the prepared LD microspheres

Batch Code	Polymer Concentration	Stirring Speed	Temperature	Volume of processing medium (ml)
LDM1	1:1	500 rpm	40 ± 0.5 °C	200
LDM2	1:2	500 rpm	40 ± 0.5 °C	200
LDM3	2:1	500 rpm	40 ± 0.5 °C	200
LDM4	1:3	500 rpm	40 ± 0.5 °C	200
LDM5	3:1	500 rpm	40 ± 0.5 °C	200
LDMS1	1:2	250 rpm	40 ± 0.5 °C	200
LDMS2	1:2	750 rpm	40 ± 0.5 °C	200
LDMT1	1:2	500 rpm	30 ± 0.5 °C	200
LDMT2	1:2	500 rpm	50 ± 0.5 °C	200
LDMV1	1:2	500 rpm	40 ± 0.5 °C	150
LDMV2	1:2	500 rpm	40 ± 0.5 °C	250

0.5 h attributed to the presence of drug particles on the surface of microspheres, followed by a slower release phase as the entrapped drug slowly diffused out into the release medium. The data obtained for *in vitro* release fitted equations for the zero order, first order, and Higuchi release models. The interpretation of data was based on the value of the resulting correlation coefficients as shown in Table 3.

The best fit order with highest correlation coefficient was shown in First order, Higuchi, and Zero order (Table 3). The drug release was proportional to time indicating that the drug release from microspheres was first order diffusion controlled. The data obtained were also fitted to Korsmeyer Peppas model in order to find out the n value. The n value of LD and CD is shown in Table 3, which indicates that the release through microspheres was obeying Fick's law of diffusion.

3. Experimental

3.1. Material

Carbidopa and levodopa were a generous gift from Cipco pharmaceutical (Pigdambar, M.P., India), hydroxypropylmethylcellulose (HPMC K15 M)

and ethyl cellulose (EC) were obtained from, Shin-etsu Chemical, Japan. Monostearin (Han-i Chemical, Japan) and polyvinyl alcohol (Sigma Chem. Co., St. Louis, Mo, USA) were used. Ethanol, dichloromethane were purchased from Rankem (New Delhi, India). The other chemicals and solvents used were of analytical grade.

3.2. Preparation of floating microspheres

Floating microparticles of (LD or CD) were prepared using the o/w emulsion-solvent diffusion technique (Kawashima et al. 1992). The drug (LD or CD), polymers (HPMC K15 M and EC) and monostearin (0.5 g) were dissolved in a mixture of dichloromethane (10 ml) and ethanol (10 ml) at room temperature. The resulting solution was then drop wise poured into an aqueous phase containing polyvinyl alcohol (0.75%w/v, 200 ml) with constant stirring at 500 rpm, employing a propeller type agitator for 3 h, at room temperature. The resulting microspheres were separated by filtration, washed with water and finally air dried over a period of 24 h at room temperature in a dessicator. The formulation scheme with the preparative variables is shown in Tables 4 and 5.

3.3. Characterization of microspheres

3.3.1. Particle size

Particle size of floating microspheres was measured using Labomed CXR III Research Microscope System.

Table 5: Batch specifications of the prepared CD microspheres

Batch code	Polymer concentration	Stirring speed	Temperature	Volume of processing medium (ml)
CDM1	1:1	500 rpm	40 ± 0.5 °C	200
CDM2	1:2	500 rpm	40 ± 0.5 °C	200
CDM3	2:1	500 rpm	40 ± 0.5 °C	200
CDM4	1:3	500 rpm	40 ± 0.5 °C	200
CDM5	3:1	500 rpm	40 ± 0.5 °C	200
CDMS1	1:2	250 rpm	40 ± 0.5 °C	200
CDMS2	1:2	750 rpm	40 ± 0.5 °C	200
CDMT1	1:2	500 rpm	30 ± 0.5 °C	200
CDMT2	1:2	500 rpm	50 ± 0.5 °C	200
CDMV1	1:2	500 rpm	40 ± 0.5 °C	150
CDMV2	1:2	500 rpm	40 ± 0.5 °C	250

3.3.2. Surface morphology and shape

The morphology of the microspheres was studied by scanning electron microscopy. SEM requires the coating of the dried sample with a conductive material usually gold. The samples for SEM were prepared by lightly sprinkling the powder on a double-sided adhesive tape stuck to a stub. The stubs were then coated with a mixture of gold and palladium to a thickness of 200–500 Å under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken with SEM (Leo 435VP Corporation Zeiss-Leica).

3.3.3. Drug entrapment efficiency

The drug content of HPMC K15 M and EC microspheres was determined by taking 50 mg formulation, thoroughly triturated and suspended in 10 ml, 0.1 N HCl followed by agitation with a magnetic stirrer for 12 h to dissolve the polymer and allow the drug to be extracted. The solution was filtered and the absorbance was measured spectrophotometrically (UV-1700 Shimadzu Corporation) at 280 nm and 282 nm. The percent drug entrapment was calculated as follows

$$\text{D.E.E.} = \frac{\text{Amount of drug actually present}}{\text{Theoretical drug load expected}} \times 100 \quad (1)$$

3.3.4. Buoyancy

Microspheres (100 mg) were weighed and placed in 300 ml of 0.1 N HCl, (pH 1.2) maintained at 37 °C. The mixture was stirred at 100 rpm for a period of 6 h using a stirrer and the floating and the settled portions of microspheres were recovered separately. The microspheres were dried and weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

$$\text{Buoyancy (\%)} = \frac{W_f}{W_f + W_s} \times 100 \quad (2)$$

Where W_f and W_s are the weights of the floating and settled microspheres, respectively.

3.3.5. In vitro drug release study (Arica et al. 2005)

Microspheres (50 mg) were enclosed in hard gelatin capsules. The enclosed microspheres were dispersed in 900 ml of the dissolution medium [0.1 N HCl (pH 1.2) containing 0.02% (w/v) Tween 80] in USP XXII dissolution apparatus 1 (rotating basket) at 37 ± 0.5 °C, rotated at 50 rpm. Sample (5 ml) was withdrawn at specified time intervals and filtered through filter paper and the volume was replaced with fresh media. Absorbance measurement was done spectrophotometrically at 280 and 282 nm for LD and CD, respectively. Data obtained from *in vitro* release studies were fitted to various kinetics equations to find out the mechanism of drug release from microspheres. The kinetics models used were zero order equation, first order equation and Higuchi model. The following plot were made Q_t vs. t (Zero order model), $\log (Q_0 - Q_t)$ vs. t (first order model) and Q_t vs. $t^{1/2}$ (Higuchi model), where Q_t is the drug release at time t and Q_0 is the initial amount of drug present in the microspheres. The rate were also calculated for the respective models further to confirm the mechanism of drug release, the drug release was fitted in Korsmeyer-Peppas equation.

$$M_t / M_\infty = Kt^n \quad (3)$$

where M_t / M_∞ is the amount of drug released at time t and k is a kinetic release rate constant and n is the release exponent. The n value is used to characterize different release mechanism and is calculated from the slope of the plot of log of fraction of drug released vs. log of time.

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