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## Design and Fabrication of Tramadol Hydrochloride Microspheres for Colon Targeted Drug Delivery System

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### ABSTRACT

Colon targeted drug delivery system is capable of protecting the drug in route to the colon i.e. drug gets released and absorbed once it reaches the colon. A multiparticulate system combining pH sensitive property and biodegradability has been investigated to prepare and evaluate Eudragit S-100 coated Sodium alginate microspheres for colon targeting of Tramadol Hydrochloride. Uncoated Tramadol Hydrochloride microspheres were prepared by ionotropic gelation technique using different ratios of Tramadol Hydrochloride and Sodium alginate. Coated Tramadol Hydrochloride microspheres were prepared by coacervation phase separation technique using different ratios of uncoated Tramadol Hydrochloride and Eudragit S-100/ Eudragit L-100. Uncoated and coated Tramadol Hydrochloride microspheres were evaluated for percentage yield, particle size, surface morphology, flow properties, drug content and entrapment efficiency and were found to be within the acceptable range. The uncoated microspheres sustained the release upto 8 hrs whereas coated microspheres sustained the release upto 12 hrs in a pH progression medium mimicking the condition of GIT. The drug release from MC4 formulation coated with Eudragit S-100 (1:4) showed desired rate as there was no drug release observed upto 4-5 hrs, while in colonic fluid controlled drug release was observed releasing about 69.66 % after 12 hrs. The release kinetics followed Peppas showing Super Case II transport. Stability studies suggested formulations were stable. It is concluded from the present investigation that Eudragit S-100 coated Sodium alginate microspheres are promising controlled release carriers for colon targeted delivery of Tramadol Hydrochloride.

**Keywords:** Colon targeted drug delivery system, Tramadol Hydrochloride, Sodium alginate, Eudragit S-100, Eudragit L-100.

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## INTRODUCTION

To date, oral delivery is still the preferred route of drug administration, especially for chronic therapies where repeated administration is required. Oral administration offers patients less pain, greater convenience, higher likelihood of compliance, and reduced risk of cross infection and needle stick injuries. Thus, formulations of oral drug delivery continue to dominate more than half of the drug delivery market share. Despite these advantages, the oral route is not amenable to the administration of most protein and polypeptide drug available today, due to their high susceptibility to digestive enzymes in the gastrointestinal tract (GIT), poor absorption, and their limited ability to transport across the intestinal epithelial barrier. As a result, new strategies of drug delivery have been developed to overcome obstacles encountered by oral delivery. Among these strategies, colon-specific delivery has been extensively studied from the last two decades<sup>1</sup>.

Irritable Bowel Syndrome (IBS) is a functional disease, that is, a disease in which the intestine (bowel) functions abnormally. Patients with IBS exhibit increased gut sensitivity because the nerves that carry information from the gut to the brain, the afferent neurons, produce a response greater than that expected to be produced by the stimuli they have received, which results in non-painful stimuli being perceived as painful (visceral hyperalgesia). The primary symptoms of IBS are constipation, diarrhea, and abdominal pain. Secondary symptoms include abnormal passage of stool, abnormal form of stool, increased amounts of mucus in the stool, and a subjective feeling of abdominal distention (bloating). Treatment of IBS consists primarily of medications to control constipation, diarrhoea, and abdominal pain<sup>2</sup>.

Tramadol Hydrochloride is a synthetic centrally acting aminocyclohexal analgesic that acts as an opioid agonist with selectivity for the  $\mu$  receptor have demonstrated that this drug is an effective agent for moderate to severe chronic pain, for the treatment of functional GI disorders such as IBS and non-cardiac chest pain. The most commonly occurring adverse side effects during treatment of pain with Tramadol preparations are gastrointestinal upsets, nausea and vomiting. It has mean plasma half-life of 5 hrs. A dose of 25-50 mg appears to provide effective relieve from the symptoms of IBS but higher frequency of administration of drug may lead to high plasma concentration, resulting into systemic side effects like decreased heart rate and blood pressure<sup>3,4,5</sup>. Controlled release oral drug delivery systems are designed to achieve therapeutically effective concentrations of drug in the systemic circulation over an extended period of time, thus achieving better patient compliance and allowing a reduction of both the total dose of drug administered and

the incidence of adverse side effects. Considering this aspect, it is desirable to develop a 12-16 hrs sustained release formulation of Tramadol Hydrochloride.

## MATERIALS AND METHODS:

Tramadol Hydrochloride, Sodium alginate, Eudragit S-100 and Eudragit L-100 were procured from Yarrow chemical, Mumbai. All the reagents used were of analytical grade.

### Preparation of uncoated Tramadol Hydrochloride microspheres<sup>6,7</sup>:

The Sodium alginate microspheres were prepared by ionotropic gelation technique. Tramadol Hydrochloride was dispersed in an aqueous solution of Sodium alginate with stirring to produce a viscous form. Then polymer drug solution was added drop wise by using syringe of 22 G in diameter from a height of about 5 cm into a beaker containing 10% w/v solution of calcium chloride with continuous stirring by magnetic stirrer at 500 rpm. Then the solution containing gel formed microspheres was filtered by using whatman filter paper no. 1. The microspheres were allowed to dry at 30-40 °C and kept in an airtight container for further studies. Sodium alginate microspheres were prepared using different ratios of Tramadol Hydrochloride: Sodium Alginate.

**Table 1: Preparation of uncoated Tramadol Hydrochloride microspheres**

Formulation Code	Drug: Polymer Ratio	CaCl <sub>2</sub> Solution	Speed of Rotation
M1	1:1	10% w/v	500 rpm
M2	1:2		
M3	1:3		
M4	1:4		

### Evaluation of uncoated Tramadol Hydrochloride microspheres<sup>8,9,10</sup>:

#### Percentage Yield:

The percentage yield of the prepared microspheres was determined by using the formula:

$$\text{Percentage yield} = \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \times 100$$

#### Particle Size:

Determination of average particle size was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of microspheres was spread on a clean glass slide and average size of 150 microspheres was determined in each batch by the given equations:

Size of Individual particles ( $\mu\text{m}$ ) = Number of Division on Eyepiece  $\times$  Calibration factor

$$\text{Average Particle Size } (\mu\text{m}) = \frac{\text{Sum of Size of Individual Particles}}{150}$$

#### Surface Morphology:

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. SEM studies

were carried out by using JEOL-JSM 6380LA analytical scanning electron microscope. The samples of SEM were prepared by lightly sprinkling the microspheres powder on a double adhesive tape, which was stuck on an aluminium stub. The photomicrographs were taken with the help of SEM analyzer.

### Flow Properties:

Flow properties of microspheres were studied by measuring the angle of repose of the formulation by employing fixed funnel method. Microspheres were weighed and passed through the funnel, which was kept at a height of 'h' from the horizontal surface. The passed microspheres formed a pile of a height 'h' above the horizontal surface and the radius 'r' of the pile was measured and the angle of repose was determined for all the batches by using the formula:

$$\theta = \tan^{-1} \frac{h}{r}$$

$\theta$  = angle of repose

h = height of the pile

r = radius of the pile

**Table 2: Angle of Repose as an Indication of Powder Flow Properties**

Sr. No.	Angle of Repose	Type of Flow
1	< 20	Excellent
2	20 – 30	Good
3	30 – 34	Passable
4	> 34	Very Poor

### Drug Content:

Microspheres were accurately weighed equivalent to 50 mg of Tramadol Hydrochloride, triturated and digested in 10 ml buffer solutions (pH 1.2, pH 4.5 and pH 7.4) separately and kept overnight for extraction of drug. The digested homogenate was centrifuged and supernatant was collected. After appropriate dilution of supernatant with same buffer solutions, aliquots were assayed by UV spectrophotometer at  $\lambda_{\max}$  274 nm for acidic buffer pH 1.2 and at  $\lambda_{\max}$  273 nm for acetate buffer pH 4.5 and phosphate buffer pH 1.2. Corresponding drug concentrations in the sample were calculated from the standard calibration curve.

### Drug Entrapment Efficiency:

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula:

$$\text{Drug Entrapment Efficiency} = \frac{\text{Practical Drug Content (mg)}}{\text{Theoretical Drug Content (mg)}} \times 100$$

The entrapment efficiency was calculated as the standard deviation in all three buffer solutions. Theoretical drug content was determined by calculation assuming that the entire drug present in the solution i.e. 50 mg gets entrapped in microspheres and no loss of drug occurs at any stage of preparation of microspheres.

### ***In-vitro* Drug Release:**

*In-vitro* release study of microspheres was performed in pH progression medium at  $37\text{ }^{\circ}\text{C} \pm 0.5\text{ }^{\circ}\text{C}$ . The drug dissolution test of microspheres was performed in the USP dissolution test apparatus by the paddle method specified in USP XXIII. Microspheres (equivalent to 50 mg of Tramadol Hydrochloride) were weighed accurately and filled into tea bags. The tea bags were tied using thread with paddle and located into the basket of the dissolution apparatus. The content was rotated at 100 rpm. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time intervals. The pH of the dissolution medium was kept 1.2 for 2 hrs, pH 4.5 for 2 hrs and pH 7.4 for next 8 hrs. The samples were withdrawn from the dissolution medium at various time intervals. The rate of drug release was analyzed using UV spectrophotometer.

Details of Dissolution test:

Apparatus: USP type II

Volume of medium: 900 ml

Temperature:  $37 \pm 0.5\text{ }^{\circ}\text{C}$

Paddle speed: 100 rpm

Dissolution medium:

Acidic buffer pH 1.2 for 2 hrs

Acetate buffer pH 4.5 for 2 hrs

Phosphate buffer pH 7.4 for 8 hrs

Aliquot taken at each time interval: 5 ml

### **Preparation of coated Tramadol Hydrochloride microspheres<sup>6,7</sup>:**

Sodium alginate microspheres were coated with Eudragit S-100 (ES-100) and Eudragit L-100 (EL-100) using coacervation phase separation method. A known amount of microspheres (equivalent to 50 mg of Tramadol Hydrochloride) was dispersed in Ethyl Acetate (25 ml) solution containing ES-100/ EL-100 (200, 250, 300 and 350 mg) and containing 0.2% w/v span 80. This mixture was agitated for 5 min at 600 rpm. Subsequently 50 ml n-hexane (as the non-solvent) was poured into the polymeric solution containing the core material with the rate of 1 ml/min. The medium was stirred for 60 min to complete the process of microparticles coating. Coated microspheres were

then washed with an excess of n-hexane, filtered and dried at room temperature and kept in an air-tight container for further studies.

**Table 3: Preparation of coated Tramadol Hydrochloride microspheres**

Formulation Code	Drug: Polymer Ratio		Conc. of Span 80	Speed of Rotation
	ES-100	EL-100		
MC1	1:4	-	0.2%	600 rpm
MC2	1:5	-		
MC3	1:6	-		
MC4	1:7	-		
MC5	-	1:4		
MC6	-	1:5		
MC7	-	1:6		
MC8	-	1:7		

**Evaluation of coated Tramadol Hydrochloride microspheres<sup>8,9,10</sup>:**

The coated Tramadol Hydrochloride microspheres were evaluated for percentage yield, particle size, surface morphology, flow properties, drug content, drug entrapment efficiency and *in-vitro* drug release similar to the procedure carried out for evaluation of uncoated Tramadol Hydrochloride microspheres as mentioned above. In addition kinetic studies were carried out.

**KINETIC STUDIES:**

In order to describe the kinetics of the release process of drug in the different formulations, models were fitted to the dissolution data of optimized formulations using linear regression analysis.

**Zero order kinetics:**

Drug dissolution from pharmaceutical dosage forms that release the drug slowly, assuming that area does not change and no equilibrium conditions and are represented by the equation:

$$Q_t = Q_0 + K_0 t$$

$Q_t$  is the amount of drug dissolved in time  $t$ ,

$Q_0$  is the initial amount of drug in the solution,

$K$  is the zero order release constant.

**First order kinetics:**

The application of this model to drug dissolution studies used to describe absorption and/ or elimination of drugs. To study the first order release rate kinetics the release rate data were fitted to the following equation:

$$\text{Log } Q_t = \text{log } Q_0 + K_1 t/2.303$$

$Q_t$  is the amount of drug released in time  $t$ ,

$Q_0$  is the initial amount of drug in the solution,

$K_1$  is the first order release constant.

**Higuchi model:**

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

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

$Q_t$  is the amount of drug released in time  $t$ ,

$K_H$  is higuchi dissolution constant.

Higuchi describes drug release as a diffusion process based in the Fick's law, square root time dependent.

**Korsmeyer and Peppas model:**

This model is generally used to analyse the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved.

$$M_t / M = K \cdot t^n$$

$M_t / M$  is the fraction of drug release

$K$  is the release constant

$t$  is the release time

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

**Table 4: Kinetic studies**

<b>Release Exponent (n)</b>	<b>Drug Transport Mechanism</b>
$n = 0.5$	Fickian diffusion or square root of time kinetics
$0.5 < n < 1$	Anomalous (non-Fickian) diffusion
$n = 1$	Case – II transport
$n > 1$	Super Case II transport

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

- Cumulative percent drug release v/s time (Zero order rate kinetics).
- Log cumulative percent drug retained v/s time (First order rate kinetics).
- Cumulative percent release v/s square root time (Higuchi matrix).
- Log of cumulative percent drug released v/s log time (Peppas exponential equation).

**Stability studies<sup>11</sup>:**

Selected formulation was subjected to stability studies for a period of 2 months at an accelerated condition at  $40 \pm 2$  °C/  $75 \pm 5\%$  RH due to lack of time to carry out for 6 months as per new ICH

guidelines. Microspheres were evaluated for drug content, entrapment efficiency and *in-vitro* drug release studies.

## RESULTS AND DISCUSSION:

### Evaluation of uncoated Tramadol Hydrochloride microspheres:

#### Percentage Yield:

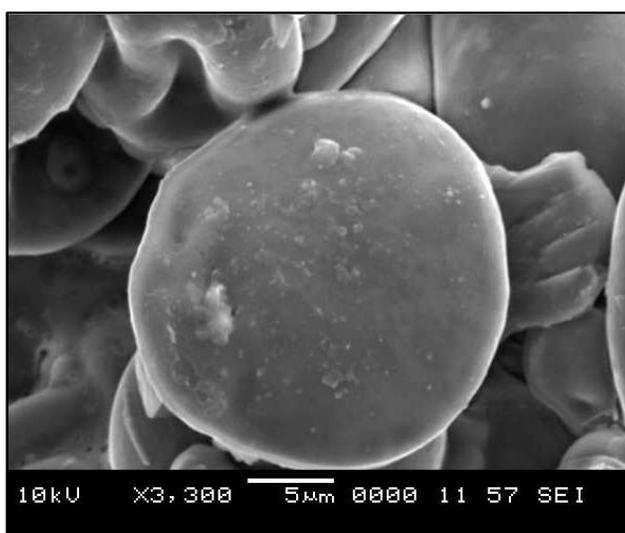
The percentage yield of formulations, M1-M4, was found to be 72.7, 76.6, 79.9 and 84.8% respectively. The results indicated that as the amount of polymer increases, the number of microspheres formed increases and hence the percentage yield increases. The results obtained are given in Table 5.

#### Particle Size:

The particle sizes of formulations, M1-M4, were found to be 25.5, 26.2, 27.6 and 29.0  $\mu\text{m}$  respectively. The results indicated that with increase in polymer concentration in the microspheres from M1-M4, the particle size of the microspheres increases. This increase in particle size can be attributed to an increase in viscosity with increase in polymer concentration, which resulted in larger emulsion droplets and finally in greater microsphere size. The results obtained are shown in Table 5.

#### Surface Morphology:

The SEM studies of uncoated Tramadol Hydrochloride microspheres showed that they were spherical in shape and with a smooth surface. Very less particulate matter of the drug were seen on the surface of the microspheres indicating uniform distribution of the drug in the polymer network. SEM photographs of formulation M4 are shown in Figure 1.



**Figure 1: SEM of M4 formulation of uncoated Tramadol Hydrochloride microsphere**

**Flow Properties:**

The values of angle of repose of formulations, M1-M4, were found to be 25° 12", 26° 34", 26° 52" and 27° 77" respectively, which indicate good flow properties of the microspheres. The results obtained are given in Table 5.

**Drug Content:**

As the polymer ratio was increased, the drug content increased with 32.85, 34.45, 36.67 and 39.02 mg, for formulations M1-M4 respectively. High drug content was observed for M4 formulation, thereby indicating that the polymer concentration plays a major role in drug content. The results obtained are depicted in Table 5.

**Drug Entrapment Efficiency:**

As the polymer ratio was increased, the entrapment efficiency increased with 65.70, 68.90, 73.38 and 78.05% for formulations M1-M4 respectively. High entrapment efficiency was observed for M4 formulation, thereby indicating that increase in polymer concentration resulted in the formation of larger microspheres entrapping greater amount of drug. The results obtained are listed in Table 5.

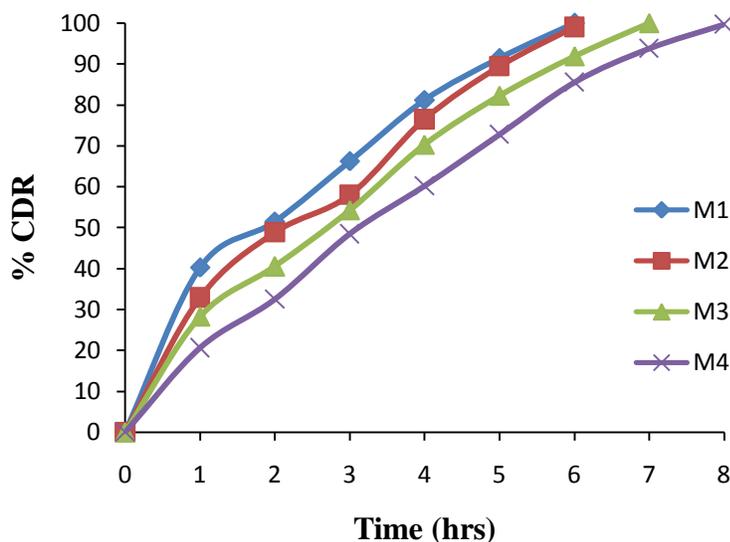
**Table 5: Evaluation of uncoated Tramadol Hydrochloride microspheres**

Formulation Code	Evaluation parameters				
	% Yield (%)	Particle Size (µm)	Angle of Repose	Drug Content (mg)	Entrapment Efficiency (%)
M1	72.7	25.5	25° 12"	32.85	65.70
M2	76.6	26.2	26° 34"	34.45	68.90
M3	79.9	27.6	26° 52"	36.67	73.38
<b>M4</b>	<b>84.8</b>	<b>29.0</b>	<b>27° 77"</b>	<b>39.02</b>	<b>78.05</b>

***In-vitro* drug Release:**

The *in-vitro* release profiles obtained for all formulations, M1-M4, are shown in Figure 2. A high burst release of 40.24% was observed from the formulation M1, which contain 1:1 drug polymer ratio; whereas a less burst release of 20.62% was observed from M4, which contain 1:4 drug polymer ratio. Within 4 hrs 60-90% of drug was released from the formulations M1-M4. These results indicated that formulation with lesser drug-polymer ratio shows higher drug release.

This type of high burst release in stomach and small intestine is not satisfactory for a formulation, which is supposed to release its contents in the colon. In order to prevent the drug release in stomach and small intestine these uncoated Tramadol Hydrochloride microspheres were coated with ES-100/ EL-100, which shows solubility at a pH  $\geq$  7. Since M4 formulation showed high drug entrapment efficiency and controlled drug release pattern, it is selected for coating process.



**Figure 2: Percentage CDR of uncoated Tramadol Hydrochloride microspheres**

### Evaluation of coated Tramadol Hydrochloride microspheres:

#### Percentage Yield:

The percentage yield of formulations, MC1-MC8, was found to be 79.8, 83.7, 88.9, 90.8, 75.6, 82.5, 85.7 and 89.0% respectively. The results indicated that as the amount of polymer increases, the number of microspheres formed increases and hence the percentage yield increases. The same are shown in Table 6.

#### Particle Size:

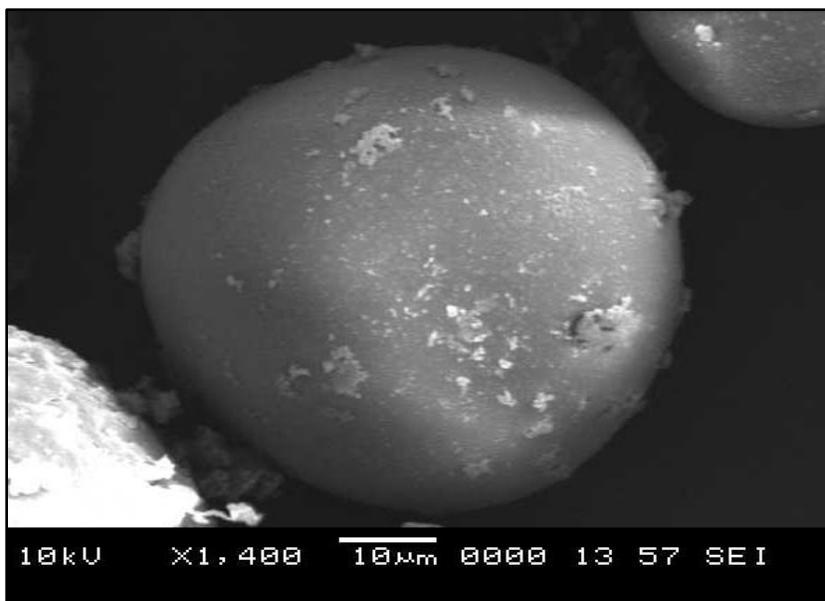
The particle sizes of formulations, MC1-MC8, were found to be 120.00, 122.47, 124.02, 126.00, 121.02, 123.00, 124.90 and 126.05  $\mu\text{m}$  respectively. The results indicated that with increase in polymer concentration in the microspheres from MC1-MC8, the particle size of the microspheres increases. This increase in particle size may be due to corresponding increase in the polymer concentration that results in larger emulsion droplets. The results obtained are shown in Table 6.

**Table 6: Evaluation of coated Tramadol Hydrochloride microspheres**

Formulation Code	Evaluation parameters					
	% Yield (%)	Particle Size ( $\mu\text{m}$ )	Angle of Repose	of Drug (mg)	Content	Entrapment Efficiency (%)
MC1	79.8	120.00	23° 67''	44.40		88.80
MC2	83.7	122.47	22° 99''	45.89		91.78
MC3	88.9	124.02	22° 00''	46.57		93.14
MC4	90.8	126.00	21° 91''	48.50		97.00
MC5	75.6	121.02	25° 94''	43.88		87.76
MC6	82.5	123.00	24° 97''	45.08		90.16
MC7	85.7	124.90	23° 02''	46.00		92.00
MC8	89.0	126.05	23° 50''	47.10		94.20

**Surface Morphology:**

The SEM studies of coated Tramadol Hydrochloride microspheres showed that coated Tramadol Hydrochloride microspheres exhibited smooth surface and spherical shape. SEM photographs of formulation MC4 is shown in Figure 3.



**Figure 3: SEM of MC4 formulation of coated Tramadol Hydrochloride microsphere**

**Flow Properties:**

The values of angle of repose of formulations, MC1-MC8, were found to be 23° 67", 22° 99", 22° 07", 21° 91", 25° 94", 24° 97", 23° 02" and 23° 50" respectively, which indicate good flow properties of the microspheres compared to uncoated Tramadol Hydrochloride microspheres. The results obtained are shown in Table 6.

**Drug Content:**

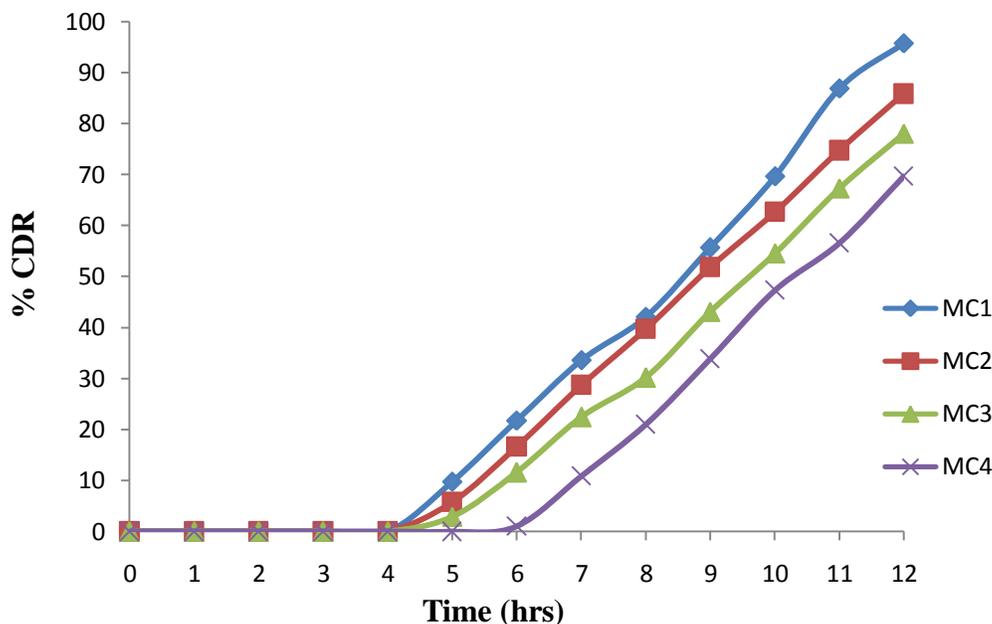
As the polymer ratio was increased, the drug content increased with 44.40, 45.89, 46.57, 48.50, 43.88, 45.08, 46.00 and 47.10 mg considering 20 mg for formulations, MC1-MC8 respectively. High drug content was observed for MC4 formulation thereby indicating that the polymer concentration plays a major role in drug content. The results obtained are depicted in Table 6.

**Drug Entrapment Efficiency:**

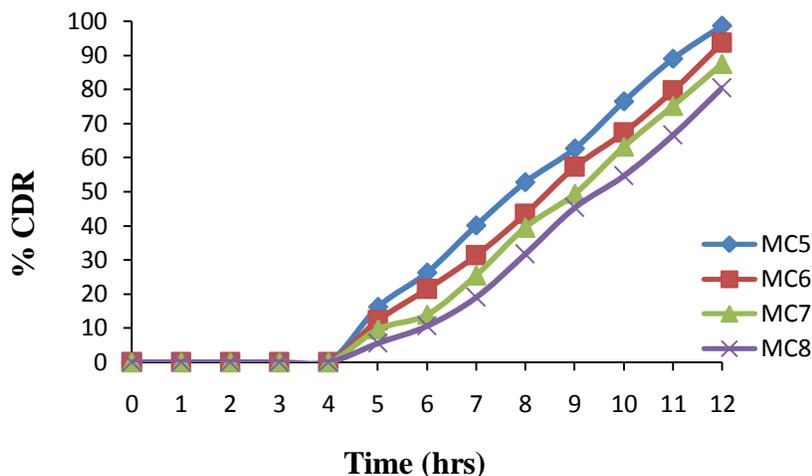
As the polymer ratio was increased, the entrapment efficiency increased with 88.80, 91.78, 93.14, 97.00, 87.76, 90.16, 92.00 and 94.20% for formulations MC1-MC8 respectively. High entrapment efficiency was observed for MC4 formulation, thereby indicating that increase in polymer concentration resulted in the formation of larger microspheres entrapping greater amount of drug. The results obtained are depicted in Table 6.

***In-vitro* drug Release:**

The *in-vitro* release profile obtained for all formulations, MC1-MC8, are shown in Figure 4 and Figure 5. The cumulative percent drug release after 12 hrs were found to be 95.73, 85.83, 78.00, 69.66, 98.62, 93.62, 87.50 and 80.40% for formulations MC1-MC8 respectively.



**Figure 4: Percentage CDR of coated Tramadol Hydrochloride microspheres coated with ES-100**



**Figure 5: Percentage CDR of coated Tramadol Hydrochloride microspheres coated with EL-100**

As expected, there was no measurable drug release observed at acidic pH 1.2 and acetate pH 4.5 for 4 hrs, this may be due to coating of uncoated Tramadol Hydrochloride microspheres with ES-

100/ EL-100 polymers. The results showed that release of Tramadol Hydrochloride slowed down as the concentration of coating polymer increased. This could be attributed to an increase in the density of the polymer matrix and the diffusional path length that the drug has to traverse.

The cumulative percentage drug release from MC4 formulation coated with ES-100 (1:7) showed the desire rate, as there was no drug release observed upto 4 hrs in acidic buffer pH 1.2 and acetate buffer pH 4.5. While in colonic fluid controlled drug release was observed due to dissolution of the Eudragit coat at pH 7.4 and the sodium alginate were degraded on exposure to the colonic fluid, thus resulting in drug release.

### Kinetic Studies:

In order to study the exact mechanism of drug release from coated Tramadol Hydrochloride microspheres, drug release data were fit into various mathematical models, zero order, first order, Higuchi matrix and Peppas and regression co-efficient are depicted in Table 7. These values were compared with each other for model fitting equation. Based on the highest regression values ( $r$ ), the best fit model for MC1-MC8 was Peppas. Further Korsmeyer and Peppas equation resulted into the values of  $n > 1$ , which appears to indicate that the release from the coated Tramadol Hydrochloride microspheres was by Super Case II transport, indicating that the release mechanism is dominated by the erosion and swelling of the polymer.

**Table 7: Kinetics release study of coated Tramadol Hydrochloride microspheres**

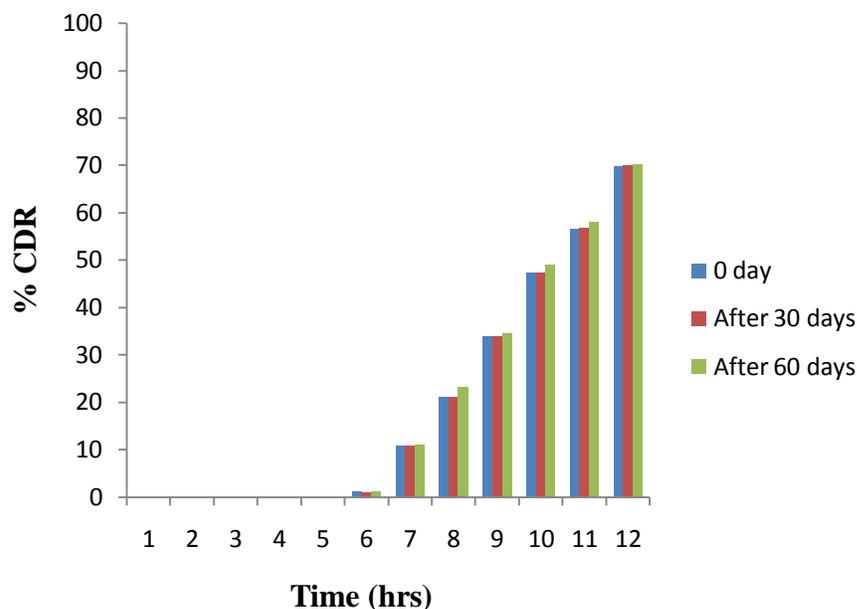
Formulation Code	Zero Order	First Order	Higuchi Matrix	Peppas Plot		Best Fit Model
				'r <sup>2</sup> ' values	'n' values	
MC1	0.9939	0.8396	0.6086	0.9942	2.1474	Peppas
MC2	0.9801	0.9324	0.6345	0.9905	2.3009	Peppas
MC3	0.9816	0.9383	0.5959	0.9961	2.5849	Peppas
MC4	0.9853	0.9352	0.5065	0.9988	2.3948	Peppas
MC5	0.9931	0.7796	0.6298	0.9954	2.2703	Peppas
MC6	0.9925	0.8483	0.5960	0.9970	2.1138	Peppas
MC7	0.9842	0.9039	0.5559	0.9926	2.5853	Peppas
MC8	0.9899	0.9182	0.5487	0.9900	2.6855	Peppas

### Stability Studies:

The results of the stability studies indicated that the microspheres did not show any changes in the drug content and entrapment efficiency during the stability study period. The percentage cumulative drug release after 6 months showed 70.23% after 12 hrs indicating no significant changes. The results obtained were depicted in Table 8 and Figure 6.

**Table 8: Evaluation of MC4 during stability study**

Evaluation Parameters	Time (Days) (Accelerated condition at 40±2 °C & 75±5 % RH)		
	0	30	60
Drug Content (mg)	48.50	48.47	48.49
Entrapment Efficiency (%)	97.00	96.94	96.98

**Figure 6: In-vitro release data of MC4 after stability study****CONCLUSION:**

The present study has been a satisfactory attempt to formulate microparticulate system for colon targeted delivery of Tramadol Hydrochloride using natural polymer (Sodium alginate) and pH sensitive polymers (ES-100 and EL-100). From experiments, it can be concluded that in order to prevent the drug release in stomach and small intestine these uncoated Tramadol HCl microspheres were coated with ES-100/ EL-100. The % CDR from MC4 formulation coated with ES-100 (1:7) showed desired rate, as there was no drug release observed up to 4 hrs, while in colonic fluid controlled drug release was observed.

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