

# Design of Microparticulated Salbutamol Sulfate pH Sensitive Pulsatile Delivery System for Chronotherapy of Nocturnal Asthma

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## Abstract

**Objective:** The main objective of this study was to prepare and evaluate pH-dependent chronotherapeutic drug delivery of salbutamol using chitosan (CS) and Eudragit polymers. **Materials and Methods:** Chronotherapeutic microparticles were prepared in two steps, in first step core CS microparticles were prepared using glutaraldehyde cross-linking and in the second step entrapped within Eudragit S 100 and Eudragit L 100. **Results:** The microparticles were spherical with size ranging from 50 to 100  $\mu\text{m}$ . Core and coated microparticles were monodisperse (uniformity index [UI] <1.2) and had wide size distribution (UI >1.2). The Eudragit S-100 coated microparticle did not release the drug in acidic pH of the stomach, and the Eudragit S-100 and L-100 coated microparticles showed burst release at pH 7.4 and pH 5.8, indicating perfect pH sensitive pulsatile drug delivery.

**Key words:** Chitosan, chronotherapeutic, Eudragit, pH-dependent, release kinetic, uniformity Index

## INTRODUCTION

Chronotherapeutic defined to treat a patient as per the daily, monthly, or yearly biological clock, with respect to increasing the health benefit and reduced adverse effect.<sup>[1]</sup> The pulsatile release system is an excellent way for chronotherapeutic drug delivery. Chronotherapeutic can be defined as the sudden release of a certain amount of drug within a short time period after a lag time.<sup>[2]</sup> The enteric coated formulation is utilized for the chronotherapeutic time-controlled system when a lag time is required.<sup>[3,4]</sup>

Asthma is the most widely recognized disease with the biggest circadian varieties. The movement of the lung shows a circadian beat with a most extreme around 12 am and at least around 4 am. In the patient of asthma, the force of variety in lung work is as much as half in a day.<sup>[5]</sup> Nighttime asthma is characterized as rest related intensifying in reversible airway route sickness. Manifestations by and large incorporate shortness of breath or wheezing during the evening. Asthma was roughly 70-crease more incessant at midnight amid indented evening rest than in the vicinity of 2 and 3 am center of the daytime movement

traverse. It has been accounted for that 94%, 74%, 64%, and 39%, persistent under asthmatic treatment aggravated their evening time rest no <1 time for each month, 1 time for every week, 3 evenings for every week, and consistently individually. It is accounted for that 13 of 19 (68%) asthma passing happened nocturnally, between 12 am and 6 am.<sup>[6-8]</sup>

Most of the current sustained release formulations have a shortcoming of not maintaining high blood levels for a long period with high disease intensity. In this manner, the patient might be unprotected against the most noticeably bad occasion of nighttime asthma. Hence, a chronotherapeutic drug delivery conveyance that is regulated before rest and keeps up high blood levels for a more drawn out period (from midnight to 8 am toward the beginning of the day amid which most extreme power of disease happens) could be especially helpful for the correct administration of nighttime asthma.

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Colonic conveyance helpful to enhance treatment of disease likewise relies on diurnal asthma, for example, asthma and joint inflammation.<sup>[9]</sup> Covering with pH disintegration subordinate polymers, for example, Eudragit S100 and L100 is one imperative approach utilized for outlining colon focused on based pulsatile drug delivery system framework Chitosan (CS) got by deacylation of chitin (a normally happening copolymer) is a biodegradable and non-lethal polymer and ended up being valuable excipients in the different adjusted detailing.<sup>[11]</sup>

The potential benefits of the multi-unit particulate system include increased bioavailability, predictable reproducible and short gastric residence time, no risk of dose dumping, reduced risk of local irritation, and the flexibility of blend pellets with different composition or release pattern due to their smaller size. These systems are capable of passing through upper gastrointestinal tract (GIT) easily leading to less intra-subject variability.<sup>[1,10]</sup>

## MATERIALS AND METHODS

### Materials

Drug sample of salbutamol sulfate and polymer CS was purchased from Yarrow chemicals (Mumbai India). Eudragit S 100 and L 100 were obtained from Loba Chemicals (Mumbai, India). Light liquid paraffin, glutaraldehyde (GA), acetone and petroleum ether were procured from Loba chemicals (Mumbai, India). All other chemicals used were of analytical grade.

### Methods

#### Preparation of core microparticle

A 25% w/v CS solution in aq. acetic acid (1% v/v) was made to which salbutamol sulfate was added, and finally, this dispersed phase was added with stirring to continuous phase (125 ml) consisting liquid paraffin containing 1% w/v span-85 to form water in oil (w/o) emulsion. The prepared emulsion was continuously stirred at 1500 rpm using a 3-blade propeller stirrer for 2.5 h. After this, a solution of a measured quantity of 2.5 ml each of toluene-saturated glutaraldehyde (2.5% w/v) was added dropwise. The core CS ([CS]-Salbutamol sulfate) - microparticles were separated by centrifugation at 1000 rpm for 30 min. The oil and untreated GA were removed completely by washing with petroleum ether. The CS - microparticles were filtered under vacuum and dried. The formulation variable was the concentration of CS (drug to polymer ratio) 1:1, 1:2, 1:3, 1:4, and 1:5.<sup>[12-15]</sup>

#### Determination of mean particle size and particle size distribution

Particle measure assurance of unloaded and medication stacked microspheres was performed by optical microscopy

utilizing a compound magnifying microscope. A couple of drug-loaded microspheres were suspended in refined water (10 ml). The suspension was ultrasonicated for 5 s. A little drop of suspension in this way got was put in a reasonable glass slide. The slide was mounted on the phase of the magnifying instrument and Ferret's width of no <300 particles was estimated utilizing an aligned visual micrometer.

### Uniformity index (UI) determination

The UI was calculated by the given formula:

$$UI = \frac{D_w}{D_n}$$

$D_w$  = weight average diameter

$D_n$  = number average diameter

$$D_n = \frac{\sum N_i D_i}{\sum N_i}$$

Where  $N_i$  = number of a particle with  $D_i$  diameter. UI = showing monodisperse and nearly monodisperse particles range (1 to 1.1 and 1.1 to 1.2).<sup>[16,17]</sup>

### Morphology studies of microspheres

Morphology of the microparticles was analyzed utilizing examining electron magnifying lens. The adequate measure of the test was mounted on metal (aluminum) stubs; the samples were mounted onto aluminum specimen stubs utilizing two-fold sided sticky tape and broke with extremely sharp steel. The examples were sputtered covered with gold/palladium for 120 s at 14 mA under argon air for auxiliary electron emissive scanning electron microscopy (SEM) and watched for morphology at speeding up the voltage of 15 KV at various amplification.

### Determination of drug entrapment, loading capacity

The drug content present in microparticles was examined through extraction of microparticles in distilled water. The known amount approximately 50 mg of the crushed and powdered microparticles were taken and extracted in distilled water and stirred for 15 min at 1500 rpm. The solution was filtered, afterward, diluted with 0.05 M NaOH and the absorbance was measured spectrophotometrically at 276 nm.<sup>[18,19]</sup>

Salbutamol encapsulation efficiency (EE) and loading capacity were calculated using the following formula:

$$EE = \frac{\text{Weight of albutamol loaded in microsphere}}{\text{Total weight of starting salbutamol}} \times 100$$

$$LC = \frac{\text{Weight of salbutamol loaded in microsphere}}{\text{Weight of dried microsphere}} \times 100$$

### Percentage yield

Percentage yield of CS microparticles was examined after entire drying from the accompanying condition:

$$\% \text{ Yield} = \frac{\text{Dried microsphere}}{\text{Drug (mg) + chitosan weight + cross linker weight}}$$

### Swelling ratio studies

The swelling proportion studies about the core microparticles were estimated by drenching the microparticles in phosphate support saline (7.4) for 24 h at room temperature with delicate shaking. At an alternate point, interim (0.5, 1, 2, 4, 6, 8, 12, and 24 h) test was gathered and flushed with Milli-Q water. The microparticles were discovered stay in place amid the procedure, and no tiny pores were obvious. Microparticles were then smudged dry, and the swollen weight ( $W_{sw}$ ) was estimated, and the swelling proportion ( $E_{sw}$ ) was computed by the condition as takes after:

$$E_{sw} = \frac{(W_{sw} - W_o)}{W_o} \times 100$$

$E_{sw}$  = The swelling ratio of microparticles

$W_o$  = Initial dry weight

$W_{sw}$  = Weight of swollen microparticles

### Drug release study

The microparticles were weighed equivalent to 50 mg of salbutamol sulfate and suspended in 900 ml of dissolution medium USP apparatus (Type I) at 37°C, stirred at 50 rpm. The drug release was studied for first 2 h using 0.1N HCl (pH 1.2) (simulated gastric fluid), then at pH 4.5 for another 2 h (simulated duodenum fluid) followed by pH 7.4 (simulated distal ileum and colon). After specified time interval, the samples were withdrawn by replacing with an equal volume of the blank. Centrifuged the samples at 2000 rpm; the supernatant was filtered through a membrane filter and analyzed at 276 nm by spectrophotometrically.<sup>[19]</sup>

### Selection and optimization of core microparticles for coating

On the basis of percentage yield, EE, swelling profile, and drug release formulation MC3 were selected for further coating with different concentration of Eudragit polymer.

### Coating of the core microparticles

Eudragit S-100 in different ratios was dissolved in 10 ml of organic solvent (Acetone:ethanol: 2:1). The optimized batches (MC3) of CS core microparticles were dispersed in

the above solution. Organic solvent dissolved the Eudragit S-100 and L-100 polymer while maintaining the integrity of CS core microparticles. The solution was then placed into 70 ml of liquid paraffin and stirred at 1500 rpm for 3 h at room temperature to evaporate the solvent. Finally, the coated microparticles were washed with n-hexane to remove liquid paraffin and then dried at 30°C for 24 h.

### Fourier-transform infrared spectroscopy (FTIR) spectroscopy

IR spectra of CS, Salbutamol, core and coated microsphere with or without drug were recorded with an FTIR spectrophotometer.

### Drug release study of coated microparticles

Coated microparticles containing 50 mg of drug were placed in 900 ml of pH 1.2 (0.1 N HCl) for 2 h, which was replaced by pH 5.8 phosphate buffer for another 2 h, then increased by the addition of  $\text{Na}_2\text{HPO}_4$  to pH 6.8 for next 2 h. Subsequently, pH of the medium was increased to 7.4. Samples were withdrawn at specified time interval replacing with a blank. The samples were centrifuged, filtered, and analyzed at 276 nm spectrophotometrically.

## RESULTS AND DISCUSSION

The purpose of the present study was to develop a new microparticulated chronotherapeutic drug delivery for the treatment of nocturnal asthma. For the development and evaluation of this system, which contains CS microparticles coated with pH-sensitive enteric polymers, we took  $\beta_2$  agonists (salbutamol sulfate) as a model drug. The system of drug delivery was developed in two steps: First, salbutamol was encapsulated within CS microparticles using glutaraldehyde cross-linking. Second, salbutamol CS microparticles were coated with Eudragit S-100 and L-100 polymer by oil in oil solvent evaporation method. Core microparticles were prepared using different drug: Polymer ratio as shown in Table 1. The formulation was coated with Eudragit S-100 (core: coat, 1:10, 1:5) and Eudragit L-100 (core: coat, 1:5) by oil in oil solvent evaporation method. It was also observed that Eudragit was not only physically coating CS microparticles but certain chemical interaction also occurred between Eudragit and CS during coating.<sup>[20]</sup>

### Determination of mean particle size and particle size distribution

The microparticles were prepared initially at a low speed of 500 rpm, then with 1000–1500 rpm followed by 2000 rpm. Based on percentage practical yield, mean particle size distribution and surface morphology study of 1:1 core microparticles, an optimum speed of 1500 rpm was used for the preparation of drug-loaded microparticles. Increase

**Table 1:** Particle size distribution and UI of different formulations

Formulation	Drug: polymer	Stirring speed (rpm)	Mean particle size ( $\mu\text{m}$ ) <sup>a</sup>	UI <sup>a</sup>
MC 1	1:1	500	49.80 $\pm$ 7.2	2.193 $\pm$ 0.14
MC 2	1:1	1000	53.30 $\pm$ 3.5	2.321 $\pm$ 0.09
MC 3	1:1	1500	51.20 $\pm$ 5.8	2.098 $\pm$ 0.13
MC 4	1:1	2000	54.60 $\pm$ 4.9	2.898 $\pm$ 0.11
MC 5	1:2	1500	56.30 $\pm$ 2.1	1.2097 $\pm$ 0.04
MC 6	1:3	1500	59.90 $\pm$ 2.1	1.005 $\pm$ 0.07
MC 7	1:4	1500	63.10 $\pm$ 1.9	0.99 $\pm$ 0.04
MC 8	1:5	1500	63.50 $\pm$ 2.9	0.9933 $\pm$ 0.11

<sup>a</sup>mean $\pm$ SD, *n*=5, UI: Uniformity index

in concentration of CS solution the mean particle size was increased due to the increase in viscosity of the droplet [Table 1]. This increase in viscosity results in difficulty in dispersion and subdivision of droplets. As the stirring speed increases, the size of dispersed droplet decreases which is due to high energy.<sup>[16,21]</sup>

The UI of different formulations is given in Table 1. UI was not affected by rotation speed, but UI was affected by drug:polymer ratio. However, the exact correlation could not be established. The UI value for MC 1-MC 4 and microparticles was  $>1.2$ . This value indicates that microparticles have a wide size distribution. The UI value for MC 5-MC 8 was below 1.2, indicating monodispersity and hence nearly monodisperse microparticles.

### Morphology studies of microspheres

The surface characteristics of the optimized formulation of salbutamol were studied by SEM. The SEM images are shown in Figure 4. The SEM images proved that the resulting microspheres were non-aggregated, spherical, smooth surfaced, and uniformly sized without any visible crystals of the drug [Figure 1].

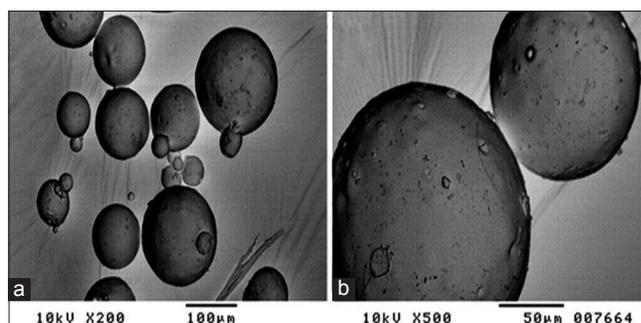
### Determination of drug entrapment, loading capacity

The value of percentage yield, loading capacity and encapsulated efficiency of different core and coated microparticles are given in Tables 2-4. The percentage yields of the different formulation were in the range 78–94%. The highest percentage yield was observed for MC7. The EE varied from 48% to 95%. As the drug-polymer ratio increases, the EE also increases. The highest value of encapsulated efficiency was observed for MC8.<sup>[21]</sup>

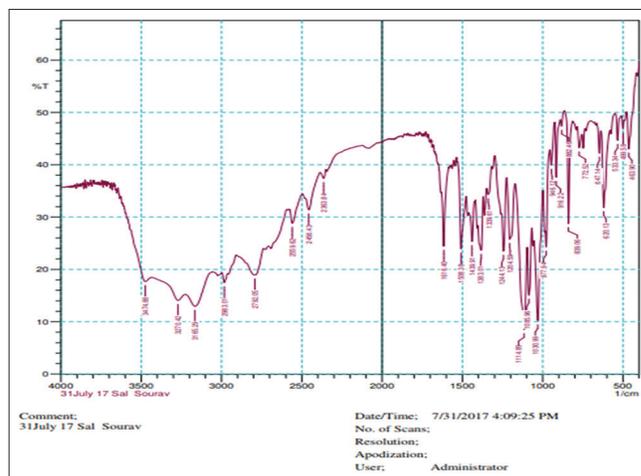
**\*Formulation MC1, MC2, and MC4 not selected for further studies due to poor percentage yield and EE**

### FTIR spectroscopy

IR spectra of CS, Salbutamol, core and coated microsphere with or without drug were recorded with an FTIR



**Figure 1:** (a and b) Scanning electron microscopy photographs of (a) optimized coated microparticle formulation EMC1 and (b) optimized core formulation MC3



**Figure 2:** Fourier-transform infrared spectroscopy spectra of salbutamol sulfate

spectrophotometer [Figures 2-4]. The IR spectra of the pure drug show characteristic peak at  $1100\text{ cm}^{-1}$  (C-O structure) and  $3478\text{ cm}^{-1}$  (OH bonding). All the characteristic peaks were also obtained in the FTIR spectra of core microparticles which indicate their compatibility [Figure 3].

### Swelling ratio studies

The observed swelling rates of crosslink microspheres followed the order  $\text{MC8} > \text{MC7} > \text{MC6} > \text{MC5} > \text{MC3}$  [Figure 5]. As the concentration of CS increases, the swelling

**Table 2:** Percentage yield, loading capacity, and EE of a different formulation

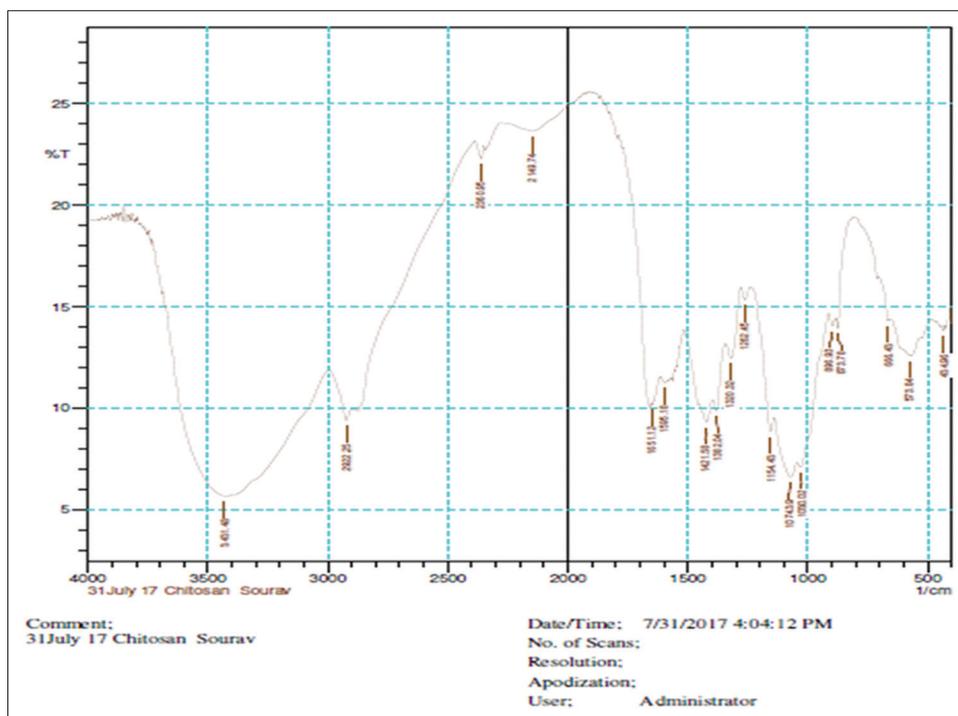
Formulation	% yield <sup>a</sup>	Loading capacity <sup>a</sup> (%)	Encapsulation <sup>a</sup> (%)
*MC 1	50.15±0.45	54.31±0.25	67.32±0.76
*MC 2	49.31±0.36	45.36±0.34	59.62±0.65
MC 3	82.98±0.92	68.62±0.31	89.68±0.84
*MC 4	56.57±0.76	48.39±0.42	51.32±0.67
MC 5	82.99±0.38	68.06±0.29	80.00±0.98
MC 6	88.46±0.35	62.84±0.06	80.00±0.69
MC 7	94.05±0.63	59.86±0.84	86.31±0.75
MC 8	92.61±0.63	57.96±0.20	89.02±0.56

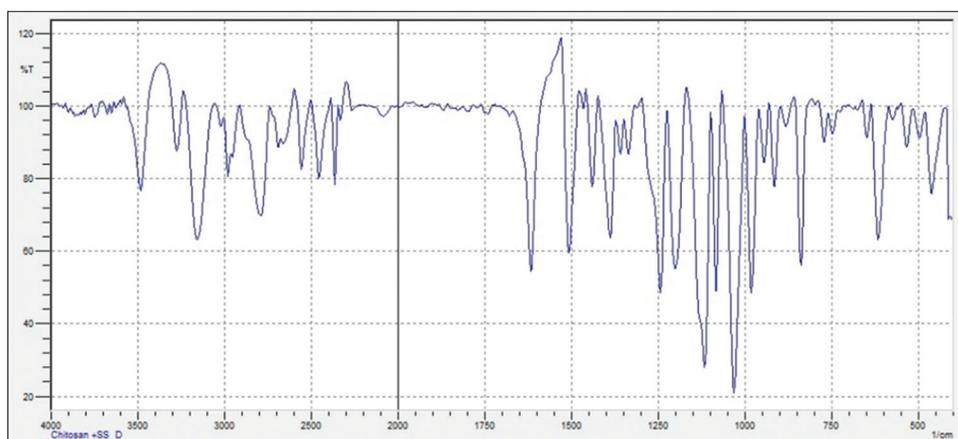
<sup>a</sup>mean±SD, n=5, EE: Encapsulation efficiency**Table 3:** Particle size distribution and UI of the coated formulation

Formulation	Drug: Polymer	Stirring speed (rpm)	Mean particle Size (µm) <sup>a</sup>	UI <sup>a</sup>
EMC1 (Eudragit S 100)	1:10	1500	102.5±10.2	1.23±0.08
EMC2 (Eudragit S 100)	1:5	1500	94.5±8.3	1.34±0.03
EMC3 (Eudragit L 100)	1:5	1500	94.3±6.5	1.29±0.07

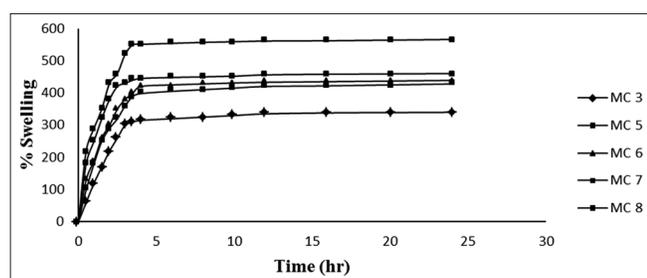
<sup>a</sup>mean±SD, n=5, UI: Uniformity index**Table 4:** Percentage yield, loading capacity, and EE of the different coated formulation

Formulation	Rotation speed (R.P.M)	% yield <sup>a</sup>	Loading capacity <sup>a</sup> (%)	Encapsulation <sup>a</sup> (%)
EMC1	1500	92.83±0.86	82.81±0.16	96.34±0.46
EMC2	1500	94.87±0.25	79.32±0.06	94.76±0.56
EMC3	1500	93.89±0.42	80.28±0.18	95.10±0.04

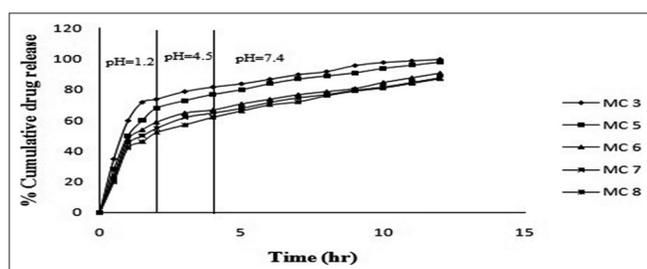
<sup>a</sup>mean±SD, n=3, EE: Encapsulation efficiency**Figure 3:** Fourier-transform infrared spectroscopy spectra of chitosan



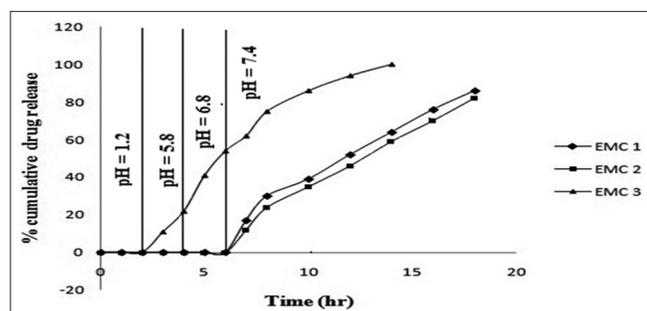
**Figure 4:** Fourier-transform infrared spectroscopy spectra of Salbutamol sulfate and Chitosan



**Figure 5:** Swelling profile of chitosan-salbutamol sulfate core microparticles



**Figure 6:** Drug release of different core microparticle at pH 1.2 (2 h), pH 4.5 (2 h), and pH 7.4



**Figure 7:** Drug release of different coated microparticle at pH 1.2 (2 h), pH 5.8 (2 h), pH 6.8 (2 h), and pH 7.4

rate increases. As the number of the available charged amino group increases, the porosity of polymer network increases. When the drug loading increases the swelling decreases, which could be attributed due to the dense network structure

at high loadings and water could not easily diffuse into the matrix microspheres.<sup>[22]</sup>

### Drug release from core microparticles

Drug release profiles of different core microparticles are given in Table 5 and Figure 3. The microparticles having more quantity of polymer, that is, MC 8 shows less rate and extent of drug release as compared to other core microparticles [Figure 6]. Thus, a change in drug:core ratio leads to modified dissolution profile, which may be attributed to the change in the density of the polymer matrix and hence the diffusional path length.<sup>[12]</sup>

The dissolution studies indicated that the core microparticles were characterized by an initial rapid release during the 1<sup>st</sup> hour which may be attributed to the solubility of CS in the acidic pH; to overcome this shortcoming and avoid the drug release in upper GIT, the core CS microparticles were coated with pH-sensitive polymer Eudragit S-100 (pH threshold > 7.0) and Eudragit L-100 (pH threshold > 6.0).<sup>[22]</sup>

### Particle size distribution of coated formulation

The selected formulations were coated with different ratio of Eudragit S-100 and L-100. All the formulations show monodisperse particle size distribution [Table 3].

### Determination of drug entrapment, loading capacity

The value of percentage yield, loading capacity and encapsulated efficiency of different coated microparticles are given in Table 2 and 4. The percentage yields of the different formulation were in the range 92–94%. The highest percentage yield was observed for EMC2.

### Drug release study of coated microparticles

Coated microparticles containing 50 mg of drug were placed in 500 ml of pH 1.2 (0.1 N HCl) for 2 h, which

**Table 5: Drug release and kinetic of different core formulation**

Formulation	% Drug release	Zero order		First order		Higuchi		Pappas	
		r <sup>2</sup>	K <sub>0</sub>	r <sup>2</sup>	K <sub>1</sub>	r <sup>2</sup>	K <sub>h</sub>	r <sup>2</sup>	n
MC 3	100	0.98	2.712	0.920	0.141	0.994	12.98	0.983	0.669
MC 5	98	0.947	3.245	0.959	0.114	0.985	15.71	0.934	0.541
MC 6	91	0.978	3.221	0.941	0.069	0.992	15.36	0.854	0.424
MC 7	88	0.976	3.308	0.945	0.062	0.993	15.92	0.868	0.418
MC 8	87	0.971	3.632	0.960	0.062	0.996	17.47	0.885	0.442

was replaced by pH 5.8 phosphate buffer for another 2 h, then increased by the addition of Na<sub>2</sub>HPO<sub>4</sub> to pH 6.8 for next 2 h. Subsequently, pH of the medium was increased to 7.4. Samples were withdrawn at specified time interval replacing with a blank. No drug release was observed in first 2 h at pH 1.2.<sup>[23]</sup> However, formulation EMC3 shows up to 15–55% drug release at pH 5.4. Formulation EMC1 and EMC2 show burst release at pH7.4 with initial 20 and 18% drug release, respectively, and maintain the drug release up to 18 h [Figure 7].

## CONCLUSIONS

The main purpose of the study was to develop a new microparticulated chronotherapeutic drug delivery for the treatment of nocturnal asthma. For the development and evaluation of this system, which contains CS microparticles coated with pH-sensitive enteric polymers, we took  $\beta_2$  agonists (salbutamol) as a model drug. The development of the drug delivery system took two steps: First, salbutamol was encapsulated within CS microparticles using glutaraldehyde cross-linking. Second, salbutamol CS microparticles were coated with a Eudragit polymer by oil in oil solvent evaporation method. Core microparticles were prepared using different drug:polymer ratio. The formulation was coated with Eudragit S-100 (core:coat, 1:10, and 1:5) and Eudragit L-100 (core: coat and 1:5) by oil in oil solvent evaporation method. From the result of the current study, it can be concluded that salbutamol sulfate microparticles containing CS as the core, coated with Eudragit S-100/L-100 could be used for chronotherapeutic delivery of salbutamol sulfate. Enteric coated CS microparticles lead to prevent drug release in stomach pH with rapid release of a certain amount of drug. However, further, *in vivo* studies are required to describe the efficacy of formulation in physiological condition.

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