International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.5, pp 1026-1041, 2015

Preparation and *In Vitro* Evaluation of Sustained Release Microcapsules Containing Theophylline

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Abstract: In view of the wide clinical use of theophylline, its narrow therapeutic index, repeated daily dosing and gastrointestinal side effects, sustained-release microcapsules of theophylline were prepared by a modified emulsion-solvent evaporation –non solvent addition technique. Two different polymers, namely, cellulose acetate butyrate (CAB) and ethyl cellulose (EC) were utilized at different polymer to drug ratios (2:1, 1:1 and 1:2). The microcapsules were evaluated ***in vitro*** for total recovery (yield %), microcapsule size (sieve analysis), surface morphology by scanning electron microscopy (SEM), drug loading (encapsulation efficiency) and drug release characteristics in simulated GIT fluids (pH 1.2 and 6.8). Results obtained revealed that spherical, free flowing microcapsules with smooth surfaces were successfully prepared with the two polymers. The percentages drug loading (encapsulation efficiency) were more than 95% for the two polymers at different polymer to drug ratios, indicating efficiency of the method. The drug release was affected by the type of polymer, polymer to drug ratios, microcapsule size and pH of the dissolution medium. The release of theophylline from CAB was slower than EC microcapsules. The release of theophylline from the microcapsules increased with decreasing microcapsules size. The release of theophylline from all the prepared microcapsules was markedly retarded as compared to commercial theophylline marketed product (**Theo SR 100 Capsules**). The release of theophylline from the prepared can be described by Zero-order release kinetic. These data clearly indicate ability of the prepared microcapsules to control and sustain the release of theophylline which is important for subsequent sustained absorption rate from GIT that can results in decreasing or eliminating gastrointestinal side effects as well as maintaining constant blood level for such drug with narrow therapeutics index, theophylline.
Keywords: Microcapsules of theophylline, modified emulsion-solvent evaporation-non solvent technique, Theo SR 100 Capsules, Cellulose acetate butyrate (CAB), Ethyl cellulose (EC), encapsulation efficiency, drug release.

1. Introduction

Microencapsulation is a process in which tiny particles or a coating to give small capsules with many useful properties surrounds droplets. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating, or membrane. Most microcapsules have diameters between a few micrometers and a few millimeters (1). Gelatin is a common wall-forming material but synthetic polymers like polyvinyl alcohol, ethyl cellulose, polyvinyl chloride and other materials also may be used. One of the advantages of microencapsulation is that the administered dose of a drug is subdivided into small units that are spread over a large area of the gastrointestinal tract, which may enhance absorption by

diminishing localized drug concentration (2-3). The reasons for microencapsulation are countless (4,5). In some cases, the core must be isolated from its surroundings, as in isolating vitamins from the deteriorating effects of oxygen, retarding evaporation of a volatile core, improving the handling properties of a sticky material or isolating a reactive core from chemical attack (6). There are several reasons why substances may be encapsulated (7-9) such as to protect reactive substances from the environment, to convert liquid active components into a dry solid system, to separate incompatible components for functional reasons, to mask undesired properties of the active components, to protect the immediate environment of the microcapsules from the active components, to control release of the active components for delayed (timed) release or long-acting (sustained) release.

Different techniques of microencapsulation (10-48) have been developed for controlled delivery of different drugs (10-48) including, encapsulation of injectable proteins (10-13, 15), human growth hormone (14), human serum albumin (19), potassium chloride (25), *in-situ* gelation for theophylline and salbutamol sulphate (27,35-41), emulsion-solvent diffusion for ibuprofen (28,34, 45) and spray drying controlled-release microparticles loaded with tramadol hydrochloride (29), Furosemide (42), Pseudoephedrine HCl (43) and Nifedipine (46).

Choosing a suitable microencapsulation method is highly dependent on the drug characteristics, type of polymer used and economic considerations. Emulsion-solvent evaporation technique is one of early methods of microencapsulation which has been widely studied for preparation of polymeric microcapsules. In this technique, a polymer solution which drug substance is dissolved or dispersed in is emulsified in the external phase. By evaporation of the solvent, polymeric capsules are formed around the drug particles. The size and state of the particle in the internal phase play an important role in the final status of the micro particles. The choice of the internal and the external phase of the emulsion, type of emulsifier and method of homogenizing the two phases will effectively determine the characteristics of the final micro particles (30). Therefore, the method is very flexible for different types of polymers and hydrophilic and lipophilic drugs, and by selecting suitable solvent and emulsifier; various combinations of drug substances and polymers could be applied. In this study ethyl cellulose (EC) was selected as the sustaining polymer since it is a water-insoluble polymer with good film forming ability, durability and low cost and extended drug release properties (31,32). Ethyl cellulose (EC) is a non-biodegradable and biocompatible and gastro-resistant polymer which has been extensively used as drug release retardant which easily forms microcapsules with a one-step encapsulation method (33,34).

Theophylline is a methyl-xanthine alkaloid which is used as bronchodilator in treatment of chronic obstructive pulmonary disorders especially asthma. Although, it is used for about 70 years, the complications associated with its use are still unsolved (35,36). Theophylline is a narrow therapeutic index drug with a short half-life. Conventional dosage forms of theophylline should be administered 3 to 4 times a day to provide effective concentration and to avoid large fluctuations in blood concentration. This leads to poor patient compliance and enhanced risk of gastrointestinal (GI) and cardiovascular adverse effects. Sustained- release formulations would provide steady blood higher therapeutic efficacy and lower risk of toxicity (37,38). Among sustained-release drug delivery systems, microcapsules have received much attention because of uniform distribution in GI tract which leads to uniform absorption and decreasing risk of local effects on GI tract. Another advantage of microparticulate systems is their feasibility to be incorporated into liquid dosage forms such as suspensions. In addition to sustain the drug release, microencapsulation of theophylline can decrease its irritating effect on GI mucosa and mask drug taste (39). Although theophylline encapsulation in cellulose acetate butyrate (CAB) and EC microspheres for sustained delivery have been reported in several studies (35-42), preparation of sustained-release microcapsules containing theophylline by a modified emulsion-solvent evaporation-non-solvent addition has not been reported.

Therefore, this study aimed at preparation and *in vitro* evaluation of sustained-release microcapsules containing theophylline as a bronchodilator. The novelty of our work was to adopt a new, rapid, efficient and reproducible emulsion-solvent evaporation-non-solvent method for microencapsulation of theophylline by utilizing two biodegradable polymers, namely, cellulose acetate butyrate 171-15s (CAB) and ethyl cellulose (EC) at different polymer to drug ratios (2:1, 1:1 and 1:2). The prepared microcapsules were evaluated *in vitro* for the total recovery (yield percentage), microcapsule size distribution (sieve analysis), surface morphology by scanning electron microscopy (SEM), drug loading (encapsulation efficiency) and drug release characteristics.

2. Material and methods

2.1. Materials

Theophylline, ethyl cellulose (EC) and Cellulose acetate butyrate 171-15s (CAB) were obtained from Sigma Chemicals (St. Louis, MO, USA). Light liquid paraffin was purchased from (S&C Chem., Germany). All organic solvents were of analytical grade and were purchased from Lab-Scan-(United Kingdom). Theo SR **100® capsules** containing 300 mg theophylline produced by GlaxoSmithKline, was purchased from a local drugstore in KSA.

2.2. Preparation of theophylline microcapsules:

Theophylline microcapsules were prepared by a newly developed modified emulsion-solvent evaporation-non solvent addition technique. Known amount of polymer was dissolved in acetone and different amounts of theophylline was added to the polymer solution to produce 2:1, 1:1 and 1:2 polymer to drug ratios. The mixture was emulsified into 100 ml of light mineral paraffin containing 2.5% magnesium stearate for one hour, followed by addition n-hexane (non-solvent) drop wise using 10 ml syringe. The formed microcapsules were separated, washed three times with 100 ml of n-hexane to remove any adsorbed oil. The microcapsules were then dried overnight at room temperature, filled into dry colored bottles for further *in-Vitro* studies. (42, 47-48).

2.3. *in-Vitro* evaluation of theophylline microcapsules:

The prepared theophylline microcapsules were evaluated for total recovery (yield %), microcapsules size distribution (sieve analysis), drug loading (encapsulation efficiency %), surface morphology was studied by (SEM), drug release characteristics and kinetics of drug release.

2.4. Total recovery of theophylline microcapsules (yield %):

The yield % was determined by dividing the weight of the recovered theophylline microcapsules by the sum initial weight of drug and polymer used.

2.5. Microcapsules size distribution (sieve analysis):

Theophylline microcapsules size distribution was determined by utilizing a set of standard sieves (Gilson Company SS-15, USA).

2.6. Drug loading (encapsulation efficiency %):

The encapsulation efficiency was determined by assaying the amount of theophylline in 100 mg of a given batch of microcapsules. A weighed 100 mg of microcapsules were dissolved in 100 ml ethyl acetate, followed by filtration and appropriate dilution. Drug concentration was measured spectrophotometrically at λ_{max} 272 nm using a double beam spectrophotometer (Shimadzu UV-160 1CP, Japan). At the specified wavelength, no interaction observed from blank microcapsules (blank polymer).

2.7. Scanning electron microscopy (SEM):

The surface characteristics of the prepared theophylline microcapsules were observed by scanning electron microscope (Jeol, JSM-5400 LV, Japan).

2.8. *In-vitro* drug release characteristics:

The in-vitro release characteristics of theophylline from the prepared microcapsules were studied in simulated gastric fluids (pH 1.2) and simulated intestinal fluids (pH 6.8) using USP dissolution apparatus type II USP 20 (Pharmatest Germany). Accurately weighed amounts of the prepared theophylline microcapsules equivalent to 100mg theophylline were suspended in 900 ml of the dissolution medium at 37 ^{o}C and 100 rpm. At specified time intervals up to 8 hrs, 5 ml samples of the dissolution fluid was withdrawn and replaced by 5 ml of fresh medium. Theophylline concentrations in withdrawn samples was assayed spectrophotometrically at λ_{max}272 nm. Each data point represents the average of three determinations.

The release data of theophylline from the prepared microcapsules was compared with that of the commercial theophylline product Theo SR®.

2.9. Kinetics of theophylline release from the prepared microcapsules:

To investigate the mechanism of theophylline release from the prepared and commercial microcapsules, all the release data were fitted to the mathematic equation of Ritger and Peppas (Equation 1):

$$Q = K t^n \quad \text{(Eq.1)}$$

By taking log scale

$$\text{Log } Q = \text{Log } K + n \text{ Log } t \quad \text{(Eq.2)}$$

Where **Q** is the fractional drug released at time **t**, **K** is a kinetic constant and **n** is an exponent indicative of the release mechanism. When **n** approximate **0.5**, a Fickian/Diffusion controlled mechanism is applied, With **0.5 < n < 1** indicating non-Fickian transport, **and n = 1** for Zero order release mechanism.

3. Results and discussion

3.1. Preliminary evaluation

The microspheres obtained under the described experimental conditions were mostly spherical, free flowing and without aggregation. The percentage yield of all the formulation was found to be satisfactory (> 96 %) and drug entrapment (encapsulation) efficiency of all formulations were found to be more than 95 %.

3.2. Effect of Stirring Rate (rpm) on Geometric Mean Diameter:

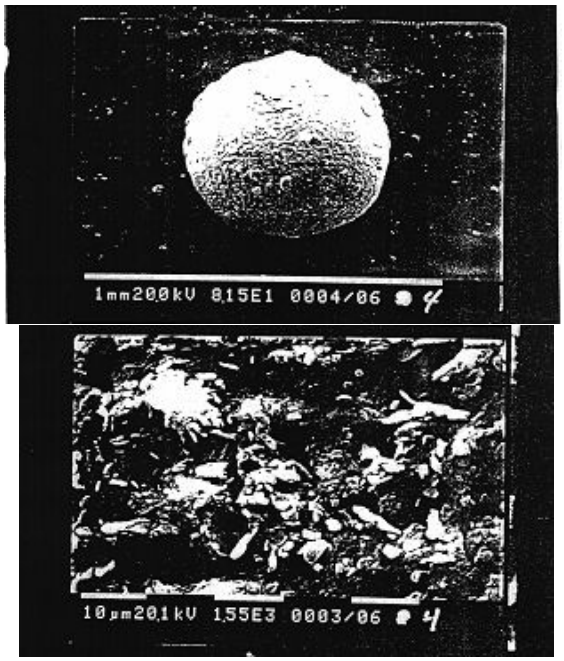
The effect of the stirring rate on the Geometric Mean Diameter (GMD), Geometric Standard Deviation (GSD) and encapsulation efficiency of theophylline were summarized in Table 1. Increasing the stirring rate was found to decrease the mean microcapsule size. At 250 rpm, the Geometric Mean Diameter (GMD) of the prepared microcapsules was 1000 µm, while at 800 rpm the GMD was 350 µm**.** However, the encacapsulation efficiency was not affected by the stirring rate (Table 1).

Table 1: Effect of the stirring rate on the Geometric Mean Diameter (GMD), Geometric standard deviation (GSD) and encapsulation efficiency of theophylline microcapsules prepared with 15% CAB at 1:1 polymer to drug ratio.

Stirring rate (rpm)	GMD (µm)	GSD	Theoretical drug content (w/w%)	Encapsulation Efficiency
250	1000	2.00	50	48.50 ± 1.20
300	910	1.80	50	47.80 ± 2.41
350	900	1.90	50	46.54 ± 3.20
400	820	1.70	50	48.50 ± 1.99
450	700	1.34	50	47.80 ± 1.99
500	600	1.10	50	48.90 ± 1.60
600	520	1.30	50	49.20 ± 0.50
800	350	1.20	50	47.30 ± 2.10

3.3. Scanning Electron Microscopy:

Figures 1 and 2 show the scanning electron microphotographs (SEM) of theophylline loaded microspheres prepared with CAB and EC, respectively. The microspheres obtained with CAB and EC were discrete, spherical and free flowing, indicating importance of the utilized technique for microencapsulation of theophylline.



a

b

Figure 1: Scanning electron microscopy of theophylline microcapsules prepared with cellulose acetate butyrate (CAB 171s) at 1:1, polymer : drug ratio
(a : low magnification, b: high magnification)

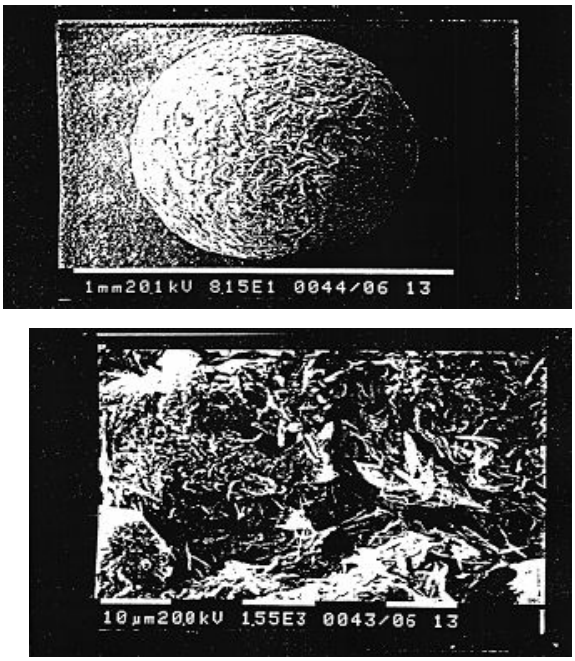


Figure 2: Scanning electron microscopy of theophylline microcapsules prepared with ethyl cellulose (EC) at 1:1, polymer: drug ratio
(a :low magnification, b: high magnification)

3.4. Total recovery (yield %) and Random Encapsulation Efficiency:

Table 2: Total recovery (yield %) and Random Encapsulation Efficiency (REE) of theophylline microcapsules prepared with CAB and EC at different polymer to drug ratio.

Polymer used	polymer to drug ratio	Initial weight of drug and polymer (g)	Yield (%)	Theoretical drug content (w/w%)	Encapsulation Efficiency	Encapsulation Efficiency (%)
CAB (15%w/w)	2:1	6.75 (4.5 +2.25)	98.50	33.33	32.50 ± 1.20	97.50 ± 1.20
	1:1	9 (4.5 x2)	97.90	50	48.80 ± 2.41	97.60 ± 2.41
	1:2	13.5 (4.5 + 9)	97.50	66.67	64.54 ± 3.20	96.80 ± 3.20
EC (6% w/w)	2:1	2.7 (1.8 + 0.9)	99.60	33.33	32.50 ± 1.99	97.50 ± 1.99
	1:1	3.6 (1.8x2)	98.80	50	48.10 ± 1.99	96.20 ± 1.99
	1:2	5.4 (1.8 + 3.6)	98.90	66.67	64.90 ± 1.60	97.34 ± 1.60

Table 2 shows total recovery (yield %) and Random Encapsulation Efficiency (REE) of theophylline microcapsules prepared with CAB and EC at different polymer to drug ratio. The total recovery (yield %) was higher than 97.50 % for the two polymers at different polymer to drug ratios (Table 2). The encapsulation efficiency (%) was higher than 96.20 % for the two polymers at different polymer to drug ratios (Table 2). These data clearly indicate the efficiency of the utilized procedure for microencapsulation of theophylline. These results are in agreement with our previous work on microencapsulation of ciprofloxacin and norfloxacin (48).

3.5. Particle size distribution of theophylline microcapsules:

Tables 3 and 4 show the particle size distribution of theophylline microcapsules prepared with CAB (Table 3) and EC (Table 4) at different polymer to drug ratios. The mean size of the microcapsules was increased by increasing the drug amount from 2:1, 1:1, and 1:2 polymer to drug ratio (Tables 3 and 4). In this study, microcapsule size range of 1000-500 µm (Average 750 µm) was of our interest. This narrow range of particle size can be attributed to the effect of stirring time, stirring speed and rate of solvent evaporation during preparation of microspheres (48).

Table 3: Particle size distribution of theophylline microcapsules prepared with Cellulose Acetate Butyrate (CAB-171) at different polymer to drug ratio.

Microcapsule Size Range (µm)	Microcapsule Average Size (µm)	Particle size distribution (% Frequency)		
		2:1	1:1	1:2
1500-1000	1250	25±1.9	26±2.5	5±1.5
1000-500	750	60±1.3	70±3.2	80±3.5
500-355	427	13±1.4	4±0.6	13±1.5
355-180	267	2±0.7	1±0.3	2±.0.5

Table 4: Particle size distribution of theophylline microcapsules prepared with Ethyl Cellulose (EC) at different polymer to drug ratio.

Microcapsule Size Range (µm)	Microcapsule Average Size (µm)	Particle size distribution (% Frequency)		
		2:1	1:1	1:2
1500-1000	1250	30±2.6	30±2.5	10±1.5
1000-500	750	45±2.0	65±3.2	80±3.5
500-355	427	20±1.4	3±0.6	8±1.5
355-180	267	5±0.5	2±0.3	2±.0.5

3.6. Effect of microcapsule average size (particle size distribution) on encapsulation efficiency:

Tables 5 and 6 show the effect of microcapsule average size (particle size distribution) on encapsulation efficiency (EE%) of theophylline microcapsules prepared with CAB and EC at different polymer to drug ratios. The encapsulation efficiency (EE%) was higher than 95% for all microcapsules prepared with the polymers at different polymer to drug ratios. Data in tables 5 and 6 clearly indicate the usefulness of the utilized technique for microencapsulation of theophylline. The principal parameters controlling the particle size are the rotational speed, equipment, and the concentration of both the polymer and drug (polymer to drug ration) in the dispersed phase (48).

Table 5: Effect of microcapsule average size (particle size distribution) on encapsulation efficiency (EE%) of theophylline microcapsules prepared with Cellulose Acetate Butyrate (CAB-171) at different polymer to drug ratio.

Microcapsule Average Size (µm)	Polymer to drug ratio								
	2:1			1:1			1:2		
	TDC[a]	ADC[b]	EE[c] (%)	TDC[a]	ADC[b]	EE[c] (%)	TDC[a]	ADC[b]	EE[c] (%)
1250	33.33	32.2	96,66	50	48.7	97.40	66.67	65.5	98.24
750	33.33	32.5	97.50	50	48.9	97.80	66.67	64.9	97.34
427	33.33	32.4	97.20	50	48.2	96.40	66.67	64.7	97.04
267	33.33	31.8	95.40	50	49,1	98.20	66.67	64.0	95.99

TDC[a] : Theoretical drug content
ADC[b] : Assayed drug content
EE[c] : % Encapsulation efficiency = **(Assayed drug content / Theoretical drug content) x100**

Table 6: Effect of microcapsule average size (particle size distribution) on encapsulation efficiency (EE%) of Theophylline microcapsules prepared with Ethyl cellulose (EC) at different polymer to drug ratio.

MicrocapsuleAverage Size (µm)	Polymer to drug ratio								
	2:1			1:1			1:2		
	TDC[a]	ADC[b]	EE[c] (%)	TDC[a]	ADC[b]	EE[c] (%)	TDC[a]	ADC[b]	EE[c] (%)
1250	33.33	32.7	9810	50	48.1	96.20	66.67	64.6	96.89
750	33.33	32.2	96.60	50	48.2	96.40	66.67	64.8	97.19
427	33.33	32.1	96.30	50	47.9	95.80	66.67	64.5	96.74
267	33.33	32.2	96.50	50	47.8	95.60	66.67	63.8	95.50

TDC[a] : Theoretical drug content
ADC[b] : Assayed drug content
EE[c] : % Encapsulation efficiency = **(Assayed drug content / Theoretical drug content) x100**

3.7. Comparison with commercial capsules (Theo SR 100):

Figures 7 a&b show the effect of pH of release fluid on % theophylline released from theophylline microcapsules (average size 750 µm) prepared with Cellulose Acetate Butyrate (CAB) and Ethyl Cellulose (EC) at 1:1, polymer to drug ratio, in comparison with commercial capsules (Theo SR 100®). The rate of release of theophylline from the prepared microcapsules was slower from the two polymers at different pH as compared to the commercial theophylline capsules. These data clearly indicate the usefulness of the prepared microcapsules in sustaining the rate of release of theophylline.

Table 7a: Effect of microcapsule average size and pH of release fluid on % Theophylline released from theophylline microcapsules prepared Cellulose Acetate Butyrate (CAB-171) at 2:1, polymer to drug ratio.

Time (h)	% Theophylline released					
	Microcapsule Average Size (µm)					
	1250		750		427	
	pH 1.2	**pH 6.8**	**pH 1.2**	**pH 6.8**	**pH 1.2**	**pH 6.8**
0.166	0.90	1. 0	1.044	1. 11	2.5	2. 7
0.33	2.07	1.6	12.07	2.6	15.07	5.6
0.5	5.51	3.5	19.51	5.5	22.51	10.5
0.75	8.05	7.2	31.05	10.2	34.05	20.2
1	11.48	10.7	32.48	13.7	37.48	34.3
1.5	15.63	13.2	35.63	15.2	40.63	40.7
2	20.18	15.1	37.18	17.9	45.18	45.8
3	31.77	20.2	51.77	23.9	55.77	48.3
4		25.2		32.7		50.4
5		31.4		41.9		53.5
6		42.5		52.9		55.5
8		50.3		60.6		64.8

Table 7b: Effect of microcapsule average size and pH of release fluid on % Theophylline released from theophylline microcapsules prepared Cellulose Acetate Butyrate (CAB-171) at 1:1, polymer to drug ratio.

Time (h)	% Theophylline released					
	Microcapsule Average Size (µm)					
	1250		750		427	
	pH 1.2	**pH 6.8**	**pH 1.2**	**pH 6.8**	**pH 1.2**	**pH 6.8**
0.166	1.00	1.5	1.54	**1.80**	3.54	4.5
0.33	11.65	3.3	17.65	3.5	19.65	6.3
0.5	13.31	9.3	23.31	5.3	26.31	9.3
0.75	17.71	12.1	35.71	14.3	37.71	19.1
1	20.7	20.7	40.7	16.1	47.7	30.7
1.5	23.13	30.5	43.13	27.7	53.13	39.4
2	27.15	35.6	47.15	35.4	57.15	42.4
3	34.96	40.5	54.96	49.4	64.96	49.2
4		45.6		55.2		55.4
5		47.8		60.4		57.1
6		50.8		67.1		62.2
8		52.9		70.2		82.2

Table 7c: Effect of microcapsule average size and pH of release fluid on % Theophylline released from theophylline microcapsules prepared Cellulose Acetate Butyrate (CAB-171) at 1:2, polymer to drug ratio.

Time (h)	% Theophylline released					
	Microcapsule Average Size (µm)					
	1250		750		427	
	pH 1.2	**pH 6.8**	**pH 1.2**	**pH 6.8**	**pH 1.2**	**pH 6.8**
0.166	1. 5	2.01	2. 9	3.01	3. 9	5.01
0.33	5.9	6.9	15.9	7.9	17.9	18.9
0.5	11.8	8.9	35.8	10.9	37.8	20.9
0.75	22.6	16.3	42.6	17.3	46.6	27.3
1	31.4	28.3	43.4	24.3	53.4	34.3
1.5	35.5	35.8	48.5	30.8	58.5	40.8
2	37.1	38.5	50.1	35.5	60.1	45.5
3	40.2	42.6	55.2	40.6	65.2	50.6
4		47.1		44.1		58.1
5		49.9		58.9		68.9
6		55.4		64.4		77.4
8		59.9		86.9		96.9

Table 8a: Effect of microcapsule average size and pH of release fluid on % Theophylline released from theophylline microcapsules prepared with Ethyl Cellulose (EC) at 2:1, polymer to drug ratio.

Time (h)	% Theophylline released					
	Microcapsule Average Size (µm)					
	1250		750		427	
	pH 1.2	**pH 6.8**	**pH 1.2**	**pH 6.8**	**pH 1.2**	**pH 6.8**
0.166	0.53	1.2	1.53	1.69	2.53	2.69
0.33	3.85	4.8	6.85	6.8	8.85	9.8
0.5	10.28	6.43	12.28	7.43	15.28	17.43
0.75	13.26	11.43	15.26	12.43	18.26	22.43
1	15.28	12.88	17.28	12.88	20.28	27.88
1.5	17.32	14.15	19.32	15.1	29.32	35.1
2	19.26	17.39	21.26	19.39	31.26	39.39
3	22.04	24.81	25.04	25.81	35.04	45.81
4		30.74		32.74		47.74
5		40.70		44.70		54.70
6		50.89		57.89		60.89
8		56.49		62.49		72.49

Table 8b: Effect of microcapsule average size and pH of release fluid on % Theophylline released from theophylline microcapsules prepared with Ethyl Cellulose (EC) at 1:1, polymer to drug ratio.

Time (h)	% Theophylline released					
	Microcapsule Average Size (µm)					
	1250		750		427	
	pH 1.2	pH 6.8	pH 1.2	pH 6.8	pH 1.2	pH 6.8
0.166	0.5	0.57	1.8	2.97	3.8	5.97
0.33	3.5	6.17	5.5	10.17	9.5	12.17
0.5	11.3	15.92	13.3	16.92	17.3	19.92
0.75	20.4	22.13	28.4	30.13	30.5	65.13
1	22.2	26.5	32.2	52.5	45.5	69.5
1.5	28.6	30.87	48.6	58.87	50.4	70.87
2	31.9	33.33	51.9	71.33	61.5	79.33
3	40.9	45.69	77.9	75.69	87.4	80.69
4		48.54		78.54		88.54
5		50.57		79.57		90.57
6		60.91		80.91		92.91
8		70.7		89.7		93.7

Table 8c: Effect of microcapsule average size and pH of release fluid on % Theophylline released from theophylline microcapsules prepared with Ethyl Cellulose (EC) at 1:2, polymer to drug ratio.

Time (h)	% Theophylline released					
	Microcapsule Average Size (µm)					
	1250		750		427	
	pH 1.2	pH 6.8	pH 1.2	pH 6.8	pH 1.2	pH 6.8
0.166	0.7	3.13	1.2	5.13	3.2	8.13
0.33	8.3	11.3	10.3	14.32	15.3	24.32
0.5	13.2	20.5	19.8	22.5	25.8	32.8
0.75	19.7	30.7	39.1	50.72	40.1	55.7
1	23.9	43.1	43.2	53.11	47.2	58.9
1.5	30.5	50.4	55.5	59.43	65.5	69.9
2	45.9	56.7	62.1	66.71	72.1	76.9
3	49.9	60.3	79.1	70.3	89.1	80.9
4		69.5		79.59		85.9
5		70.7		80.71		90.6
6		74.5		84.51		94.8
8		82.5		92.54		96.9

3.8. Relative dissolution rate of theophylline (RDR):

Table 9 shows the relative dissolution rate (RDR) of theophylline released from theophylline microcapsules (average size 750 µm) prepared with Cellulose Acetate Butyrate (CAB) and Ethyl Cellulose (EC) at 1:1, polymer to drug ratio, in comparison with commercial capsules (Theo SR 100®). The RDR always lower than one (<1) indicating good excellent retardation of the release rate of theophylline.

Table 9: Relative dissolution rate of theophylline released from theophylline microcapsules (average size 750 µm) prepared with Cellulose Acetate Butyrate (CAB-171) and Ethyl Cellulose (EC) at 1:1, polymer to drug ratio, in comparison with commercial capsules (Theo SR 100).

Time (h)	Relative dissolution rate of theophylline **(RDR)***			
	CAB **(1:1)**		EC (1:1)	
	pH 1.2	**pH 6.8**	**pH 1.2**	**pH 6.8**
0.166	0.36	0.35	0.43	0.57
0.33	0.14	0.24	0.53	0.71
0.5	0.34	0.16	0.59	0.52
0.75	0.43	0.28	0.92	0.59
1	0.44	0.24	0.90	0.78
1.5	o.50	0.38	0.96	0.81
2	0.50	0.46	0.78	0.94
3	0.46	0.62	0.97	0.95
4		0.66		0.94
5		0.69		0.91
6		0.74		0.90
8		0.75		0.95

***(RDR)** = % Theophylline released at any time from the prepared microcapsules divided by the amount released from the commercial capsules at the same time.

3.9. Kinetics of theophylline release:

Tables 10a&b show the release kinetics of theophylline release from the prepared microspheres in comparison with the commercial capsules (Theo SR 100®). In which r = Linear correlation coefficient, r^2 = Determination coefficient, K = Kinetic release constant, n = Diffusion release constant, which it is an indicative of the release mechanism. When n approximates 0.5, a Fickian/diffusion controlled mechanism implied, with 0.5 > n< indicating non-Fickian transport, and n ≥1 for zero-order release. Each point represents the mean of three determinations. The results obtained revealed that theophylline release can be described by zero order release kinetics (n ≥ 1). These data clearly demonstrate the ability to control the release rate of theophylline through the utilized modified emulsion-solvent evaporation-non solvent addition technique.

Table 10a: Kinetic parameters of theophylline released from theophylline microcapsules (average size 750 µm) prepared with Cellulose Acetate Butyrate (CAB-171) and Ethyl Cellulose (EC) at 1:1, polymer to drug ratio, in comparison with commercial capsules (Theo SR 100®).

Time (h)	Log t+1	% Kinetic of Theophylline released (Log Q = Log K + n Log t)											
		CAB **(1:1)**				EC (1:1)				commercial			
		pH 1.2	**Log pH 1.2**	**pH 6.8**	**Log pH 6.8**	**pH 1.2**	**Log pH 1.2**	**pH 6.8**	**Log pH 6.8**	**Log pH 1.2**	**Log pH 1.2**	**pH 6.8**	**Log pH 6.8**
0.16	0.22	**1.50**	0.17	**1.80**	0.255	1.8	0.255	2.97	0.47	4.13	0.61	5.13	0.71
0.33	0.519	1.54	0.40	3.5	0.54	5.5	0.74	10.17	1.00	10.32	1.01	14.32	1.15
0.5	0.699	7.65	0.88	5.3	0.72	13.3	1.12	16.92	1.22	22.50	1.35	32.5	1.51
0.75	0.875	13.31	1.12	14.3	1.18	28.4	1.45	30.13	1.47	30.72	1.48	50.72	1.70
1	1	15..71	1.19	16.1	1.20	32.2	1.50	52.5	1.72	35.60	1.55	66.50	1.82
1.5	1.176	25.7	1.40	27.7	1.40	48.6	1.68	58.87	1.76	50.50	1.70	72.50	1.86
2	1.301	33.13	1.50	35.4	1.55	51.9	1.71	71.33	1.85	66.10	1.82	75.70	1.88
3	1.477	37.15	1.56	49.4	1.70	77.9	1.89	75.69	1.87	80.00	1.90	79.60	1.90
4	1.602			55.2	1.74			78.54	1.89			83.40	1.92
5	1.699			60.4	1.78			79.57	1.90			86.60	1.94
6	1.778			67.1	1.82			80.91	1.91			89.50	1.95
8	1.903			70.2	1.84			89.7	1.95			93.50	1.97
A		-0.060		0.128		0.115		0.420		0.503		0.829	
B		1.199		1.00		1.299		0.798		1.02		0.70	
r		0.977		0.975		0.974		0.919		0.979		0.896	

A = intercept B= slope r = linear correlation coefficient

Table 10b: Kinetic parameters of theophylline released from theophylline microcapsules (average size 750 µm) prepared with Cellulose Acetate Butyrate (CAB-171) and Ethyl Cellulose (EC) at 1:1, polymer to drug ratio, in comparison with commercial capsules (Theo SR 100®).

Polymer	pH	Kinetic parameters ($Q = Kt^n$)				
		r	r^2	K	$K_{relative}$	n
CAB	1.2	0.977	0.954	0.870	0.273	1.199
	6.8	0.975	0.951	1.342	0.198	1.00
EC	1.2	0.974	0.948	1.304	0.410	1.299
	6.8	0.919	0.844	4.393	0.650	0.798
Commercial Capsules (Theo SR 100)	1.2	0.979	0.958	3.180	-------	1.020
	6.8	0.896	0.802	6.753	-------	0.703

*r = Linear correlation coefficient
r^2 = determination coefficient
K = Kinetic release constant
n = Diffusion release constant, it is an indicative of the release mechanism. When n approximates 0.5, a Fickian/diffusion controlled mechanism implied, with 0.5 > n< indicating non-Fickian transport, and n ≥ 1 for zero-order release. Each point represents the mean of three determinations.

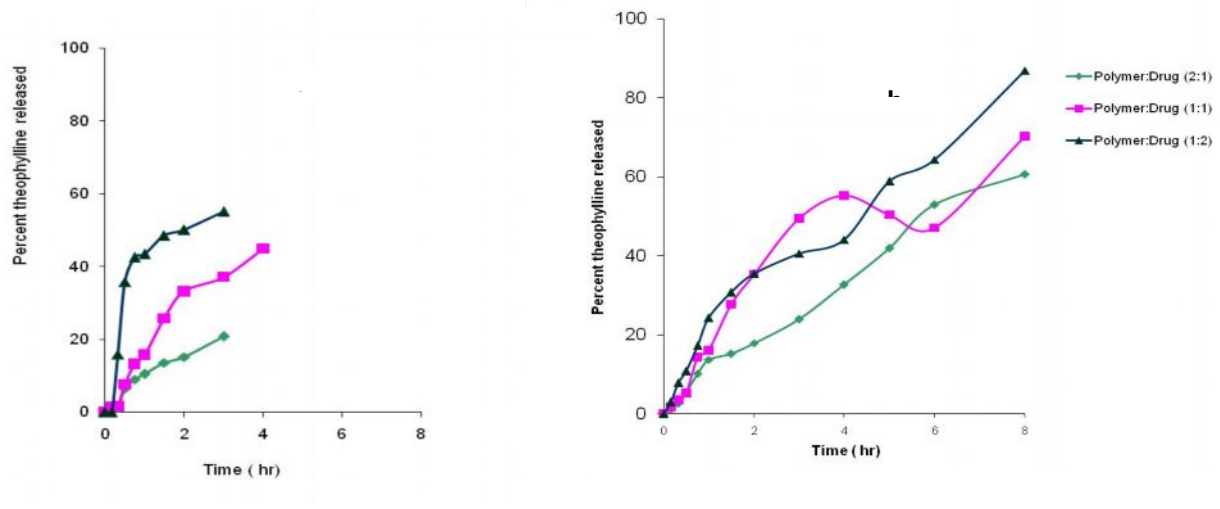


Figure 3: Effect of polymer to drug ratio and pH of release fluid on % Theophylline released from the prepared Cellulose Acetate Butyrate (CAB-171) theophylline microcapsules (Average Size 750 µm). a: pH=1.2 b: pH=6.8.

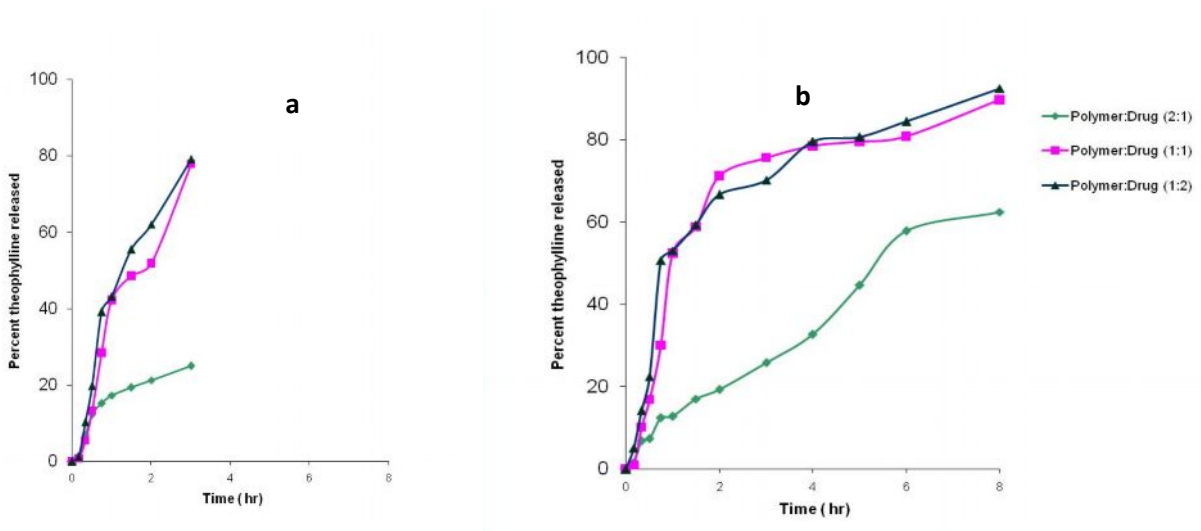


Figure 4: Effect of polymer to drug ratio and pH of release fluid on % theophylline released from the prepared EC theophylline microcapsules (Average Size 750 µm) a: pH=1.2, b: pH=6.8.

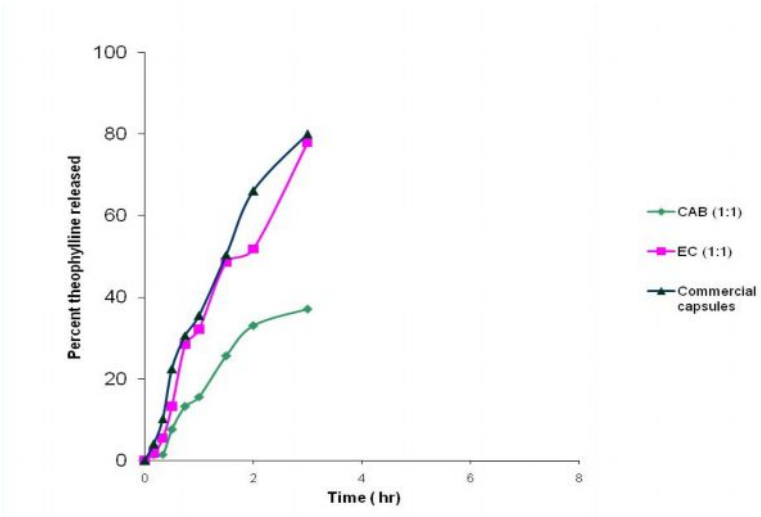


Figure 5a: Release of theophylline into simulated intestinal fluids (pH 1.2) from microcapsules (average size 750 µm) prepared with (EC) and (CAB) at 1:1, polymer: drug ratio in comparison with commercial formulation.

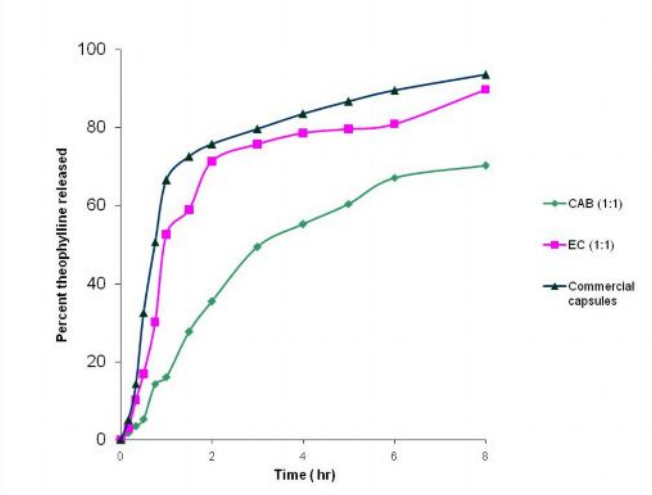


Figure 5b: Release of theophylline into simulated intestinal fluids (pH 6.8) from microcapsules (average size 750 µm) prepared with (EC) and (CAB) at 1:1, polymer: drug ratio in comparison with commercial formulation.

Conclusion:

Spherical, free flowing microcapsules with smooth surfaces of theophylline were successfully prepared with the two polymers, CAB and EC. The encapsulation efficiency percentages were more than 95% for the two polymers at different polymer to drug ratios, indicating efficiency of the method. The drug release was affected by the type of polymer, polymer to drug ratios, microcapsule size and pH of the dissolution medium. The release of theophylline from CAB was slower than EC microcapsules. The release of theophylline from CAB and EC microcapsules was dependent on size of the microcapsules, increase with decreasing microcapsules size. The release of theophylline from all the prepared microcapsules was markedly retarded as compared to commercial theophylline marketed product (Theo SR 100® Capsules). The kinetics studies of the release data indicated that theophylline release can be described by Zero order release kinetics ($n \geq 1$). These data clearly indicate ability of the prepared microcapsules to control and sustain the release of theophylline which is important for subsequent sustained absorption that can results in decreasing or eliminating gastrointestinal side effects as well as maintaining constant blood level for such drug with narrow therapeutics index, theophylline.

References

1. Hammad U., Hemlata N., Asif M. T., Sundara M. N., Microencapsulation: Process, Techniques and Applications, International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011 , 2, 474 – 481.
2. Ansel H.C., Pharmaceutical dosage forms and drug delivery systems. Lippincott Williams and Wilkins, 2000, pp. 233-234.
3. Yazici E., Oner, Kas H.S., Hincal A.A., Phenytoin sodium microcapsules: bench scale formulation, process characterization and release kinetics, Pharmaceutics Dev Technol., 1996, 1,175-183.
4. Blair H.S., Guthrie J., Law T., Turkington P., Chitosan and modified chitosan membranes I, preparation and characterization, J. App. Poly. Sci, 1987, 33, 641-656.
5. Nack H., Microencapsulation techniques, application and problems, J.Soc.Cosmetic Chemists, 1970, 21, 85-98.
6. Swapan Kumar Ghosh, Functional Coatings and Microencapsulation: A General Perspective. WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim. ISBN 3- 527-31296-X. 2006
7. Finch C.A., Polymers for microcapsule walls, Chem. Ind., 1985, 22, 752-756.
8. Li S.P., Kowarski C.R., Feld K.M., Grim W.M., Recent advances in microencapsulation technology and equipment, Drug Dev. Ind. Pharm., 1988, 14, 353-376.
9. Lehman L., Lieberman A. Herbert, Kanig L. Josep. (Ed), The Theory and Practice of Industrial Pharmacy, 3rd edition, Vargehese Publishing House, 1976, p. 412.
10. Schwendeman S.P., Recent advances in the stabilization of proteins encapsulated in injectable PLGA delivery systems, Crit Rev Ther Drug Carrier Syst, 2002, 19, 73-98.
11. Lu W., Park T.G., Protein release from poly (lactic-co-glycolic acid) microspheres: protein stability problems, PDA J. Pharm. Sci. Technol., 1995, 49, 13-19.
12. Blanco M.D., Alonso M.J., Development and characterization of protein-loaded poly (lactideco-glycolide) nanospheres, Eur. J. Pharm. Biopharm., 1997, 43, 287-294.
13. Zhu G., Mallery S.R., Schwendeman S.P., Stabilization of proteins encapsulated in injectable PLGA, Nat. Biotechnol., 2000, 18, 52- 57.
14. Capan Y., Jiang G., Giovagnoli S., Na K-H, DeLuca P.P, Preparation and characterization of poly (D, L-lactide-co-glycolide) microspheres for controlled release of human growth hormone, AAPS PharmSciTech., 2003, 4, E28.
15. Perez C., Castellanos I.J., Costantino H.R., Al- Azzam W., Griebenow K., Recent trends in stabilizing protein structure upon encapsulation and release from bioerodible polymers, J. Pharm. Pharmacol., 2002, 54, 301-313.
16. Yeo Y., Basaran O.A., Park K., A new process for making reservoir-type microcapsules using inkjet technology and interfacial phase separation, J. Control. Release, 2003, 93,161-173.
17. Yeo Y., Park K., A new microencapsulation method using an ultrasonic atomizer based on interfacial solvent exchange, J. Control. Release, 2004, 100, 379-88.
18. Berger H.L. (Ed), Ultrasonic Liquid Atomization, 1st edition, Hyde Park, NY: Partridge Hill
19. Publishers, 1998.
20. Hora M.S., Rana R.K., Nunberg J.H., Tice T.R., Gilley R.M., Hudson M.E., Release of human serum albumin from PLGA microspheres, Pharm. Res., 1990, 7, 1190-1194.
21. Murtaza G., Ahamd M., Akhtar N., Rasool F., A comparative study of various microencapsulation techniques: effect of polymer viscosity on microcapsule characteristics, Pak. J. Pharm. Sci., 2009, 3, 291- 300.
22. James S. (Ed), Encylopedia of Pharmaceutical Technology. 3rd edition., Marcel Dekker, 2002, pp.1325-1333.
23. Jain N.K. (Ed), Controlled and Novel drug delivery, 4th edition, CBS Publisher & Distributors,New Delhi, 2010, pp. 236-237.
24. Vyas S.P., Khar R.K., Targeted and Controlled drug delivery, 7th edition, CBS Publisher & Distributors,New Delhi, 2001, 418.
25. Schugens C., Larvelle N., Nihantn, Grandfils C., Jerome R., Teyssie P., Polylactide microparticles prepared by double emulsion/evaporation technique, I. Effect of primary emulsion stability, Pharm. Res., 1994, 11, 1479-84.
26. Pao-Chu Wua, Yaw-Bin Huanga, Jui-Sheng Changa, Ming-Jun Tsaib, Yi-Hung Tsaia., Design and evaluation of sustained release microspheres of potassium chloride prepared by Eudragit. Eur. J. Pharm. Sci. 2003, 19, 115-122.

27. Alagusundaram M., Madhu Sudana C., Umashankari C., Microspheres as a Novel drug delivery system - A review, International J. of chem. Tech., 2009, 526- 534.
28. Miyazaki S, Kubo W, Attwood D (2000). Oral sustained delivery of theophylline using *in-situ* gelation of sodium alginate. J. Control Release 67:275-280.
29. Kawashima Y., Iwamoto T., Niwa T., Takeuchi H., Itoh Y., Preparation and characterization of a new controlled release ibuprofen suspension for improving suspendability, Int. J. Pharm., 1991,75, 25-36.
30. Naeem Aamir M., Ahmad M., Akhtar N., Murtaza G., Khan S.A., Shahiquz Z., Nokhodchi A., Development and *in vitro-in vivo* relationship of controlled-release microparticles loaded with tramadol hydrochloride, Int. J. Pharm., 2011, 407, 38-43.
31. Matsumoto A., Matsukawa Y., Nishioka Y., Harada M., Horikiri Y., Yamahara H., A new method of preparing TRH derivative-loaded poly(dl-lactide-co-glycolide) microspheres based on a solid solution system, Drug Discov. Ther., 2008, 2, 45-51.
32. Shi P., Li Y., Zhang L., Fabrication and property of chitosan film carrying ethyl cellulose microspheres, Carbohydr. Polym., 2008, 72, 490-499.
33. Shi P., Zuo Y., Zou Q., Shen J., Zhang L., Li Y., Morsi Y.S., Improved properties of incorporated chitosan film with ethyl cellulose microspheres for controlled release, Int. J. Pharm., 2009, 375, 67-74.
34. Das M.K., Rao K.R., Evaluation of zidovudine encapsulated ethylcellulose microspheres prepared by water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion technique, Acta Pol. Pharm. Drug Res., 2006, 63, 141-148.
35. Sudhamani T., Noveenkumar reddy K., Ravi Kumar V.R., Revathi R., Ganesan V., Preparation and evaluation of ethyl cellulose microspheres of ibuprofen for sustained drug delivery, Int. J. Pharm. Res. Dev. Online, 2010, Publication Ref No.:I JPRD/2010/PUB/ARTI/VOV-2/ISSUE-8/OCT/019.
36. Obeidat W., Obeidat S., Alzoubi N., Investigations on the physical structure and the mechanism of drug release from an enteric matrix microspheres with a near-zero-order release kinetics using SEM and quantitative FTIR, AAPS Pharm. Sci. Tech., 2009, 10, 615-623.
37. Sinko P.J. (Ed), Martin's physical pharmacy and pharmaceutical sciences. 5th ed., Lippincott Williams & Wilkins, Philadelphia, 2006, pp. 502-503.
38. Roy C., Vega-González A., Subra-Paternault P., Theophylline formulation by supercritical antisolvents, Int. J. Pharm., 2007, 343, 79-89.
39. Zhang W., Chen X., Li P., He Q., Zhou H., Preparation and characterization of theophylline loaded chitosan/β-cyclodextrin microspheres, J. Mater. Sci. Mater. Med., 2008, 19, 305-310.
40. Lavasanifar A., Ghalandari R., Ataei Z., Zolfaghari M.E., Mortazavi S.A., Microencapsulation of theophylline using ethylcellulose: *In vitro* drug release and kinetic modeling, J. Microencapsul., 1997, 14, 91-100.
41. Pachuau L., Sarkar S., Mazumder B., Formulation and evaluation of matrix microspheres for simultaneous delivery of salbutamol sulphate and theophylline, Trop. J. Pharm. Res., 2008, 7, 995-1002.
42. Emami J., Varshosaz J., Amirsadri M., Ahmadi F., Preparation and evaluation of a sustained-release suspension containing theophylline microcapsules, African Journal of Pharmacy and Pharmacology, 2012, 6, 2091-2099.
43. Akbuga J., Furosemide-loaded ethyl cellulose microspheres prepared by spherical crystallization technique: Morphology and release characteristics, Int. J. Pharm., 1991, 76, 193-198.
44. Bodmeier R., Chen H., Tyle P., Jarosz P., Pseudoephedrine HCl microspheres formulated into an oral suspension dosage form. J. Control Release, 1991, 15, 65-77.
45. Cuña M., Vila Jato J.L., Torres D., Controlled-release liquid suspensions based on ion-exchange particles entrapped within acrylic microcapsules, Int. J. Pharm., 2000, 199, 151-158.
46. Dalal P.S., Narurkar M.M., *In vitro* and *in vivo* evaluation of sustained release suspensions of ibuprofen, Int. J. Pharm., 1991, 73, 157-162.
47. Guyot M., Fawaz F., Nifedipine loaded-polymeric microspheres: Preparation and physical characteristics, Int. J. Pharm., 1998, 175, 61-74.
48. Halder A., Sa B., Preparation and *in vitro* evaluation of polystyrene-coated diltiazem-resin complex by oil-in-water emulsion solvent evaporation method, AAPS Pharm. Sci. Tech., 2006, 7, E105-E112.
49. Mohamed F.A, Hassan M. A., Formulation and evaluation of ciprofloxacin hydrochloride and norfloxacin microspheres prepared by an enhanced emulsion-solvent evaporation process, S.T.P PHARMA SCIENCES, 2003, 13, 319-327.

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