International Journal of Research and Development in Pharmacy and Life Sciences
Available online at http//www.ijrdpl.com
June - July, 2016, Vol. 5, No.4, pp 2280-2290
ISSN (P): 2393-932X, ISSN (E): 2278-0238

Research Article

DEVELOPMENT AND IN VITRO EVALUATION OF CHRONOMODULATED DELIVERY SYSTEMS OF TERBUTALINE SULPHATE

PatilShrishailgouda S*[1,3], Gupta Venkatadri Ram Mohan[2,3]

1. Department of Pharmaceutics, N.E.T Pharmacy College, Raichur, Karnataka, India.
2. Department of Pharmaceutics, Pulla Reddy Institute of Pharmacy, Dommadugu, Dundigal, Hyderabad, Telangana, India.
3. Department of Pharmaceutical Sciences, Jawaharlal Nehru Technological University, Kukatpally, Hyderabad, Telangana, India.

***Corresponding author's Email:** shailpharma@gmail.com

(Received: March 17, 2016; **Accepted:** May 29, 2016**)**

ABSTRACT

Objective: In view of chronobiological considerations of nocturnal asthma, the present study deals with the development and evaluation of multiparticulate system for the chronomodulated delivery of terbutaline sulphate.

Methodology: The basic design is based on the Pulsincap technology and consisted of formaldehyde treated insoluble hard gelatin capsule body filled with glutaraldehyde cross-linked carboxymethyl chitosan microspheres of terbutaline sulphate and sealed with a hydrogel tablet plug. The entire device was enteric coated, so as to prevent the variable gastric emptying time.

Results: The glutaraldehyde cross-linked carboxymethyl chitosan microspheres appeared to be roughly spherical with the size range of 4.63±0.48 to 11.75±0.92µm. The prepared microspheres possessed good yield and high encapsulation efficiency. Particle size, encapsulation efficiency and release rate are dependent on the fabrication conditions of the microspheres. FT-IR and XRD studies revealed the compatibility of the drug both in the physical mixture and the formulation form. Drug release from the microspheres depended on the core: coat ratio, reaction time and the rotational speed used in the preparation of microspheres. Formaldehyde treatment efficiently rendered the hard gelatine capsule bodies water insoluble. The ejection of the plug from the chrnomodulated delivery system depended on the nature and concentration of polymer used in the preparation of table plug. A lag time of 3-8hrs was observed for the chronomodulated delivery systems prepared with different hydrogel plugging materials. Among the different polymers studied, HPC showed highest lag time compared to HPMC K4 M and sodium alginate.

Conclusion: The results of the study conclusively proved the suitability of carboxymethyl chitosan microspheres and the adopted Pulsincap technology in the development of chronomodulated delivery systems for terbutaline sulphate in the treatment of nocturnal asthma.

Keywords: Nocturnal asthma; terbutaline sulphate; chronomodulated systems; Tablet plug; Lag time.

INTRODUCTION

With the advancement of the technologies in the pharmaceutical field, drug delivery systems have drawn an increasing interest over the last few decades. Nowadays, the emphasis of pharmaceutical galenic research is turned towards the development of more efficacious drug delivery systems with already existing molecules rather than going for new drug discovery because of the inherent hurdles posed in drug discovery and development process[1].

Nowadays, the concept of chronopharmaceutics has emerged which is a branch of pharmaceutics devoted to the design and evaluation of drug delivery systems that release a bioactive agent at a rhythm that ideally matches the biological requirement of a given disease therapy. Ideally,

chronopharmaceutical drug delivery systems (ChrDDS) should represent time-controlled and site-specific drug delivery systems[2].

Compared to single-unit dosage forms, multiparticlate systems like microspheres exhibit more uniform distribution and absorption of the drug in the gastrointestinal tract, reduced local irritation, higher colonic residence time, more predictable gastric emptying and also eliminates unwanted intestinal retention of polymeric material[3,4]. Carboxymethyl chitosan, a water soluble derivative of chitosan, with enhanced biological and physicochemical properties compared to chitosan, has emerged as a promising candidate for different biomedical applications. The biodegradability and biocompatibility of carboxymethyl chitosan along with its permeation enhancing property makes it an ideal polymer for the development of drug delivery systems[5,6].

Asthma is a disease characterized by chronic inflammation of the airways and linked with airway hyper-responsiveness resulting in episodes of wheezing, shortness of breath, chest tightness, and cough, particularly at night or in the early morning.

The worsening of asthma particularly at night is commonly referred to as nocturnal asthma (NA). Nocturnal asthma is a variable exacerbation of the underlying asthma condition associated with increases in symptoms, need for medication, airway responsiveness, and/or worsening of lung function. Approximately two-thirds of total asthmatics suffer from night time symptoms. Lung function (e.g., peak expiratory flow rate or FEV1) is usually highest at 4 PM and lowest at 4 AM[7].

Terbutaline sulphate is a potent β-adrenoreceptor agonist widely used in the treatment of asthma[8,9]. The absorption of terbutaline sulphate form the gastrointestinal tract is variable and only 33-50% of the total administered oral dose is believed to be absorbed of which 60% is metabolized by the liver under the first pass effect[10]. The drug also undergoes gut wall metabolism[11]. Due to these factors the oral bioavailability of the drug is only 15% of the total administered dose[12]. Further the drug is also having short half life of 3-4hrs requiring frequent administration[13]. Thus, development and evaluation of chronomodulated delivery systems of terbutaline sulphate has been undertaken since the advantages of such systems also includes the better utilization of drugs having short half life with extensive first pass metabolism thereby providing better therapeutic outcome for nocturnal asthma.

MATERIALS AND METHODS:

Terbutaline sulphate pure drug was generously supplied by Shimoga Chemicals, Sangli, Maharashtra. Carboxymethyl chitosan was obtained as gift sample from Pelican Biotech Pvt Ltd, Kuthiathode, Kerala. Sodium Alginate and HPMC K4M were purchased from Himedia Lab Pvt Ltd, MumbaiandS.D Fine Chemicals, Mumbai respectively.Hard gelatine capsules were generously supplied by Elegant Drugs Pvt Ltd, Hubli, Karnataka. All other chemicals and reagents were of analytical grade and were purchased from SD fine chemicals, Mumbai.

Preparation of microspheres

Carboxymethyl chitosan microspheres loaded with terbutaline sulphate were prepared by emulsion cross linking technique. Accurately weighed quantity of carboxymethyl chitosan (3%w/v) was dissolved in distilled water by stirring on a magnetic stirrer. Thereafter, known quantity of terbutaline sulphate was added to the above polymeric solution. The resulting solution was added drop wise into 40 ml of light and heavy liquid paraffin (1:1) containing 1% w/w of span-8o (needle no: 22G) at 1000 rpm using digital overhead stirrer. The system was allowed for emulsification for 30 minutes and then 2 ml of glutaraldehyde (25% v/v aqueous solution) was added and stirring was continued for the specified time period. Microspheres thus obtained were filtered and washed several times with petroleum ether to remove traces of oil and then they were finally washed with ethanol to remove excess amount of glutaraldehyde. The microspheres were then dried at room temperature for 24 hrs[14,15]. The formulation details of terbutaline sulphate loaded carboxymethyl chitosan is given in Table No: 1

CHARACTERIZATION OF MICROSPHERES

Particle size and surface topography

The size of the prepared microspheres was analyzed using optical microscopy fitted with a calibrated eye piece micrometer. The mean of 100 microspheres was noted as average particle size[16]. The surface topography of the microspheres was studied using scanning electron microscopy (JEOL, JSM - 6360, Japan). Microspheres were mounted on

Table 1: Formulation and Characterization of terbutaline sulphate loaded carboxymethyl chitosan microspheres.

Code	Variables		Physicochemical characterization			
	Core: coat		Yield (%)	Actual Drug content(mg)*	Encapsulation Efficiency (%)	Particle Size (µm)
C1	1:2	constant:	85.05±1.67	3.90±0.15	78.13±3.98	5.03±0.57
C2	1:4	1000 rpm	88.95±1.42	4.06±0.25	81.33±5.07	6.38±0.35
C3	1:6	1% span	91.15±1.20	4.31±0.17	86.26±3.52	9.04±0.71
C4	1:8	6 hrs	92.96±1.51	4.40±0.14	88.06±2.94	11.11±1.14
	Reaction time					
R1	5hrs	constant:	89.28±0.93	4.14±0.15	82.93±3.0	10.13±1.10
R2	7hrs	1:6;	92.03±1.06	4.35±0.23	87.06±4.62	7.95±0.86
R3	8hrs	1000 rpm; 1% span	93.07±1.67	4.46±0.17	89.2±3.53	6.57±0.61
	Speed					
S1	1200	constant:	92.21±1.11	4.44±0.14	88.8±2.82	7.51±0.75
S2	1400	1:6;	89.16±1.49	4.38±0.18	87.73±3.75	6.87±0.38
S3	1600	1% span 6hrs	86.84±1.52	4.13±0.11	82.73±2.33	4.63±0.48

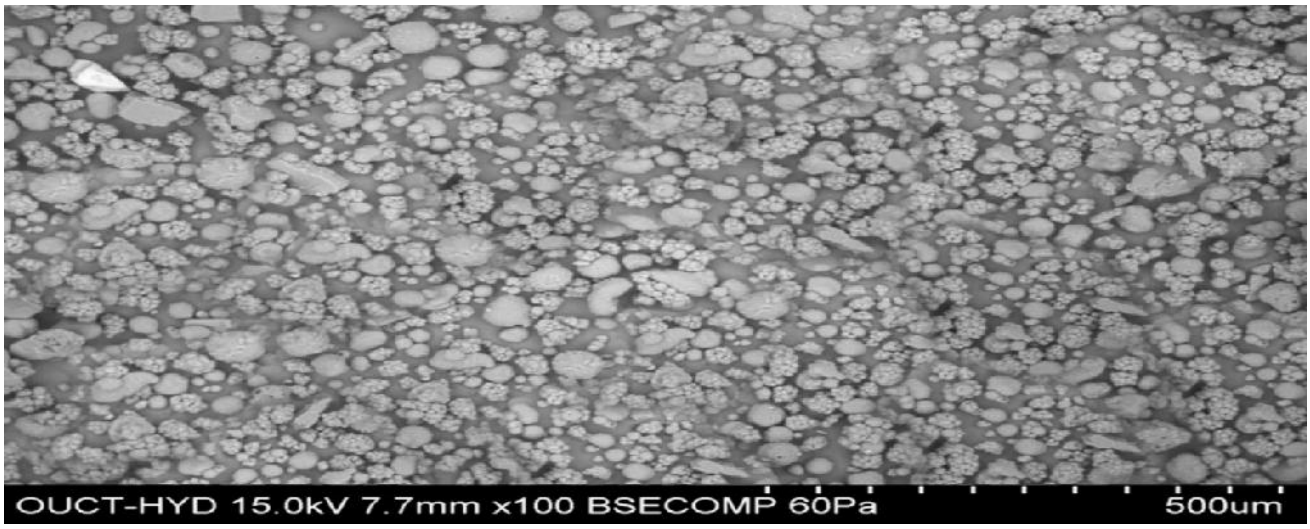
Table 2: Composition of different hydrogel tablet plug for the chronomodulated delivery systems of terbutaline sulphate.

S. No	Batch code	Sodium Alginate (mg)	HPMC (mg)	HPC (mg)	Sodium CMC (mg)	Spray Dried Mannitol (mg)	Mg. Stearate (mg)	Total weight (mg)
1	SA1	60	---	---	---	38	2	100
2	SA2	50	---	---	---	48	2	100
3	SA3	40	---	---	---	58	2	100
4	SA4	30	---	---	---	68	2	100
5	HM1	---	60	---	---	38	2	100
6	HM2	---	50	---	---	48	2	100
7	HM3	---	40	---	---	58	2	100
8	HM4	---	30	---	---	68	2	100
9	HC1	---	---	60	---	38	2	100
10	HC2	---	---	50	---	48	2	100
11	HC3	---	---	40	---	58	2	100
12	HC4	---	---	30	---	68	2	100

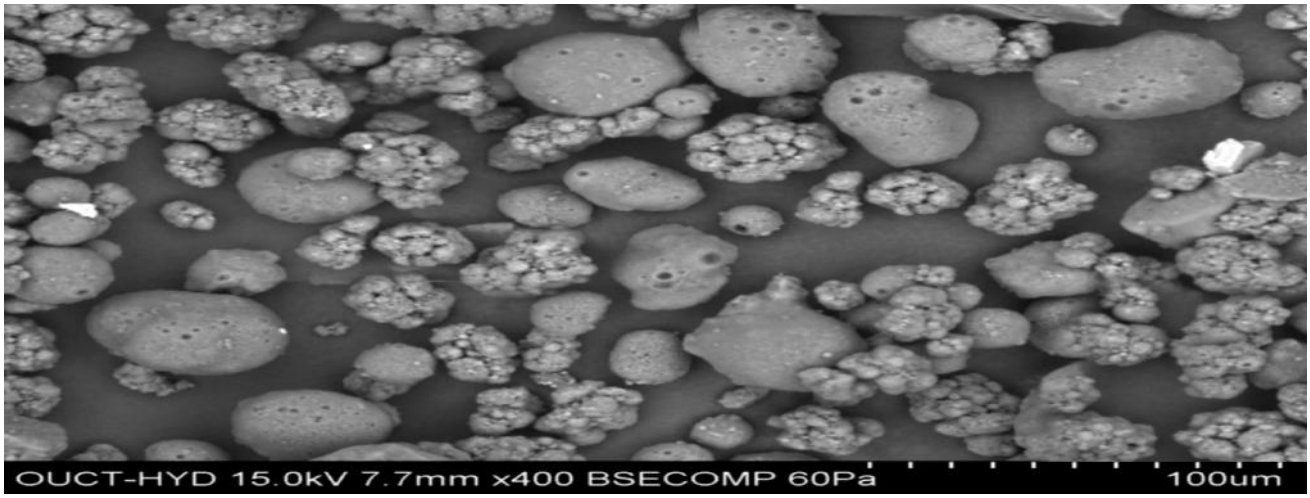
Table 3: Composition of chronomodulated drug delivery systems of terbutaline sulphate

Code	Weight of empty capsule (mg)	Weight of microspheres* (mg)	Weight of tablet Plug (mg)	Total weight of capsule (mg)	Weight after enteric coating (mg)
TSA1	60.73±0.42	39.41	100.69±0.62	200.83±0.69	216.92±2.41
TSA2	60.76±0.40	39.41	100.36±0.46	200.53±0.61	218.35±2.06
TSA3	61.14±0.55	39.41	99.96±0.48	200.51±0.80	217.83±2.36
TSA4	60.91±0.53	39.41	100.54±0.39	200.86±0.78	220.37±2.09
THM1	60.93±0.51	39.41	99.81±0.30	200.15±0.67	217.63±1.77
THM2	61.1±0.44	39.41	100.54±0.59	201.05±0.52	219.37±1.23
THM3	60.74±0.55	39.41	100.33±0.61	200.48±0.84	218.49±2.27
THM4	60.81±0.51	39.41	100.74±0.57	200.96±0.58	221.09±2.15
THC1	61.09±0.28	39.41	100.57±0.48	201.22±0.47	220.32±1.56
THC2	60.88±0.34	39.41	99.98±0.62	200.27±0.84	218.83±1.77
THC3	59.97±0.32	39.41	100.17±0.24	199.55±0.41	217.33±2.23
THC4	60.86±0.41	39.41	100.92±0.66	201.19±0.92	220.51±2.11

* Weight of microspheres equivalent to 5mg of terbutaline sulphate



(A)



(B)

Fig No 1: SEM Photographs of optimized carboxymethyl chitosan microspheres of terbutaline sulphate (S1). A. 100X magnification and B. 400X magnification.

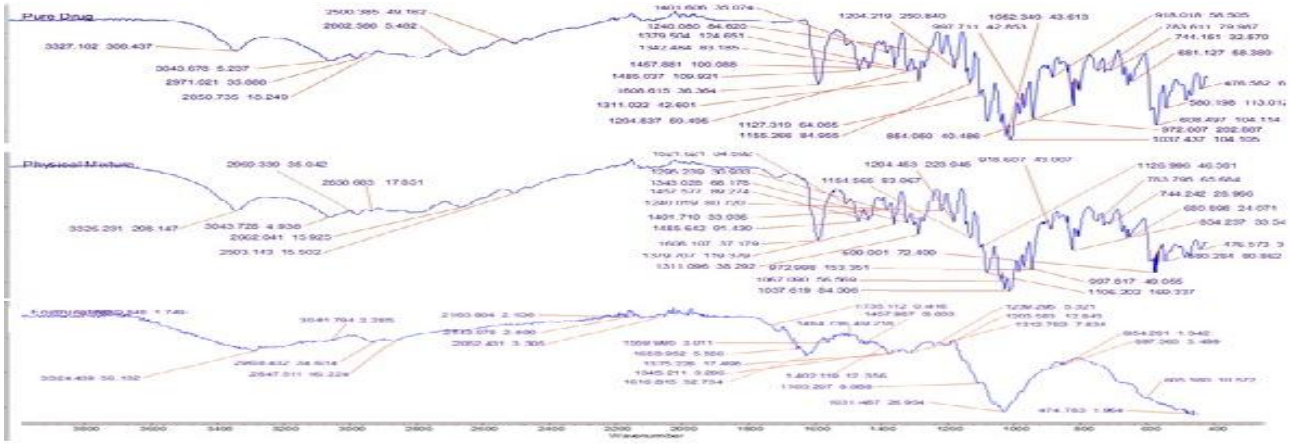


Fig No2: FT-IR spectra of pure drug terbutaline sulphate, Physical mixture and optimized microsphere formulation.

aluminium specimen studs using double sided adhesive tape and coated with platinum under vacuum. The morphology of the microspheres was observed at acceleration voltage of 10 kV at different magnifications. The results of the particle size analysis and surface topography is given Table No 1 and Fig No 1 respectively.

Percentage Yield

The percentage yield (PY) was calculated based on the dry weight of drug and the polymer used in the preparation of microspheres and total quantity of product obtained. The following equation was used in the calculation of percentage of yield[17]:

Pecentage Yield = (Obtained mass of microspheres)/(Initial mass of drug + Initial mass of polymer) X100

Encapsulation efficiency

Crushed microspheres equivalent to 5 mg of terbutaline sulphate was accurately weighed and transferred to a 100ml volumetric flask containing 50ml of phosphate buffer of pH 7.4 and the volume was made up to the mark using the same buffer solution. The flask was stirred on a thermostatic water bath at room temperature for 24h to extract the entrapped drug. The content was filtered and after suitable dilution, the absorbance was noted on a UV spectrophotometer at 281.0 nm using phosphate buffer of pH 7.4 as blank. Triplicate readings for each batch were noted and the average was determined as drug content of the microspheres[18].

The encapsulation efficiency was calculated using the below formula:

Encapsulation Efficiency= (Actual drug content)/(Theoretical drug content) X100

Fourier transform–infrared (FT-IR) spectral studies:

Pure drug, drug-excipients physical mixture and drug loaded microspheres of optimized formulation were analyzed using Fourier transformer infrared spectrophotometer (Agilent Cary 630 FT-IR Spectrometer). The samples were triturated with KBr and scanned over wave number range of 4000 to 400 cm-1. FT-IR spectra were analyzed for functional groups and drug polymer interactions.

X-ray diffraction (XRD) analysis

The effect of microencapsulation process on drug crystallinity was studied using XRD analysis. XRD patterns of pure drug, physical mixture and optimized microspheres were recorded on X-ray diffractometer (XRD 6000, Shimadzu Corporation, Japan) using Ni-filtered, CuK radiation, a voltage of 40 Kv and current of 30mA. The scanning speed employed was 4°/min over the 10° to 80° diffraction angle range. Microspheres were triturated to fine powder before performing the analysis.

In vitro drug release studies

The release of terbutaline sulphate from the microspheres was studied using USP type II dissolution apparatus. Microspheres equivalent to 5 mg of terbutaline sulphate were taken into the basket and the release studies were carried out under the following conditions; media: 400 ml of phosphate buffer of pH 6.8; temperature: 37 ±0.5 °C; speed: 100 rpm. At fixed interval of time, aliquots were withdrawn and replaced with fresh dissolution media to maintain the constant volume. The concentration of drug released at different time intervals was then determined by measuring the absorbance at 281.0 nm against blank using UV spectrophotometer.

Kinetic modelling of drug release

To investigate the drug release mechanism from the microspheres, the in-vitro release data was fitted into various kinetics models like zero order, first order, Higuchi's equations. Further, the drug release mechanism was also analysed by Korsemeyer-Peppas equation.

Development of chronomodulated drug delivery systems:

In the present investigation, PulsincapTMtechnology was used for the development of chronomodulated systems of terbutaline sulphate with some modifications[19-21]. Briefly, the hard gelatine capsule bodies were treated with formaldehyde vapours for 12 hrs to render them water insoluble. The treated capsule bodies were filled with optimized microspheres containing 5 mg of terbutaline sulphate and sealed with a hydrogel tablet plug. The tablet plugs were prepared with various semisynthetic polymers like sodium alginate, HPMC K4M and HPC by direct compression technique using spray dried mannitol and magnesium stearate as diluent and flow promoter respectively. The joint of the microspheres loaded capsule bodies were sealed with a small amount of 5% ethyl cellulose ethanolic solution. The sealed capsules were enteric coated by dip coating method with 4 % HPMCP 55 in 4 :1 (v/v) mixture of

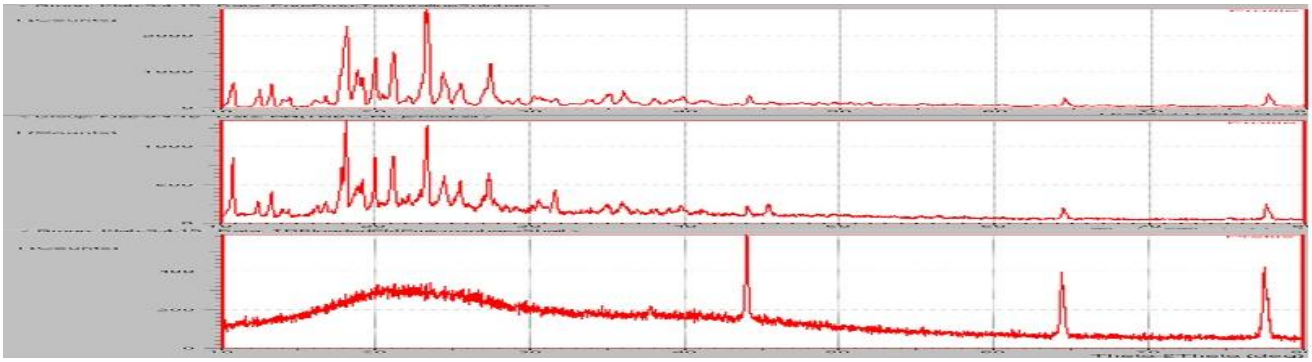


Fig No 3: XRD patterns of pure drug terbutaline sulphate, Physical mixture and optimized microsphere formulation.

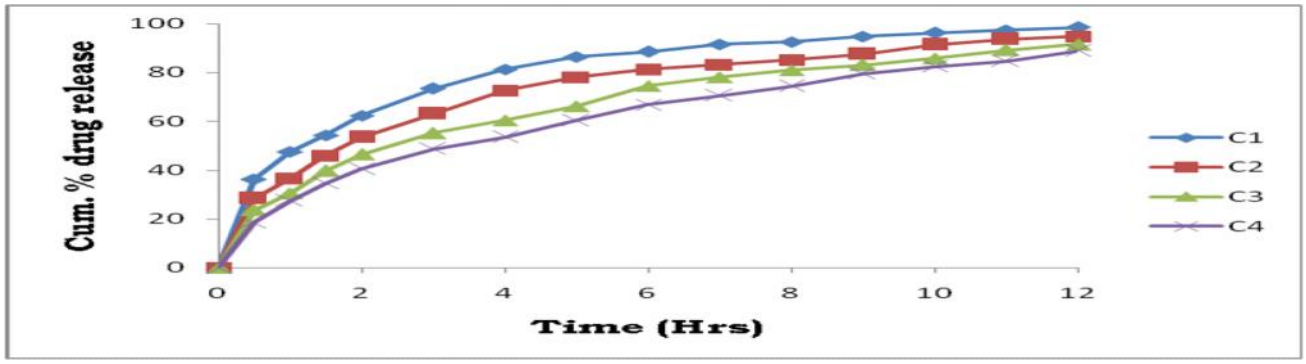


Fig No 4: Effect of core: coat ratio on in vitro release of terbutaline sulphate from carboxymethyl chitosan microspheres (C1-C4)

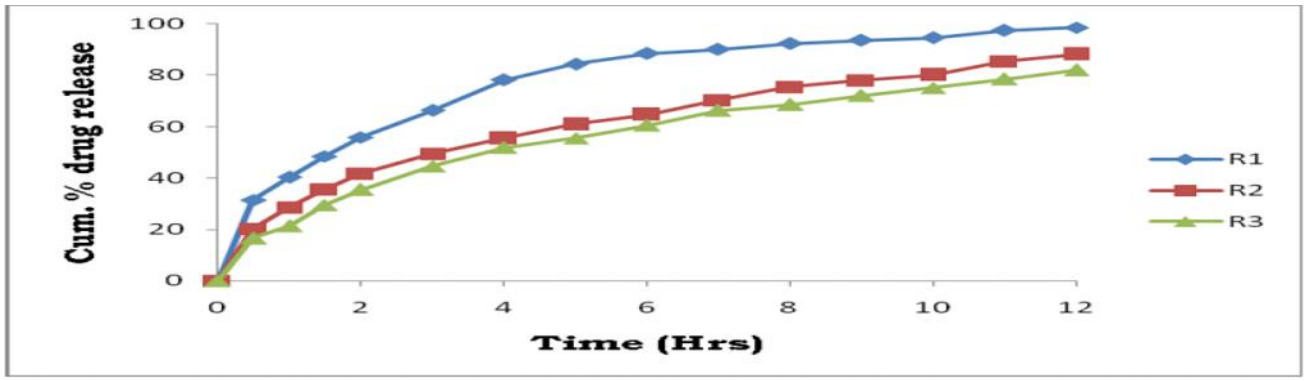


Fig No 5: Effect of reaction time on in vitro release of terbutaline sulphate from carboxymethyl chitosan microspheres (R1-R3).

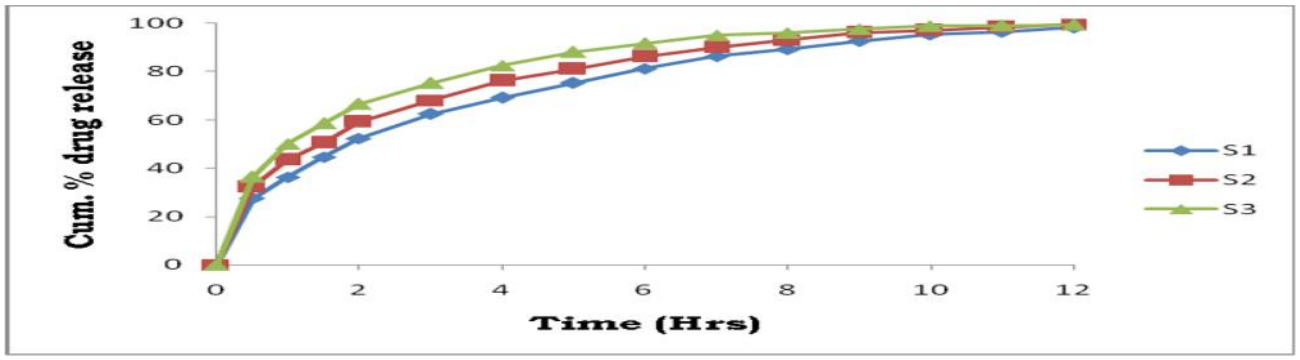


Fig No 6: Effect of rpm on in vitro release of terbutaline sulphate from carboxymethyl chitosan microspheres (S1-S3)

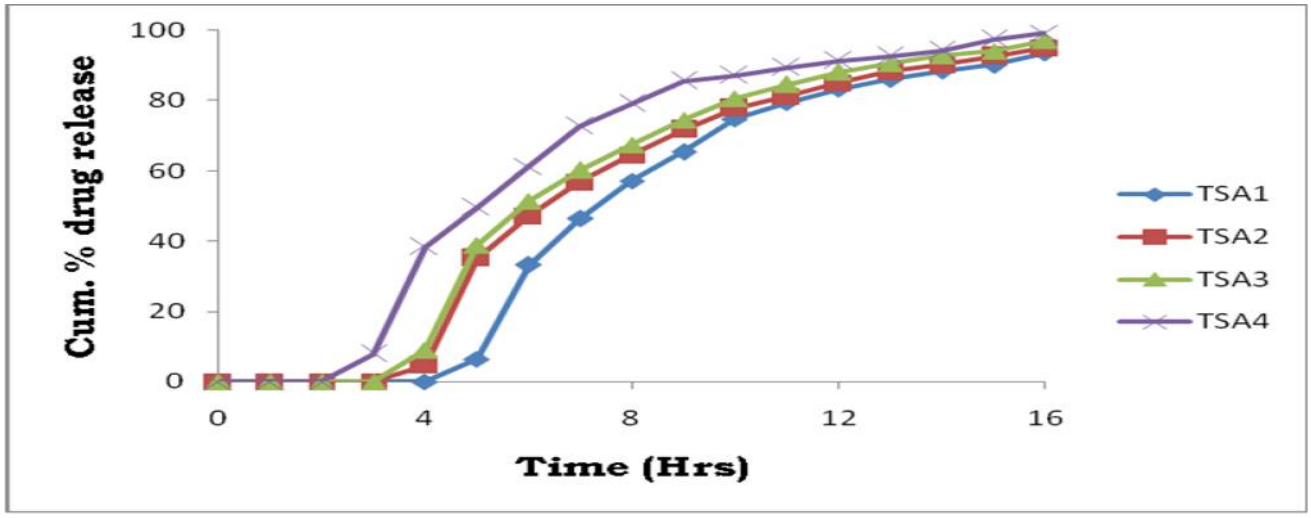


Fig No 7: In vitro release of terbutaline sulphate from chronomodulated delivery systems with sodium alginate as tablet plug

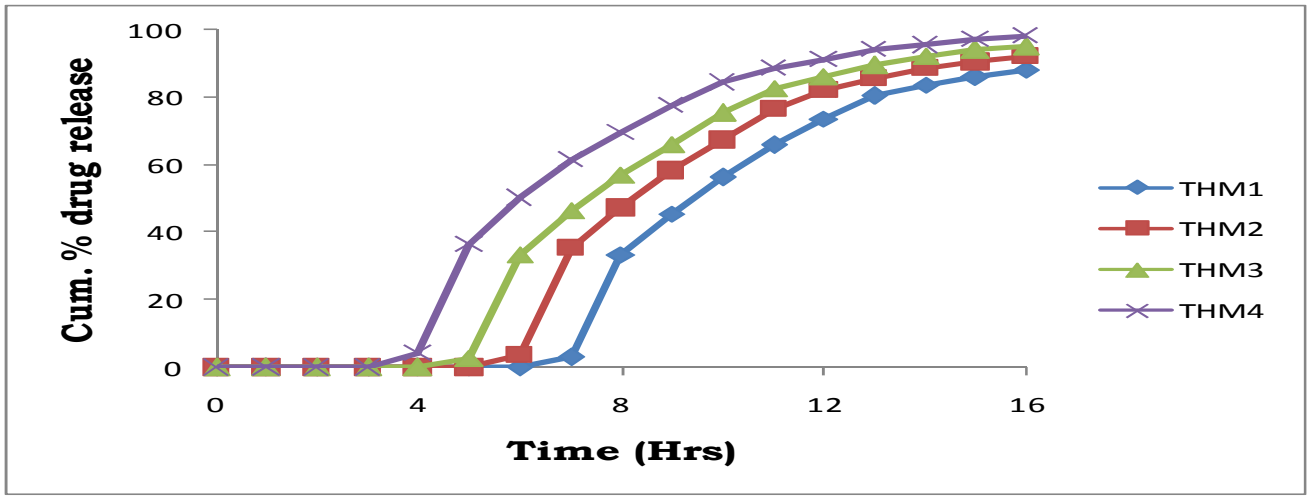


Fig No 8: In vitro release of terbutaline sulphate from chronomodulateddelivery systems with HPMC as tablet plug

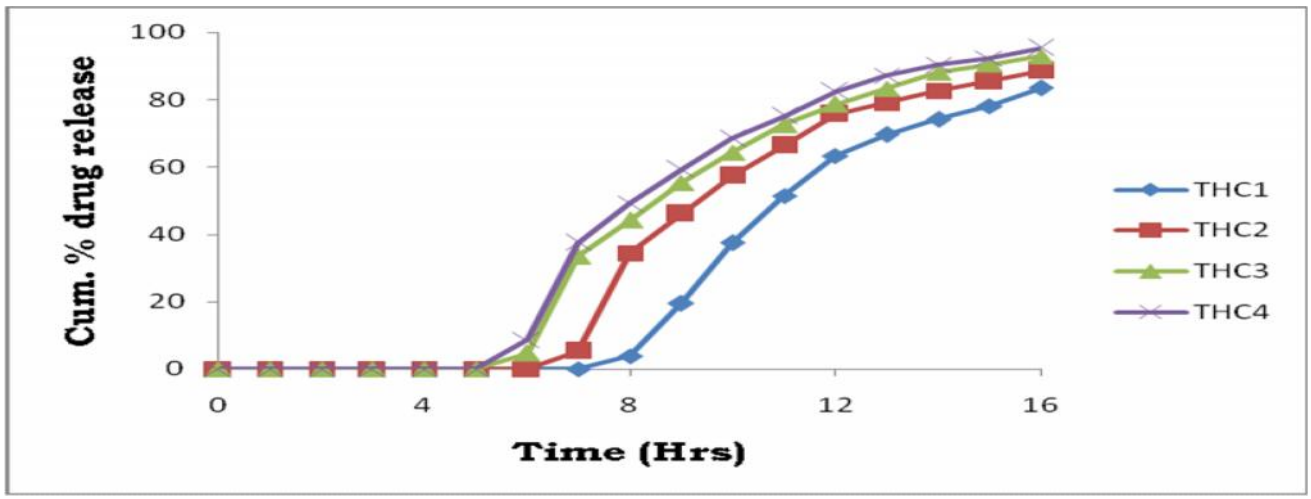


Fig No 9: In vitro release of terbutaline sulphate from chronomodulateddelivery systems with HPC as tablet plug

Dichloromethane : acetone, plasticized with dibutylphthalate (0.75%), to prevent variable gastric emptying.

Evaluation of chronomodulated delivery systems:

Formaldehyde treated gelatin capsule bodies

The formaldehyde treated empty hard gelatin capsule bodies were evaluated for the presence of free formaldehyde[19], physical changes like deformations, shrinkage, perforations and solubility.

In vitro dissolution studies

Dissolution studies were carried out by using USP XXIV dissolution apparatus (paddle method) to analyze the release of drug from the developed chronomodulated systems of terbutaline sulphate. Capsules were tied to paddle with a cotton thread so that the capsule should be immersed completely in dissolution media. In order to simulate the pH changes of the GI tract, sequential pH change method was adopted in the dissolution studies. Dissolution media of pH 1.2 was first used for 2 h (since the average gastric emptying time is 2 h), then removed and the fresh pH 7.4 phosphate buffer saline (PBS) was added. After 3 h (average small intestinal transit time is 3 h), the medium was removed and fresh phosphate buffer of pH 6.8 was added for subsequent hours. Four hundred milliliters of the dissolution medium was used at each time. Rotation speed was 100 rpm and temperature was maintained at 37±0.5 ^{0}C. Five milliliters of dissolution media was withdrawn at predetermined time intervals and fresh dissolution media was replaced. The withdrawn samples were analyzed at 276.5 nm for acidic medium and at 281.0 nm for phosphate buffer of pH 6.8 and 7.4 by UV absorption spectroscopy and the cumulative percentage release was calculated. The study was carried out in triplicate and the average readings were used to know the drug release.

Kinetic modelling of drug release

To investigate the drug release mechanism from the chronomodulated delivery systems, the in-vitro release data was fitted into various kinetics models like zero order, first order, Higuchi's equations. Further, the drug release mechanism was also analysed by Korsemeyer-Peppas equation.

DISCUSSION:

Particle size and surface topography: The average particle size of the microspheres was found to be in the range 4.63±0.48 to 11.75±0.92 µm and is given in Table No 1. Particle size increased with increase in core: coat ratio which could be due to more amount of coat material in same volume of liquid droplet. The particle size decreased with increase in the reaction time which could be due to fact that, at greater extent of cross-linking, formation more densely cross-linked polymeric chains occurs leading to reduced particle size. The particle size also reduced with increase in the rpm which could be to higher turbulence or mechanical shear created within the dispersion medium leading to decreased particle size. The microspheres appeared to be roughly spherical with few pores on the external structure and the tendency of adhering to each other as revealed by the SEM images. The SEM images also showed the presence of some irregular particles which could be due mechanical stress during the stirring process or the movement of the moisture during the drying period. The SEM images of the optimized microspheres is given in Fig No 1.

Encapsulation efficiency

The encapsulation efficiency of terbutaline loaded carboxymethyl chitosan microspheres was in the range of 78.13±2.86 to 89.2±3.53%. It was observed that, as the amount of coat material increased, more efficient entrapment of the drug within the polymeric matrix of the microspheres occurred thereby leading to higher encapsulation efficiency. The encapsulation efficiency also increased with extensive cross-linking conditions. This could be due to denser cross-linking of polymeric chains that prevented the migration of drug into the dispersion medium resulting in higher encapsulation efficiency[22]. The results of the encapsulation efficiency are given in Table No: 1.

FT-IR Studies:

The pure drug terbutaline sulphate exhibited a broad peak at 3327.16 cm^{-1} due to OH stretching and aromatic C-H stretching was observed at 3043.67 cm^{-1}. C-H stretching of CH_3 groups, asymmetric and symmetric was observed at 2971.02 cm^{-1} and 2850.73 cm^{-1}.A sharp, intense band of N-H bending was witnessed at 1608.61 cm^{-1}. Bands at 1485.03 cm^{-1} and 1457.88 cm^{-1} indicated the C=C ring stretching, whereas bands at 1458.03 cm^{-1} and 1342.48 cm^{-1} indicated the C-H bending of CH_3 groups, asymmetric and symmetric. The bands at 1240.08 cm^{-1}, 1204.21 cm^{-1} are due to the t-butyl characteristic absorbances. Bands appeared at 1401.60 cm^{-1}, 1294.53 cm^{-1} and 854.05 cm^{-1} are due to C-N stretching, O-H and substituted phenyl ring

respectively. The FT-IR spectra of terbutaline sulphate in the physical mixture as well as in the optimized microspheres showed all the characteristic absorption bands without any significant variations. The FT-IR spectra of pure drug terbutaline sulphate, physical mixture and optimized microsphere formulation is given in Fig. no 2.

The X-Ray powder diffractogram of pure drug exhibited a series of intense peaks which is indicative of its crystalline nature. The typical crystalline peaks of the drug in the physical mixture were clearly visible without any significant changes in the positions and relative intensities, thereby ruling out any interaction between the drug and the excipients. However, the diffractogram of the terbutaline sulphate loaded microspheres showed peaks of diminished intensities indicating that the drug is molecularly dispersed in the polymeric matrix or might have undergone amorphization during themicrosphere preparation.The XRD spectra of pure drug, physical mixture and optimized microspheres is given in Fig. no 3. Overall, the results of the FT-IR and XRD studies revealed the integrity of the drug both in its physical mixture and formulation.

In vitro drug release studies

Dissolution studies revealed that with increase in core: coat ratio the resulted in the reduced drug release. The retardation of drug release could be to the formation of thick gel at higher polymeric concentration that created a greater diffusion path length for the diffusion of the drug. It was observed that, as the cross-linking time of the microspheres increased there was a proportionate decrease in the drug release which could be due to reduction of the macromolecular chain mobility and the formation of more stable and rigid spheres. It is well known fact that, the particle size is controlled by the agitation speed and indeed particle size has marked effect on drug release. Hence in the present study, microspheres were prepared at various speeds (S1-S3) and were subjected for dissolution studies. It was observed that, as the rotation speed increased there was a proportionate increase in the drug release. This could be due to the fact that, at higher rotational speeds, the size of the microspheres was reduced leading to higher surface area which further resulted in higher drug release. The effect of core: coat ratio, reaction time and rotation speed on the drug release is given in Fig. no 4, 5 and 6 respectively. Microspheres of the batch S1 which showed good encapsulation efficiency and also gave satisfactory drug release profile suitable for chronotherapeutic delivery of terbutaline sulphate and hence was selected for the further development of chronomodulated delivery systems.

Mechanism of drug release:

The release profile of all the microspheres batches was best fitted in to the first order equation as observed from the highest correlation coefficient values (0.9659-0.9959), indicating the first order drug release mechanism. Plots of percent drug released versus square root of time were found to be linear with high correlation coefficient values (0.9039-0.9967) indicating that the drug release from the microspheres was diffusion controlled. Further, the release data was also analyzed by Korsmeyer-Peppas equation. The release exponent 'n' was in the range of 0.2748-0.5124 indicating diffusion controlled Fickian drug release mechanism.

Evaluation of chronomodulated delivery systems:

Formaldehyde treated capsule bodies:

In about 100 capsule bodies treated with formaldehyde, about 8-10 bodies were found to be shrunk or distorted. The capsule bodies which were shrunk or distorted after the formaldehyde treatment were discarded for the further studies. The formaldehyde capsules were also tested for the presence of free formaldehyde. The sample solution was not more intensely colored than the standard solution inferring that less than 20µg/ml of free formaldehyde per 25 capsules, taken for test.

In vitro dissolution studies:

From the results of the in vitro release studies, it is clear that, all the capsules remained intact in the acidic pH for the initial 2 hrs of the dissolution studies, indicating the integrity of the enteric coating with HPMCP. Further, when the dissolution medium was changed to pH 7.4, the enteric coating along with the soluble cap dissolved thereby exposing the hydrogel plug to the dissolution medium. The exposed hydrogel plug then, absorbed the surrounding fluid, swelled and ejected thereby releasing the drug loaded microspheres into the dissolution media. With all the formulations, there was absolutely no drug release in pH 1.2, thus indicating the efficacy of enteric coating with HPMCP.

In order to assess the release of terbutaline sulphate from the chronomodulated delivery systems, various semisynthetic

polymers like sodium alginate, HPMC and HPC in the concentrations of 60, 50, 40 and 30% w/w were used in the preparation of tablet plugs. Chronomodulated delivery systems of batches TSA1-TSA4 were prepared with sodium as plugging material. The cumulative drug release at the end of 5th was found to be 6.38% for TSA1, whereas TSA2 and TSA3 released 4.63 and 8.97% at the end of 4th hr respectively. Formulation TSA4 with the lowest sodium alginate concentration (30%) released 7.89% of terbutaline at the end of 3rd hr of dissolution study. After the complete ejection of the tablet plug, the release of terbutaline sulphate was found to be 93.63, 95.07, 97.12 and 99.31% for TSA1, TSA2, TSA3 and TSA4 respectively. The results of the drug release studies from the chronomodulated delivery systems prepared using sodium alginate as hydrogel tablet plug is given in Fig No 7.

HPMC K4M in the concentration of 60, 50, 40 and 30% w/w was used as tablet plugging material in the chronomodulated delivery systems of THM1, THM2, THM3 and THM4 batches respectively. The cumulative drug release for THM1, THM2, THM3 and THM4 were found to be 2.98 and 3.69, 2.71 and 4.25% at the end of 7, 6, 5 and 4hrs respectively. The cumulative amount of drug release after the complete ejection of the tablet plug was found to be 88.35, 92.16, 94.88 and 98.39 % for THM1, THM2, THM3 and THM4 respectively at the end of 16hrs dissolution study. The results of the drug release studies from the chronomodulated delivery systems prepared using HPMC K4M as hydrogel tablet plug is given in Fig No 8.

In another set of formulations, hydroxypropyl cellulose (HPC) as a hydrogel plugging material in four different concentrations like 60, 50, 40 and 30 mg were used in chronomodulated delivery systems of THC1, THC2, THC3 and THC4 respectively. The results of the drug release studies from the chronomodulated delivery systems prepared using HPC as hydrogel tablet plug is given in Fig No 9. The cumulative drug release from THC1 and THC2 was found to be 3.87 and 5.33% at the end of 8th and 7th hrs respectively, whereas 4.73 and 8.69% drug was released at the end of 6th hr for THC3 and THC4 respectively. The cumulative amount of drug release after the complete ejection of the tablet plug was found to be 83.35, 88.54, 92.87 and 95.28% for THC1, THC2, THC3 and THC4 respectively at the end of 16hrs dissolution study. From all the formulations, it was observed that, as the polymer concentration in the hydrogel plug was increased, there was a proportionate delay in the ejection of the hydrogel plug. This could be attributed to delayed wetting and swelling of the hydrogel material at the higher polymeric concentration that resulted in higher lag time. However, the lag time observed in case of formulations of HPC was higher when compared to that of sodium alginate and HPMC. This could be probably due to slower hydration and swelling of the HPC leading to delayed ejection of the plug. The rank order of drug release sustaining ability of different semisynthetic polymers used in the preparation of tablet plug was in the following order: HPC > HPMC > sodium alginate.

Mechanism of drug release:

The 'r' values for zero order and first order kinetics were found in the range of 0.7974-0.9268 and 0.9787-0.9975 respectively. Higher correlation coefficient values were found for first order compared to zero order kinetics, indicating first order release mechanism. When the data was fitted to Higuchi's model, high correlation coefficient values ranging from 0.8742-0.9496 were observed indicating the diffusion controlled release mechanism. Further, to analyze the nature of the diffusion controlled release mechanism, the release data was also fitted into Korsmeyer and Peppas equation. The 'r' values for Korsmeyer and Peppas were in the range of 0.9346-0.9840 and the 'n' values were found between 0.4591-0.9185 indicating non-Fickian diffusion controlled drug release. Overall, the kinetic analysis of the drug release data from various chronomodulated delivery systems of terbutaline sulphate revealed first order diffusion controlled non-Fickian release mechanism.

CONCLUSIONS:

The glutaraldehyde cross-linked carboxymethyl chitosan microspheres appeared to be roughly spherical with the size range of 4.63±0.48 to 11.75±0.92µm. Particle size, encapsulation efficiency and release rate are dependent on the fabrication conditions of the microspheres. FT-IR and XRD studies revealed the compatibility of the drug both in the physical mixture and the formulation form. Drug release from the microspheres depended on core: coat ratio, reaction time and the rotational speed used in the preparation of microspheres. Formaldehyde treatment efficiently rendered the hard gelatine capsule bodies water insoluble. The ejection of the plug from the chrnomodulated delivery system

depended on the nature and concentration of polymer used in the preparation of table plug. Among the different polymers studied HPC showed highest lag time compared to HPMC K4 M and sodium alginate. The results of the study conclusively proved the suitability of carboxymethyl chitosan microspheres and the adopted Pulsincap technology in the development of chronomodulated delivery systems for terbutaline sulphate in the treatment of nocturnal asthma.

Acknowledgments:

The authors are thankful to shimoga Chemicals, Sangli, Maharashtra and Pelican Biotech Pvt Ltd, Kuthiathode, Kerala for proving pure drug terbutaline sulphate and carboxymethyl chitosan respectively. The authors are also thankful to principal and management of N.E.T Pharmacy College, Raichur, Karnataka for providing the facilities to carry out the research work.

REFERENCES

1. Davis SS, Illum L. Drug delivery systems for challenging molecules. Int J Pharm 1998; 176: 1-8.
2. Youan B. Chronopharmaceutics: gimmick or clinically relevant approach to drug delivery?.Journal of Control Rel 2004; 98: 337– 53.
3. Anil K. Anal, Willem F. Stevens, Carmen Remu˜n´an-L´opez. Ionotropic cross-linked chitosan microspheres for controlled release of ampicillin. Int J Pharm 2006; 312: 166–73.
4. Asghar ALF, Chandran S. Multiparticulate formulation approach to colon specific drug delivery: current prosspectives. J Pharm PharmSci 2006;9:327-38.
5. Mourya VK, Inamdara NN, Tiwari A. Carboxymethyl chitosan and its applications. Adv Mat Lett 2010; 1(1): 11-33.
6. Upadhyaya L, Singh J, Agarwal V, Tewari RP. Biomedical applications of carboxymethylchitosans. Carbohydrate Polymers 2013; 91(1):452-66.
7. Masoli M, Fabian M, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee Report. Allergy 2004; 59(5): 469–78.
8. Mahajan AN, Pancholi SS. Formulation and evaluation of timed delayed capsule device for chronotherapeutic delivery of terbutalinesulphate.Ars Pharm 2010; 50(4): 215-23.
9. Rao NGR, Panchal H, Hadi MA. Formulation and evaluation of biphasic drug delivery system of terbutalinesulphate for chronotherapy.Int J Pharm Bio Sci 2012; 3(3):626 – 37.
10. Emami J, Boushehri MAS, Varshosaz J, Eisaei A. Preparation and characterization of a sustained release buccoadhesive system for delivery of terbutalinesulphate. Research in Pharmaceutical Sciences2013; 8(4): 219-231.
11. Kola R, Ramani ND, Pragatikumar B. Formulation and in-vitro evaluation of terbutalinesulphate sustained release tablets. Indian Journal of Research in Pharmacy and Biotechnology 2013; 1(5): 621-24.
12. Pramodkumar TM, Shivakumar HG. Novel core in cup buccoadhesive systems and films of terbutalinesulphate–development and in vitro evaluation. Asian J Pharm Sci 2006, 1 (3-4): 175-187.
13. Gobade NG, Koland M, Harish KH. Asymmetric membrane osmotic capsules for terbutalinesulphate. Indian J Pharm Sci 2012; 74 (1): 69-72
14. Liu H, Yan H, Zhaoying, Peng K. Preparation of ambroxol hydrochloride carboxymethyl chitosan microspheres without burst release.Afr J Pharm Pharmacol 2011; 5(8): 1063-69.
15. Li GY, Zhong M, Zhou ZD, Ping D, Li YJ. Novel Carboxymethyl Chitosan Microspheres for Controlled Delivery of Chelerythrine. Journal of Macromolecular Science, Part A: Pure and Applied Chemistry 2011; 48(11):904-11.
16. Srinatha S, Pandit JK, Singh S. Ionic cross-linked chitosan beads for extended release of ciprofloxacin: in vitro characterization. Indian J Pharm Sci 2008; 70 (1): 16-21.
17. Souto EB, Jose S, PremaMT, Chacko AJ, Thomas AC. Colon specific chitosan microspheres for chronotherapy of chronic stable angina. Colloids and Surfaces B: Biointerfaces 2011; 83: 277–83
18. The effect of polymer and CaCl2 concentrations on the sulfasalazine release from alginate-N,Ocarboxymethyl chitosan beads. Farahani EV, Tavakol M, Najafabadi SH. Progress in Biomaterials 2013: 2:10
19. Mastiholimath VS, Dandgi PM, Jain SS, Gadad AP, Kulkarni AR. Time and pH dependent colon specific, pulsatile delivery of theophylline for the treatment of nocturnal asthma. Int J Pharm 2007; 328: 49-56.
20. Bhat A, ChowdaryKPR ,Shobharani RH, Narasu L. Chronopharmaceutical release of theophylline from pulsatile capsular device based on programmable erodible plug: influence of various poylmers and plug position. Int J Pharm &Techn2011; 3(1):1419-1432.
21. Tekade AR, Gattani SG. Development and evaluation of pulsatile drug delivery system using novel polymer. Pharm DevTechnol 2009;14(4):380-7.
22. Kotadiya R, Patel V, Patel H, Koradiya H. Effect of cross-linking on physicochemical properties of chitosan mucoadhesive microspheres: A factorial approach. Int J Green Pharm 2009: 58-62.

