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RESEARCH ARTICLE

DESIGN, IN VITRO AND IN VIVO EVALUATION OF CHRONOMODULATED DELIVERY SYSTEMS OF TERBUTALINE SULPHATE FOR NOCTURNAL ASTHAMA

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ABSTRACT:

The present study deals with the design and evaluation of chronomodulated delivery of terbutaline sulphate as a chronotherapeutic approach in the treatment of nocturnal asthma. 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. 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. 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. The Roentgenographic studies revealed the predicted in vivo performance of the developed chronomodulated delivery systems of terbutaline sulphate. Pharmacokinetic analysis revealed the significant increase in t_{max}, AUC and MRT of optimized chronomodulated system of terbutaline sulphate compared to that of pure drug. 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.

Key words: Nocturnal asthma; Terbutaline sulphate; Chronomodulated systems; Lag time; Roentgenography; In vivo pharmacokinetics

INTRODUCTION:

Chronomodulated delivery systems have attracted because of their multiple benefits over conventional dosage forms. These systems deliver the drug at the right time, at the right site of action and in the right amount, which provides increased patient compliance and more benefits compared to conventional dosage forms. These systems are designed to release the drug after a predetermined lag time that matches the chronobiological requirements of the given disease condition. Thus, these systems are beneficial for drugs which are used in the treatment of certain diseases like asthma, rheumatoid arthritis and various cardiovascular conditions that exhibit circadian rhythmicity. Chronomodulated delivery systems are also useful where nocturnal dosing is required, and for drugs that show the first-pass effect[1,2].

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 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]:

$$Pecentage\ Yield = \frac{Obtained\ mass\ of\ microspheres}{Initial\ mass\ of\ drug + Initial\ mass\ of\ polymer} X100$$

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 24 h 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:

$$Encapsulation\ Efficiency = \frac{Actual\ drug\ content}{Theoretical\ drug\ content} X100$$

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

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.

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

Sl 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

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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

Development of chronomodulated drug delivery systems

In the present investigation, Pulsincap™ technology 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 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 ^{o}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.

In vivo Roentgenography studies:

Roentgenography study, a comparatively safer technique was carried out in healthy male albino rabbits to assess the in vivo behaviour of the optimized chronomodulated delivery systems of terbutaline sulphate. The investigation was performed after obtaining approval from the institutional animal ethical committee (576/02/bc/CPCSEA,22/03/2002) of N.E.T Pharmacy College, Raichur, Karnataka. To closely mimic the human physiological environment of the gastro intestinal tract, rabbits were selected as animal model for evaluating the chronomodulated drug delivery systems. For this purpose, young and healthy male albino rabbits weighing 2.5 to 3.0 kg were selected (n=3). The animals were housed in individual

cages, and the experiments were conducted in a sanitized room at a temperature maintained at around 24 °C. To render the chronomodulated delivery system visible in the X-ray images, the drug loaded microspheres were replaced with the barium sulphate loaded microspheres. The barium sulphate loaded microspheres were prepared using the same methodology as stated in the preparation of drug loaded microspheres. Rabbits were fasted overnight before the start of the study and the optimized chronomodulated delivery systems were administered with 10ml of water. The chronomodulated delivery system (capsule) was kept behind the tongue to avoid biting by rabbit. Free access to water was provided to the animals during the study period. At different time intervals (0, 2, 4, 6 and 8hrs) X-ray images were taken to follow the movement, location and the behaviour of the chronomodulated systems in different parts of gastro intestinal tract[22-24].

In vivo Pharmacokinetic studies: A reverse phase HPLC method with PDA detection was used for the estimation of terbutaline sulphate[25,26].

Instrumentation and chromatographic conditions: A Shimadzu Prominence HPLC system provided with DGU-20A3 degasser, LC-20AD binary pumps, SIL-20AHT auto sampler, and SPD-M20A PDA detector was used. The chromatographic analysis was performed on Phenomenex C18- RP aqueous column (250 × 4.6mm, 5µ). Mobile phase consisting of 15mM ammonium acetate: methanol (70:30% v/v) was used in isocratic mode and the mobile phase was filtered through nylon disc filter of 0.45µm and sonicated for 3 min before use. The flow rate was 1 mL/min and the injection volume was 20µL. PDA detection was performed at 230nm and the separation was achieved at ambient temperature.

Construction of HPLC calibration curve: The stock solution of terbutaline sulphate 1mg/ml was prepared by dissolving 10 mg of drug in methanol and volume was adjusted to the mark with the same solvent in a 100ml volumetric flask. Calibration standards were prepared in rabbit plasma by adding appropriate volumes from the stock solution to drug free blank plasma and then serially diluting it with blank plasma to attain the concentration range of 5-500ng/ml.

Plasma Extraction:

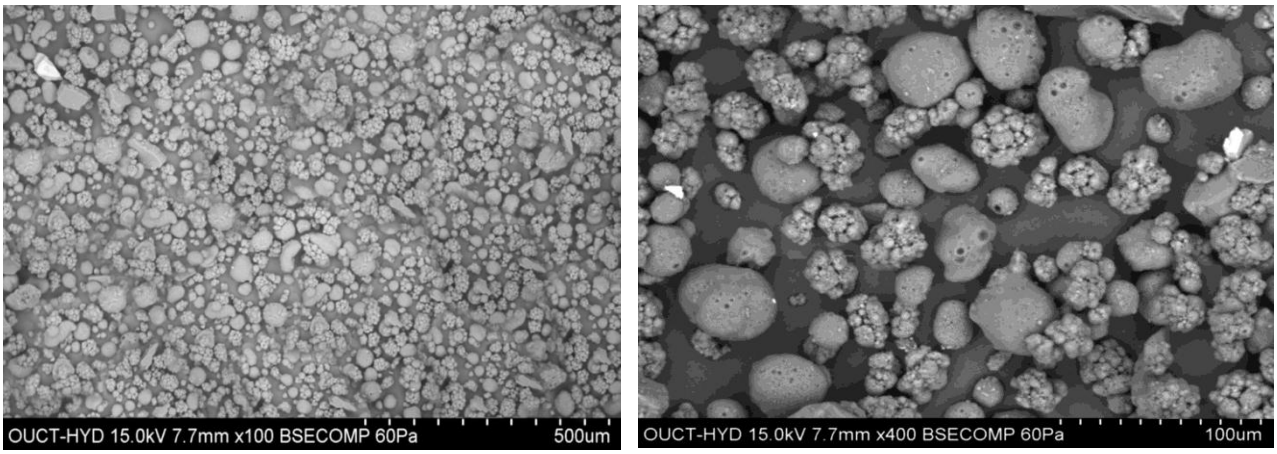
The 200 µL aliquot of each samples were taken into polypropylene tube, 10 µL of 100 µg/mL IS was added in each sample and further 500 µL of formic acid (5% w/v) was incorporated to mixture and vortexed for 5 min. Plasma samples were extracted with 2 mL of ethyl acetate for 5 minutes. Supernantant 1.6ml was transferred to evaporation tubes and dried gently under nitrogen gas at 40 ˚C and reconstituted with 500 µL of mobile phase. An aliquot of 20 µL was injected onto an analytical column to perform the HPLC analysis.

Study Design: The approval of the Institutional Animal Ethics Committee was obtained before starting the study. A total of nine healthy albino rabbits of either sex weighing 3.0-3.5kg were used for the study. The animals were kept in individual cages under well-defined and standardized conditions (humidity and temperature controlled room) and fed with standard food and water access. Three groups consisting of three rabbits in each group was taken for the study. Paget and Barners table was used for the calculation of terbutaline sulphate dose in rabbits[27,28]. Accordingly, 2.25 mg of terbutaline sulphate was fixed for rabbits taken for the study. The pure drug terbutaline sulphate and its optimized chronomodulated delivery system containing this dose were given to the animals. The rabbits were fasted overnight before the administration of the products. One group was kept as control and the other two groups were administered with terbutaline sulphate pure drug in suspension form and the optimized chronomodulated delivery system of terbutaline sulphate respectively. After collecting the zero hour blood sample (predose), the products were administered orally with 10 ml of water. The chronomodulated delivery system (capsule) was kept behind the tongue to avoid biting by rabbit. Blood samples (200µl) were withdrawn from the marginal ear vein at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 16 hrs for the pure drug and 2, 4, 6, 8, 10, 12, 14, 16, 18 and 24hrs for the optimized chronomodulated delivery system. Blood samples were collected in heparinised tubes and were centrifuged at 3000rpm for 10 minutes. Plasma was separated and collected into fresh tubes and stored at -20˚C until further used for analysis. The serum concentration of terbutaline sulphate from both pure drug and its optimized chronomodulated delivery systems were measured by reverse phase HPLC method as stated earlier. Various pharmacokinetic parameters like Cmax, tmax, AUC, MRT and Kel were determined by using Kinetica 5.0 software.

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±0.92µm 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.



(A) **(B)**

Figure 1: SEM Photographs of optimized carboxymethyl chitosan microspheres of terbutaline sulphate (S1). A. 100X magnification and B. 400X magnification.

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 more denser cross-linking of polymeric chains that prevented the migration of drug into the dispersion medium resulting in higher encapsulation efficiency[29]. The results of the encapsulation efficiency is given in Table No:1.

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 2, 3 and 4 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.

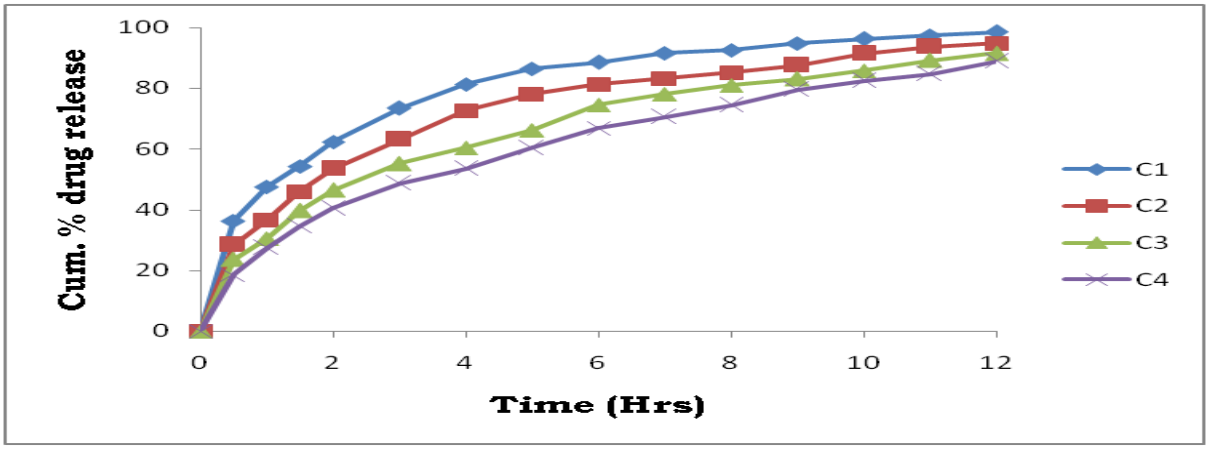


Figure 2: Effect of core: coat ratio on in vitro release of terbutaline sulphate from carboxymethyl chitosan microspheres (C1-C4)

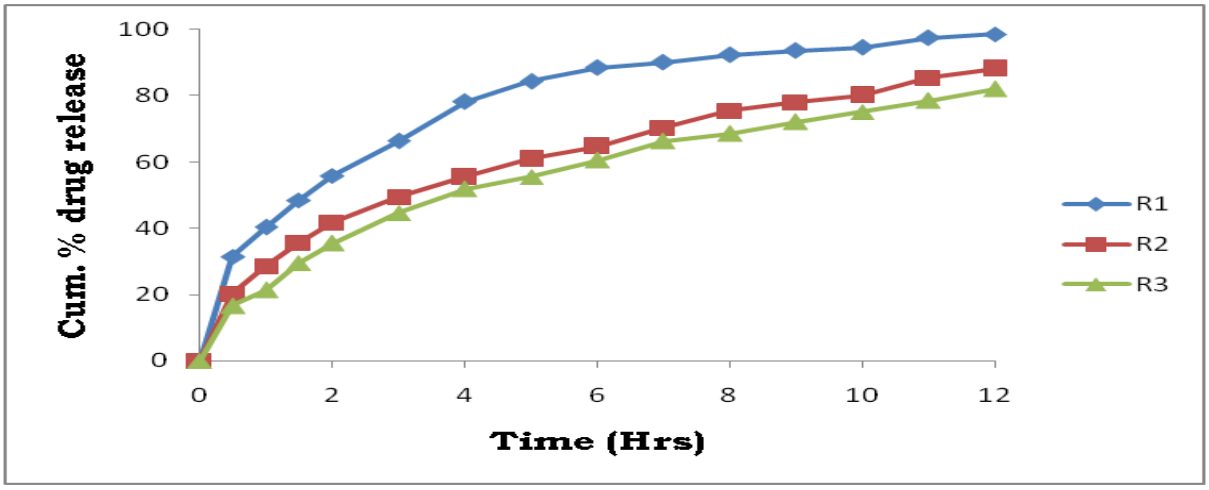


Figure 3: Effect of reaction time on in vitro release of terbutaline sulphate from carboxymethyl chitosan microspheres (R1-R3).

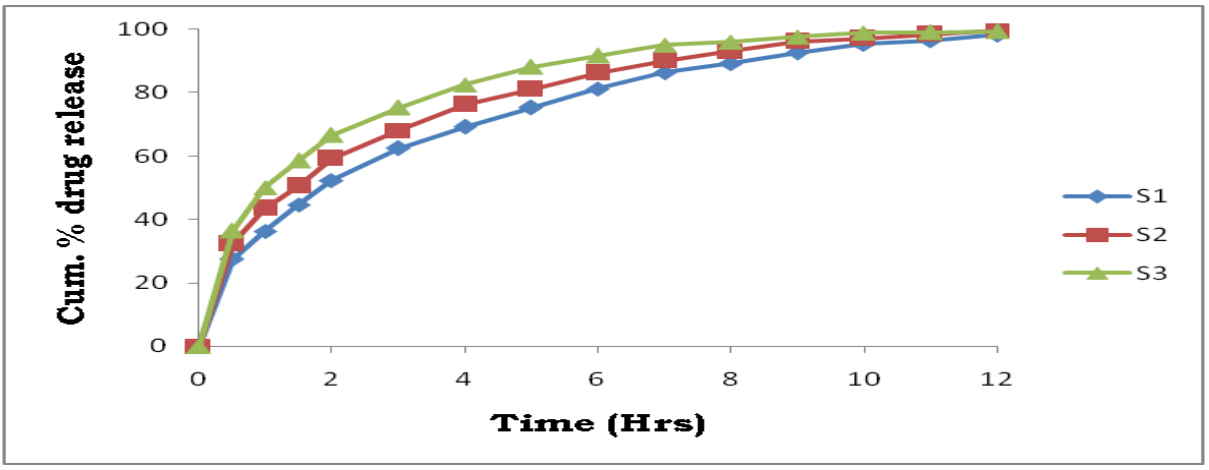


Figure 4: Effect of rpm on in vitro release of terbutaline sulphate from carboxymethyl chitosan microspheres (S1-S3)

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 5.

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 6.

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 7. 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.

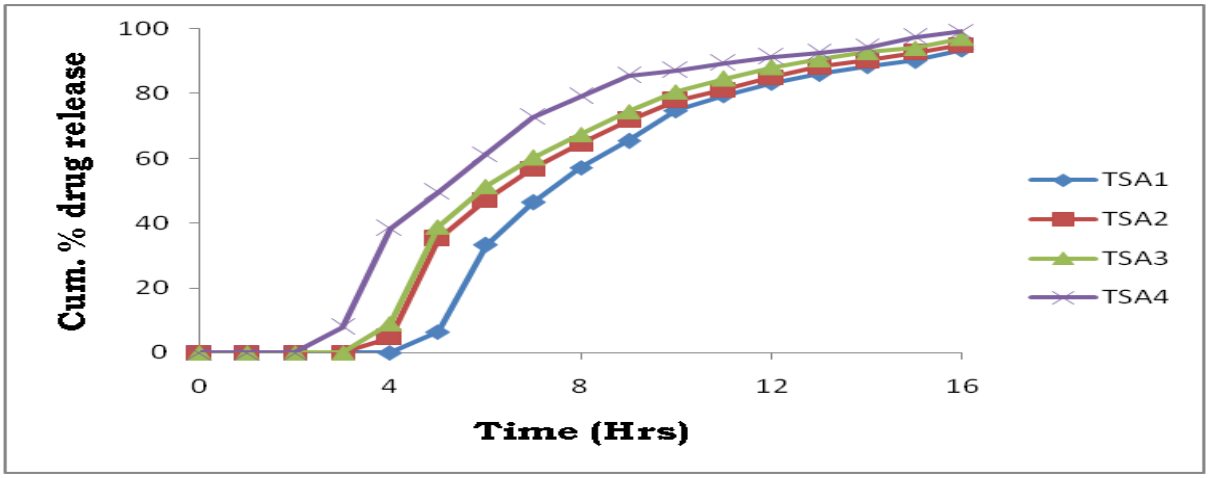


Figure 5: In vitro release of terbutaline sulphate from chronomodulated delivery systems with sodium alginate as tablet plug

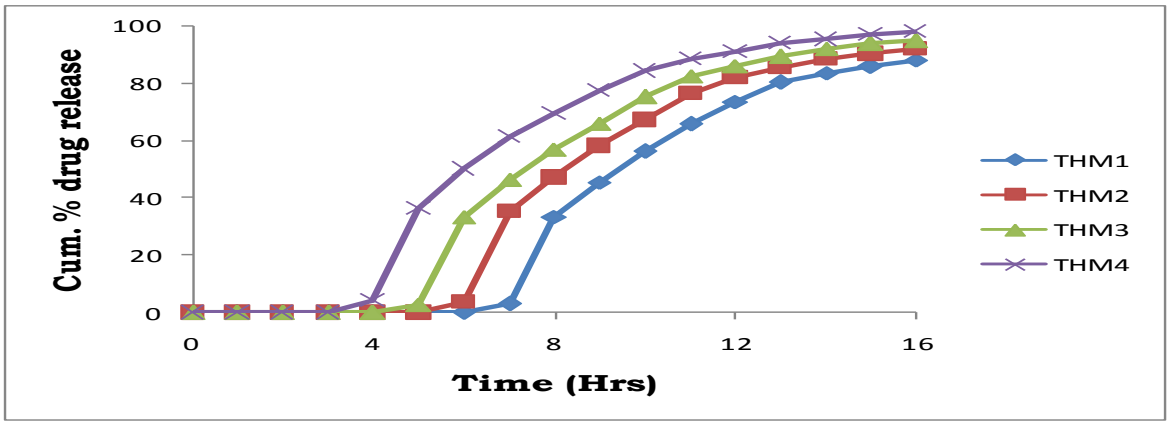


Figure 6: In vitro release of terbutaline sulphate from chronomodulateddelivery systems with HPMC as tablet plug

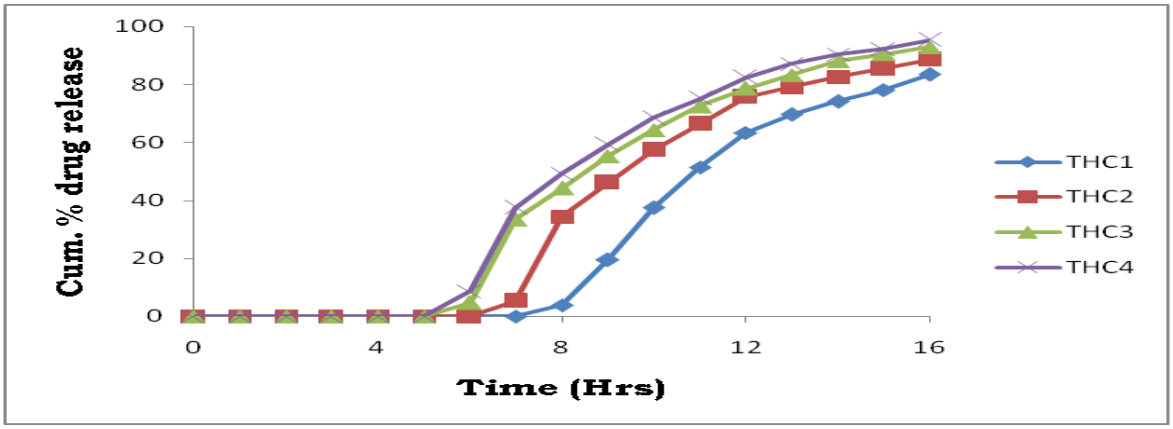


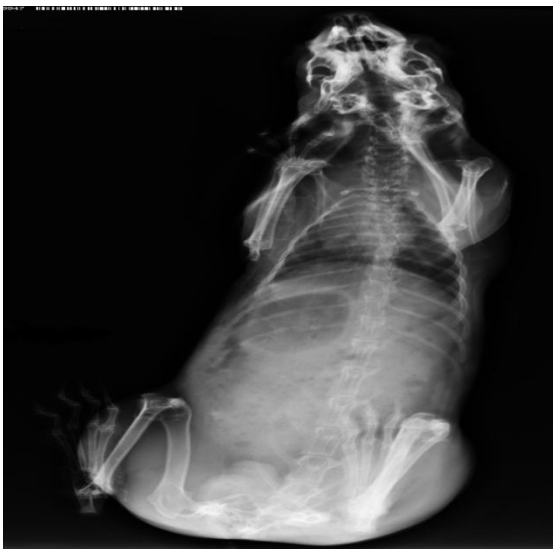
Figure 7: In vitro release of terbutaline sulphate from chronomodulateddelivery systems with HPC as tablet plug

Mechanism of drug release:

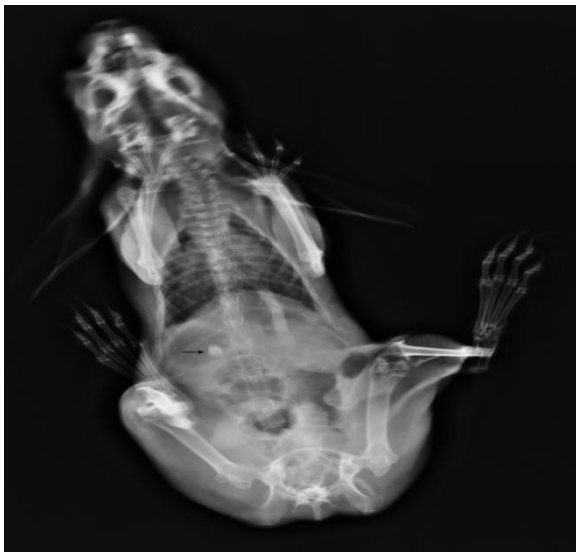
The 'r' values for zero order and first order kinetics were found in the range of 0.7974-0.9268 and 0.9787-0.9975 respectively. Higher correlation coefficient values were found for first order compared to zero order kinetics, indicating first order release mechanism. When the data was fitted to Higuchi's 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.

Roentgenographic studies:

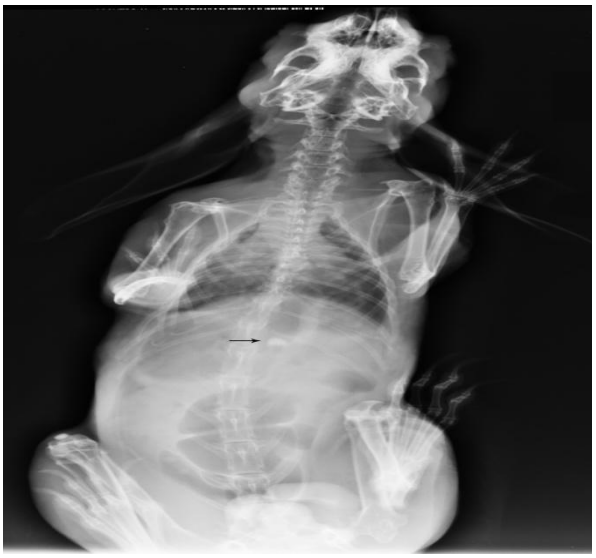
Roentgenographic studies were carried out on healthy male albino rabbits to assess the in vivo behaviour of the optimized chronomodulated delivery systems of terbutaline sulphate (THM4). At different time intervals (0, 2, 4, 6 and 8hrs), X-ray images were taken to follow the movement, location and the integrity of the chronomodulated systems in different parts of gastro intestinal tract. X-ray photographs taken after 2hrs, indicated the presence of chronomodulated delivery system in the stomach which further moved to the small intestinal region as observed from the photograph taken after 4hrs of the study. The chronomodulated system remained intact both in the stomach and small intestine of the rabbit as revealed from the x-ray photographs. The photographs taken at the end of 6^{th} hr revealed the release of barium sulphate loaded microspheres in to the small intestine due to the ejection of the tablet plug. The microspheres remained adhered to the small intestinal region due to the mucoadhesive nature of the chitosan used in the preparation of microspheres as observed from the x-ray photograph taken at the end of 8^{th} hr study. The results of the in vivo Roentgenographic study were in accordance with the in vitro dissolution studies in which the chronomodulated delivery system remained intact for first 4hrs after which the ejection of the tablet plug occurred with the liberation of microspheres. Overall, the Roentgenographic studies revealed the predicted in vivo performance of the developed chronomodulated delivery systems of terbutaline sulphate. The results of the in vivo Roentgenographic studies for the optimized chronomodulated delivery systems of terbutaline sulphate (THM4) is given Fig No 8.



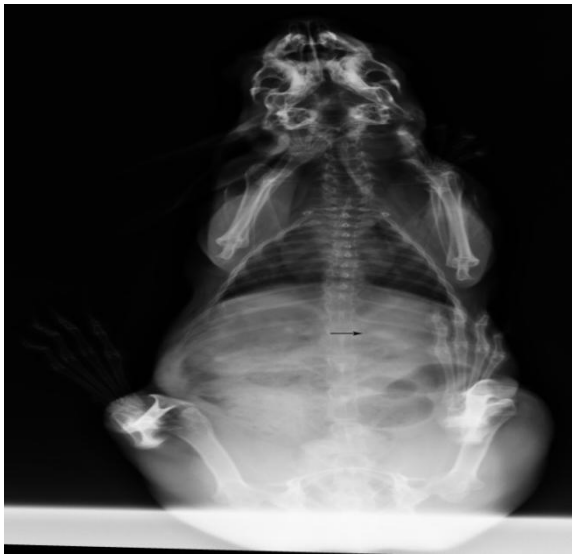
I. X-Ray image taken at 0 hrs



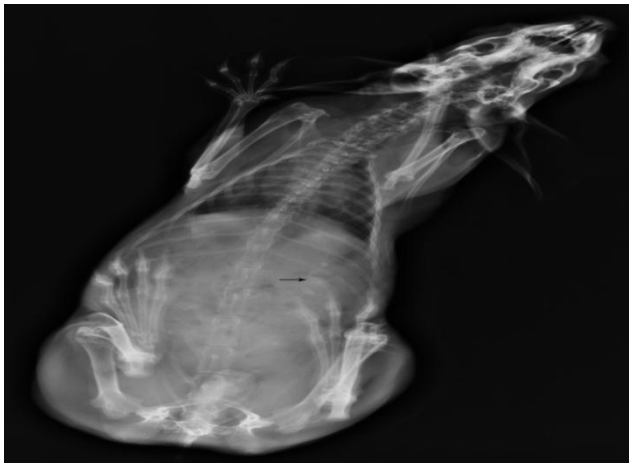
II. X-Ray image taken after 2 hrs



III. X-Ray image taken after 4 hrs



IV. X-Ray image taken after 6 hrs



V. X-Ray image taken after 8 hrs

Figure 8: In vivo Roentgenographic evaluation of optimized chronomodulated delivery systems of terbutaline sulphate (THM4) in rabbits.

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In vivo Pharmacokinetic studies

The plasma concentration versus time profile of pure drug terbutaline sulphate and its optimized chronomodulated drug delivery system is given in Fig No 9. The data obtained from the in vivo HPLC analysis of rabbit plasma samples of both terbutaline sulphate pure drug and its optimized chronomodulated delivery system (THM4) was used to determine various pharmacokinetic parameters using Kinetica 5.0 software.

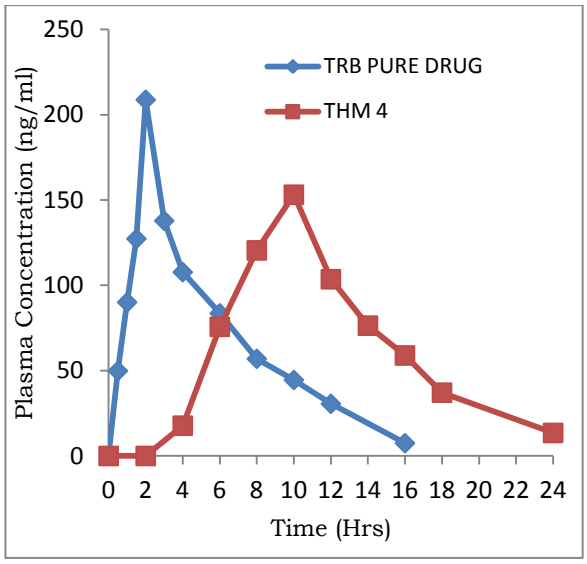

Figure 9: Mean serum concentrations versus time curve of terbutaline sulphate pure drug and its optimized chronomodulated delivery system (THM4)

The pure drug terbutaline sulphate showed the mean C_{max} of 208.74±9.82ng/ml, t_{max} of 2hrs, AUC_{total} of 1074.78±31.1 ng/ml/hr, $t_{1/2}$ of 2.27±0.95 hrs, MRT of 5.689±0.37 hrs and K_{el} of 0.3052±09 hr^{-1}. The optimized chronomodulated system of terbutaline sulphate (THM4) exhibited the mean C_{max} of 153.11 ±9.44 ng/ml, t_{max} of 10 hrs, AUC_{total} of 1461.133±105.0 ng/ml/hr, $t_{1/2}$ of 4.01±0.156 hrs, MRT of 12.614±0.249hrs and K_{el} of 0.1728±0.007 hr^{-1}. Pharmacokinetic analysis clearly revealed the significant increase in t_{max}, AUC and MRT of optimized chronomodulated system of terbutaline sulphate compared to that of pure drug. The study demonstrated the time delayed drug release and improved pharmacokinetic parameters of the terbutaline sulphate from its optimized chronomodulated delivery systems.

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. 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 Roentgenographic studies revealed the predicted in vivo performance of the developed chronomodulated delivery systems of terbutaline sulphate. Pharmacokinetic analysis revealed the significant increase in t_{max}, AUC and MRT of optimized chronomodulated system of terbutaline sulphate compared to that of pure drug. 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.

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