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Preparation and Characterization of Terbutalin Sulphate Microsphere

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ABSTRACT

Advancement in drug delivery could come from innovate improvement to existing drug delivery system. Because of reduce frequency of administration, sustain release dosage from enjoy convenience and ambulatory patient compliance. Ethyl cellulose is a unique non-biodegradable, water soluble and pH independent polymer used in sustained release preparations. Different viscosity grade of Ethyl cellulose use for the preparation of sustained drug release from dosage form. Terbutalin sulphate is a model drug use for the treatment of Asthma. To optimize the process variables in the preparation of terbutalin sulphate loaded different viscosity grade 10 cps (EC10) and 45 cps (EC45) ethyl cellulose microspheres were prepared by w/o/w emulsion solvent evaporation technique for the sustained release effect. The results showed that the effect of different viscosity grade of ethyl cellulose polymer and drugs polymer ratio was found to be significant on different dependent parameters. Higher viscosity grade (EC45) found having excellent sustain release effect for longer period of time than lower viscosity grade (EC10) micro spheres. Therefore it can be conclude that TBS loaded microspheres made up of EC 45 viscosity grade may useful in promoting the prolonged release of drug for effective treatment of bronchial asthma.

Key words: *Asthma, Terbutalin Sulphate, Microsphere, encapsulation efficiency*

INTRODUCTION

Asthma is a chronic lung condition that can develop at any age. It is most common in childhood and occurs in approximately 10% of the pediatric population [1]. It accounts for ¼ of school absenteeism. It affect twice as many boys as girls in childhood more girls than boys develop asthma as teenagers and in adulthood the ratio become 1:1 males to female[2].

It is characterized by difficulty in breathing. People with asthma have extra sensitive or hyper responsive airways. The airways react by narrowing or obstructing when they become irritated. There are two factors that provoke asthma. Triggers result in tightening of the airways and other would be inflammation of the airways. Inflammation due to allergens. Probably 75-80% of young asthmatics are allergic. The common inhaled allergen includes pollens animating secretion tends to be most allergen causing and another would be respiratory viral infection [3].

It affects children varying degree from very mild to very severe. There is a general trend of increased deaths and hospitalization from asthma recorded in an entire all the industrialized countries of the world. In Canada, approximately 20 children and 500 adult die each year due to asthma [4-6].

Terbutaline sulphate (TBS) is widely used in the treatment of bronchial asthma, chronic bronchitis and emphysema. The usual does of TBS for oral adults is 5mg taken every 6 hours 3 times a day. In children 12 to 15 year age the used does is 2.5 mg 3 times a daily. However due to short biological half life and low bioavailability of the drug high frequency dosing is necessary for the effective therapy [7]. Its short biological half life and thus frequent administration create necessity to development of long acting formulation is desirable to improve not only the treatment of lung disorder but also the patients compliance.

In the present investigation it was tried to develop long acting formulation of micro particulate system of terbutaline sulphate to improve itself. Micro spheres were prepared using two different type of viscosity grade of ethyl cellulose polymer. Micro spheres prepared with both viscosity grade of ethyl cellulose was formed free flowing and spherical. EC45 ethyl cellulose micro spheres containing 45 cps viscosity showed more prolong release of terbutaline sulphate compare to EC10 ethyl cellulose containing 10 cps viscosity grade of micro spheres.

Attempt was made to prepare ethyl cellulose micro sphere using emulsion solvent evaporation method. Non-spherical shaped micro spheres were obtained when prepared with w/o single emulsion solvent evaporation technique. Therefore micro spheres were prepared with double emulsion solvent evaporation method [8-10].

For both EC10 and EC45 ethyl cellulose micro spheres 3^2 factorial designs was developed to optimized the formulation of ethyl cellulose micro spheres. There are two independent variable such as Drug content, Polymer content at three different levels of studies, drug content at 50 mg, 75 mg and 100 mg and polymer content at 150 mg, 200 mg and 250 mg. Effect of these two independent variables were studied on dependant parameters of ethyl cellulose micro sphere such as percentage yields, particle size, % Encapsulation Efficiency and In-vitro drug release of both EC10 and EC45 micro sphere and comparison of % drug release after 12hrs. For EC10 ethyl

cellulose micro spheres dependant parameters which may help to formulate new formulation with required properties , that found to favors highest % yield of. % Encapsulation Efficiency and smallest average particle size. For EC45 microspheres that found favor highest % yield,% Encapsulation Efficiency and smallest average particle size. For the comparison of % drug release after 7 hr. lower % drug release was found to EC10 micro spheres of and for EC45 micro spheres was found to favor long acting release effect of micro spheres drug polymer ratio was found to effect dependant parameter significantly [11,12].

The effect of drug content and polymer content on Both EC10 and EC45 ethyl cellulose micro spheres. Increase in drug content of EC10 and EC45 micro sphere would increase in particle size, percentage yield and, % Encapsulation Efficiency of microspheres and also increase in polymer content of EC10 and EC45 microspheres would increase in particle size, percentage yield and % Encapsulation Efficiency of microspheres. For dissolution study ethyl cellulose microspheres prepared with lower viscosity had a faster dissolution rate than those prepared with higher viscosity ethyl cellulose.From this study it was concluded that micro spheres prepare using ethyl cellulose may prove useful for long acting of TBS for the effective treatment of bronchial asthma.

MATERIALS AND METHODS

2.1 Determination of solubility of the drug

Solubility of Terbutalin Sulphate was determined in different solvents. Excess of Terbutalin Sulphate was added to 10 ml of different solvents. The samples were stirred in a conical flask for 24 hour at 37ºC. The suspensions were filtered using a 0.45-micron whattman filter paper. The concentration of Terbutalin Sulphate in the filtrate was determined spectrophotometrically by measuring absorbance at 272 nm. The studies were repeated in triplicate (n=3) and mean was calculated [13].

2.2 Determination of absorpation Maxima (λ_{max}):

Spectrophotometry method is very useful method to estimate drug content *in-vivo* and *in-vitro* studies as well as for the assay of various drugs. In the present study terbutaline sulphate was estimated by U.V spectrophotometric method. Terbutaline sulphate was dissolved in distilled water and 0.1 N hydrochloride acid and separately to prepare 6 µg/ml solutions. Scans of both the solutions were taken separately for the concentration 6 µg/ml of terbutaline sulphate between 400 nm to 200 nm. Terbuatline sulphate exhibited U.V absorption maxima at 272.0 nm and 278.0 nm in distilled water and 0.1N HCL respectively.

2.3 Preparation of calibration curve in distilled water:

A stock solution of terbutaline sulphate (100 µg/ml) in distilled water was prepared by dissolving 25 mg of drug in 25 ml of distilled water. Series of different concentration 0.2 µg/ml, 0.4 µg/ml, 0.6 µg/ml, 0.8 µg/ml, 1 µg/ml were prepared from stock solution. Absorption of these solution were taken at 272.0 nm using distilled water as a blank.

2.4 Preparation of Calibration Curve:in Phosphate Buffer pH 6.8

A stock solution of terbutaline sulphate (100 µg/ml) in Phosphate Buffer pH 6.8 was prepares by dissolving 25 mg of drug in 25 ml of Phosphate Buffer pH 6.8. Series of different concentration

0.2 µg/ml, 0.4 µg/ml, 0.6 µg/ml, 0.8 µg/ml, 1 µg/ml, 2 µg/ml, 4 µg/ml, 6 µg/ml, 8 µg/ml and 10 µg/ml were prepared from stock solution. Absorbance of these solution were taken at 272.0 nm

2.5 Preparation of Calibration Curve:in 0.1 N HCl:

A stock solution of terbutaline sulphate (100 µg/ml) in 0.1 N HCl was prepared by dissolving 25 mg of drug in 25 ml of 0.1 N HCl. Series of different concentration 0.2 µg/ml, 0.4 µg/ml, 0.6 µg/ml, 0.8 µg/ml, 1 µg/ml were prepared from stock solution. Absorption of these solutions was taken at 278.0 nm.

2.6 Viscosity measurement of EC10 AND EC45:

Viscosity of polymer was measured by Brookfield viscometer. The viscosity of ethyl cellulose was measured typically at 25^0 C using 5 % ethyl cellulose dissolved in blend of 80% toluene and 20% ethanol [14].

2.7 Preparation of Microspheres

The microspheres were prepared by w/o/w emulsion solvent evaporation method. A specific grade of ethyl cellulose was dissolved in dichloromethane and terbutaline sulphate was dissolved in water. The terbutaline sulphate solution was added to ethyl cellulose solution and the mixture was sonicated in sonicator for 2 minute to prepare a primary (W/O) emulsion. Subsequently the resulting primary emulsion was gradually added to an aqueous solution of 1% PVA with continues stirring at maximum speed on a magnetic stirrer to get a W/O/W emulsion. The stirring was continued at maximum speed 2000 RPM for 1.5 hr. at room temperature to allow solvent evaporation [14,15].

The dispersion of microspheres was centrifuged at 2000 rpm for 10 min. The supernatant containing non-encapsulated terbutaline sulphate was discarded. To remove traces of non-encapsulation drug remaining in the palette, the palette was re-suspended in 25 ml. of distilled water and centrifuged at 2000 rpm for 10 min. This procedure was repeated two times; finally the palette was transferred to whattman filter paper and subjected to drying in a incubator at 37 ± 0.5 ^oC for 16 hr. Microspheres were preserved in a desiccators until the time of evaluation [16].

2.8 Evaluation of prepared Microspheres

2.8.1 Percentage Yield

Percentage yield was calculated for each batch using following equation:

$$\%\text{yield} = \frac{\text{Wt. of microsphere}}{\text{Wt of polymer + drug}} \times 100$$

2.8.2 Particle Size and Size Distribution

The simple optical microsphere was used for particle size measurement to measure particle size of individual microspheres; optical micrometer was calibrated using standard stage micrometer. According to microscopic method of particle size analysis, slides of various batches of microspheres were prepared using dilute suspension of microspheres in liquid paraffin. Particle

size of 100 microspheres from each batch was measured for calculating size distribution and average particle size [17].

2.8.3 Encapsulation Efficiency

The encapsulation efficiency of terbutaline sulphate in microspheres was calculated. Actual drug loading of microspheres of each batch of EC10 and EC45 was determined by following method. 25 mg of microspheres were weighed accurately and dissolved in dichloromethane to prepare 10 ml solution. Terbutaline sulphate was extracted three times from dichloromethane using 25 ml of Distilled water. Each time extraction was carried out using separating funnel. Each time separating funnel was hand shaken for 15 min. and then allowed to equilibrate for 10 min. The absorption of each aqueous extract was measured at 272.0 nm using UV spectrophotometer [18,19].

2.8.4 In-Vitro Release Study

Drug release study was carried out at 37 C in 900 ml distilled water using a USP type I dissolution apparatus. Accurately weighted microspheres (50 mg) were placed into the beaker containing 900 ml 0.1 N HCl for first 2 hours and phosphate buffer pH 6.8 for last hours rotated at speed of 100 rpm. At different time intervals 5 ml of aliquots of the dissolution medium were withdrawn and analyzed at 272.0 nm for drug content using an U.V visible spectrophotometer. An equal volume of the D.W was immediately replaced to maintain a constant volume of 900 ml within the dissolution beaker [20].

2.8.5 Scanning Electron Microscopy (SEM)

Surface morphology was carried out by Scanning Electron Microscopy (SEM). SEM studies were carried out with Phillips (FEL), ESEM- XL-30 TMP model and gaseous secondary electron detector was used for the detection with 20 KW accelerating speed. Microspheres sample was fixed on aluminum specimen stub by adhesive strip.

2.8.6 Factorial Design

Factorial design provides a means whereby the factors involved in a reaction or a process can be evaluated simultaneously and their relative importance assessed. It is thus a mean of separating those factors which are important from those which are not. The technique can be applied to many pharmaceutical problems, and it forms the basis for many tests which seek to find an optimum solution [21, 22]**.** Polynomial Equation was used in the optimization procedure is to carry out multiple regression analysis. This involve fitting the values of a dependent variable and the independent variable into polynomial equation of the form-

$$Y = b_0 + b_1X_1 + b_2X_2$$

Where Y = Dependent variable, X_1 = Independent variable-1 (amount of drug)
X_2 = Independent variable-2 (amount of polymer), b_0, b_1 and b_2 = Constants
The values of constants (b_0, b_1 and b_2) were obtained by multiple regression analysis by mathematical treatment of data.

RESULT AND DISCUSSION

To find out the drug release, the calibration curves of Terbutalin Sulphate (TBS) in distill water, 0.1 N HCl solution and Phosphate buffer pH 6.8 were plotted. Figure 1 showed the calibration curve of Terbutalin Sulphate in distill water. The Calibration curve were linear and regration co-efficient was found to be 0.9902 in distill water, which was 0.9853 in case of 0.1 N HCl while 09891 in case of phosphate buffer solution (pH 6.8) as shown in figure 2 and figure 3.

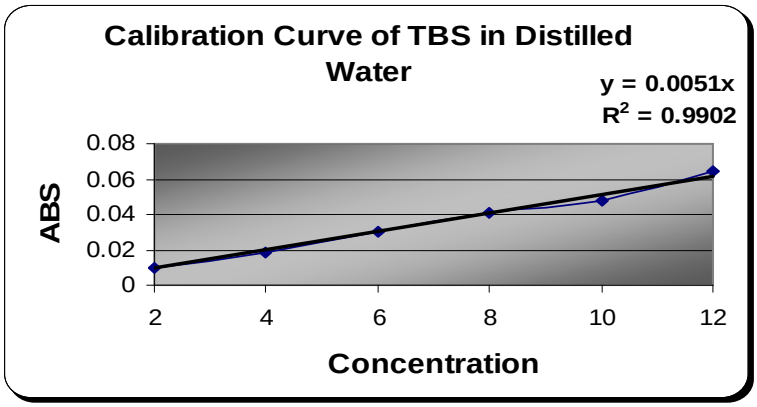

Figure 1. Calibration curves of TBS in Distilled water

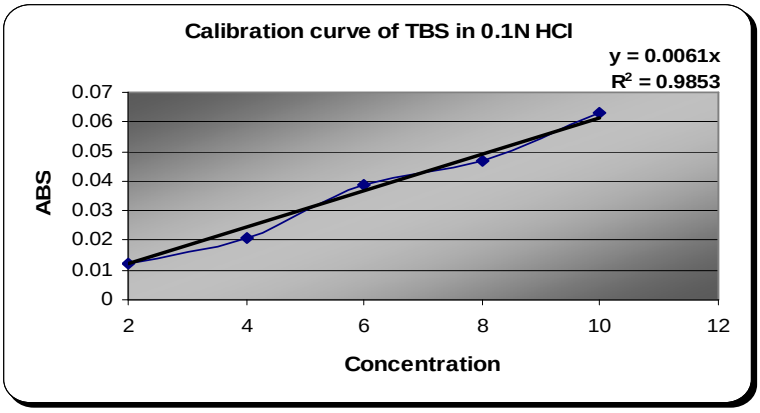

Figure 2:Calibration curves of TBS in 0.1 N HCl

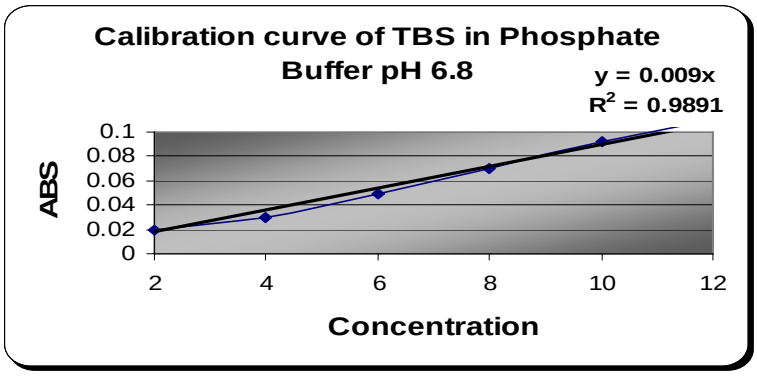

Figure 3:Calibration curves of TBS in Phosphate buffer pH 6.8

Ethyl Cellulose (EC10 and EC 45) polymers were used for the preparation of microsphere. Factorial design ware applied for the preparation of the different batches of ethyl cellulose. Nine batch for EC10 and nine batches of EC45 (Total 18 Batches) were prepared with the help of factorial design.

The percentage yield and particle size of different formulations were found. In EC 10 batches % yield increases with increased in the amount of drug loading. As shown in Table 1, batch No. B9 contained higher % yield than that of the other batches, but having higher particle size. The same phenomena were observed In EC 45 formulations. The % yield increases with increase in amount of drug loaded and Batch No. BC9 showed the higher % yield but higher particle size than other batches as observed in Table 2.

Table 1: Percentage yield of EC10 Batches

Batch No.	Batch description		% yield	Particle Size	Encapsulation Efficiency (%)
	Drug Content (mg)	Polymer content (mg)			
B1	50	150	48.40	17.62	15.48
B2	75	150	63.81	17.8	18.81
B3	100	150	67.75	18.3	21.43
B4	50	200	67.35	21.03	24.85
B5	75	200	63.75	21.83	28.45
B6	100	200	66.07	22.8	33.89
B7	50	250	61.32	23.14	38.65
B8	75	250	68.14	23.2	40.98
B9	100	250	76.14	23.22	44.7

Table 2: Percentage yield of EC45 Batches

Batch No.	Batch description		% yield	Particle Size	Encapsulation Efficiency(%)
	Drug Content (mg)	Polymer content (mg)			
BC1	50	150	53.27	18.36	17.81
BC2	75	150	60.00	18.69	19.98
BC3	100	150	63.51	19.1	22.87
BC4	50	200	54.88	22.03	27.68
BC5	75	200	58.41	22.98	30.89
BC6	100	200	64.25	23.6	34.66
BC7	50	250	57.25	24.66	39.12
BC8	75	250	61.30	25.6	42.76
BC9	100	250	67.45	26.89	46.8

Figure 4 and Figure 5 showed the Scanning Electron Microscope studies for the optimized formula at 35 X and 1500 x.

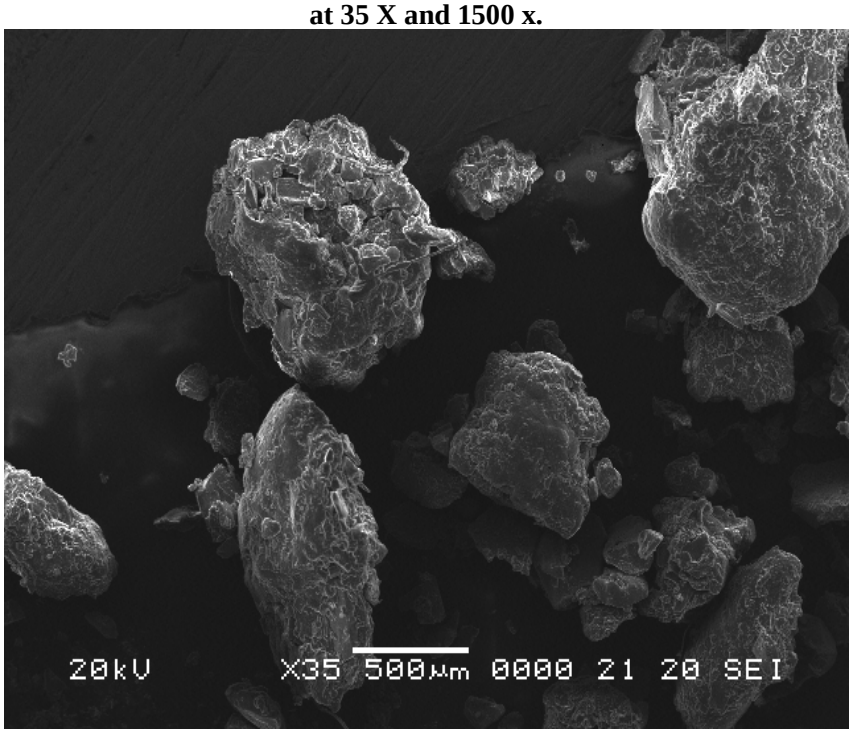

Figure 4. SEM image of optimized batch at 35x.

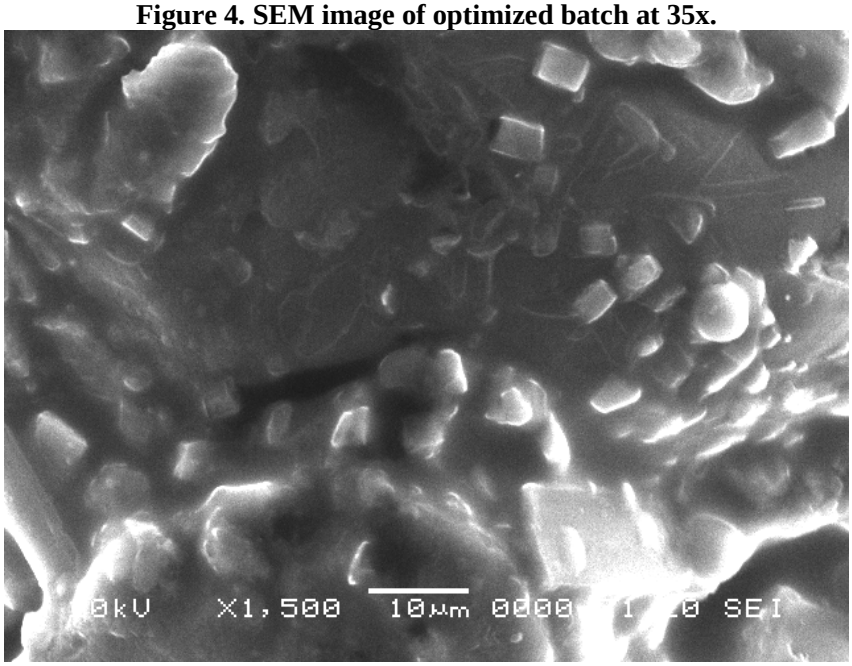

Figure 5:SEM image of optimized batch at 1500x

In vitro permeation studies were carried for the different formulations. For % Drug Release amount of polymer played a major role. Higher amount of polymer gave better sustained release

effect than lower amount of polymer. In EC 10 Formulation, Batch B1, B2 and B3 gives maximum % release upto 10 hours only as shown in Figure 6. They contained 150mg polymer. When batches B7, B8 and B9 gives good sustained release effect. Batch B9 gave 85.16% release within 12 hours.In Figure 7, it seems that In EC 45 formulation, Batch BC1, BC2 and BC3 gives maximum % release upto 11 hours. They contained 150mg polymer. When batches BC7, BC8 and BC9 gives good sustained release effect which contain 250mg polymer. Batch BC9 gave 80.63% release within 12 hours.

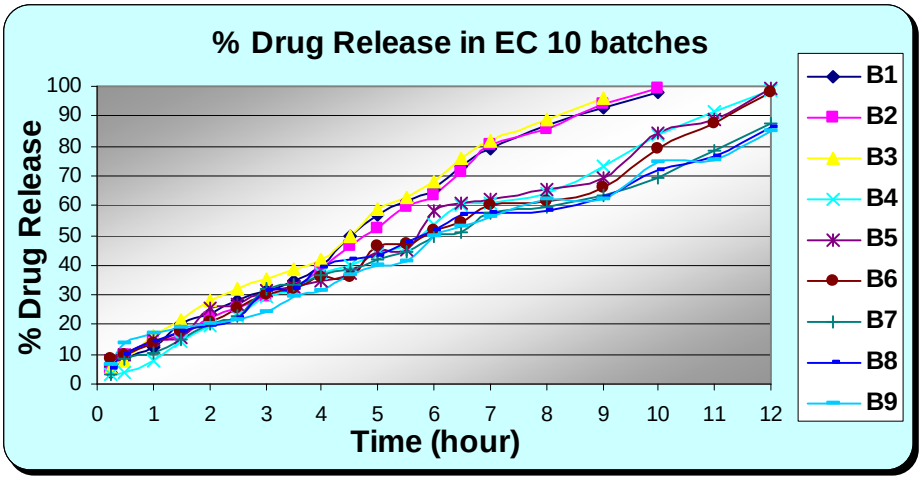

Figure 6: % Drug Release of EC10 Batches

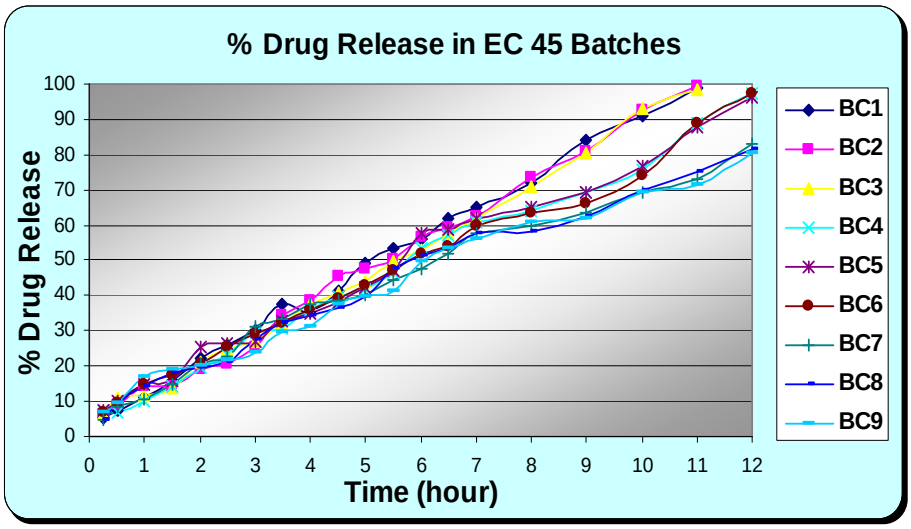

Figure 7: % Drug Release of EC45 Batches

CONCLUSION

The experimental work was carried out to prepare ethyl cellulose microspheres demonstrate the use of 3^2 factorial designs for preparation of long acting turbutaline sulphate microspheres. This statically technique allows examining effect of more than one independent variable at a time on

dependent variable such as percentage yield, particle size, % encapsulation efficiency and in vitro dissolution study. By using this factorial design desirable goal can be obtained in shortest possible time. Prolonged release effect obtained for ethyl cellulose microspheres way found to be significant with T50 value 270.32 min. In this study, effect of different viscosity grade of ethyl cellulose polymer and drugs polymer ratio was found to be significant on different dependent parameters. Higher viscosity grade (EC45) found having excellent sustain release effect for longer period of time than lower viscosity grade (EC10) micro spheres. Therefore it can be conclude that TBS loaded microspheres made up of EC 45 viscosity grade may useful in promoting the prolonged release of drug for effective treatment of bronchial asthma.

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