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RESEARCH ARTICLE

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EFFECT OF DIFFERENT POLYMERS AND STORAGE TEMPERATURE ON DRUG RELEASE FROM SALBUTAMOL SULPHATE LOADED MICROSPHERES PREPARED BY USING W/O EMULSION SOLVENT EVAPORATION TECHNIQUE

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Abstract: This study was aimed to formulate and evaluate controlled release preparations of salbutamol sulphate microspheres by w/o emulsion technique using ethyl cellulose as the rate retardant material. Microspheres were prepared by water in oil emulsion technique using methanol solvent system and span 80 was used as the surfactant. Different polymers [hydroxypropyl methyl cellulose (HPMC15 cps) and methacrylic polymers (Eudragit E100, Eudragit L100)] were used to modify the release characteristics of salbutamol sulphate. Scanning electron microscopy (SEM) was performed to study the size, shape and surface morphology of the prepared microspheres. The size distribution of microsphere batches generally ranged from 204 µm to 582.3 µm with geometric means close to 600 µm. The best fit release kinetics was first order when hydroxyl propyl methyl cellulose (HPMC15) was used and Higuchi plot was followed in case of eudragid E100 and eudragid L100. Korsmeyer equation was used to calculate the release exponent value (n) which indicates the drug release behavior and the mean dissolution time (MDT) for release rate. The release of salbutamol sulphate was influenced by altering the polymer where drug release was found to be fickian diffusion controlled. The drug release profile of different formulations was changed with temperature while kept under two different storage temperatures of 60ºC and 100ºC.

Keywords: Salbutamol sulphate, Ethyl cellulose, Emulsion Solvent Evaporation Technique, Microsphere, Temperature**.**

INTRODUCTION

Polymeric microparticles are widely studied in the pharmaceutical field for the purpose of obtaining better results in the oral administration of drugs by the production of multiple-unit drug delivery systems[1-3]. The literature review showed the different criteria for the designing of microparticles according to polymer properties[4, 5]. Ethyl cellulose is a water insoluble cellulosic polymer and commonly used for the formulations of microparticles, pellets and beads for the delivery of prolonged and sustained therapy[6]. In the present work ethyl cellulose was chosen as suitable polymer for encapsulating the salbutamol sulphate by solvent evaporation technique.

Salbutamol sulphate is a potent β-2 adrenoceptor stimulant which is used for the treatment of reversible airways obstruction. It is readily absorbed from the gastrointestinal tract and its biological half life is about 4 to 6 hrs[7].

In previous studies salbutamol sulphate was microencapsulated into various polymers by different techniques e.g. coacervation-thermal change and solvent evaporation[8, 9]. Ethyl cellulose is also used in microcapsules preparation of salbutamol sulphate. Microspheres of salbutamol sulphate were also prepared with poly lactic acid-co-glycolic acid (PLGA 85115) by the modified solvent evaporation method using a w/o/w double emulsion [10]. Huq *et al.*, 2011 used kollidon SR as rate modifying polymer for preparing salbutamol sulphate microcapsules[11, 12].

The aims of this study are to investigate, by solvent evaporation, the production of microcapsules of salbutamol sulphate with a cellulose polymer and the effectiveness of different polymers in improving the quality of the microcapsules and to investigate the temperature effect on release of salbutamol sulphate as drug release rate can be significantly impressed by the thermal effect[13].

MATERIALS AND METHODS

Salbutamol sulphate was a generous gift from Sanofi-aventis, ethyl cellulose (Ethocel 20 cps) was collected from Colorcon, USA and liquid paraffin oil light was from MERCK, Germany. Span 80 was from Loba Chemicals, India. All other chemicals or ingredients were of analytical grade.

Stirrer (Heidolph No. 5011) (Heidolph, England), UV-visible Spectrophotometer-1240(Shiamdzu, Japan), Scanning Electron Microscope (SEM) S-3400 N (Hitachi, Japan), sonicator (POWER SONIC 505, HWASHIN TECHNOLOGY CO., Seoul, Korea.)Tablet dissolution machine tester (USP Type II dissolution Apparatus, VEEGO, INDIA) for dissolution, oven (BINDER, Germany) and Digital Photo Tachometer (DT 623413) Circuit Specialist Inc, USA was used in this study.

Preparation of salbutamol sulphate microspheres

Emulsion solvent evaporation is the most frequently used microencapsulation method[14]. So we used this method for the preparation of microspheres. The Water in Oil (W/O) type of emulsion formation technique was adopted as the ideal technique for the study. Microspheres were formulated as per formulation in table 1 by using hydroxypropyl methyl cellulose (HPMC15 cps) and methacrylic polymers (Eudragit E100, Eudragit L100) along with ethyl cellulose at a concentration of 10 % of drug as additives with the help of span 80 as surfactant. Another batch was formulated containing only ethyl cellulose keeping the surfactant same which was taken as a blank formulation.

At first polymer solution was prepared by dissolving polymer in methanol which acts as internal phase. Liquid paraffin oil was emulsified using span 80 with the help of stirrer at 1000 rpm. Then salbutamol sulphate was dispersed in the emulsified external phase. A volume of previously prepared polymeric internal phase containing ethyl cellulose was slowly added to the external phase and the stirring was raised to 3000 rpm and kept for about 1 hour until hard, uniform shaped microspheres were formed. After 1 hour the prepared microspheres were washed by n-hexane and were allowed to dry in the natural air for about 2 or 3 days. The prepared microspheres were then sieved, weighed and transferred to glass vials and stored in desiccators. The formulations were named as E S80 for blank, ES H15 for formulation with HPMC15 cps, ES E100 for eudragit E100 and ES L100 for the formulation with eudragit L100 as shown in table 1.

Assay of microspheres

Microspheres were taken in a mortar and triturated properly until fine powder formed. 10 mg of fine powder was taken in a 100 ml volumetric flask with the help of a funnel. Purified water was added with the powdered microspheres, sonicated for 30 minutes in a sonicator (POWER SONIC 505, HWASHIN

TECHNOLOGY CO., Seoul, Korea.) to make a clear solution and then volume was adjusted to 100 ml with distilled water and it was filtered finally.

Absorbance value was determined using UV spectrophotometer (UV mini-1240, SHIMADZU CORP., Kyoto, Japan) at a wavelength of 276.5 nm. Using the absorbance value, the amount of salbutamol sulphate entrapped was determined with the help of standard curve.

Following calculations used for determining different analytical parameters as shown in Table 1

% yield = (Actual yield/Theoretical yield) × 100.

Theoretical content (%) = (Theoretical salbutamol sulphate loading / Theoretical batch yield) × 100.

% loading = (Actual salbutamol sulphate loading / Theoretical batch yield) × 100.

Loading efficiency (%) = (% loading / % Theoretical content) × 100.

In-vitro dissolution study of ethyl cellulose-salbutamol sulphate microspheres:

In-vitro dissolution study was performed in a paddle type dissolution apparatus (USP Type II Dissolution Apparatus, VEEGO, INDIA). A fixed amount of microspheres (containing 8 mg of Salbutamol sulphate) from each batch was calculated for dissolution purpose. 500 ml distilled water was used as dissolution media, paddle speed was set at 50 rpm and temperature was maintained at 37ºC. The fixed amount of microspheres from each batch was weighed and transfer in each dissolution basket. The dissolution process was carried out for 8 hour and 7 ml of dissolution sample from each batch was withdrawn at a predetermined intervals of 0.25 hour, 0.50 hour, 0.75 hour, 1 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 7 hour, 8 hour. Each and every time 7ml dissolution sample was compensated by fresh 7 ml distilled water. The dissolution samples were then analyzed spectrophotometrically in a UV-VIS spectrophotometer (UV mini-1240, SHIMADZU CORP., Kyoto, Japan). The dissolution study for each batch was performed in triplicate. Each value of the dissolution curve is percent release ± standard deviation (SD).

Release kinetic study

After linear transformation of dissolution curves, the results were tested with the following mathematical models.

- The zero order equation assumes that drug release is constant:

$$M=M_o-K_ot \text{ …… (I)}$$

In this equation M is the amount of drug remaining undissolved at time t, M_o is the amount of drug undissolved at t=0 and K_o is the corresponding release rate constant.

- A form of the Higuchi Square Root Law is given by equation:

$$Q=K_s \sqrt{t} \text{ ……. (II)}$$

Where Q (Q=100-M) is the amount of drug dissolved at time t and K_s is the corresponding rate constant.

- Release behavior generally follows the following first order release equation:

$$\ln M=\ln M_oK_1t \text{ ……. (III)}$$

Where M is the amount of drug undissolved at t=0 and K_1 is the corresponding release rate constant.

- The Korsmeyer's equation which derived from the linear line of log cumulative percentage versus log time curve is:

$$M_t/M_\alpha = K_kt \text{ …….. (IV)}$$

Where K_k is the Korsmeyer release rate constant.

- The Hixson- Crowell equation which derived from the cubic root of percent release versus log time curve is:

$$Q_0^{1/3} - Q_t^{1/3} = K_{HC}\, t\text{…….. … (V)}$$

Where, Q_t is the amount of drug released in time t, Q_0 is the initial amount of the drug in the microcapsules and K_{HC} is the rate constant for Hixson-Crowell rate equation. The Hixson-Crowell equation describes the release from systems where there is a change in surface area and diameter of particles or tablets [14].

Particle morphology (Size, Shape) analysis

Surface nature of selected batches of microspheres (based on comparatively better dissolution profile) was examined with help of scanning electron microscope (S-3400N, Hitachi). The microspheres were dried completely before examination.

Annealing effect on microspheres

Microspheres of different formulas were kept in an oven at 60^o C and in another oven at 100 ^{o} C for 24 hours. A few amount (arbitrarily taken) of microspheres from all batches were taken in a small airtight glass vials. Then all the vials were taken in a large glass container arranged inside in such a way that the heat could dissipate properly

through the vials. They were kept in oven for 24 hours. After thermal treatment, microspheres were analyzed by *In-vitro* dissolution and scanning electron microscope (For selected batches based on comparatively better dissolution profile) to verify fusion properties of polymers on the microspheric surface.

Statistical analysis

The data obtained from the particle size, encapsulation efficiency and percent release determination studies of salbutamol sulphate microcapsules were statistically evaluated by using one way ANOVA and Student *t* test for comparison of sample groups with the blank group (ES80).Values with $p<0.1$ were considered as significant.

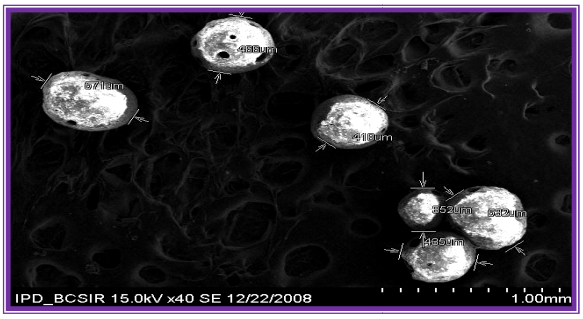
RESULTS AND DISCUSSION

Quantitative analysis of microspheres

The theoretical loading of salbutamol sulphate was assumed to be 50% for all thee formulations. Table 2 showing the loading efficiency of prepared microspheres where within 93.72±0.5-96.44±1.08%.

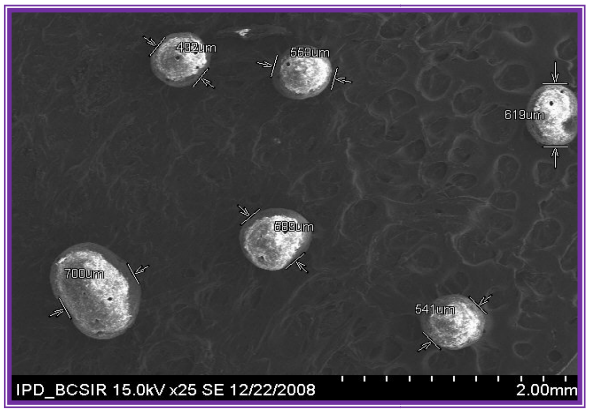
Surface morphology study and particle size of microspheres

Microspheres of Ethyl cellulose containing salbutamol sulphate were prepared using different polymer variation. Images at different magnifications of 15.0 kV x 25 SE, 15.0 kV x 207 BSE, 15.0 kV x 503 BSE and 15.0 kV x 1.02 k BSE were taken to examine the size and surface morphology of the microspheres. The average particle size ranged from 204±22.1 µm -582.3±44.3 µm (Table 2).

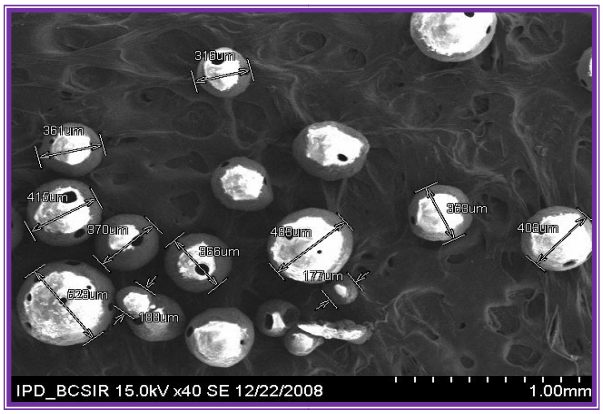


A. E S80

B. ES H15



C. ES E100



D. ES L100

Figure 1. A, B, C and D shows the scanning electron microscopy of different batches of salbutamol sulphate containing ethyl cellulose microspheres using different polymers.

In SEM pictures, the formulations of microspheres are seen with some holes on its body. The overall body seems very fluffy as viewed individually. The boundary and edges of big holes are not so rigid. It indicates that these holes would be more broadened against rush of fluid flow onto it. Moreover, the microspheres of all the formulations were relatively round and spherical in shape having different size range. The surface of the microspheres is somewhat rough except ES H15 (Figure 1). Its overall surface is very much polished and no rough surface is found. This may confer retarded release from the microspheres.

In vitro dissolution study

Fig 2 illustrates the percent release profile of salbutamol sulphate from different formulations of ethyl cellulose microspheres

prepared by using HPMC 15 cps (ES H15), Eudragit E100 (ES E100) and Eudragit L100 (ES L100).

From this zero order release curve it is seen that almost 50% drug release was observed for ES H15 and ES L100 after 2 hour (52.41% and 48.03% respectively) and 3 hour for ES E100 (47.58%) in comparison to the release of drug (47.97%) from the blank E S80 which occurred after 30 min. After completion of 8 hour of dissolution ES E100 showed the most delayed drug release profile (81.05%) among all the formulations where ES H15 and ES L100 were found to release about 87.16% and 88.37% respectively. The figure clearly indicates that all the formulation prepared with different polymers showed slower drug release profile in compared to the blank ES80. The experimental data were statistically found to be significant while evaluated by using Hartley F_{max}, Cochran C and Bartlett Chi-square test where the F values were found to be 0.0051, 0.024 and 0.0318 for ES H15, ES L100 and ES E100 respectively and p level were 0.995, 0.968 and 0.999. Again, in all the cases the F_{crit} value and p value were 8.5785 and 0.001 respectively.

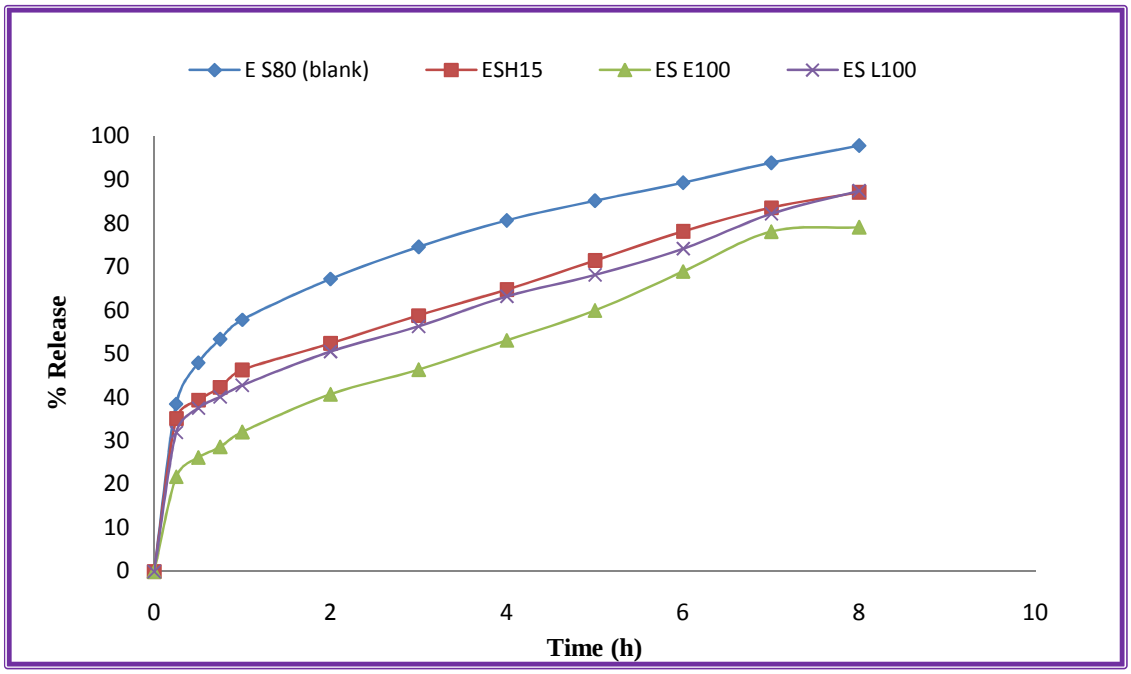


Figure 2. Zero order release of salbutamol sulphate.

Moreover, the study accomplished with putting the data into other various kinetic models first-order, Higuchi and Korsmeyer to obtain more meaningful information

about drug release. Table 3 summarizes the release exponent (*n*), mean dissolution time (MDT) and the correlation coefficient (r^2) for the different release kinetic models of salbutamol sulphate microcapsules. Among these formulations the blank E S80 and ES H15 best fitted with first order release kinetic (r^2= 0.95 and 0.96 respectively). But in case of ES E100 and ES L100 (r^2= 0.98 and 0.97 respectively) the highest correlation coefficient was observed in Higuchi model indicating diffusion controlled principle[15]. The values of diffusion exponent *n* and the MDT of controlled release of water soluble drugs from the polymers [16, 17] were found out for microspheres and matrices by using the Korsmeyer-Peppas equation which is summarized in table 3.

For spherical matrices, if $n \leq 0.43$, a fickian diffusion, $0.43 \leq n < 0.85$, a non-fickian diffusion transport and $n \geq 0.85$, a case-II transport (Zero order) drug release mechanism dominates. All the experimental formulations showed fickian diffusion mechanism (table 4). The dissolution data were also plotted in accordance with the Hixson-Crowell cube root law.

Determination of release rate

Release rate of different formulations are shown in following bar diagrams. In each case, their rates of burst and sustained release are also shown. The curves are plotted by varying mode of release against different mode of time taken.

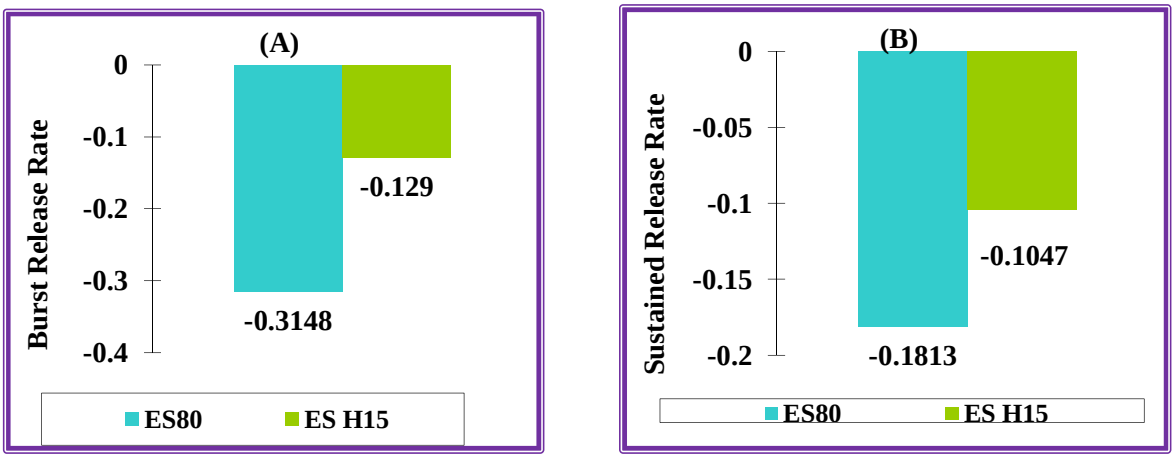


Fig 3.1. A and B shows the comparative burst and sustained release rate of formulation ES H15 with the blank using first order release kinetic.

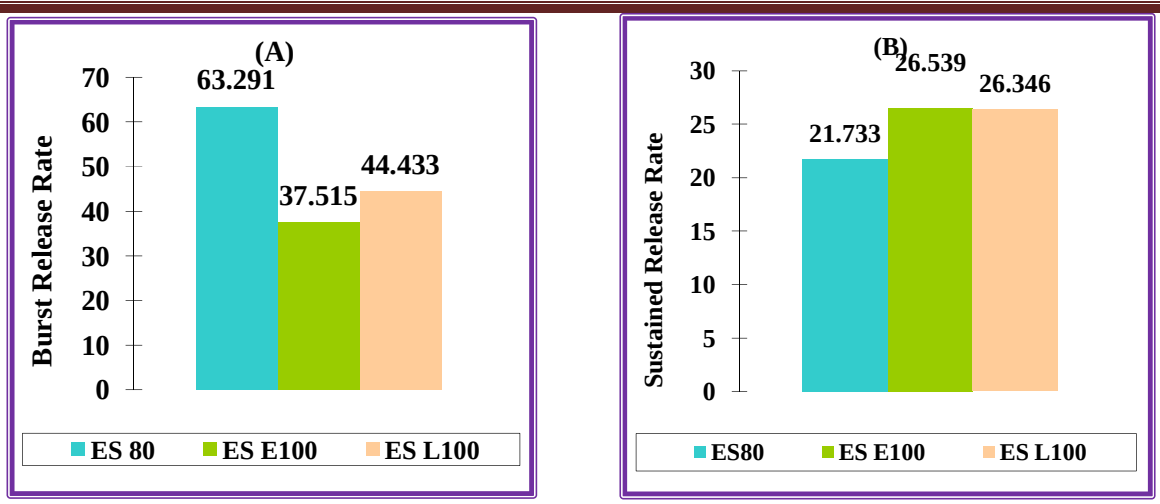


Fig 3.2. A and B shows the comparative burst and sustained release rate of formulation ES E100 and ES L100 with the blank using higuchi release kinetic.

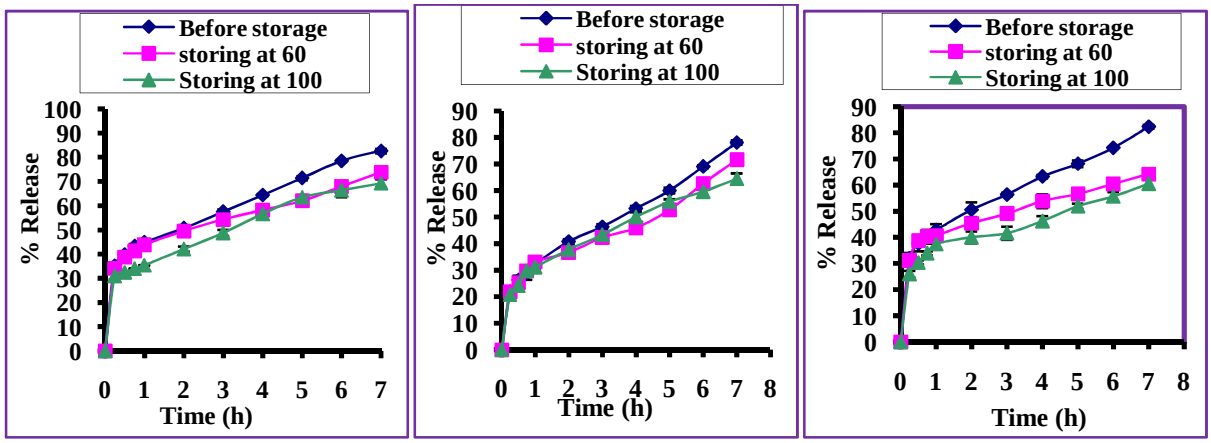
Effect of storage temperature on salbutamol sulphate-ethyl cellulose microspheres

Storage of microspheres was observed at 60^0 C as well as at 100^0 C by keeping them under same temperature inside oven for 24 hours (BINDER, Germany). This storage of microspheres affected the 7 hour dissolution test immediately after removal of samples from oven. The microspheres were checked with naked eyes for finding whether any change in color was occurred before subjected to dissolution (Table 4).

***In vitro* dissolution study after storage at different temperature**

After storage at 60^o and 100^o C the prepared microspheres except ES80 were subjected to dissolution study using the same condition and dissolution medium as previous. The change in release characteristics were observed by measuring the percent release of salbutamol sulphate. In 7h dissolution study, it was observed that the release of drug was reduced significantly along with the increased storage temperature (Table 5 and Figure 4) and this may be due to removal of moisture from the microspheres and thus the compactness of the surface. The experimental data were statistically calculated by using one way ANOVA. For ES H15, the F values were found to be 0.0017, 0.0005 and 0.0001 before storage, after 60ºC and 100 ºC respectively where it was 0.0016, 0.0003, 0.004 and 0.0018, 0.0123 and 0.0003 for ES E100 and ES L100 respectively at respective storage conditions. At room temperature (before storage), the p level was 0.9983 for ES H15

while it was 0.9995 and 0.9999 at 60 ºC and 100 ºC respectively for the same formulation. Moreover, the p level for ES E100 and ES L100 was 0.9984 and 0.9982 at room temperature (before storage), 0.9997 and 0.9878 at 60 ºC and 0.9878 and 0.9997 at 100 ºC. Again, the F_{crit} value and p value were 3.2849 and 0.05 in cases at respective storage temperatures



(A) ES H15 **(B) ES E100** **(C) ES L100**

Figure 4. A-C represents the percent release of salbutamol sulphate from different formulations of microspheres.

CONCLUSION

Salbutamol sulphate was successfully encapsulated into ethyl cellulose microspheres by w/o emulsion solvent evaporation method using different polymers. The drug release pattern was found satisfactory with eudragit E100 although the dissolution rate of salbutamol sulphate was slowed down by other two polymers. The temperature effects ensured more retained drug release which may indicate that this curing treatment can be useful to delay the drug release. But more stability testing is required in this point. The ethyl cellulose-eudragit E100 microspheres were thus found suitable for controlled and sustained release products *In vitro*. However, a formulation of salbutamol sulphate cannot be considered as sustained

release entity unless the *in vivo* study demonstrates a concentration/response relationship together with a short plasma elimination half-life and inactive or uncharacterized activity of any generated metabolites and produce transient side effects associated with dissolution and/or peak concentrations. Therefore, further both extensive *In vitro* and *in vivo* study should be carried out to make this sustained-release microsphere formulation of salbutamol sulphate to be used clinically.

Table 1

Formulations microspheres of salbutamol sulphate with ethyl cellulose

Formulation	Materials					
	SBS* (g)	EC** (g)	Span80 (g)	HPMC 15cps (g)	Eu E100	Eu L100
E S80	2	2	0.5	-	-	-
ES H15	2	1.8	0.5	0.2	-	-
ES E100	2	1.8	0.5	-	0.2	-
ES L100	2	1.8	0.5	-	-	0.2

**SBS= Salbutamol sulphate, **EC= Ethyl cellulose,*

Table 2

size and loading efficiency of microspheres

Formulation	Avg. Particle size (µm)	Loading eficiency (%)
E S80	470.7±88.5	96.44±1.08
ES H15	204±22.15	95.04±0.65
ES E100	421.3±70.95	96.00±0.09
ES L100	582.3±44.3	93.72±0.5

Table 3

Release of salbutamol sulphate from ethyl cellulose microspheres

Time (h)	ES80(Blank)	ES H15	ES E100	ES L100
0.0	0.0	0.0	0.0	0.0
0.25	38.5±0.9	35.1±0.6[d]	21.8±0.2[b]	32.2±1.3[a]
0.5	47.9±0.6	39.7±0.01[b]	26.2±1.3[b]	37.6±1.1[a]
0.75	53.4±0.7	43.3±0.1[b]	28.6±1.2[b]	40.2±1.4[a]
1	57.9±0.9	44.9 ±0.7[a]	32.1±0.2[b]	42.8±0.6[a]
2	67.2±0.9	50.7±0.02[b]	40.7±0.06b	50.5±0.3[a]
3	74.6±0.6	57.5±0.1[b]	46.4±0.4[b]	56.3±0.7[b]
4	80.7±0.6	64.4±0.4[b]	53.1±0.06[b]	63.2±0.7[b]
5	85.2±0.3	71.4±0.3[b]	60.0±0.02[b]	68.1±0.5[b]
6	89.4±0.3	78.5±0.1[b]	69.0±0.3[b]	74.2±0.07[b]
7	93.9±0.4	82.6±0.3[b]	78.1±0.08[b]	82.2±0.2[b]
8	97.9±0.3	87.2±1.3[c]	79.1±0.1[a]	87.5±0.6[b]

The values are expressed as Mean ±SD; SD = standard deviation; BS= before storage;

a=p<0.0005, b= p<0.0001, c= p<0.005, d= p<0.05

Table 4.

Release kinetics of salbutamol sulphate from ethyl-cellulose microspheres of different polymers.

Formulation	Zero order		First order		Higuchi		Hixon-Crowell		*n*	MDT (h)
	r^2	K_0	r^2	K_1	r^2	K_2	r^2	K_3		
E S80(blank)	0.75	8.64	0.95	-0.16	0.92	29.12	0.38	0.27	0.26	1.92
ES H15	0.82	7.93	0.96	-0.09	0.94	25.98	0.40	0.26	0.27	3.48
ES E100	0.91	8.18	0.97	-0.08	0.98	25.94	0.50	0.29	0.36	4.89
ES L100	0.88	8.38	0.96	-0.09	0.97	26.95	0.45	0.28	0.31	3.60

Table 5.

Characteristic color change of microspheres of different formulations with thermal effect

Formulation	Color before curing (room temperature)	Color after curing at 60^0 C	Color after curing at 100^0 C
E S80(blank)	White	Unchanged (white)	Dark Red
ES H15	White	Unchanged (white)	Light Brown
ES E100	White	Unchanged (white)	Off White
ES L100	White	Unchanged (white)	Off White

Table 6

Release of salbutamol sulphate from ethyl cellulose microspheres after curing at different temperature

Time (h)	ES H15			ES E100			ES L100		
	BS	**60°C**	**100°C**	**BS**	**60°C**	**100°C**	**BS**	**60°C**	**100°C**
0.25	35.1±0.1^{d}	34.1±0.2^{e}	31.4±0.4^{a}	21.8±0.2^{b}	21.8±0.2^{b}	20.7±0.6^{b}	32.0±1.3^{a}	30.5±0.4^{c}	25.9±0.2^{c}
0.5	39.7±0.01^{b}	38.6±0.1^{a}	32.5±0.1^{b}	26.2±0.3^{b}	25.2±0.01^{b}	24.1±0.05^{b}	37.6±1.1^{a}	38.6±0.5^{b}	30.3±0.3^{b}
0.75	43.3±0.1^{B}	41.2±0.006^{a}	33.9±0.3^{b}	28.6±1.3^{b}	29.6±1.0^{b}	29.6± 0.3^{a}	40.2±1.4^{a}	40.4±0.4^{b}	33.8±0.5^{a}
1	44.9±0.7^{a}	43.8±0.2^{a}	35.4±0.1^{a}	32.1±0.2^{b}	33.1 ±0.09^{b}	31.0±0.7^{b}	42.8±0.6^{a}	40.7±1.5^{b}	37.4±0.6^{b}
2	50.7±0.02^{b}	49.6±0.1^{b}	42.0±0.08^{b}	40.7±0.06^{b}	36.6±0.07^{b}	37.6±0.2^{b}	50.5±0.3^{a}	45.4±2.2^{b}	39.9±0.1^{b}
3	57.5±0.1^{b}	54.3±0.7^{b}	48.8±0.7^{b}	46.4±0.4^{b}	42.2±0.6^{b}	43.2±0.07^{b}	56.3±0.7^{b}	49.0±0.1^{b}	41.5±0.1^{b}
4	64.4±0.4^{b}	58.1±0.04^{b}	56.6±0.1^{b}	53.1±0.06^{b}	45.9±0.1^{b}	49.9 ±0.2^{b}	63.2±0.7^{b}	53.8±0.1^{b}	46.1±0.1^{b}
5	71.4±0.3^{b}	62.0±0.08^{b}	63.5±0.1^{b}	60.0±0.02^{b}	52.6±0.02^{b}	55.7±0.4^{b}	68.1±0.5^{b}	56.5±0.1^{b}	51.8±0.1^{b}
6	78.5±0.1^{b}	67.9±0.2^{b}	66.4±0.2^{b}	69.0±0.3^{b}	62.5±0.2^{b}	59.5±0.3^{b}	74.2±0.07^{b}	60.4±1.1^{b}	55.6±1.1^{b}
7	82.6±0.05^{b}	73.9±0.1^{b}	69.3±0.6^{b}	78.1±0.08^{b}	71.5±0.5^{b}	64.4±0.1^{b}	82.2±0.2^{b}	64.2±0.2^{b}	60.4±0.4^{b}

The values are expressed as Mean ±SD; SD = standard deviation; BS= before storage (room temperature); a=p<0.0005, b= p<0.0001, c= p<0.005, d= p<0.05, e= p< 0.1

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