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Preparation and In-vitro Characterization of Mucoadhesive Microspheres of Terbutaline Sulphate

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Abstract **Original Research Article**

In the present investigation is with respect to plan and assessment of terbutaline sulfate mucoadhesive microspheres by solvent evaporation method. The mucoadhesive microspheres were effectively created by w/o emulsion solvent evaporation method. A total of nine formulations were prepared i.e MM1, MM2, MM3, MM4, MM5, MM6, MM7, MM8 and MM9. The particle size of all the formulations were ranged between 128.80 ± 11.12 and 152.42 ± 17.13. The entrapment efficacy was ranged between 71.54 And 64.48. Based on above parameters three formulation were selected i.e. MM3, MM5 and MM8 for further studies like micromeritic property swelling index and *in vitro* release study. The optimized batch MM3 was found to release the drug for 16h (94.4%) and follows higuchi matrix model and korsmeyer-peppas equation in dissolution study. Stability studies showed almost negligible changes in particle size, entrapment efficiency and drug release throughout the study period.
Keywords: Terbutaline sulphate, Microspheres, Higuchi model, Korsmeyer-peppas equation and Stability studies.

INTRODUCTION

The term microsphere is defined as a spherical particle with size from 1 um - 1000um. The microsphere are typical free-flowing powder consist of synthetic polymer which is Biod e.g. radable in nature and having particle size less than 200 um. The microspheres are made from highly transparent glass can perform as much high-quality optical microcavities or micro resonators. The success of these microspheres is limited having provided intimate contact of the of the drug delivery system with the absorbing membranes [1, 2]. Mucoadhesion or bioadhesion can be characterized as the state in which two material (something like one of which is biological in nature) are held together for a delayed time period and by means of interfacial force. Mucoadhesive dosage form might be intended to delayed the retention time at the site of application, giving a controlled rate of medication discharge for enhanced therapeutic outcome [3, 4].

Asthma is one of the leading disease in the world and needs some serious attension. It can lead to various complications. Like bronchospasm, Respiratory failure, chronic obstructive pulmonary diseases [5].

Terbutaline stimulates β-adernergic receptors of the sympathetic nervous system and has little or no effect on α-adrenergic receptors [6]. Despite its low and stereoselective bioavailability, terbutaline is widely used as a bronchodilator for the treatment of bronchial asthma, chronic bronchitis and emphysema. On other hand, terbutaline has not been approved and should not be used without permission on the patient in preterm labor [7].

Terbutaline is a synthetic β2-adrenoceptor stimulant that is used as a bronchodilator in the treatment of bronchial Asthma. It is known chemically as –α-[(tert-butylamino)] methyl 1]-3,5-dihydroxy-benzyl alcohol ($C_{12}H_{19}NO_3$) Mol. Wt. 225.29. It exits as a racemic mixture [(+) and (-) terbutaline] and has the chemical structure shown below [8].

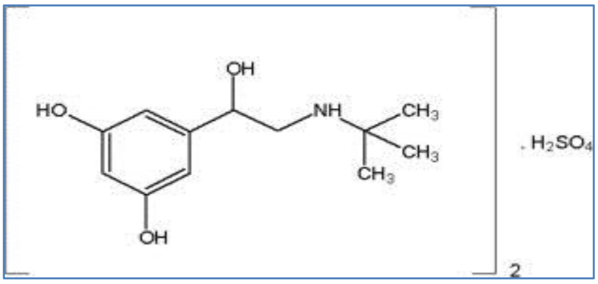

Fig-1: structure of terbutaline sulphate

Terbutaline given as the sulfate (TBS). It is a white to gray-white, crystalline, powder; odorless or with a faint odor of acetic acid; and slightly bitter. It is unstable in light and melts at about 247 ºC.

MATERIALS AND METHOD

Materials: terbutaline sulphate was obtained from the Mumbai. The polymers hydroxyl methyl cellulose (HPMC) and carbopol 934p (Cp) were obtained from Mumbai. Dicholoromethane, methanol and sodium lauryl sulphate were also obtained from Mumbai.

Formulation of Terbutaline Sulphate Microspheres

Drug loaded microsphere were prepared by water in oil (w/o) emulsification solvent evaporation method. For this, 50 mg of drug dissolved in 5 ml dimethyl sulfoxide, and then it was dispersed into 45 ml of 2% aqueous polymer solution. A vortex homogenizer was used for rapid mixing of the drug solution into the aqueous polymer solution for 3 min. Then drug and polymer solution was added dropwise to 400 ml of the liquid paraffin containing 0.5% span 20 as an emulsifying agent with constant stirring at 500 rpm. The constant stirring was carried out using magnetic stirrer. The beaker and its content were heated at 80 ºC with constant stirring for 4.5 h until the aqueous phase was completely removed by evaporation. The liquid paraffin was decanted and collected microsphere were washed 5 times with 100 ml of n-hexane, filtered through Whatman's filter paper, dried in hot air oven at 50 ºC for 2 h and stored in a desiccater at room temperature. For each formulation, 3 batches of microspheres were prepared for the purpose of assessing the reproducibility of drug loading, particle size, % mucoadhesion and *in-vitro* drug release by this method.

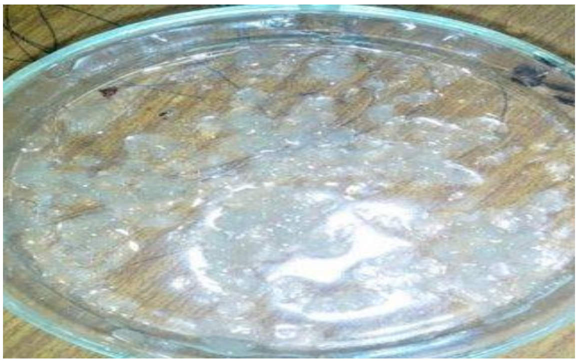

Fig-2: Microspheres preparation

Table-1: preparation of mucoadhesive microspheres

S. no.	Formulation code	(Terbutaline sulphate)	HPMC (mg)	Carbopol 934p (mg)	Sodium alginate (mg)
1	MM 1 (1:1)	50 mg	50 mg	-	-
2	MM 2 (1:1.5)	50 mg	75 mg	-	-
3	MM 3 (1:2)	50 mg	100 mg	-	-
4	MM 4 (1:1)	50 mg	-	50 mg	-
5	MM 5 (1:1.5)	50 mg	-	75 mg	-
6	MM 6 (1:2)	50 mg	-	100 mg	-
7	MM 7 (1:1)	50 mg	-	-	50 mg
8	MM 8 (1:1.5)	50 mg	-	-	75 mg
9	MM 9 (1:2)	50 mg	-	-	100 mg

Where, Drug = Terbutaline Sulphate
HPMC = Hydroxypropyl methylcellulose
MM = Mucoadhesive microsphere
MM = Mucoadhesive microsphere

Particle Size Analysis

Particle size of different batches of microspheres was determined by optical microscopy. The projected diameter of microspheres from each batch was determined using ocular micrometer and stage micrometer equipped with optical microscope. Analysis was carried out by observing the slide containing microspheres under the microscope. The average particle size of the microspheres was expressed as diameter. The mean microsphere size was calculated by measuring 100 particles with the help of a calibrated ocular micrometer [9].

D Mean = Number of microspheres observed / Mean size range

Drug Entrapment Efficiency

To determine the drug entrapment efficiency or incorporation efficiency the microspheres were crushed in glass mortar and powered, then suspended in 10 ml of methanol, after 24 hrs. The solution was filtered and filtrate was analyzed for drug content. The drug incorporation efficiency was calculated by the following formula [10].

$$\textbf{Incorporation efficiency} = \frac{b}{a} \times 100$$

b = calculated amount of drug present in the formulation,
a = theoretical amount of drug present in the formulation

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was done to study morphology of microspheres. Surface morphology of microspheres was studied under scanning electron microscope (JEOL, JSM 5760 LY). Samples were mounted on stubs and coated for 120 sec with a layer of gold using a sputter coater (polaron SC 502). SEM photographs were taken at room temperature (25 °C) using a low beam voltage of 20 kV and 15 kV [11].

Micromeritic Properties

Bulk Density

Bulk density of formulated microspheres was determined by taking a known mass of microspheres in a 5 ml graduated measuring cylinder. The cylinder was dropped three times from a height of one inch at an interval of two seconds. The bulk density was calculated by following equation [12].

Bulk density = Total weight of powder/Total bulk volume

Tapped Density

Tapped density is the volume of powder determined by tapping using measuring cylinder containing weighed amount of sample. Tapped density of microspheres was calculated by following equation.

Tapped density = Total weight of powder/ Total tapped volume

Compressibility Index

It is indirect measurement of bulk density, size and shape, surface area, moisture content, and cohesiveness of materials since all of them can influence the consolidation index. It is also called as compressibility index.

Compressibility index % = Tapped density – Bulk density × 100 / Tapped density

Hausner's Ratio

Hausner's ratio of microspheres is determined by comparing the tapped density to the bulk density using the equation.

Hausner's ratio = Tapped density / Bulk density

***In vitro* Drug Dissolution**

USP 23 type-2 rotating paddle dissolution test apparatus (electrolab, EDT-08Lx) was used to study the *in vitro* drug dissolution. 900 ml phosphate buffer (pH 6.8) at 37.5 ºC stirred at 100 rpm was used as the dissolution medium. 100mg equivalent of drug samples of microspheres were placed in the dissolution medium. Samples (1 mL) were withdrawn at pre-determined time intervals (1,2,3,4,5,8,10,12 and 14 hrs) and replaced with equal volumes of dissolution medium. Samples were filtered through 0.46µm filter and appropriately diluted with phosphate buffer (PH 6.8) and analysed UV spectrophotometrically at 276nm. Drug release mechanism was determined by finding the best fit of the release data of Higuchi and Korsmeyer-peppas plots[13].

Stability Studies

The stability of a drug product is the ability of a particular formulation, in a specific container, to remain within its physico-chemical, therapeutic and toxicological specifications. Stability testing provides evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity, and light and enables recommended storage conditions, retest periods, and shelf lives to be established [14].

RESULT AND DISCUSSION

A total of 9 formulations, MM1, MM2, MM3, MM4, MM5, MM6, MM7, MM8, and MM9 were prepared using HPMC and Carbopol by solvent evaporation technique using distilled water as continous phase. Use of water as a solvent was the reason for the long duration of drying time during the formulation step.

Partical Size Analysis, Percentage Yield, Entraptment Efficiency and Scanning Electron Microscopy

The particle size of Terbutaline Sulphate microspheres was analyzed by optical microscopy. The average particle size was found to be in the range of 128.80 ± 11.12 to 152.42 ± 17.13 µm. The average particle size of microspheres was found to be increased as the concentration of the polymer was increased. This may be due to increased coat thickness with increasing polymer proportion. Particle size of the microspheres was large. The particle size shown in table.

Micromeritic Properties

The Micromeritic studies revealed that the microspheres have better flow properties which indicate the microspheres produced are spherical and non-aggregated. The bulk density, tapped density, compressibility index, angle of repose and hausner's ratio for all formulations i.e. MM1 to MM9 were found to be in the range of 0.63±0.03 to 0.82±0.07, 0.131±0.004 to 0.212±0.007, 28.31±5.09 to 17.78±8.89 and 35.78±1.30 to 28.30±0.43 and 0.20±0.133 to 0.25±0.10 respectively. All the formulations showed excellent flowability as expressed in term of Micromeritic parameters. The results are shown in table.

Table-2: Micromeritic properties

Batch	Mean Particle[a] (µm)	Bulk Density[b] (g/cm^3)	Tapped Density[b] (g/cm^3)	Compresiibility Index [b] (%)	Angle of Repose (Θ)	Hausners Ratio
MM 1	128.80±11.12	0.63±0.03	0.131±0.004	28.31±5.09	35.78±1.30	0.20±0.133
MM 2	140.28±12.15	0.83±0.05	0.210±0.006	23.54±2.08	31.68±2.56	0.25±0.12
MM 3	165.15±15.32	0.91±0.06	0.221±0.003	14.32±5.00	34.64±2.23	0.24±0.05
MM 4	135.18±12.82	0.75±0.02	0.154±0.002	25.56±4.67	35.84±1.78	0.20±0.10
MM 5	160.16±17.87	0.81±0.06	0.231±0.006	10.23±7.03	32.89±0.07	0.28±0.10
MM 6	170.21±19.17	0.89±0.01	0.221±0.004	19.87±8.90	23.36±0.24	0.24±0.40
MM 7	154.52±15.18	0.91±0.02	0.276±0.011	26.58±2.09	37.32±1.45	0.30±0.55
MM 8	167.56±15.40	0.83±0.04	0.209±0.019	13.23±1.56	32.27±1.87	0.25±0.47
MM 9	152.42±17.13	0.82±0.07	0.212±0.007	17.78±8.89	28.30±0.43	0.25±0.10

Entrapment efficacy

Table-3: Determination of Entrapment Efficiency

Formulation code	Theotical drug content (mg)	Practical drug content (mg)	% Drug content
MM1	7.31	5.23	71.54
MM2	5.12	5.11	99.80
MM3	3.61	1.66	46.12
MM4	4.19	1.52	36.42
MM5	2.70	1.24	46.03
MM6	2.44	1.52	62.48

Surface Morphology by Scanning Electron Microscopy (SEM)

SEM was performed for morphological characterization of microspheres. The microspheres were found to be discrete, spherical and free flowing. The SEM photographs indicated that the microspheres were spherical and completely covered with the coat polymer.

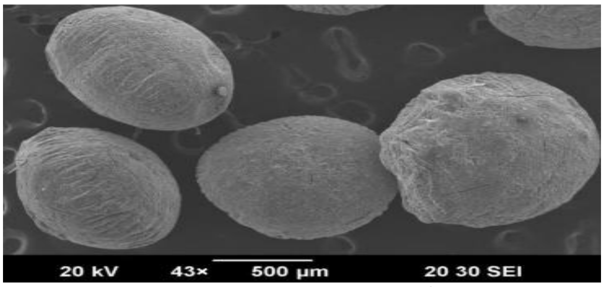

Fig-1: Scanning Electron Micrographs of Terbutaline

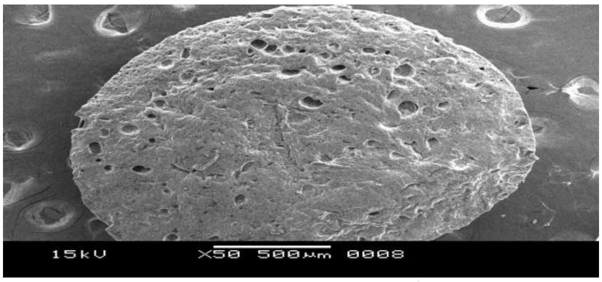

Fig-2: Scanning Electron Micrographs of Mucoadhesive Microshperes MM1

Fig-3: Scanning Electron Micrographs of Muco-Adhesive Microshperes MM2

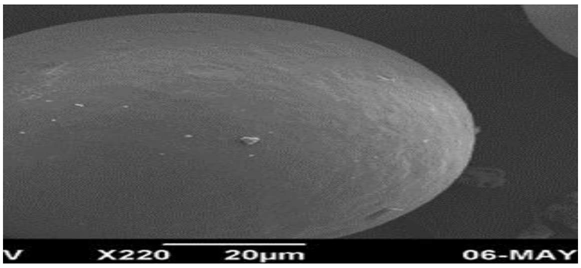

Fig-4: Scanning Electron Micrographs of Mucoadhesive Microhperes MM3

In-vitro Drug Release Study

Terbutaline Sulphate release from the microspheres was studied at pH 6.8 buffer solution for 16 hours. Terbutaline Sulphate release from the microspheres was slow and depended on the composition of the coat. Microspheres of a sodium alginate carbopol 934 P gave relatively slow release when compared to others. The order of increasing release rate observed with various microspheres was sodium alginate-carbopol 934 P < sodium alginate-chitosan < sodium alginate-HPMC. It has been also found that the release rate was decreased with the increasing ratio of sodium alginate in the coat composition.

Table-4: Comparative drug release profile of MICROSPHERES MM1, MM2, AND MM3

S. No	Time (Hours)	%Cumulative drug release MM1	%Cumulative drug release MM2	%Cumulative drug release MM3
1	0	0	0	0
2	1	2.0	2.8	8
3	2	16.0	16.7	23.5
4	3	28.1	24.6	31.4
5	4	36.4	32.5	40.4
6	5	45.3	41.4	45.6
7	6	53.7	46.7	49.4
8	7	60.4	53.0	56.3
9	8	67.4	58.2	60.5
10	9	75.0	65.2	64.3
11	10	83.1	70.4	70.4
12	12	92.3	86	78.7
13	14	95.6	87.1	86.5
14	16	97.6	91.3	94.4

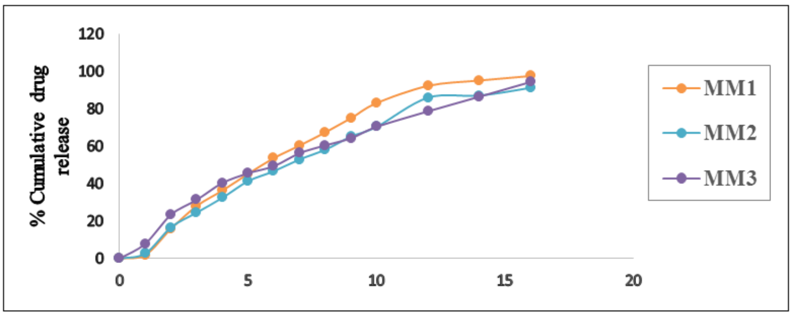

Fig-5:Cumulative Drug Release of Microspheres MM1, MM2, MM3

Stability Study

Terbutaline Sulphate microspheres were filled in high-density polyethylene HDPE containers at 30˚C / 60% RH, 35˚C / 60% RH and 45˚C / 75% RH for 2 months as per ICH specifies the length of study and storage conditions. After each month microspheres were evaluated for their drug entrapment efficiency and drug release study. From the studies it was found that the formulation was stable since there was no difference in the drug entrapment efficiency and drug release pattern of the formulation.

Table-5: Incorporation efficiency data of formulation MM3 for stability study

S. NO.	Condition	Time		
		Zero month	**First month**	**Second month**
1	30 ºC / 60% RH	71.00	71.8	72.6
2	35 ºC / 60% RH	71.00	70.3	71.7
3	45 ºC / 75% RH	71.00	70.5	71.3

CONCLUSION

The terbutaline sulphate microspheres were prepared successfully by solvent evaporation technique using combination of novel polymer and the in-vitro release studies have shown that better release profile with combination of polymers especially with increase in carbopol concentration.

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