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Research Article

PREPARATION, CHARACTERIZATION AND OPTIMIZATION OF THIOPECTIN BASED MUCOADHESIVE MICROSPHERES OF SALBUTAMOL SULPHATE

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ABSTRACT

The present research aimed at formulation and evaluation of the mucoadhesive microspheres forsalbutamol sulphate using thiolated pectin as a polymer for controlled release dosage form by optimization technique using software-based response surface methodology. In current investigation preparation of salbutamol based mucoadhesive microspheres was optimized using two important process variables i.e. drug: polymer ratio and volume of glutaraldehyde. The desired responses are particle size and drug entrapment efficiency. Optimization was done by fitting experimental data to the software programme (Design Expert® 9 trial version). The microspheres were evaluated for physicochemical parameters as shape, size, Fourier Transform-Infrared (FT-IR), XRD and% entrapment efficiency. SEM studies showed that the microspheres were in a spherical form. The Entrapment efficiency of salbutamol microspheres was found to be 61 ± 1.34%. Further it was found that optimum % Entrapment efficiency and Particle size can be achieved by using best possible combination of Drug: Polymer ratio and volume of cross-linking agent. The *in vitro* drug release estimated in 0.1N HCl (pH 1.2) after 8 hours was found to be 81.65 ± 1.301%. The release studies showed the drug release was increased with thiolated pectin formulation as compared to the formulation prepared by pectin.

Keywords: Salbutamol sulphate, thiolated pectin, mucoadhesive, microspheres

INTRODUCTION

Mucoadhesive drug delivery system has recently gained great concern in pharmaceutical sciences[1]. The concept of mucoadhesive was introduced in the early 1980s[2]. The key goal of using mucoadhesive formulations is to enhance the bioavailability of drug by achieving a significant increase in the residence time of the drug at the site of absorption hence decreasing chances of first pass metabolism. The rapid absorption because of enormous blood supply and good blood flow rates. Biodegradable polymers are utilized for development of mucoadhesive sustainable drug delivery system. A large number of recent literature reports has indicated the importance of target specific drug delivery systems in revolutionizing the medical and pharmaceutical field[3-5]. Microspheres as novel drug delivery systems can be prepared using various methods and has an advantage of target specific drug delivery[3]. However, their short residence time at the site of absorption limits its application in product development. So, it would be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes[6-9]. Thus, bio adhesive microspheres offer better absorption and improved bioavailability due to a high surface-to volume ratio and specific targeting of drugs to the absorption site is an additional advantage.[10-13].

Salbutamol sulphate is a direct-acting β2 selective sympatho mimetic used in the management of asthma as bronchodilators and is given to patients having swallowing difficulties accompanied by nausea and vomiting[14]. The plasma half-life of the drug has been estimated to range from 4 to 6 hours and having 40% bioavailability[15] The rationale behind this work was to design a sustained release formulation which remains at the absorption site for an extended period of time by developing mucoadhesive microspheres of Salbutamol sulphate using Thiolated pectin as polymer and to increase the bioavailability and reduce dosing frequency and improve patient compliance.

MATERIALS AND METHODS

Salbutamol Sulphate, Pectin, Thioglycolic acid, HCL, Glutaraldehyde, NaOH, Span 60, Cyclohexane, Chloroform

Synthesis of thiolated pectin by esterification of pectin

Thiolated pectin was carried out by esterification of pectin with thioglycolic acid in the presence of HCL. Pectin (16 g) was dissolved in hot water. In this solution 80% v/v thioglycolic acid and 2 ml of 7 N HCL was added. After that above reaction was allowed to react 150 min at 80^0C. The above reaction mixture was poured in excess of methanol. White precipitates of Thiolated pectin were obtained. Precipitates were washed twice with methanol and dried at room temperature[4].

Preparation of microspheres by emulsion cross linking method

Salbutamol loaded microspheres were prepared by emulsion cross linking method. The solution of Salbutamol Sulphate and pectin (1:4) and 5 g of 2 M NaOH was prepared and kept under mechanical stirrer for uniform mixing. To this mixture cyclohexane and chloroform (4:1v/v) was added containing span 60v/v and this emulsion was stirred for 3 min under homogenizer. After this add glutaraldehyde (3 ml) and kept this mixture under magnetic stirrer at 1000 rpm for 18 hours at 40^0C. Prepared microspheres were isolated by centrifugation and washed twice

with cyclohexane and distilled water. Microspheres were dried under desiccators[16].

Experimental design

For optimization of microspheres response surface methodology was used, total sixteen formulations were prepared in which drug: polymer and volume of cross liking agent were varied, and all other parameters were kept same. The formulations were based on 2^2 factorial design. The design includes two factors which were evaluated at two levels. In this study, drug: polymer ratio (X1) and volume of cross-linking agent i.e. glutaraldehyde (X2) were selected as independent variables. The % entrapment efficiency and particle size were selected as dependent variables as shown in Table 1.

Table 1: Variables in 2^2 factorial design

Variables	Low (-1)	High (+1)
Drug : polymer	1:2	1:4
Volume of cross-linking agent glutaraldehyde (ml)	2	4

Table 2: Formulation of mucoadhesive microspheres of Salbutamol: F1-F16

S. No.	Formulation Code	Drug (mg)	Polymer (mg)	Volume of glutaraldehyde (ml)
1	F1	150	289	4
2	F2	150	328	2.2
3	F3	150	232	3.3
4	F4	150	312	3.1
5	F5	150	256	2
6	F6	150	400	4
7	F7	150	400	2
8	F8	150	200	4
9	F9	150	400	2.9
10	F10	150	312	3.1
11	F11	150	256	2
12	F12	150	312	3.1
13	F13	150	400	4
14	F14	150	266	2.6
15	F15	150	200	2.6
16	F16	150	200	2.6

Characterization of optimized formulation

Surface morphology

Surface morphology of Salbutamol microspheres was studied by using scanning electron microscope (Jeol JSM 6360, Japan). Dried powders were mounted onto separate, adhesive coated aluminum stubs. Excess powder was removed by tapping the stubs sharply and then gently blowing a jet of particle free compressed gas across each. The SEM was operated at high vacuum with accelerate voltage of 10 kV [17].

Attenuated Total Reflectance (ATR)

ATR spectroscopy (Bruker alpha USA) determines the confirmation of drug in the formulation. The procedure consists of placing the powdered sample (pure Salbutamol, Thiolated Pectin and Salbutamol microspheres preparation) and the spectrum was recorded in the region of 3800-600 cm^{-1}.

Particle Size

Particle sizes of the prepared microspheres were determined by an optical microsphere and the mean particle size was calculated by measuring 50 microspheres with the help of calibrated ocular microscopy.

Determination of drug encapsulation efficiency

10 mg microspheres were crushed and dissolved in 10 ml of water. This solution was kept for 3 hours stirring. After 3 hours solution was filtered by what-man filter paper. The filtered solution was analyzed spectrophotometrically at 276 nm. Each experiment was repeated in triplicate. Percentage drug entrapment was determined by the following formula[18].

$$\% \text{ Entrapment efficiency} = \frac{\text{Actual Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

X-Ray Diffraction (XRD)

The X-Ray Diffraction was done to monitor the changes in crystallinity characteristics of the drug when the drug was loaded into the microspheres. An XRD pattern was measured using X-Ray Diffractometer (Bruker, Germany). The diffracted intensities were recorded from 5 to 80^0 at 2 θ angles.

***In-Vitro* drug release**

The *In-Vitro* release study of the microspheres was determined by USP rotating basket method at 50 rpm at 37°C. Dissolution study was performed in 0.1 N HCL taking 900 ml for each study. 500 mg of the microspheres was placed in the dissolution medium and test samples were taken from the medium at predetermined time intervals over a period of 8hours and the samples were analyzed for drug release by UV spectrophotometer at 276 λmax.

RESULTS AND DISCUSSION

Results of different formulation of Salbutamol microspheres

The effects of drug: polymer ratio and volume of glutaraldehyde on selected variables from the respective experimental formulations are as shown in Table 3.

Table 3: Particle size and drug entrapment efficiency of mucoadhesive microspheres of Salbutamol: F1-F16

Formulation code	Particle size (µm)	% entrapment efficiency (%)
F1	0.402	60
F2	0.288	68
F3	0.342	52.5
F4	0.486	65.3
F5	0.432	65
F6	0.234	71
F7	0.342	64
F8	0.258	63
F9	0.240	56.5
F10	0.360	55.5
F11	0.594	51.5
F12	0.366	57.5
F13	0.324	57.5
F14	0.636	51.5
F15	0.174	63.5
F16	0.336	61

Effect of drug polymer ratio on responses

Response plots (Figure 1 and Figure 2) showed that on increasing the concentration of drug-polymer ratio, the particle size was decreased. It means greater the drug polymer ratio smaller will be the particle size of microspheres. On the other hand, as the ratio of drug-to-polymer increases, encapsulation efficiency increased. So, F6 formulation is having high drug polymer ratio resulting in small particle size (0.234 µm) and high drug entrapment efficiency (71%). This is due to higher ratio of drug- to-polymer would produce small size droplets with increase surface area.

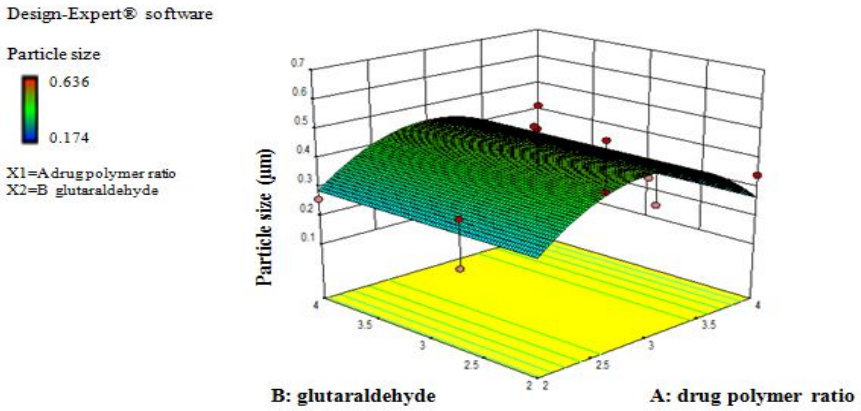

Figure 1: Response Surface Plot for Particle size

Effect of volume of glutaraldehyde on responses

Response plots (Figure 1 and Figure 2) showed that % drug entrapment was increased with increasing the volume of cross-linking agent i.e. glutaraldehyde. This can be due to higher degree of crosslinking occurred by higher concentration of glutaraldehyde. On the other hand, as the volume of glutaraldehyde was increased particle size of microspheres was decreased. So, F6 formulation is having high concentration of glutaraldehyde resulting small particles size and high drug entrapment efficiency. This can be due to an increase in the volume of glutaraldehyde increase the efficiency of the stirrer and decrease the viscosity of due oil phase that may result in decreasing the particle size.

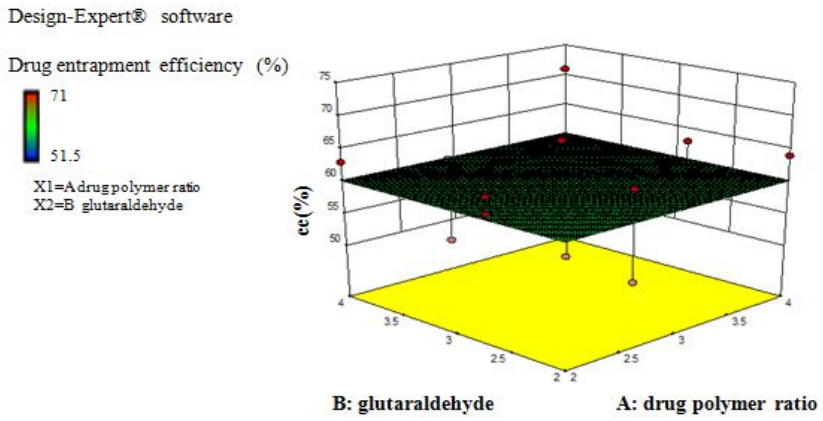

Figure 2: Response Surface Plot for Drug Entrapment Efficiency

Attenuated Total Reflectance (ATR)

The confirmation of the drug in the formulation was carried out by ATR (as shown in Figure 3) (A) spectra of pure drug (Salbutamol), (B) Blank Microspheres, (C) Drug loaded Microspheres ATR of Salbutamol shown peaks at 3464 cm^{-1} (O-H bonding), 3144 cm^{-1} (N-H stretching), 1616 cm^{-1} (C=C aromatic ring stretching), 2949 cm^{-1} (aromatic C-H stretching), 1496 cm^{-1} (C-N stretching). Similar peaks were seen in Salbutamol loaded microspheres but these peaks are not found in blank microspheres as shown in Table 4.

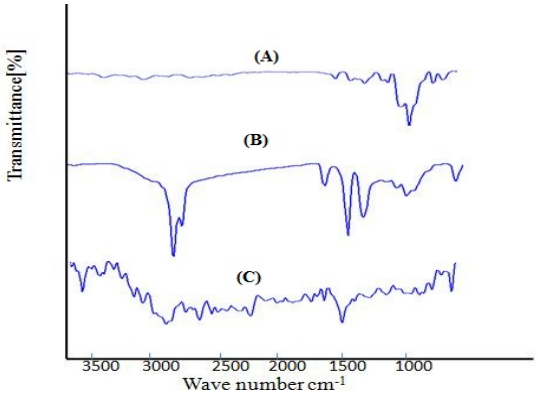

Figure 3: IR Spectra of (A) Pure Salbutamol (B) Blank Microspheres (C) Salbutamol Loaded Microspheres

Table 4: Interpretation of IR Peaks

Sample	Peaks cm^{-1}	Assignment
(A) Salbutamol	3464	O-H
	3144	N-H
	1616	C=C
	2949	C-H
	1496	C-N
(B) Blank Microspheres	3696	O-H
	1737	C=O
	1093	C-O
(C) Salbutamol Loaded microspheres	3423	O-H
	3186	N-H
	1689	C=C
	2991	C-H
	1435	C-N
	2619	S-H

Percentage Entrappment Efficiency

The encapsulation efficiency of the optimized formulation was calculated to be 61.34 ± 1.34%.

Surface morphology

Surface Morphology of Salbutamol microspheres was examined by using a Scanning Electron Microscopy (SEM) (Hitachi, Japan MSW-301). The result showed that the microspheres were in a spherical form and had an average size less than 100 µm. Salbutamol microspheres have smooth spherical shaped appearance (Figure 4).

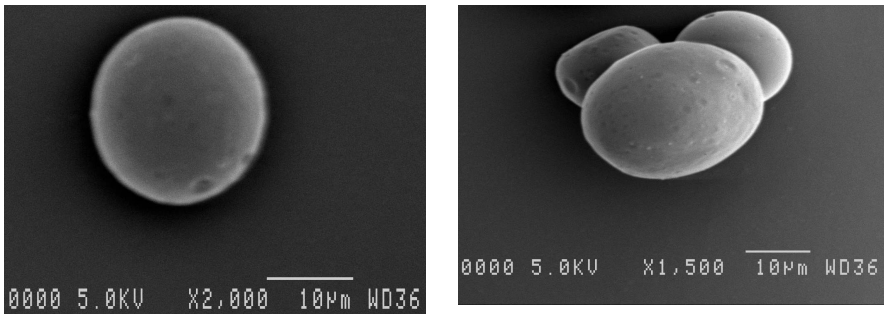

Figure 4: Scanning Electron Microscopy of Salbutamol loaded microspheres showing the spherical morphology and size range less than 100 µm

Particle size

The Average Particle size of Salbutamol loaded microspheres were calculated as 23.52 µm (Figure 5)

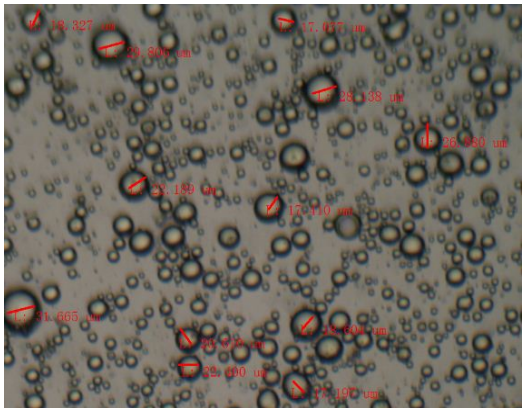

Figure 5: Salbutamol loaded microspheres showing average particle size of 23.52 µm

X-Ray Diffraction (XRD)

Figure 6 shows X-ray diffraction patterns of (A) Salbutamol Sulphate, (B) Blank microspheres and (C) Salbutamol loaded Thiolated pectin microspheres. The peaks on X-ray diffractogram indicate the crystalline form of Salbutamol Sulphate pure form, but these peaks were not observed in Thiolated pectin microspheres. The microspheres showed peaks of Salbutamol Sulphate having less intensity than pure form representing complete entrapment of drug within the polymer.

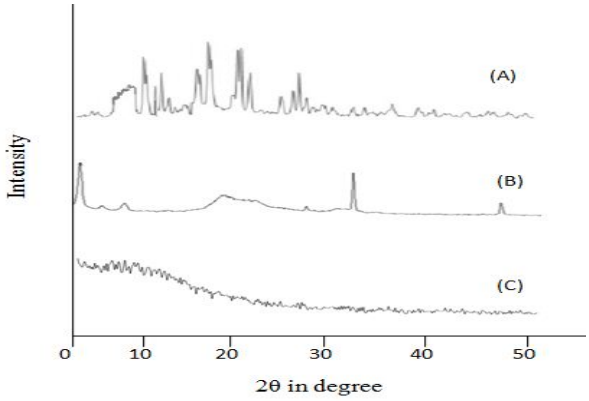

Figure 6: X-Ray Diffractograms of (A) Salbutamol Sulphate (B) Blank microspheres (C) Drug loaded Thiolated pectin microspheres

***In-vitro* drug release**

The *In-Vitro* release study of the microspheres was determined by USP rotating basket method at 50 rpm at 37ºC. *In vitro* drug release with pectin showed 66.98 ± 1.207% of drug release within 8 hours. On the other hand, *in vitro* release with thiolated pectin showed 81.65 ± 1.265% of drug release within 8 hours. The formulation developed from thiolated pectin enhanced the bioavailability of Salbutamol sulphate. Thus, results suggested thiolated pectin controlled the drug release.

Table 5: Salbutamol released from the microspheres prepared using pectin and thiolated pectin

S. No.	Time (hours)	% Drug Release	
		Salbutamol loaded pectin microspheres	Salbutamol loaded thiolated pectin microspheres
1	0	0 ± 0.00	0 ± 0.00
2	30 min.	4.57 ± 0.245	9.36 ± 0.454
3	1	10.65 ± 0.479	14.41 ± 0.587
4	2	19.63 ± 0.404	23.71 ± 0.371
5	3	27.41 ± 0.532	31.78 ± 0.644
6	4	39.96 ± 0.735	43.06 ± 1.210
7	5	46.34 ± 0.976	52.01 ± 1.011
8	6	55.14 ± 1.076	59.12 ± 1.375
9	7	60.64 ± 1.107	71 ± 1.276
10	8	66.98 ± 1.143	81.65 ± 1.301

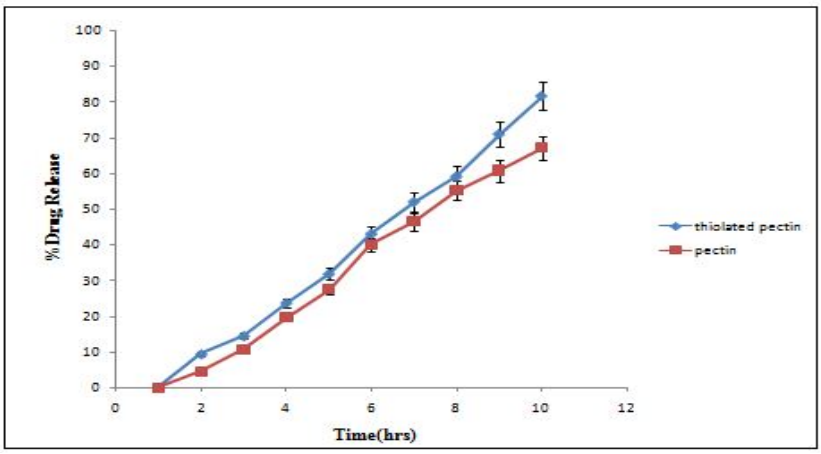

Figure 7: Dissolution profile of Salbutamol microspheres (values represent mean ± SD)

CONCLUSION

This study focused on formulation of controlled release microspheres using Thiolated Pectin polymers to enhance its stability and bioavailability and *In-Vitro* release of Salbutamol. The microspheres were evaluated like particle size, entrapment efficiency, XRD, SEM, stability study and percent drug release after 8 hours. Based on these parameters, it was concluded that formulation with Thiolated Pectin showed superior results compared to other formulations.

In addition to this, optimization was carried out by using design of experiments (Design expert 9.0). This optimized formulation was filled into tightly closed glass vials and subjected to stability testing according to ICH guidelines. The packed container of the microspheres was kept under accelerated condition of 40 ± 2°C/75 ± 5% RH in a programmable stability chamber (Tanco manufacturer and exports) for a period of six months. The result of stability study indicated that the formulation was stable at accelerated condition. Hence the product shows good stability at room temperature.

The comparison of dissolution profiles of pure drug, Pectin and thiolated Pectin in 0.1 N HCl shows significant effect on %drug release. *In-Vitro* release from pure drug is found to be 99.53 ± 0.594% within 6 hours which results the faster elimination of the drug through the body and having short half-life. *In-Vitro* release with pectin shows 81.65 ± 1.301% of drug release within 8 hours due to its less mucoadhesive property. While *In-Vitro* drug release with Thiolated Pectin is found to be 66.98 ± 1.143% of drug release within 8 hrs. Thiolation of Pectin polymer enhances the mucoadhesive property by attaching thiol side chain in the polymer structure. On the basis of this study it can be concluded that Thiolated Pectin is promising mucoadhesive polymer for oral controlled delivery of Salbutamol sulphate.

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