Carbohydrate Polymers 102 (2014) 529–536

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation, characterization and pulmonary pharmacokinetics of xyloglucan microspheres as dry powder inhalation

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ARTICLE INFO

Article history:
Received 8 August 2013
Received in revised form 19 October 2013
Accepted 27 November 2013
Available online 4 December 2013

Keywords:
Xyloglucan
Mucoadhesion
Spray drying
Microspheres
Pulmonary

ABSTRACT

The purpose of the present investigation was to prepare microspheres using xyloglucan as the polymer and to evaluate them for pulmonary delivery of Montelucast. Xyloglucan microspheres were prepared using spray-drying technique employing lactose monohydrate as carrier for dry powder inhalation. The formulation of microspheres was optimized using response surface methodology. Multiple response surface simultaneous optimizations using desirability approach were use to find optimal formulation. In vitro characterization of optimized microsphere formulation demonstrates its suitability as dry powder inhalation. It found to provide high and prolonged drug concentration within lungs after pulmonary administration in animal model.

1. Introduction

Currently, there is particular research interest in pulmonary delivery of drugs, specially peptides, proteins and genes, not only for local, but also for systemic effect. This is due to the large alveolar surface area suitable for drug absorption, the low thickness of the epithelial barrier, extensive vascularization and relatively low proteolytic activity in the alveolar space compared to other routes of administration and the absence of the first-pass metabolism (Patton & Platz, 1992; Clark, 2002; Courrier, Butz, & Vandamme, 2002). In general, microparticle delivery to the lungs is an attractive concept because it can cause retention of the particles in the lungs accompanied with a prolonged drug release (Edwards et al., 1997). Despite of these advantages, the success of the pulmonary delivery of any therapeutic molecules depends on various factors such as the effect of the airways structure, mucocilliary clearance and phagocytosis by alveolar macrophages (Courrier et al., 2002; Hastings, Folkesson, & Matthay, 2004). Thus, to assure optimal drug delivery to the desired site considering all above factors, it is quite difficult to develop the appropriate drug carriers. In this respect, specific characteristics in drug delivery are require which provide it the ability to reach the alveolar region, if a systemic effect is desired, or another specific site, if a local action is intended (Pandey & Khuller, 2005).

The use of polymers in medicine is not new and undoubtedly, natural polymers have used as herbal medicine since ages. Natural polysaccharide represents a group of polymers. The selection of suitable biocompatible materials used for the preparation of lung carriers has shown to be an essential consideration and, in this context, the polysaccharides are particularly attractive polymers. Xyloglucan is natural polysaccharide isolated from seed kernel of *Tamarindus indica*. It possesses properties like high viscosity, broad pH tolerance and adhesivity. This leads to its application as stabilizer, thickener, gelling agent in food and binder in pharmaceutical industries (Glicksman, 1986). In addition to these, other important properties of xyloglucan have identified recently. They include non-carcinogenicity, mucoadhesivity, biocompatibility, high drug holding capacity and high thermal stability. This leads to its application as excipients in hydrophilic drug delivery system (Avachat, Gujar, & Wagh, 2013). The recent advances in the dry-powder inhalation (DPI) technology have addressed some of the limitations associated with inhaled formulations, including unwanted loss of drug due to oropharyngeal deposition (Bell & Newman, 2007). However, the limitations associated with poor deposition of particles larger than 5 μm yet to overcome. To address these limitations, we have proposed the microencapsulation of drug in polymer. In the present study, a novel polymeric microparticle formulation has developed using the polysaccharide polymer xyloglucan, for prolonging the retention of anti-asthmatic agent Montelukast sodium in lungs. Spray drying technique used to produce Montelukast sodium loaded microspheres system. SEM and

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http://dx.doi.org/10.1016/j.carbpol.2013.11.036

Table 1
Factorial design (3^2) using formulation variables influencing characteristics of microspheres.

Formulation	X_1 (%)	X_2 (%)	Y_1 (%)	Y_2 (θ)	Y_3 (%)	Y_4 (min)
F1	1	0.5	79.16	34.58	77.82	109
F2	2	0.5	85.92	36.87	86.9	139
F3	3	0.5	89.40	40.23	91.78	161
F4	1	1	78.26	31.76	76.08	111
F5	2	1	88.06	29.2	86.37	139
F6	3	1	87.24	34.08	89.14	163
F7	1	1.5	75.45	30.16	74.45	115
F8	2	1.5	83.87	28.96	85.66	141
F9	3	1.5	85.39	32.04	88.59	167

X_1 – concentration of xyloglucan, X_2 – concentration of lactose; Y_1 – % entrapment efficiency, Y_2 – angle of repose, Y_3 – mucoadhesion strength, Y_4 – time for 90% drug dissolution.

XRD characterized microspheres. The optimized microspheres formulation further evaluated for in vitro dry powder inhalation (DPI) performance and pulmonary pharmacokinetics.

2. Materials and methods

2.1. Materials

Xyloglucan (47% galactose removal ratio) obtained as gift sample from DSP Gokyo Food and Chemicals, Japan. Montelukast sodium, obtained as a kind gift from Alkem Pharmaceutical Pvt. Ltd., Mumbai, India. Acetonitrile and methyl t-butyl ether were HPLC grade solvent purchased from Merck Pvt. Ltd., Mumbai, India.

2.2. Methods

2.2.1. Experimental design

A 3^2 factorial design employed for designing formulations. The concentration of polymer (xyloglucan) and dry powder inhaler carrier (lactose monohydrate) were selected as formulation variables and studied at 3 levels (i.e. High, low, and medium). All other formulation and processing variables kept invariant throughout the study. Table 1 summarizes an account of the 9 experimental runs studied, Entrapment efficiency (%) (Y1), angle of repose (θ) (Y2), mucoadhesion strength (%) (Y3) and $t_{90\%}$ (min) (Y4), were taken as response parameters as the dependent variables. The experimental design and statistical analysis of the data was done using the Design Expert® software (version 8.0.1, Stat-Ease Inc., Minneapolis, MN).

2.2.2. Preparation of microspheres using spray-drying technique

The microspheres of xyloglucan were prepared employing spray drying technique. To optimize formulation and process variables employed in formulation of microspheres, various formulations of microspheres were prepared in triplicate employing 3^2 factorial designs as depicted in Table 1. Briefly, xyloglucan was added in distilled water and dissolved by gentle heating to 50–60 °C with continuous stirring at 1000–1200 rpm for 3 h. Drug, lactose monohydrate (Kawashima, Serigona, Hino, & Yamamoto, 1998; Staniforth, 2000) was added to above polymer solution with continuous stirring using magnetic stirrer for 30 min. Microspheres were obtained by spraying the feed with spray drier (LU222, Labultima, India) using a standard 0.7 mm nozzle. The dispersion was fed to nozzle with a peristaltic pump, atomized by the force of compressed air and blown together with heated air to the drying chamber where the solvent in the droplets is evaporates. The dried microspheres were harvest from the apparatus collector. The process conditions of the spray drying were inlet temperature 100–120 °C; outlet temperature 80–90 °C; feed pump setting of 4–6 ml/min; spray pressure 2 kg/cm^2 and aspiration 45–50%.

2.2.3. Evaluation of xyloglucan microspheres

The evaluation of xyloglucan microspheres were evaluated for their angle of repose (θ), particles size, entrapment efficiency, in vitro mucoadhesion and % in vitro drug dissolution (t_{90}%).

2.2.4. Angle of repose

The angle of repose determine by the funnel method. The accurately weighed powder kept in funnel. Height of the funnel adjusted in such a way that the tip of the funnel just touches the apex of the heap of the powder. The powder allowed to flow-through the funnel freely onto the surface. The diameter of the powder cone was measured (Lachmann, Lieberman, & Kanig, 1990).

The angle of repose calculated using the following Eq. (1).

$$\tan\theta = \frac{h}{r} \quad (1)$$

where, h height and r radius of the powder blend.

2.2.5. Particle size analysis

The microspheres were placed on the glass slide, Particle size of microspheres was measured using a Motic DMWB2-223 digital microscope fitted with a 1/3 CCD Camera imaging accessory and using Motic Images 2000 (1.3 Version) image analysis software. The images of microspheres were analyzed for their average diameter and shape.

2.2.6. Entrapment efficiency

The weighed amount of microspheres were dissolved in distilled water and kept overnight in orbital shaker. The drug content was measured spectrophotometrically (UV 1700, Shimadzu, Japan) at 285.5 nm for Montelukast sodium. The entrapment efficiency (%) calculated using following Eq. (2).

$$\text{Entrapment efficiency}\,(\%) = \frac{M_{\text{actual}}}{M_{\text{theoritical}}} \times 100 \quad (2)$$

2.2.7. In vitro mucoadhesion

In vitro mucoadhesion test was performed using modified falling liquid film technique to determine the mucoadhesive property of microspheres (Saraparn, Vimolmas, Narueporn, & Garnpimol, 2006). A freshly cut piece, 5 cm long, of sheep lung mucosa obtained from a local abattoir within1 h of killing the animal was cleaned by washing with isotonic saline solution. An accurate weight (100 mg) of microspheres were placed on mucosal surface, which was attached over a polyethylene plate that fixed in an angle of 45° relative to the horizontal plane, and distilled water at 37 °C was pumped at a rate of 5 mL/min over the tissue. After 1 h, the concentration of the drug in the collected perfusate was determined spectrophotometrically.

$$\%\,\text{Mucoadhesion} = \frac{A-B}{A} \times 100 \quad (3)$$

where *A*, actual amount of drug in applied microspheres; B, amount of drug in wash out liquid.

2.2.8. In vitro drug dissolution

The in vitro drug release from all spray-dried formulations investigated using dissolution study. An accurately weighed amount of microspheres equivalent to 10 mg of Montelukast sodium were added to dialysis bag (average molecular weight – 12 kDa) suspended in 700 ml of dissolution medium containing 0.5% SDS in water at 50 rpm and 37 °C using the USP rotating paddle dissolution apparatus (Electrolab TDT-08L plus, Dissolution tester USP Mumbai, India) (Shah, 2011). The samples were withdrawn from the dissolution medium at various time intervals up to 3 h. About 5 ml of sample collected and diluted to 10 ml with dissolution medium and subjected to UV spectrophotometric analysis (UV Shimadzu 1700) at λ_{max} 285.5 nm.

2.3. Characterization of optimized microspheres formulation

2.3.1. Scanning electron microscopy

The surface morphology of microspheres was examined by scanning electron microscopy (JSM 6390, Japan). Samples of microspheres were dusted on double sided tape on an aluminum stub and coated with gold using a cold sputter coater to a thickness of 400 Å, and then imaged using a 20 kV electron beam.

2.3.2. Zeta potential measurement

The microspheres dispersed in deionized water. This dispersion was filled in zeta cell and zeta potential was determined using Zeta Sizer (Nano ZS 90, Malvern Instruments, UK) with the help of software.

2.3.3. Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) of pure drug, blank microspheres and drug loaded microspheres were conducted using differential scanning calorimeter (DSC 822c, Mettler Toledo,) at heating rate of 10 °C/min over a temperature range of 30–300 °C under an inert atmosphere flushed with nitrogen at a rate of 20 ml/min.

2.3.4. X-ray diffraction study (XRD)

The X-ray diffraction (XRD) pattern of pure drug, blank microsphere and drug-loaded microsphere recorded on an X-ray diffractometer (Brucker AXS, D8 Advance, Germany). The samples irradiated with monochromatized Cu *K* radiation and analyzed between 3° and 80° (2θ). The voltage and current used were 30 kV and 30 mA, respectively (Jain, Chauk, Mahajan, Tekade, & Gattani, 2009).

2.3.5. In vitro assessment of developed dry powder inhaler

Formulations were subjected to Andersen cascade impactor (ACI, SS 316, Copley Scientific Ltd.) studies to determine mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) (De Boer, Hagedoorn, Gjaltema, Goede, & Frijlink, 2003; Jensen et al., 2010; Muttil et al., 2007). ACI utilizes eight jet stages enabling classification of aerosols from 9 μm and above to 0.4 μm (at 60 L/min) and allows airborne particulates to impact upon stainless steel impaction surfaces. A final filter collects all particles smaller than 0.4 μm. The dry powders of microspheres aerosolized using a dry powder inhalation device (Rotahaler®) containing 10 mg/capsule of Montelukast sodium. Test conducted at flow rate of 60 L/min for 4 s. For each test formulation, amount particle was determined and analyzed for drug content by UV spectrophotometer (UV-170, Shimadzu, Japan) at 285.5 nm. Starting at the filter, a cumulative mass deposition (undersize in percentage) vs. cut-off diameter of respective stages was derived, the mean mass aerodynamic diameter (MMAD), geometric standard diameter (GSD), fine particle dose (FPD), % fine particle fraction (%FPF) and % mass balance was calculated (Kundawala, Vishnu, & Patel, 2011; Naikwade & Bajaj, 2009). FPF considered as directly proportional to the amount of drug able to reach the pulmonary tract in vivo: consequently, higher the percentage of FPF, deeper the estimated lung deposition will be.

2.3.6. Pulmonary pharmacokinetics

All animal experiments were approved and performed in accordance with the guidelines of by Institutional Animal Ethics Committee of R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur (Registration No: 651/02/C/CPCSEA under CPCSEA, India).

The rats (Male Wistar, average weight 250–270) were divided in two groups were anesthetized with an intraperitoneal injection of urethane (1 g/kg, 25% (w/v)) and kept on a heating pad to maintain the body temperature. For administration trachea was exposed by blunt dissection sternohyoideus muscle and small midline incision was made over the trachea between the fifth and sixth tracheal rings using a 20 gauze needle followed by cannulated with PE200 tubing (5–7 cm) with the tip positioned approximately at the bifurcation of trachea. Formulation (dose equivalent to 1 mg/kg) was administered to the group I by inserting PEG tubing into, cannula which is attached with the micro syringe having 16 gauge needle. The powder was place at the tip of needle and 4–5 actuations of air pump made to ensure the complete delivery of dose. The rats held at 45° position during intratracheal administration. Pure drug (1 mg/kg) was administered intratracheally to group II in similar manner.

After administration, the rats were sacrifice humanely at different time intervals and then lungs harvested by surgical resection. Both lung lobe was quickly rinsed with saline and blotted up with filter paper get rid of blood-taint and macroscopic blood vessels as much as possible and weighed. At each time point, 3 rats were taken for measurements. Lungs homogenate samples were stored for up to 48 h in a deep freezer (−70 °C) until HPLC analysis (Misra, Chougule, & Padhi, 2007; Beinborn, Wiederhold, & Hugh, 2012). The lung tissue samples were homogenized with 1% (w/w) of saline in a tissue homogenizer (Teflon homogenizer). To 200 μl of lung homogenate, 50 μl of the IS (40 ppm) was spiked and vortex mixed for 30 s. Then 0.5 ml of acetonitrile was added and vortex-mixed for 1 min. The sample was centrifuge at 50,000 rpm for 10 min at 25 °C in an ultracentrifuge (Mukherjee, Sinha, & Pattnaik, 2013). The supernatant layer (0.75 ml) was transferred to a 15 ml glass test tube, and then 4.5 ml of extraction solvent, methyl t-butyl ether were added (Chauhan, Rani, Nivsarkar, & Padh, 2006). The sample was vortex-mixed for 3 min using a multi-tube vortex mixer and evaporated to dryness under a stream of nitrogen at room temperature. The dry residue reconstituted with mobile phase and analyzed by HPLC.

The chromatographic separations perform at ambient temperature with a reversed-phase; 150 mm × 4 mm base specific column packed with 5 μm C18 silica reversed-phase particles (Eclipse XDB, 5 μm, 4.6 mm × 150 mm, Agilent 1200 series, Singapore). The mobile phase was a mixture of ammonium acetate buffer–acetonitrile (20:80, v/v) pumped at a flow-rate of 1.0 mL/min (Quaternary Pump, Model G1354A, Agilent 1200 series). Detection performed at a wavelength of 285 nm (Ultra Violet variable wavelength detector, Model G1315D, Agilent 1200 series Diode Array Detector) (Bharathi, Hotha, Jagadeesh, Mullangia, & Naidub, 2009).

2.4. Statistical analysis of the data and validation of optimization model

Various RSM computations for the current optimization studies perform employing Design Expert Software (Version DX 8, Stat-Ease Inc, Minneapolis, MN). Fitting a multiple linear regression model to a 3^2 factorial design gives a predictor equation incorporating interactive and polynomial term to evaluate the responses. The general form of multiple linear regression analysis represented in Eq. (4):

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2 \quad (4)$$

where Y is the measured response associated with each factor level combination; b_0 is an intercept representing the arithmetic average of all quantitative outcomes of nine runs; bi (b_1, b_2, b_{11}, b_{12} and b_{22}) are regression coefficients computed from the observed experimental values of Y and X_1 and X_2 are the coded levels of independent variables. The terms X_1X_2 represent the interaction terms. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms show how the response changes when two factors were change simultaneously. The polynomial equation was used to draw conclusions after considering the magnitude of coefficients and the mathematical sign it carries, i.e. positive and negative. A positive sign signifies a synergistic effect, whereas a negative sign stands for an antagonistic effect. Statistical validity of the polynomials established on the base of ANOVA provisions of the design expert software. To study the combined effect of different variables on the response, 3-D response surface plots were generate using the Design Expert Software. The optimum values of dependent variables to achieve the desired response calculated using the numerical optimization tool along with desirability approach.

3. Results and discussion

Xyloglucan is an anionic polysaccharide, which is soluble in water, and shows temperature induced gelation in range 30–40 °C. Dry powders based on xyloglucan were usually prepared by spray-drying using GRAS (generally recognized as safe) excipients. Lactose is the only excipient that is FDA-approved for inhalation because it is a safe pharmaceutical excipient, readily available, physico-chemically stable and compatible with the majority of small molecular weight drugs.

Earlier studies on development preparation of microspheres as dry powder inhalation by spray drying technique have demonstrated that entrapment efficiency, flow property, mucoadhesion and drug dissolution are significantly influenced by the concentration of polymers and carrier. Entrapment efficiency is important factor, which governs to drug content in DPI. Angle of repose (θ) of powder is important parameters, which determine its flow property. Good flow properties are desirable for handling dry powder inhalation and for good flow; the angle of repose of should be in range of 25–35. DPI removed from its primary site of action, the lower airways, by mucociliary clearance prior to dissolution and absorption, and eventually, swallowed into the gastrointestinal (GI) tract Therefore, prolonging DPI's retention time in the lungs may be beneficial. Mucoadhesion determines retention of DPI in lung. Amount of drug dissolution signifies the extent of drug absorption. Thus, considering the same concentration of xyloglucan and lactose was selected as the variables for optimization while entrapment efficiency, angle of repose, mucoadhesion and % drug dissolution were selected as dependent variable. Selection of concentration of xyloglucan was based on its ability to form gel at specified temperature (2–4%) and concentration of lactose monohydrate selected on the basis of quantity sufficient to improve flow properties (0.5–1.5%). The optimization study conducted using response surface methodology employing numerical and graphical optimization method (Table 1). The results of response generated using experimental design fitted into polynomial models and ANOVA test applied to polynomial models to estimate the significance of the model. The results of this analysis revealed that the response entrapment efficiency (Y_1), angle of repose (Y_2), mucoadhesion strength (Y_3) and t_{90} (Y_4) best fitted into quadratic model. The polynomial models for the responses Y_1, Y_2 and Y_3 represented in Eqs. (5)–(8), respectively.

$$Y_1 = +87.68 + 4.86X_1 - 1.63X_2 - 0.075X_1X_2 - 3.98{X_1}^2 - 1.83{X_2}^2 \quad (5)$$

$$Y_2 = +29.53 + 1.64X_1 - 3.42X_2 - 0.94X_1X_2 + 2.57{X_1}^2 + 2.56{X_2}^2 \quad (6)$$

$$Y_3 = +86.28 + 6.86X_1 - 1.30X_2 + 0.045X_1X_2 - 3.45{X_1}^2 + 0.22{X_2}^2 \quad (7)$$

$$Y_4 = +139.00 + 26.00X_1 + 2.33X_2 + 0.00X_1X_2 - 2.00{X_1}^2 + 1.0{X_2}^2 \quad (8)$$

The polynomial equations comprise the coefficient for intercept, first order main effect, interaction terms, and higher order effects. The sign and magnitude of the main effect signify the relative influence of each factor on the response. In addition, a negative sign signifies antagonistic effect while the positive sign indicates a synergistic effect.

Table 2 presents the result of ANOVA test on the quadratic regression model and details the model summary statistic for the selected significant models, which indicated that the response surface model developed for four responses, were significant and adequate. It observed that all responses bearing R^2 value > 0.9, which indicates a good correlation between the experimental and predicted responses. In addition, the predicted R^2 value is in reasonable good agreement with adjusted R^2 value, resulting in reliable adequate signal. The relatively lower values of coefficient of variation indicated better precision and reliability of the experiments carried out. The results of factor effects and associated p-values for the responses Y_1, Y_2, Y_3 and Y_4 were presented in Table 2. The combination effects of X_1 and X_2 were favorable (positive) for response Y_1, Y_2, Y_3 and Y_4.

Fig. 1, portray the 3-D response surface plot constructed using the models generated by the response surface methodology. Fig. 1A depicts response surface plot of xyloglucan concentration (X_1) and lactose monohydrate concentration (X_2) on entrapment efficiency (Y_1), which indicates a linear effect. This explains that higher the amount of polymer, the more will be the Y_1 because of the more availability of polymer to encapsulate the drug. Factor amount of X_1 had higher values of "F" than X_2 (Table 2), which indicated that factor amount of X_1 more significantly affect response variable then X_2 factor as shown in Table 2.

Fig. 1B exhibits response surface plot of the effect of X_1 and X_2 on angle of repose (Y_2). This explains that higher the amount of carrier, the more will be the Y_2. Factor amount of X_2 had higher values of "F" than X_1 (Table 2), which indicated that factor amount of X_2 more significantly affect response variable than X_1. Lactose monohydrate significantly imrpoves flowability of powder blend revealing its suitability as carrier for DPI formulation.

Fig. 1C shows combined effect of concentration of xyloglucan and lactose monohydrate on the mucoadhesive strength of microspheres which indicates that X_1 and X_2 shows that increased in xyloglucan concentration (X_1) from lower to higher the mucoadhesive strength(Y_3) was also increased. Factor amount of X_1 had higher values of "F" than X_2 (Table 2) which indicated that factor amount of X_1 more significantly affect response variable than X_2 factor as being anionic polymer xyloglucan has sufficient mucoadhesive strength and lactose monohydrate had lack of mucoadheisve property.

Table 2
Summary of results of ANOVA for measured responses.

Source of variation	Sum of square	DF	Mean of square	*F* ratio	*P*-summary	
					Value	Significant
Entrapment efficiency (%)						
Quadratic model	237.43	5	47.49	49.06	<0.0001	
X_1	141.72	1	141.72	146.42	<0.0001	Significant
X_2	15.91	1	15.91	16.44	<0.0001	
Angle of repose (θ)						
Quadratic model	148.72	5	29.74	38.07	<0.0001	
X_1	16.17	1	16.17	20.70	<0.0001	Significant
X_2	70.18	1	70.18	89.82	<0.0001	
Mucoadhesion strength (%)						
Quadratic model	329.28	5	65.86	230.91	<0.0001	
X_1	282.36	1	282.36	990.04	<0.0001	Significant
X_2	10.14	1	10.14	35.55	<0.0001	
$t_{90\%}$ (min)						
Quadratic model	4099.9	5	819.98	1076.22	<0.0001	
X_1	4056.0	1	4056	5323.5	<0.0001	Significant
X_2	32.67	1	32.67	42.88	<0.0001	

Fig. 1D represents response surface plot of the combined effect of X_1 and X_2 on $t_{90}\%$ (Y_4) which indicates a linear effect. This explains that higher the amount of polymer, the more will be the time requied for dissolution of drug because of the more availability of polymer will retard the drug dissolution. Factor amount of X_1 had higher values of "*F*" than X_2 (Table 2) which indicated that factor amount of X_1 more significantly affect response variable then X_2.

A numerical optimization technique using desirability approach was employed to develop a new formulation with desired responses. Desirability is an objective function that ranges from zero outside of the limits to one at the goal. The numerical optimization finds a point that maximizes the desirability function. The characteristics of a goal may be altered by adjusting the weight or importance. For several responses and factors, all goals get combined into one desirability function. The goal of optimization is to find a good set of conditions that will meet all the goals. The optimal calculated parameters were concnetration of xyloglucan (2%, w/v) and concentration of lactose monohydrate (1%, w/v).

3.1. Characterization of optimized microspheres formulation

3.1.1. Zeta potential measurement

The xyloglucan microspheres prepared were negatively charged (zeta potential value −18.2 mV); indicating the presence of xyloglucan at the surface of all microspheres. The studies have proved

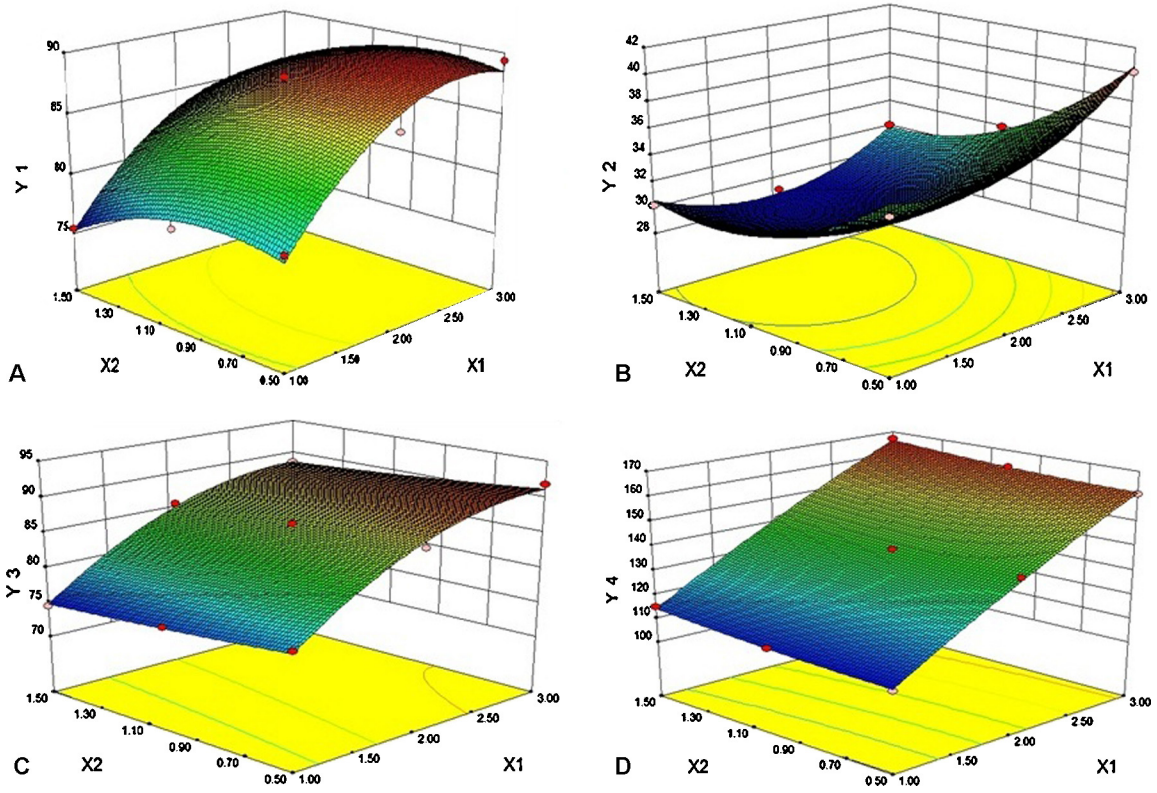

Fig. 1. Response surface plots for the xyloglucan (X_1) and lactose monohydrate (X_2) on entrapment efficiency (Y_1) – A, on angle of repose (Y_2) – B, on mucoadhesion strength (Y_3) – C and t_{90} (Y_4) – D.

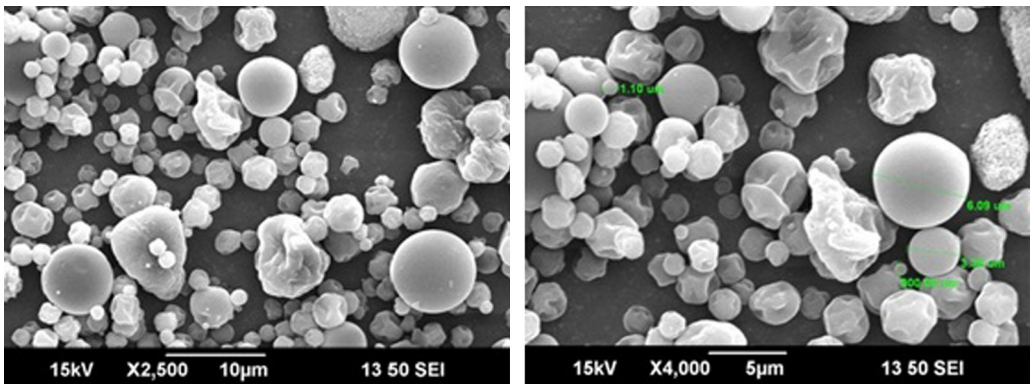

Fig. 2. Scanning electron microphotograph of xyloglucan microspheres.

that polymers with charge density can serve as good mucoadhesive agents. It has reported that poly anion polymers are more effective mucoadhesive than poly-cation or nonionic polymers.

3.1.2. Scanning electron microscopy

As evidenced by scanning electron microphotographs displayed in Fig. 2, microspheres showed a spherical morphology with smooth surface. Particles with range of particle size between 900 nm and 6 μm, which are suitable for inhalation drug delivery. Such DPIs reported to be more capable of escaping inertial and gravitational deposition in the oropharyngeal region and targeted to the deep lung that is necessary for effective pulmonary delivery (Vanbever, Mintzes, & Wan, 1999).

3.1.3. Differential scanning calorimetry

DSC thermograms of (A) pure Montelukast sodium, (B) xyloglucan blank microspheres and (C) Montelukast sodium loaded microspheres represented in Fig. 3. The xyloglucan blank microsphere has shown low intensity endothermic peak at 277 °C, indicating the decomposition of the polymer without melting. For pure Montelukast sodium, an endothermic peak observed at 139.44 °C due to melting of the drug, but absence of this in the Montelukast-loaded microspheres, indicating an amorphous dispersion of drug into the polymer matrix. It is also suggestive of encapsulation of Montelukast sodium in xyloglucan.

3.1.4. X-ray diffraction study (XRD)

X-ray diffraction pattern of pure drug, blank microspheres and drug loaded microspheres presented in (Fig. 4). X-ray diffractogram spectra of Montelukast sodium (A) illustrate the crystalline nature of drug and xyloglucan blank microspheres demonstrate amorphous nature of polymer (B). Whereas, X-ray diffraction pattern of drug loaded xyloglucan microspheres show low intensity peaks indicates amorphous molecular dispersion of drug in polymer.

3.1.5. In vitro assessment of dry powder inhaler

The optimized formulation batch containing Montelukast sodium loaded xyloglucan microsphere with DPI carrier lactose monohydrate taken for the in vitro performance and aerodynamic property using Anderson cascade impactor. The HPMC capsules containing 15 mg equivalent of Montelukast sodium was loaded to Anderson cascade impactor. The data obtained for various aerodynamic properties demonstrates that 10–11% drug retained in the capsule and device that has given indication of good

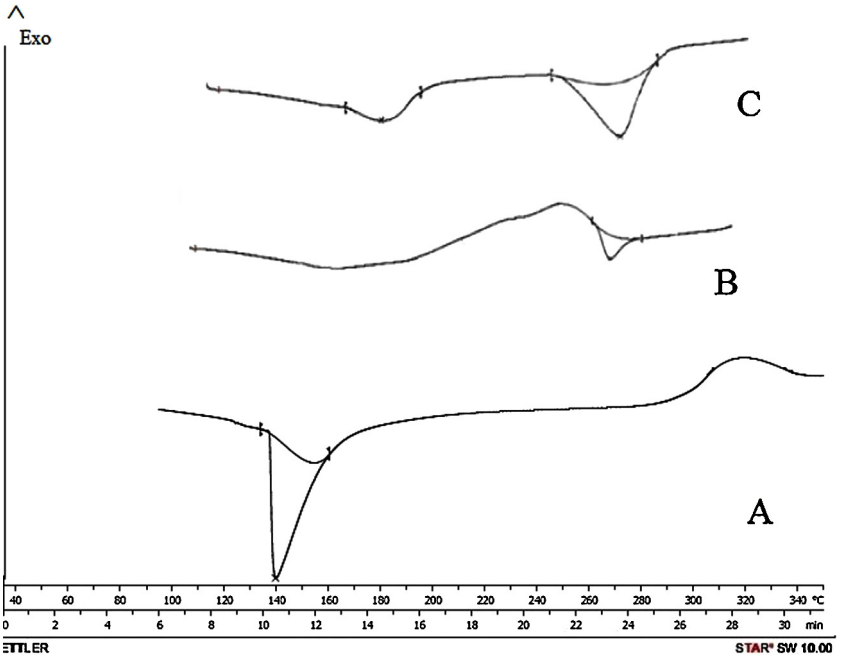

Fig. 3. DSC thermograms of pure Montelukast sodium (A), xyloglucan blank microspheres (B) and Montelukast sodium loaded microspheres (C).

Table 3
Pulmonary pharmacokinetic parameters of Montelukast sodium following intratracheal administration dry powder inhaler formulation and plain drug.

Pharmacokinetic parameter	Plain drug	DPI
C_{max} (ng/ml)	347.51 ± 45.6	406.21 ± 35.6
T_{max} (min)	15	60
$AUC_{0-360min}$ (ng/ml min)	22,038.08 ± 10,044.84	103,385.73 ± 9825.21
$AUC_{0-\infty}$ (ng/ml min)	23,441.91 ± 9445.27	234,987.23 ± 11,247.35

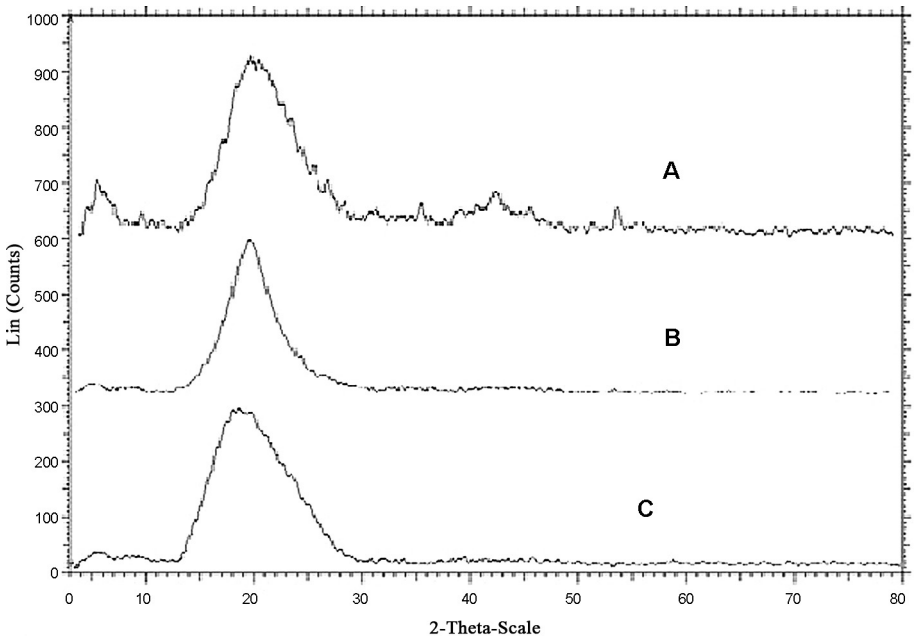

Fig. 4. XRD pattern of pure Montelukast sodium (A), xyloglucan blank microspheres (B) and Montelukast sodium loaded microspheres (C).

dispersibility of formulated DPI and about 89.86% of drug emitted from DPI. Fine particle fraction (FPF) of drug-loaded microspheres found to be 43.8%. The Mass Median Aerodynamic Diameter (MMAD) of the microsphere was less than 5 μm is prerequisite to have an inhalation of powders into the lower region of the lung and it found to be 2.53 μm. The geometric standard diameter (GSD) found to be 1.74 μm, which was depending on aerodynamic behavior. The surface properties support the value obtained for MMAD, GSD and % FPF xyloglucan contributes to these desired properties of DPI.

3.1.6. Pulmonary pharmacokinetics

The in vivo studies conducted in this investigation to test the possibility of obtaining prolonged localized actions of Montelukast from spray dried xyloglucan microspheres as DPIs in lungs. The pulmonary pharmacokinetic parameters were calculated and are represented in Table 3. The C_{max} was 406.21 ± 35.6 ng/ml at T_{max} of 15 min for plain drug and 347.51 ± 45.6 ng/ml at T_{max} of 60 min for formulation, respectively. Eventually, there was an increase in $AUC_{0-360min}$ for DPIs compared with the $AUC_{0-360min}$ of plain drug thereby confirms the maintenance of effective drug concentration with DPIs in lung tissue for prolonged period as compared to plain drug because of prolonged residence time of xyloglucan microspheres in the lungs.

4. Conclusion

Montelukast loaded xyloglucan microspheres were successfully prepared by spray drying technique. The spray-dried particles possess desired properties suitable for lungs delivery as DPI. Further, the microspheres formulations were optimize by statistical screening design considering xyloglucan and lactose as independent variables. Optimization study revealed that, concentrations of xyloglucan and lactose had prominent effect on entrapment efficiency, flow ability, mucoadhesion and release of drug from microspheres. The optimized microsphere formulation was further characterized for zeta potential, surface morphology, and DSC and XRD studies demonstrate its suitability as dry powder inhalation system. A promising in vitro aerosol performance observed for developed formulation of DPIs suggesting high deep lung deposition of drug. The data of in vivo studies showed drug residence up to 6 h. Within the prolonged and high drug, residence within lungs after pulmonary administration of xyloglucan microspheres expected to provide prolonged local action and necessitating less frequent administration and/or dose of Montelukast sodium. The results of present study clearly indicated promising potential of xyloglucan containing microspheres for delivering drug to deep lung and could be view as alternative to conventional dosage form. In conclusion, the formulated polymeric microspheres as DPI is promising approach for delivering antiasthmatic agents through pulmonary route.

Acknowledgment

Authors thankful to DSP Gokyo Food and Chemical Co. Ltd. (Fukusima, Japan) for providing xyloglucan as gift sample.

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