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Preparation and evaluation of microspheres of xyloglucan and its thiolated xyloglucan derivative

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ABSTRACT

Xyloglucan is a natural polymer reported to possess mucoadhesive properties. To enhance the mucoadhesion potential, xyloglucan was thiolated with cysteine. The microspheres of xyloglucan were prepared using a biocompatible crosslinker sodium trimetaphosphate and it was optimized for formulation variables, namely polymer concentration, internal:external phase ratio and stirring speed using a Box–Behnken experimental design. The formulation was also optimized for performance parameters like entrapment, t_{80} and % mucoadhesion. The microspheres were characterized by Fourier transform infrared spectroscopy, DSC and SEM for the optimum formula and then were reproduced by replacing the xyloglucan with thiomer. The microspheres formed showed entrapment efficiency of about 80%, t_{80} of about 400 min and % mucoadhesion of 60% while same for thiomer were 90%, 500 min and 80% respectively. In oral glucose tolerance test protocol the thiomer microspheres showed significant reduction in blood glucose levels. Thus thiolated xyloglucan offers a better polymer for multiparticulate drug delivery.

1. Introduction

Polysaccharides with low concentration can be used in increasing stability and mechanical strength of the drug delivery systems. This functional property of polysaccharides is because of its ability to form 3D network structure when comes in contact of water (gelation) [1,2]. Xyloglucan (XG) is a structural polysaccharide having gelation property. This is a polymer isolated from the seeds of *Tamarindus indica* Linn. having natural mucoadhesive property. In the food industry refined XG is used as a thickening, stabilizing and gelling agent [3].

The polysaccharide is composed of glucose, xylose and galactose units present in the ratio of 2.8:2.25:1.0 [4,5]. Use of XG has been reported as mucoadhesive agent in eye preparations [6] and as sustained release matrix for oral drug delivery systems [7]. Attempt has been made to improve the mucoadhesion of natural gums by immobilizing thiol groups onto the polymer that are capable of covalent interaction with cysteine rich subdomains of mucus glycoproteins via disulfide exchange reactions [8].

It is important to emphasize the high availability of xyloglucan and its renewable origin. Therefore, the cost-effectiveness of XG based products is another important factor for its use in the drug delivery field as well as in other biomedical applications.

Use of XG as carrier material in multiparticulates system is reported in literature such as Indomethacin-loaded XG beads [9], XG and sodium alginate beads containing glipizide by the orifice-ionic gelation method [10], multiparticulates of glipizide are also cited in literature [11,12]. Sodium trimetaphosphate (STMP) is a biocompatible crosslinker reported widely in the crosslinking applications such as crosslinked xanthan networks [13], in the development of starch microspheres as a drug delivery carriers in tissue engineering applications [14], and preparation of soluble starch-based biodegradable and microporous microspheres by emulsion chemical crosslinking [15].

The objective of present work is to prepare and optimize sustained release mucoadhesive microspheres of glipizide using XG and its thiolated derivative using STMP as crosslinking agent and evaluate them in vitro release studies.

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2. Materials and methods

XG was obtained as a gift sample from Encore Polymer Pvt. Ltd., Ahmadabad, India. Glipizide was obtained from Shri Krishna Pharma, Ltd., India. STMP was purchased from Sigma–Aldrich. All other chemicals used in the study were of analytical grade.

2.1. Preparation of thiomer

Thiomer (xyloglucan–cysteine) was synthesized by modifying the procedure reported in literature [16]. Briefly, XG (2 gm) was mixed in 250 mL of de-mineralized water with constant mechanical stirring for 15 min. This solution was stirred further for 30 min within a weight-ratio of 1:2 (polymer:cysteine).

2.1.1. Infrared spectrophotometry

The prepared thiomer was subjected to Fourier transform infrared spectroscopy to identify the changes in functional groups caused due to thiomerization over the infrared frequency range 4000–400 cm^{-1} using Jasco 460 plus instrument.

2.2. Formulation of microspheres [11]

Weighed amount of XG (1–4%) was dispersed in 50 mL freshly prepared 1 M NaOH solution. The mixture was slowly homogenized by a mechanical stirrer for 15 min. An appropriate amount of glipizide was then dispersed into the resulting solution. Desired amount of STMP was added and stirring was continued further for 15 min. The formed dispersion was extruded drop-wise through a disposable syringe into corn oil, which was placed on a magnetic stirrer and preheated to 50 °C. Adequate amount of surfactant i.e. Span 80 and Tween 80 were added in corn oil to avoid aggregation of droplets. Stirring rate and temperature were maintained constant throughout the curing time (30 min). The microspheres thus obtained were separated by centrifugation followed by filtration and washed with acetone to remove excess corn oil. The separated microspheres were air dried for 48 h.

2.3. Evaluation of microspheres

2.3.1. Drug entrapment efficiency (EE)

Microspheres (50 mg) were crushed in a glass mortar and pestle, and the powdered microspheres were suspended in 50 mL of phosphate buffer (pH 7.4). The solution was sonicated in bath sonicator for 30 min and kept overnight. After 24 h, the solution was filtered and the filtrate was analyzed spectrophotometrically at 276 nM. The drug entrapment efficiency was calculated using Eq. (1):

$$EE = \frac{\text{practical drug content}}{\text{theoretical drug content}} \times 100 \quad (1)$$

2.3.2. Drug release study

The drug release study was performed using *USP* XXIV basket apparatus at 37 ± 0.5 °C and at 100 rpm using 900 mL of phosphate buffer (pH 7.4) as a dissolution medium [17]. Microspheres equivalent to 20 mg of glipizide were used for the test. Five mL of aliquots were withdrawn at predetermined time intervals, filtered through a 0.45 μm membrane filter, diluted suitably, and analyzed spectrophotometrically at 276 nM. An equal amount of fresh dissolution medium was replaced immediately after withdrawal of the test sample.

2.3.3. In vitro mucoadhesion test for microspheres

The mucoadhesive properties of the microspheres were evaluated by in vitro wash-off test as reported by Lehr et al. [18]. A 1-cm by 1-cm piece of rat stomach mucosa was tied onto a glass slide (3-in. by 1-in.) using thread. Microspheres were spread (350) onto the wet, rinsed, tissue specimen and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in a beaker containing the simulated gastric fluid USP (pH 1.2). At the end of 30 min, 1 h and at hourly intervals up to 10 h, the number of microspheres still adhering onto the tissue was counted.

Table 1
Factor combination as per the 3^3 Box–Behnken design for microsphere of glipizide by using XG.

Factors	Levels		
	Minimum (−1)	Intermediate (0)	Maximum (+1)
% XG concentration (X_1)	1	2	4
Stirring speed (rpm) (X_2)	200	400	600
Internal:external phase ratio (X_3)	1:10	1:5	1:2

2.4. Optimization using response surface methodology [19]

Box–Behnken design was used for the optimization of variables affecting the formulation. Three factors, namely polymer concentration, stirring speed and internal to external phase ratio were optimized to achieve the desired release attributes and mucoadhesive strength. Three different levels response surface designs, specially made to require only 3 levels, coded as −1, 0 and +1 (Table 1) are available for 3–10 factors. A Box–Behnken experimental design has the advantage over full factorial design of few experimental batches [18]. The factors and levels were suitably coded and were indicated in Table 1. The three independent variables for experimental designs were X_1 = concentration of XG, X_2 = stirring speed and X_3 = internal to external phase ratio. The dependent variables were Y_1 = % entrapment efficiency and $Y_2 = t_{80}$ (time at which 80% of drug get released) and Y_3 = mucoadhesive strength. Using the data obtained equations for prediction of Y_1, Y_2 and Y_3 were obtained. These were validated by making experimental batches S_1, S_2 and S_3 of combination suggested by software as per desirability criteria (Y_1 – 72–90%, Y_2 – 302–540 min and Y_3 – 48–71%) and comparison of predicted and observed results.

2.5. Preparation and evaluation of optimum formulations using thiomer

Optimized batches S_1, S_2, and S_3 were reformulated replacing xyloglucan with thiomer (batches T_1, T_2, and T_3) and these were evaluated for % drug entrapment, % drug release and % mucoadhesion as described earlier.

2.5.1. Scanning electron microscopy (SEM)

Scanning electron microscopy was performed for morphological characterization of microsphere (Jeol JSM-6350, Tokyo, Japan). The samples for SEM were prepared by mounting microsphere onto an aluminum stub prior to coating for 70 s. The stubs were then coated with gold palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. The coated samples were then randomly scanned and photomicrographs were taken.

2.5.2. Thermal properties

Differential scanning calorimetric (DSC) measurements were carried out using differential scanning calorimetry instrument (DSC 823^e, Mettler Toledo, Melbourne, Australia). The instrument was calibrated using Indium (156°), Tin (232°) and Zinc (419.5°) as