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Evaluation of tropicamide-loaded tamarind seed xyloglucan nanoaggregates for ophthalmic delivery

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ABSTRACT

The present study was aimed to prepare tamarind seed nanoaggregates and its evaluation for ophthalmic delivery. The preparation of tropicamide-loaded tamarind seed xyloglucan nanoaggregates was optimized using face centred central composite experimental design, employing the concentrations of tamarind seed xyloglucan and Poloxamer-407, as independent variables. The results revealed that concentration of TSX has a significant antagonistic effect on particle size, while poloxamer displayed a significant synergistic effect on encapsulation efficiency. The optimal concentrations of TSX and poloxamer were found to be 0.45% (w/v) and 0.5% (w/v) respectively. The optimized formulation of tropicamide-loaded TSX nanoaggregates showed a significantly higher corneal permeation of tropicamide across the isolated goat cornea compared to commercial conventional aqueous formulation. The results revealed excellent mucoadhesive properties of TSX nanoaggregates. Further, the tropicamide-loaded TSX nanoaggregates formulation showed excellent ocular tolerance and biocompatibility as determined by hen's egg test chorioallantoic membrane and resazurin assay on Vero cell lines.

1. Introduction

Polysaccharides are composed of repetitive monosaccharide units linked *via* glycosidic linkages having a highest capacity for carrying biological information. Natural polysaccharides are the most abundant and cheap biomolecules in nature. Most of the polysaccharides are exogenous metabolites of bacteria out of which few are the product of total biochemical synthesis and some of them are modified by partial organic synthesis. They represent one of the most abundant industrial raw material due to their sustainability, biodegradability and biosafety. Their diverse properties and non-toxic nature make them suitable for use in pharmaceutical applications.

Xyloglucans are linear polysaccharides present widely in cell wall of higher plants. Viscosity of xyloglucan depends upon its molecular weight, the presence of glycosyl and non glycosyl subunits and their backbone (Gidley et al., 1991). Xyloglucans are present in many plants but most of the plants contain only structural xyloglucans, whereas storage xyloglucans are rarely present in plants. Storage xyloglucans differ in their molecular mass, substitution levels and distribution. Tamarind is the most common plant for storage xyloglucans (Hayashi, 1989). Tamarind seed xyloglucan (TSX) obtained from the trees of *Tamarindus indica* Linn is a non-ionic and neutral polysaccharide. TSX is a high molecular weight (720–880 kDa) galactoxyloglucan comprising of glucose:xylose:galactose:arabinose in the ratio of 4:3.4:1.5:0.3 (Freitas et al., 2005). TSX consist of β (1→4) linked glucan backbone chain which is partially substituted with α-D-xylose at O-6 position. Some of these xylose residues are substituted with β (1→2) linked galactosyl units at O-2 position. The xylose units are reported to be more hydrophobic than galactose and glucose units, as a result of the presence of these hydrophilic and hydrophobic groups the xyloglucan chain shows substantial stiffness (Buckeridge, Rocha, Reid, & Dietrich, 1992). Due to the balancing of hydrophilic and hydrophobic character xyloglucan shows solubility in water but the individual macromolecules do not hydrate fully leading to the presence of aggregated species in water. These species are present even in very dilute solutions. Thus aqueous solutions of xyloglucan show a structure-function relationship. The random coil overlap and entanglement of polymer backbone depend on the concentration of xyloglucan and its intrinsic viscosity respectively. The random-coil structure of xyloglucan is formed at higher concentrations in aqueous solutions (Morris, Cutler, Ross-Murphy, Rees, & Price, 1981).

Xyloglucan present in TSP is permitted for the use as food additive, thickening, stabilizing and gelling agent in the food industry (Glicksman, 1986). TSP has also been explored for potential commercial applications in pharmaceutical industry for controlled

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drug release (Sumathi & Alok, 2002). During earlier studies the xyloglucan nanoaggregates were loaded with anticancer drug camptothecin and were found to release the drug by first order kinetics (Tatiane, Petri, Denise, Lucyszyn, & Sierakowski, 2010).

Tropicamide is poorly water soluble antimuscarinic drug. It is commonly used for producing mydriasis with its aqueous solution (1%, w/v) during eye surgery and dilated funduscopic examination. It works by blocking the muscarinic receptors of the eye thereby controlling the pupil size and lens shape (Behar-Cohen, 2004; Blessel, Rudy, & Senkowski, 1974; Dey & Mitra, 2005; Duvvuri, Majumdar, & Mitra, 2003; Koevary, 2003). It is weakly basic drug. To increase its solubility its solutions are buffered to acidic pH (5.0). But this increases its irritation potential and lowers its bioavailability due to induced lacrimation.

In the present study formulation of tropicamide-loaded tamarind seed xyloglucan (TSX) nanoaggregates was optimized by Central Composite Design using two independent variables. The optimized batch of tropicamide-loaded xyloglucan nanoaggregates was employed for preparing ophthalmic formulation of tropicamide (1%, w/v). Tropicamide-loaded ophthalmic nanosuspension was evaluated comparatively with the commercial conventional aqueous formulation of tropicamide for *in vitro* corneal permeation. Further, the ocular tolerance and biocompatibility of nanoformulation was studied using HET-CAM study and resazurin assay, respectively. Mucoadhesive properties of the formulation were studied using mucin glycoprotein assay.

2. Experimental

2.1. Materials

Tamarind kernel powder (TKP), Poloxamer-407 and tropicamide were obtained as gift samples from Hindustan Gums and Chemicals Pvt. Ltd. (Bhiwani, India), Jubliant Phamaceuticals (Noida, India) and Optica Pharmaceuticals (Yamunanagar, India), respectively. Mucin, Schiff reagent and periodic acid were purchased from Hi-Media Laboratories Pvt. Ltd. (Mumbai, India). Tropicacyl® (Sunways Pvt. Ltd., Mumbai, India) was purchased from local pharmacy, Hisar. Vero cell lines were procured from National Research Centre of Equines (Hisar, India). Freshly excised goat eye was obtained from the local butcher shop (Hisar, India). Ten-day old fertilized eggs were obtained as gift samples from Indovax Pvt. Ltd. (Hisar, India). All other chemicals were of analytical grade and were used as such.

2.2. Extraction of TSX

TSX was isolated from TKP which is soluble in water as reported earlier (Rao, Ghosh, & Krishna, 1946). In brief, TKP was dissolved in cold distilled water, which was then added to 400 ml of boiling distilled water under magnetic stirring and further boiled for 20 min. The resulting suspension was kept overnight to allow settling of fibres and proteins. The above viscous solution was centrifuged at 6000 rpm (Cooling centrifuge, 4K-15, Sigma, Germany) for 20 min. The supernatant so obtained was added to twice the volume of ethanol under continuous stirring. The precipitate so obtained was lyophilized in laboratory freeze dryer (Alpha 2-4-LD Plus, Martin Christ, Germany) for 24 h at −90 °C, at 0.0010 m bar.

2.3. Optimization of formulation of tropicamide-loaded TSX nanoaggregates

A central composite design with $\alpha = 1$ was employed as per the standard protocol. The two factors, concentration of TSX and Poloxamer-407 were varied and the factor levels were suitably coded. Particle size and encapsulation efficiency were taken as response variables. Throughout the study all other processing variables were kept invariant. The present investigation involves evaluation of two factors each at three levels. In all, 13 experimental runs were carried out as shown in Table 1. The central point (0,0) was studied in pentet. All the experiments were carried out using systematic design of experiments employing Design Expert Software (Version 8.0.4, Stat-Ease Inc., Minneapolis, MN).

Table 1
Central composite design used to study effect of formulation variables on particle size (Y_1) and % encapsulation efficiency (Y_2).

S. no.	TSP (%) (X_1)	Poloxomer (%) (X_2)	Particle size (nm) (Y_1)	Encapsulation efficiency (%) (Y_2)
1	0.55	0.50	678.5	86.57
2	1.00	0.00	1260.3	36.78
3	0.55	0.50	580.2	83.23
4	1.00	1.00	745.6	88.62
5	0.10	0.50	390.8	67.89
6	0.10	1.00	445.6	64.32
7	0.55	0.50	698.7	85.49
8	0.10	0.00	399.8	27.89
9	0.55	0.00	996.7	32.61
10	0.55	1.00	675.4	76.54
11	0.55	0.50	634.5	84.41
12	0.55	0.50	608.7	86.46
13	1.00	0.50	800.8	91.62

Briefly the TSX nanoaggregates were formulated by adding tropicamide (75%, w/v of TSP) to a dispersion of TSX (0.1–1%, w/v) and Poloxamer-407 (0–1%, w/v) under magnetic stirring. Nanosuspension thus obtained was analyzed for particle size. The optimized batch of formulation was freezed at −80 °C for 4 h followed by lyophilization in laboratory model freeze dryer (Alpha 2-4 LD Plus, Martin Christ, Germany) for 24 h at −90 °C, at 0.0010 mbar using mannitol (1%, w/v) as cryoprotectant.

2.4. Characterization of tropicamide-loaded TSX nanoggregates

2.4.1. Particle size analyzer

Photon correlation spectroscopy was used for particle size determination in a suspension. Particle size distribution was also inferred. The mean particle size of optimized tropicamide-loaded TSX nanoaggregates was analyzed at 25 °C. One millilitre of the nanosuspension was scanned with 11 runs in disposable cuvette with an equilibrium time of 120 s in particle size analyzer (Zetsaizer Nano ZS90, Malvern, UK).

2.4.2. Encapsulation efficiency

The encapsulation efficiency of nanoaggregates was determined by separating the nanoparticles from aqueous medium by centrifuging the suspension at 12,000 rpm for 30 min at 4 °C (Cooling centrifuge, 4K-15, Sigma, Germany). The amount of encapsulated tropicamide in the suspension was measured by dissolving the pellet formed after centrifugation in ethanol and was sonicated (Sonoplus Bandelin) for 1 min. The solution was then analyzed for the contents of tropicamide at 257 nm by UV–vis spectrophotometer (UV 2450, Shimadzu). Encapsulation efficiency (%) was calculated by the formula given below:

$$\%\ \text{Encapsulation efficiency} = \frac{\text{Actual drug loading}}{\text{Theoretical drug loading}} \times 100 \quad (1)$$

2.4.3. Morphology

Transmission electron microscopic analysis (TEM) was done using Hitachi H7500 machine. The prepared nanosuspension was dropped onto carbon coated copper grid, extra solution was removed using a blotting paper. The grid was allowed to dry for 5 min and loaded in the goniometer. The TEM micrograph was taken by applying accelerating voltage of 80 kV.