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# Zinc alginate-carboxymethyl cashew gum microbeads for prolonged drug release: Development and optimization

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ABSTRACT

Isoxsuprine HCl-loaded microbeads using sodium alginate (SA)-carboxymethyl cashew gum (CMCG) polymer-blends were developed through ionotropic-gelation technique using $ZnSO_4$ as cross-linker. Effects of polymer-blend ratio and cross-linker concentration on drug encapsulation efficiency (DEE) and cumulative drug release at 7 h ($R_{7h}$) were optimized by $3^2$ factorial design. Optimized microbeads were of excellent combination of high DEE (79.92 ± 2.51%) and suitable sustained drug release pattern over a prolonged period of 7 h (58.67 ± 2.26%). The microbead surface morphology was analyzed by SEM. The physical state of isoxsuprine HCl within the optimized microbead matrix was analyzed by FTIR and DSC. *In vitro* isoxsuprine HCl release from alginate-CMCG microbeads in phosphate buffer (pH, 6.8) showed prolonged sustained drug release and Korsmeyer-Peppas model ($R^2$ = 0.9959–0.9992) over 7 h.



## 1. Introduction

Currently, natural polysaccharides have evoked tremendous interest due to their abundance in nature, biodegradability, biocompatibility, environmental friendliness and cheap isolation cost [1–3]. Besides having many advantages, these exhibit some limitations like hydration, low mechanical property, chemical stability, reduction in viscosity on storage, *etc* [4]. These limitations can be conquered following physical polymer-blending [5–10], chemical cross-linking [11,12], polymer-grafting [13,14], carboxymethylation [15–18], thiolation [2], esterification [19], interpenetrating polymer networkings (IPNs) [19–21], *etc*.

Sodium alginate (SA) is an anionic, hydrophilic natural polysaccharide obtained from brown marine algae [22]. It is the monovalent form of alginic acid, composed of β-D-mannuronic acid monomers (M-unit), regions of ∞-L-guluronic acid residues (G–unit) and regions of interspersed M and G units [23]. SA undergoes ionotropic-gelation in presence of divalent cations like $Ca^{2+}$, $Zn^{2+}$, *etc*., due to ionotropic interaction between carboxylic acid groups of SA and these cations [24]. Major disadvantages of ionotropically gelled alginate beads are that drugs can be leaked during gel-formation due to long immersion time, which decreases the encapsulation efficiency and the premature burst release of drugs due to quick degradation in alkaline pH [6,23]. To overcome these limitations, beads made of SA with other polymer-blends through ionotropic-gelation have been investigated for sustained drug delivery applications [5,25–28]. Recently, formulation of ionotropically gelled beads using SA-carboxymethylated gums blends have been investigated for the use in sustained drug release delivery [16,17]. Ray et al. have reported the development of beads of SA and carboxymethyl xanthan gum for sustained ibuprofen release [15]. Dey et al. have also investigated beads of SA and carboxymethyl locust bean gum for sustained glipizide release [16].

Cashew gum (CG) is an exudate polysaccharide obtained from *Anacardium occidentale* (Family: Ancardiaceae) trees [29]. It is a branched acidic heteropolysaccharide of low viscosity [33]. CG is composed of β-D-galactose (72%), α-D-glucose (14%), arabinose (4.6%), rhamnose (3.2%) and glucuronic acid (4.7%) [30]. Literature survey reveals that CG had been studied as excipients in various pharmaceutical formulations [31–34]. CG has been modified by carboxymethylation with monochloroacetic as an etherifying agent and carboxymethyl cashew gum (CMCG) possesses high hydrophilic property [35]. Literature survey also reveals the utilization of CMCG in pharmaceutical formulation [36]. In an investigation, Magalhães Jr. et al., have formulated polyelectrolyte microspheres for bovine serum albumin release using cationic polymer, chitosan and anionic polymer, CMCG [36]. In the present investigation, we made an attempted to formulate and evaluate beads using blends of two anionic polymers, namely SA and CMCG for sustained drug release.

Isoxsuprine HCl was investigated as model drug in the present investigation. It is used as a vasodilator and uterine relaxant [37].

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At high doses, it can decrease blood viscosity and reduce platelet aggregation. The short biological half-life (2.5–3 h) and fast clearance make it a suitable candidate for the development of sustained release formulation [38]. Furthermore, isoxsuprine HCl is required to be taken for a long period by the patients. The use of sustained isoxsuprine HCl release formulation is associated with less nausea and dizziness at the initiation of therapy. Hence, to improve the patient compliance as well as to reduce side-effects, isoxsuprine HCl needs to be formulated in sustained release dosage form [38].

## 2. Materials and methods

### 2.1. Materials

Isoxsuprine HCl (Albert-David Pvt. Ltd., India), SA (Central Drug House, India), zinc sulphate ($ZnSO_4$, Qualigens Fine Chemicals, India), sodium hydroxide (NaOH, Qualigens Fine Chemicals, India) and monochloroacetic acid (Loba Chemie Pvt. Ltd., India) were used. CG was isolated locally from *Anacardium occidentale* tree. The procedure of CG isolation has been described by Kumar et al. [33]. All other chemicals and reagents were commercially available and of analytical grade.

### 2.2. Synthesis of CMCG

CG was carboxymethylated in aqueous alkaline medium using monochloroacetic acid as etherifying agent. The carboxymethylation reaction was performed using the methodology reported by Magalhães Jr. et al. [36] with little modification. The isolated cashew gum (5 g) was mixed with water (5 ml) until a homogeneous paste was formed. NaOH solution (10 M, 2.7 ml) was added and the mixture was kneaded for 10 min. This was followed by the mixing of monochloroacetic acid (MCA) thoroughly with the paste. The mixture was heated at 55 °C, for 3 h. The system was neutralized with HCl (1 M) and finally oven-dried at 40 °C. The dried CMCG samples were milled, washed with 80% (v/v) ethanol and again oven-dried at 40 °C. The condition applied to obtain CMCG with degrees of substitution (DS) of 0.44 was molecular ratio of CG/MCA/NaOH of 1:1:2. The degree of O-carboxymethylation in CMCG was $0.42 \pm 0.09$.

### 2.3. Preparation alginate-CMCG microbeads containing isoxsuprine HCl

The alginate-CMCG microbeads containing isoxsuprine HCl was prepared by ionotropic-gelation technique using $ZnSO_4$ as cross-linker. Briefly, SA and CMCG aqueous dispersions were prepared separately using distilled water. These dispersions were well mixed with stirring for 10 min at 1000 rpm using a magnetic stirrer (Remi Motors, India). Afterwards, isoxsuprine HCl was added to the polymer-blend dispersion mixtures. The ratio of drug to polymer was maintained 1:2 in all formulations and mixed thoroughly using a homogenizer (Remi Motors, India). The resulting dispersions were extruded through 24 G flat-tip needle into $ZnSO_4$ solutions. Added droplets were retained in $ZnSO_4$ solutions for 30 min. The wet beads were collected by decantation, washed two times with distilled water and dried at 37 °C for overnight. The dried beads were stored in a desiccator until used.

### 2.4. Experimental design for statistical optimization

For the $3^2$ factorial design, 9 trial formulations were proposed by Design-Expert 8.0.6.1 software (Stat-Ease Inc., USA) for two independent variables: amount of SA to CMCG ratio ($A$), and $ZnSO_4$ (cross-linker) concentration (% w/v) ($B$), which were varied at three levels: low level (−1), medium level (0), and high level (+1). The drug encapsulation efficiency (DEE, %), and cumulative drug release after 7 h ($R_{7h}$, %) were evaluated as dependent variables (responses). According to this trial proposal, various alginate-CMCG microbeads containing isoxsuprine HCl were prepared by ionotropically gelation technique. The design matrix including investigated factors, responses and levels are also shown in Table 1. The mathematical model generated by $3^2$ factorial design is following [39]:

$$Y = b_0 + b_1A + b_2B + b_3AB + b_4A^2 + b_5B^2$$

where $Y$ is the response, $b_0$ the intercept, and $b_1$, $b_2$, $b_3$, $b_4$, $b_5$ are regression coefficients. $A$ and $B$ are individual effects; $A^2$ and $B^2$ are quadratic effects; $AB$ is the interaction effect. One-way ANOVA was applied to estimate the significance of the model ($p < 0.05$). Individual response parameters were evaluated using the $F$-test. The surface response plots and contour plots were analyzed to reveal the effect of independent factors on the measured responses. Design-Expert® Version 8.0.6.1 software was used for the generation and evaluation of the statistical experimental design.

### 2.5. Determination of DEE

100 mg of microbeads were crushed using pestle and mortar. The crushed powders of drug containing beads were placed in a 250 ml volumetric flask and the volume was made up to 250 ml by phosphate buffer, pH 6.8 and kept for 24 h with occasionally shaking at $37 \pm 0.5$ °C. After the stipulated time, the mixture was stirred at 500 rpm for 20 min using a magnetic stirrer (Remi Motors, India). The polymer debris formed after disintegration of bead was removed by filtering through Whatman® filter paper (No. 40). The drug content in the filtrate was determined using a UV–vis spectrophotometer (Shimadzu, Japan) at 269 nm against appropriate blank. DEE (%) of these prepared beads was calculated by the following formula:

$$\text{DEE}\,(\%) = \frac{\text{actual drug content in beads}}{\text{theoretical drug content in beads}} \times 100$$

### 2.6. Bead size measurement

Particle size of 100 dried beads from each batch was measured by optical microscopic method for average particle size using an optical microscope (Olympus). The ocular micrometer was previously calibrated by stage micrometer.

### 2.7. Scanning electron microscopy (SEM) analyses

The gold-coated samples were photographed under a scanning electron microscope (JEOL JSM-6360, JEOL Ltd., Japan) at an acceleration voltage of 20 kV.

### 2.8. Fourier transform-infrared (FTIR) spectroscopy analyses

Samples were reduced to powder and analyzed as KBr pellets by using a Fourier transform-infrared (FTIR) spectroscope (Shimadzu, Japan). The pellet was placed in the sample holder. Spectral scanning was taken in the wavelength region between 4000 and 400 $cm^{-1}$ at a resolution of 4 $cm^{-1}$ with scan speed of 1 cm/s.

### 2.9. Differential scanning calorimetry (DSC)

Samples were heated to remove the moisture. Then, the samples (7 mg) were placed into a platinum crucible 40-μl aluminium pan in hermetically sealed condition, where ∞-alumina powder was used as a reference. Thermograms were recorded from 32 to 400 °C at the heating rate of 10 °C/min under a constant flow of an inert nitrogen