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Research Article

TRIVALENT ION CROSS-LINKED AND ACETALATED GELLAN GUM MICROSPHERES OF GLIMEPIRIDE

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ABSTRACT

Objectives: A novel formulation was developed with glimepiride loaded trivalent ion Al^{+3} cross-linked and acetalated gellan gum microspheres.

Methods: The glimepiride loaded microspheres were formulated using sodium alginate and gellan gum. Cross-linking agents used for the microspheres were aluminum chloride ($AlCl_3$) and glutaraldehyde (GA). The evaluation processes of prepared microspheres were carried out by *in-vitro* release study, swelling index, microscopic analysis, and entrapment efficiency.

Results: All the formulations show good entrapment efficiency and the maximum entrapment 84.6% was governed by the formulation (F3) cross-linked by $AlCl_3$ and GA and their obtained mean particle size were 12.46±3.21 µm. Release profile of the formulations revealed the sustained design of the drug, particularly this formulation (F3), releasing approximately 40% over 4 h.

Conclusions: From this experiment, it can be accustomed that F3 possesses higher standard formulation than the rest due to good release profile and entrapment efficiency. Therefore, the long term stability study is required for future development of this formulation.

Keywords: Microspheres, Glimepiride, Gellan gum, Drug release.

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INTRODUCTION

Microspheres are solid approximately spherical particles made up of polymeric, waxy, or other protective materials (such as starches, gums, proteins, fats, and waxes) and ideally having a particle size <200 (µm), which are used as drug delivery matrices for drug delivery. It is a reliable mean of delivering a drug to target a specific site and providing maintenance of desired concentration at that site [1]. There occurs a huge difficulty in research and developmental work of new oral controlled drug delivery systems to target the drugs to particular body parts. For this reason, the drug has to shift the biological membrane, i.e., other organs, tissues, or biological compartment where drug degradation can happen [2]. The colon is selected as the area or sites of interest for the treatment purpose of much disease mainly some inflammatory diseases and colon cancer because it introduces prolonged transit time, decreased proteolytic activity as well as neutral pH, among different organs [3]. Drugs which have permeability and/or stability issues in the upper gastrointestinal tract, for that colon, become a promising site of drug release [4]. Now different types of synthetic, semi-synthetic, or natural polymers are incorporated in the formulation of controlled drug release systems [5-7].

The main objective of the study was to formulate microspheres by the ionotropic gelation method using gellan gum and sodium alginate with trivalent Al^{+3} ions as well as covalent cross-linking with glutaraldehyde (GA). Glimepiride was chosen for the sustained release therapy and this drug is having plasma half-life 5–7 h, duration of action 24 h. The drug is administered in a dose of 1–6 mg once or twice a day [8]. Hence, glimepiride is an ideal candidate for the development of sustained-release formulation which could result in increased clinical efficacy, decreased frequency of administration, and less side effects.

Glimepiride is an oral hypoglycemic drug which is used in the treatment of type 2 diabetics, but not in type 1 diabetics. It helps to decrease blood glucose levels and is effective orally. It is a second-generation sulfonylurea (KATP Channel blockers) which acts by enhancing Insulin secretion [9]. These kinds of drugs act by blocking the sulfonylurea receptor which is a significant part of ATP-sensitive K+ channel (KATP) in the pancreatic β cells membrane. The inward flow of K+ ions is thus reduced or inhibited which may be the cause of reduced intracellular K+ concentration. The cell membrane is partially depolarized, increasing the opening of Ca^{2+} channel and also the releasing of Ca^{2+} from intracellular stores. The Ca^{2+} ions induce the fusion of insulin-containing intracellular granules with the plasma membrane and immediately cause the exocytotic release of insulin.

Gellan gum is a negatively charged exopolysaccharide which is having a high tendency to mix with dissolve in or be wetted by water. It is derived by the aerobic method from bacteria called *Sphingomonas elodea*. This polymer is comprised of repeated units of glucose, glucuronic acid, and rhamnose in 2:1:1 molecular ratio and two acetyl substituent, acetate and glycerate, linked on glucose residue adjacent of the glucuronic acid [10].

Sodium alginate is derived from alginic acid, a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively [11].

MATERIALS AND METHODS

Materials required

Glimepiride and gellan gum were purchased from Yarrow Chem Pvt. Ltd. Mumbai. Aluminum chlorides ($AlCl_3$) were obtained from Loba Chemie, Mumbai. All chemicals and reagents used were of analytical grade.

Methodology

Preparation of glimepiride microspheres

The glimepiride loaded microspheres were made of sodium alginate and gellan gum. Cross-linking agents used for the microspheres were $AlCl_3$ and GA. Sodium alginate and gellan gum were accurately weighed on a digital balance and dissolved in 15 ml of distilled

water. The mixture was stirred properly until the polymer mixture becomes bubble-free or uniformly mixed. In a separate beaker, glimepiride was first dissolved in methanol and then dispersed uniformly into 15 ml aqueous dispersion of gellan gum and sodium alginate to form a drug-polymer solution. Then, stirred it properly to get a homogeneous mixture. On a separate beaker, the counter ion solution was prepared by weighing $AlCl_3$ and tween 80 and mixed in 100 ml of water for F1 and F2 and also added GA for F3. The drug-polymer dispersion was added dropwise through flat-tipped needle into slightly agitated 100 ml of 1% (w/v), 3% (w/v) $AlCl_3$ solution containing 0.08% (w/v) Tween 80, and also in the 3% (w/v) $AlCl_3$ containing 0.08% (w/v) Tween 80 and 4.5% GA. Following an incubation period of 10 min, the microspheres were separated by filtration, washed with double distilled water, and allowed for air-drying. Then, the formulated cross-linked microspheres were withdrawn from the beaker and cleaned them properly by washing with distilled water and make them free from non-reacted GA [12]. The composition of different formulations is shown in Table 1.

Drug entrapment efficiency estimation

Specified amounts of dried microspheres were properly weighed, crushed with a mortar-pestle, and were taken into the buffer solution. It is kept for a time period so that the time should allow the maximum release of drug from the formulation. Then, the suspension was filtered and sample was taken to be analyzed with a spectrophotometer [13].

Equation	Entrapment efficiency (%) = (actual drug content/theoretical drug content) × 100

***In vitro* drug release study**

The paddle-type dissolution test apparatus is useful for the experiment. First, prepared microspheres were properly weighed and taken them in 500 ml buffer solution and temperature of the system was maintained at 37±0.5°C. The paddle rotation was fixed at 50 rpm. After a specific time interval, a specific amount of aliquot was withdrawn from the dissolution medium and then that volume was replaced by the fresh medium of equal volume. There was no filtration or further dilution. The samples were analyzed using a spectrophotometer [14].

Microsphere size analysis

Randomly select a specific number of microspheres and their size was measured using an optical microscope. A standard stage micrometer had to calibrate the optical micrometer. The mean diameter was calculated from each batch [15].

Swelling study

This study is carried out in a buffer solution. A predetermined known weight sample was taken, dissolved into 50 ml buffer media and allowed to swell for a specific time. Then swollen microspheres were periodically removed, blotted with tissue paper and reweighed.

Equation	Swelling ratio = (final weight – initial weight)/initial weight.

RESULTS AND DISCUSSION

***In vitro* release studies**

The USP type II dissolution test apparatus was used for the *in vitro* drug release study of the prepared glimepiride loaded microspheres. These formulations contain gellan gum and sodium alginate as polymer, counter-ion solutions of $AlCl_3$, and GA in various ratios as cross-linkers. From the *in vitro* release study, it is found that 68.7328±0.63% drug release was obtained by the formulation (F1) cross-linked with 1% $AlCl_3$ followed by 57.4724±1.32% release by (F2) cross-linked with 3% $AlCl_3$, then 40.7098±1.68% by (F3) cross-linked with 3% $AlCl_3$ and 4.5% GA over a period of 4 h. The *in vitro* drug release profile of different formulation is shown in Fig. 1. The extent of drug release varies for different formulations depending on the degree of cross-linking between the ingredients used in each formulation.

It is estimated that the amount of drug released from each formulation over the same period of time decreases gradually with the increase of $AlCl_3$ concentration. The instinctive strength and hydrogel network microspheres can also be modified by the cross-linkage with the use of GA. Further suppression of drug release for the same formulation can be obtained by the GA treatment. The *in vitro* drug release is decreased to a great extent and the $t_{1/2}$ value is comparatively higher for the GA treated microspheres.

Drug entrapment study

Drug entrapments in the formulations were fairly good, the maximum entrapment being exhibited by the formulation (F1). The formulation (F3) also showed good entrapment efficiency. It can be predicted that the lower entrapment of formulation (F3) might be due to the cross-linking reaction of $AlCl_3$ as well as GA. It is found that the entrapment capability decreases with the increase of $AlCl_3$ concentration in the salt solution as it causes a higher degree of cross-linking. It is thought that more the gelation, greater will be the expulsion of water which is directly responsible for the convective loss of drug molecule from the microspheres during the incubation into the gelation medium. The entrapment efficiency of GA treated microspheres is lower than the untreated ones. The result of drug entrapment study is mentioned in Table 2.

Average particle size determination

The size of the particles was measured with the help of an optical microscope taking six particles from each batch. An ocular micrometer was previously attached and the particles were placed on a slide and their size was measured. From the experiment, it is observed that formulation (F1) cross-linked with 1% $AlCl_3$ is having average particle size 17.23±1.59 µm, formulation (F2) cross-linked with 3% $AlCl_3$ is having average particle size 15.58±2.67 µm, and formulation (F3) cross-linked with 3% $AlCl_3$ and 4.5% GA is having average particle size 12.46±3.21 µm. It is found that the mean particle size decreases with the increase of $AlCl_3$ concentration in the salt solution. It is thought that more the gelation, greater will be the expulsion of water due to cross-linking. The higher degree of cross-linking causes higher water loss accordingly. The effect of GA on particle size is also important. The

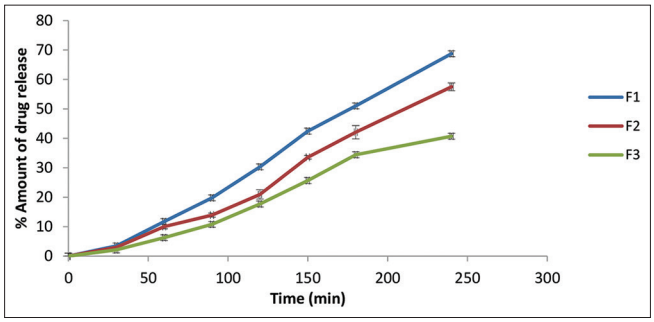

Fig. 1: Release study of glimepiride microsphere

Table 1: Composition of formulation

Formulation code	Sodium alginate (mg)	Gellan gum (mg)	Glimepiride (mg)	Aluminum chloride (%w/v)	Glutaraldehyde (%w/v)
F1	250	250	25	1	-
F2	250	250	25	3	-
F3	250	250	25	3	4.5

Table 2: % Entrapment of different batch

Batch number	% Entrapped
F1	84.6±4.72
F2	81.2±3.61
F3	78.6±5.23

Each value is represented as a mean±SEM, n=3

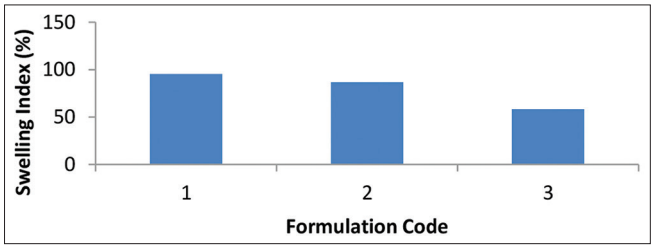

Fig. 2: Swelling indices of various formulations of microspheres

formulation (F3) treated with 4.5% GA possesses a ridged and uneven surface due to the greater degree of cross-linking of the gelation matrix.

Swelling behavior

From the swelling study, it is evident that maximum swelling (95.5%) is exhibited by the formulation (F1) cross-linked with 1% $AlCl_3$ followed by 86.7% swelling by (F2) cross-linked with 3% $AlCl_3$, then 58.3% by (F3) cross-linked with 3% $AlCl_3$ and 4.5% GA. It is also noticeable that the percentage of swelling increased gradually with decreasing concentration of $AlCl_3$. At the same time, the percentage of swelling of the microspheres decreases with the enhancement of the $AlCl_3$ concentration in all types of swelling medium having different pH value. Estimation found that when $AlCl_3$ concentration was gradually increased to 5%, then the swelling index was reduced to nearly 17% and GA treatment in those reduced the ratio further by 60%. The result is shown in Fig. 2.

CONCLUSIONS

Glimepiride is an oral hypoglycemic drug which is prescribed to treat the hyperglycemic condition in the human body by reducing the sugar level. This project was conducted to represent the activity of glimepiride as a model drug by attempting to prolong its release for a longer period of time. To achieve the design of sustained delivery of glimepiride, microspheres were prepared from where the rate of release was reduced by the incorporation of sodium alginate and gellan gum as polymer and $AlCl_3$ and GA served as counterion solutions for cross-linking of the drug. Both sodium alginate and gellan gum have the ability to undergo ionotropic gelation in aqueous solution in the presence of multivalent cations like Al^{3+}. Cross-linking occurs by the formation of covalent bonds between two or more molecules. The cross-linking agents contain two or more reactive ends to chemically attach with functional groups. This phenomenon is known as cross-linking. After the formulation of microspheres, they were evaluated to estimate their microscopy, swelling, yield, and entrapment and release. From this experiment, we have come to know that F3 is a better formulation than the rest formulated. Swelling of F3 was least while the entrapment percent was well. About 78.6% entrapment efficiency was recorded by the formulation F3. Release profile of the formulations revealed the sustained design of the drug, particularly formulation F3 releasing approximately 40% over 4 h. This study further assures us the chance and possibility of further development and optimization of the drug taken as a candidate for further investigation.

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AUTHORS' CONTRIBUTIONS

Each author is equally contributed.

CONFLICTS OF INTEREST STATEMENT

There are no conflicts of interest.

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