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Original Research Article

Effect of hydrophobic polymer on release profile of Diltiazem HCl loaded gelatinous microsphere cross-linked with glutaraldehyde

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Abstract: In this study, gelatin microspheres containing diltiazem hydrochloride (DTZ HCl) were prepared by the polymerization technique using glutaraldehyde as the cross-linking agent. The prepared microspheres were examined for its practical yield, drug content, and release kinetic. The study also includes the effect of processing variables on the result of evaluation. The shape and surface topology of prepared microspheres of various formulations were spherical as investigated by SEM studies. Pure drug and the drug polymer mixture showed similar peak in FTIR study which indicated no interaction between drug and polymer. From dissolution study of formulations, it was concluded that the formulation containing high amount of hydrophobic polymer gave high sustaining effect i.e. 8 hrs. So, the present work successfully achieved the objective of designing of controlled release microspheres of DTZ HCl.
Keywords: Diltiazem hydrochloride, gelatin microspheres, glutaraldehyde, polymerization

INTRODUCTION

An ideal drug delivery system should deliver a specified amount of drug to the site of action at an appropriate time and rate as required by the body. This idea is commonly realized in humans as feedback mechanisms and is used to control the amount and time of endogenous chemical release for optimal therapeutic activity. Similarly, the effects of dosage forms and dosing interval on the therapeutic efficacy of drugs have long being recognized as important factor for the successful treatment of diseases. Controlled drug delivery technology represents one of the frontier areas of pharmaceutical science that involves multidisciplinary scientific approach, contributing to human healthcare. These delivery systems offer numerous advantages compared to conventional dosage forms, which include improved efficacy, reduced toxicity, and improved patient compliance and convenience. Such systems often use macromolecules as carriers for the drugs [1].

Several approaches are currently utilized in the prolongation of the release factor. Encapsulation of drug molecules in particulate carriers as a method of controlled delivery of molecules has been studied extensively. In recent years, a number of different particulate systems have been proposed [2-4].It has been shown that reduction of either the applied dose or the frequency of administration give better pharmacological results compared to administration of conventional doses of drugs. The controlled release become an additional advantage for drugs that are absorbed slowly from the lower intestine.

Controlled release microspheres are especially effective in delivery of drug having short half-life by preventing the easy exposure of drug to the G.I.T. medium. The controlled release or sustained release will alter beneficially the absorption profile of the active agent, thus enhancing its bioavailability. Drugs that have poor bioavailability because of their limited absorption to the intestinal tract can also be delivered efficiently thereby maximizing their absorption and improving the bioavailability.

The present research work is an attempt to formulate controlled drug delivery system for DTZ HCl in the form of microsphere. So by increasing the release time of the drug, the availability of the drug can be lengthened. Microspheres were prepared using the mechanism of crosslinking procedure in which the drug was encapsulated within the matrixed gelatin polymer which was further made sustained using various water insoluble polymer such as ethyl cellulose and Eudragit RSPO, so that the drug will not soluble quickly in the gastric fluid, hence increasing the residence time within a suitable therapeutic range.

Glutaraldehyde was used as a crosslinking agent for gelatin which prevents the drug to release out quickly. The prepared microspheres were examined for its practical yield, drug content, and release kinetic. The study also includes the effect of processing variables on the result of evaluation. The shape and surface topology of prepared microspheres of various formulations were also investigated by SEM studies. So, the present work has been under taken with the objective of designing and evaluation of controlled release microspheres of DTZ HCl by using gelatin, with a ethyl cellulose varying properties of eudragit and a cross- linking agent glutaraldehyde.

Diltiazem HCl (2s-sis)-3-(acetyloxy-5-[2-(dimethylamino)ethyl]2,3-dihydro-2-(4-methoxy-phenyl)-1,5-benzothiazepine-4 (5H)-one monohydrochloride) is a calcium ion flux inhibitor (slow Cachannel blocker or Cachannel antagonist). It has generally been indicated for the treatment of angina and hypertension.

MATERIALS AND METHODS

MATERIALS

Diltiazem HCl and Eudragit RSPO were obtained as free gift from Torrent Pharmaceuticals, Waddi, India. Gelatin, Ethyl Cellulose and Span 80 were purchased from Merck Industry ltd, Mumbai. Glutaraldehyde and Light liquid paraffin were procured from Loba chemical, Mumbai. All other chemicals used in the study are of analytical grade.

METHODS

Preparation and Isolation of Microsphere

Controlled release microspheres of diltiazem HCl were prepared by the cross linking gelatin applying glutaraldehyde. The required amount of gelatin was taken in a 50ml beaker; to it 8ml of distilled water was added. Then the mixture was heated for 3-4 min at 40°C. After the polymer mixture was visible uniform then ethyl cellulose/eudragit RSPO was added as per the formulation shown in table-1. Then the specified amount of drug (i.e. 500mg) was dispersed thoroughly to the polymer solution.

In a 500ml beaker, 200ml of light liquid paraffin was taken and 0.1mL of span 80 was added to it. The mixture was maintained at 4°C with ice bath and stirred at 200 rpm. Then the previously prepared polymeric drug solution was added through a syringe with 22 gauge niddle. After 15 min, 2ml of glutaraldehyde was added drop wise to it. The stirring was continued for 3 hr. and the microspheres inside the beaker shown reddish in colour. After this condition the stirring was stopped and the microspheres were collected by filtration. Then the microspheres were washed by iso-propyl-alcohol 3-4 times till removal of liquid paraffin. Finally, microspheres were allowed to dry at room temperature (25 °C). Then the microspheres were collected, weighed, stored and taken for the evaluation [5, 6].

Table-1: Formulation design

Composition	Formula						
	F_0	F_1	F_2	F_3	F_4	F_5	F_6
Diltiazem HCl (mg)	500	500	500	500	500	500	500
Gelatin (mg)	1000	950	900	850	950	900	850
Ethyl Cellulose (mg)	0	50	100	150	0	0	0
Eudragit RSPO (mg)	0	0	0	0	50	100	150
Span 80 (ml)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Glutaraldehyde (ml)	2	2	2	2	2	2	2

Percentage yield

The percentage yield of different formulations were calculated using the following formula.

$$\% \text{ yield} = \frac{\text{The amount of microsphere obtained (mg)}}{\text{The theoretical amount (mg)}} * 100$$

Particle morphology

Scanning electron microscope (JEOL, JSM-6360, Japan) was used to characterize surface topography of the microsphere. The microsphere were placed on a metallic support with a thin adhesive tape and the samples were coated with gold under vacuum (fine coat, ion sputter, JFC-1100) to render them electrically conductive. The surface was screened and photomicrographs were taken at 15kV and 20kV for the drug-loaded microsphere.

Particle Size determination of microspheres

Microspheres were separated into different size fractions by sieving for 5 minutes using standard sieves having nominal mesh apertures of 1.0 mm, 0.71 mm and 0. 5 mm (sieve no.16, 22 and 30respectively). The particle size distributions of the microsphere were determined and mean particle sizes of microsphere were calculated using following formula.

$$\text{Mean particle size} = \frac{\sum(\text{Mean particle size of the fraction} \times \text{weight})}{\sum(\text{weight fraction})}$$

Determination of the swelling ratio

Gelatin microspheres were left in swelling medium (Double distilled water) for 60 min to reach maximum swelling. Volumetric measurements were

made by measuring the increased volume in the swelling medium at appropriate time intervals. The swelling ratio was calculated from the ratio of the volume of swollen particles to that of dry particles.

Drug Entrapment Efficiency

About 50 mg of accurately weighed drug-loaded microspheres were added to 50ml of phosphate buffer pH 7.4. The resulting mixture was kept shaking on mechanical shaker for 24 hrs. Then the solution was filtered and the drug content was estimated at 236.2 nm spectrophotometrically after appropriate dilution with phosphate buffer solution pH 7.4 using the standard calibration curve. All experiments were carried out in triplicate [1].

The drug entrapment efficiency was determined using the following relationship

$$\text{Drug entrapment efficiency} = \frac{\text{Experiment al drug content}}{\text{Theoretica l drug content}} \times 100$$

FTIR study

The IR spectra of pure drug (DTZ HCl), blank polymer and drug loaded polymeric microsphere cross-linked was obtained separately at room temperature in KBr pellets using a Simadzu Prestige spectrophotometer between the range of 400 to 4000 cm^{-1} and resolution was 2 cm^{-1} [7].

In-Vitro Drug Release Study

The in-vitro release of DTZ HCL from the controlled release microsphere and the pure drug was monitored in pH 7.4 phosphate buffer at 37 ± 1º C using USP peddle type dissolution rate test apparatus (UNILAB). Accurately weighed amounts of prepared microsphere of different formulations (wt. equivalent to 50mg of formulation) were stirred in 900ml dissolution medium at 50rpm. Aliquots of 5ml were withdrawn at predetermined time interval in 10ml volumetric flask, volume adjusted by the buffer and replenished immediately with the same volume of fresh medium. Aliquots following suitable dilution were analysed spectrophotometrically at 236.2nm. The concentration of drug in test samples was corrected for sampling effect. The drug release experiments were conducted in triplicates.

RESULT AND DISCUSSION

The prepared microspheres were a free flowing andreddish-browncolored. The color of the microspheres changed from yellow to yellowish orange and to reddish-brown when cross-linking time goes on increasing. This phenomenon may be caused by increasing the degree of gelatin cross-linking.

Scanning Electron Microscopy (SEM):

SEM was used to investigate the physical appearance of microspheres before dissolution study. The SEM micrographs as shown in Figure-1 revealed that the resulting microspheres were spherical in nature with almost smooth surfaces. But the SEM photographs which are taken in case of after dissolution showed rough surfaces containing cracks and holes over its surface.

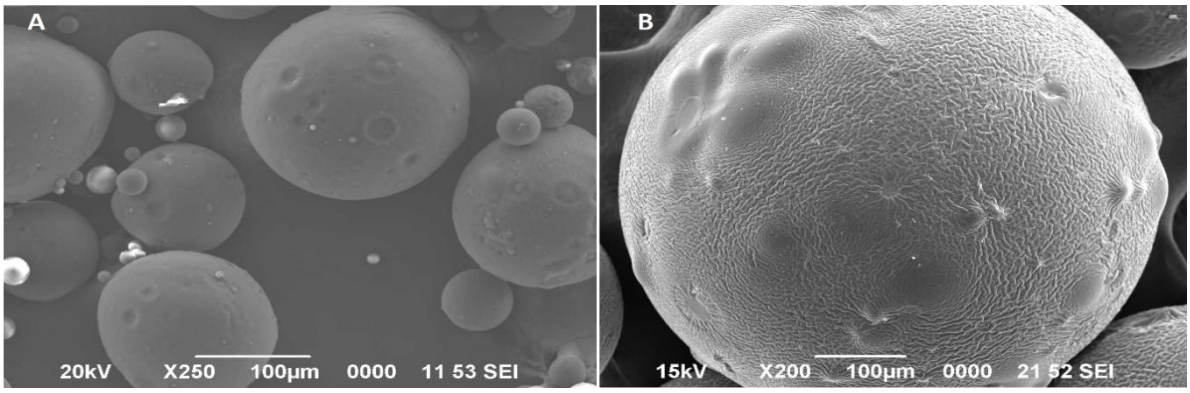

Fig-1: SEM of microspheres before dissolution (A); after dissolution (B).

FTIR

The interaction between the drug and the polymer often lead to identifiable change in the IR profile of drug in formulation. So the formulation was subjected to IR analysis in order to evaluate possible interaction between drug and polymer. Pure drug and the drug polymer mixture (formulation) shows similar peak (Table-2) which indicates that there is no interaction between drug and polymer. Hence the formula for preparing DTZ HCl loaded gelatin microsphere can be reproduced in the industrial scale without any apprehension of possible drug- polymer interaction.

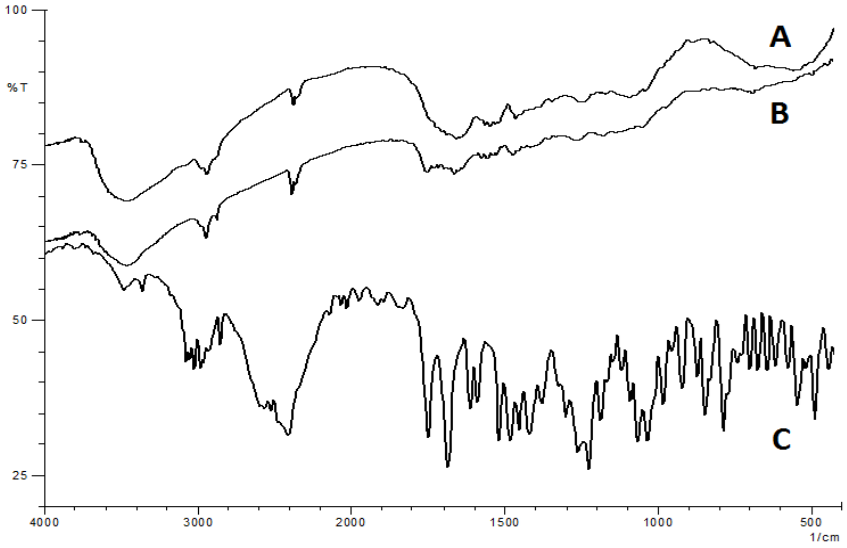

Fig-2: FTIR spectra of F_3 (A), F_6 (B) and pure drug (C)

Table-2: FTIR peaks identification

Frequency	Assignment
3056,3035-	Aromatic C-H stretch
2966--------	Aliphatic C-H stretch
2837--------	O-CH_3 C-H stretch
2393--------	Amine HCl N-H stretch
1743--------	Acetate C=O stretch
1679--------	Lactam C=O stretch
839---------	O-substituted aromatic C-H out-of-plane deformation
781---------	p-substituted aromatic C-H out-of-plane deformation

Yield and Mean particle size

The percentage yield and Mean particle size of the formulation were depicted in table-2. The particle size of the formulations were found to be between 320-351.11µm. It was found that the mean particle size was decreased with increasing the amount of ethyl cellulose in the formulation i.e. F1>F2>F3 and was increased with by increasing the amount of eudragit RSPO in the formulation i.e. F6>F5>F4

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Drug Entrapment Efficiency

Drug entrapment efficiency of all the formulation was found to be between 77.25±2.36 to 89.01±0.92 (table-3). The high entrapment efficiency suggests better drug content uniformity. This high drug content was due to the practically insoluble nature of hydrophobic polymer in the processing medium. Ethyl cellulose and eudragit, both are insoluble in water so the drug entrapment was increased as the amount of hydrophobic polymer was increased in the formulation.

Table-3: Particle Size of different formulations:

Formulation Code	Yield (%)	Mean particle size (µm)	%drug Entrapment
F_0	82.53	351.11	84.49±1.04
F_1	80.33	347.53	85.97±1.17
F_2	77.06	336.42	87.69±2.01
F_3	73.33	331.53	89.01±0.92
F_4	80.46	320	77.25±2.36
F_5	81.2	338.9	80.19±1.09
F_6	82.33	345.32	82.02±0.84

In vitro release behaviour of drug

In general, the release mechanism from a swellable hydrophilic system containing within a cross – linking agent will be influenced by a number of parameters. This included the rate of fluid infusion into matrix, the rate of matrix swelling, the molecular

diffusion of drug through swollen gel layer polymer relaxation, chain disentanglement, dissolution, erosion & total disentanglement at the dissolution front.

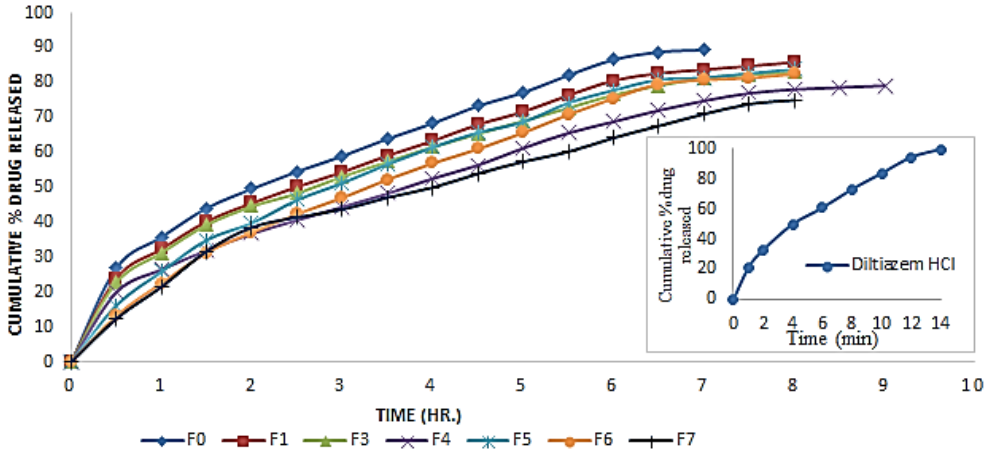

Fig-3: In vitro release behaviour of drug

The release profiles of DTZ HCL from gelatin microspheres cross-linked using glutaraldehyde- are displayed in Figure-3. A biphasic pattern of drug release was observed for all samples. Thus, regardless of the conditions of the cross-linking of gelatin microspheres, an initial burst release was observed. Within the first hour, about 30% of the loaded drug was released. This burst release was followed by a prolonged period, during which slower drug release took place. This type of release profile is of interest because the initial burst release can provide the initial penetration of the drug, and the sustained release phase supplies drug over a prolonged period of time [8]. The initial burst drug release may be attributed to the release of drug molecules held loosely into or just beneath the surface of microspheres. Such a burst effect was reported previously in literature for gelatin microspheres [9, 10].

From the pure drug dissolution, we found the t_{50} of the drug that was nearer to 4.1 min. But all the formulations prepared in this experiment had the t_{50} values lies between 2.5 to 3 hrs. From that we concluded that the rate of drug release from all the formulations were sustained for much more time in comparison to the pure drug release kinetics (which were many times sustained release in comparison to pure drug release pattern). In all formulation the rate of release was different due to their differences composition. With increasing amount of Ethyl cellulose from 50-150 mg for different formulations the drug dissolution rate decreased from F1 to F3 .This could be attributed to the hydrophobic behaviours of EC in the formulation as increasing the amount of Ethyl cellulose leading to slow drug release. Similar drug release pattern also observed in case of Eudragit replacing ethyl cellulose in the formulation. However eudragit is found to be more effective in sustaining the release as compared to ethyl cellulose at same concentration. Glutaraldehyde as cross linking agent have a tendency to form a rigid hydro gel so which restrict the leach out hence decrease the dissolution. Microspheres maintained their spherical shape during the release period (Figure-1).

CONCLUSION

The present work described a study on formulation and evaluation of controlled release microspheres of Diltiazem HCl by using different polymers (gelatin, ethyl cellulose, Eudragit) cross – linked with glutaraldehyde. From the result it is found that all the formulations shows excellent sustained property. The microspheres of all the formulations were more or less spherical in nature. From dissolution study of above formulations, it was concluded that the formulation containing high amount of hydrophobic polymer gave high sustaining effect i.e. 8 hrs. It revealed a distinct biphasic release pattern that may be desirable for oral drug delivery, since a therapeutic loading dose can be provided initially and the sustained drug release could maintain the therapeutic drug level. Thus the prepared gelatin microspheres may prove to be potential candidate for multiple unit delivery, may result in new therapeutic possibilities with substantial benefit to the patient.

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