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Development and evaluation of acyclovir loaded chitosan microspheres and cross linked with glutaraldehyde

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Abstract

The guanine derivative antiviral drug acyclovir (ACV) is one of the oldest molecules put downing triumphant market until date, being commercially accessible in a variety of dosage forms for oral, topical and parenteral administrations. Clinical purpose of this drug is better to new antiviral agents due to its potential values such as suppression of recurrence, security profile, negligible drug interactions and being inexpensive. ACV is slightly water soluble, less permeable and poorly bioavailable, yet further potential antiviral molecule, the physicochemical alterations and new dosage form approaches resulted with more than 100 research efforts within a decade. The current study endeavored at the formulation of chitosan microspheres loaded with ACV to conquer the poor bioavailability and recurrent dose administration. Chitosan microspheres were prepared by emulsification technique by glutaraldehyde cross-linking. A variety of formulation and process variables such as polymer, glutaraldehyde, drug, span 80 concentrations, effect of stirring speed and stirring time were optimized. Formulated microspheres were characterized for its drug loading, *invitro* drug release, entrapment efficiency, surface morphology (SEM), particle size analysis and FTIR spectroscopy. The characterization of the fabricated microspheres demonstrated smooth surface with thin particle size allocation and entrapment efficiency of 80.8% for stirring speed batch. The prepared microspheres showed a controlled drug release of 93.2% over a period of 8 hrs with initial burst release of 56.7 % in the first 2hrs. The FTIR showed that there was no possible drug interaction among the drug and polymer. From the data's obtained it can be concluded that the chitosan microspheres could be believed as a possible carrier for controlled drug delivery of ACV.

Keywords: Acyclovir, Antiviral drug, Microspheres, Chitosan, Glutaraldehyde.

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INTRODUCTION

Oral route is most suitable and preferable route for drug administration to arrive at systemic circulation because of its low cost and simple administration. But achievement of conventional dosage form is bounded due to its residence time. Hence mucoadhesive microsphere drug delivery systems are employed to extend the residence time at the site of application, preserve therapeutically efficient plasma drug concentration levels for a longer duration, reducing the dosing occurrence and reduce variations in the plasma drug concentration at the steady state in controlled and reproducible way. Mucoadhesive microspheres happen to adhesive on hydration and therefore employed for localizing the drugs to a scrupulous target site of GIT for prolonged period of time. Moreover, it is simple for administration, no patient compliances and suppleness in the formulation. One of the most possible approaches for attaining a prolonged and unsurprising drug delivery in GIT is to control gastro retentive drug delivery system which will give significant therapeutic alternatives. Mucoadhesive microspheres delivery system is an attractive due to aptitude of adherence to the mucosal surface and discharges the entrapped drug in a sustained release. Bio adhesion occurrence is connected with biological surface and mucoadhesion connected with i.e. mucin layer of a mucosal tissue. Mucoadhesive microspheres have benefits like competent absorption, improved bioavailability of the drugs, highest use of drugs and much more close contact with intestinal cells, enhanced patience compliance and targeting to precise absorption site [1-4]. Chitosan is a biodegradable, biocompatible and nontoxic natural polymer thus it has a great potential for biomedical and pharmaceutical applications. Chitosan is cationic in nature, so it has superior mucoadhesive and membrane permeability enhancing properties. Chitosan has previously been demonstrated to improve the mucosal absorption of a variety of compounds in a drug delivery system and have adjuvant activity in the mucosal immune response. Chitosan is a famous rate controlling polymer for drug release which assists in prolongation of the duration of action and bringing the drug to the precise sites in the body. Also, chitosan does not cause any hypersensitivity or allergic reactions with living tissues [5]. It breaks down gradually to amino sugars which is harmless and totally absorbed by the human body.

There are so a lot of accounts that established the efficiency of chitosan microspheres as a vehicle for convey of drugs in the body. Thus, this show to be safe, extensively available and cost-effective. ACV is an agent used for the treatment of infections caused by herpes viruses. The low oral bioavailability of ACV (20%) and short half-life (2-5hours) has persuaded its dosing frequency 5 times a day [6, 7]. The plan of this study was to expand mucoadhesive microspheres of ACV for gastric retention. Mucoadhesive microspheres so produced will prolong gastric residence time at or above the absorption site, thus enhancing the absorption and bioavailability of ACV. Thus consequence system could be employed for controlled release of ACV.

MATERIALS AND METHODS

MATERIALS

ACV was obtained as a gift sample from Macleods Pharmaceuticals, Mumbai. Chitosan, glutaraldehyde (25% aqueous), liquid paraffin light, span 80, petroleum ether were obtained from Loba Chem Pvt Ltd, Bombay. All other chemical used were purchased from S.D Fine Chemical Limited, Mumbai and Qualigens Fine chemicals, New Delhi. Double distilled water was prepared freshly and used whenever required. All other chemicals used in this study including those stated were of analytical reagent (A.R.) grade.

Preformulation studies

Physical characteristics

By visual examination, the drug was recognized for physical characters like colour, texture, odour etc.

Solubility

Solubility of the drug was indomitable by taking some amount of drug (about 1-2 mg) in the test tube discretely and added the 5 ml of the solvent (water, ethanol, methanol, 0.1N HCL, 0.1N NaOH, chloroform and 7.4 pH buffer) shake vigorously and kept for some time. Note the solubility of the drug in a variety of solvents (at RT).

Melting point

A minute quantity of powder was placed into a fusion tube. That tube was placed in the melting point determining apparatus (Chemline) containing castor oil. The temperature of the castor oil was slowly increased automatically and read the temperature at which powder started to melt and the temperature when all the powder gets melted.

FTIR spectroscopy

Recognition of ACV was done by FTIR spectroscopy with respect to marker compound. ACV was obtained as white or almost white crystalline powder. It was identified from the result of IR spectrum as per specification.

Determination of λ_{max} of ACV

Exactly weighed 10 mg of drug was dissolved in 10 ml of 6.8 pH buffer solution in 10 ml of volumetric flask. The resulted solution 1000µg/ml and from this solution 1 ml pipette out and transfer into 10 ml volumetric flask and volume make up with 6.8 pH buffer solution prepare suitable dilution to make it to a concentration range of 5-25µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V. spectrophotometer (Shimadzu-1800). A graph of concentration Vs absorbance was plotted.

Preparation of chitosan microspheres

Chitosan-based microspheres are prepared by the accounted method with some modifications. 4 % solution of chitosan in 5% aqueous acetic acid containing 2%Nacl was prepared and the drug was loaded by mixing the required amount of drug with 6gm of chitosan paste and it was detached in a mixture of 35ml liquid paraffin and 25ml of petroleum ether containing 0.85gm of sorbiton sesquioleate in a 100ml round bottomed flask at room temperature [8]. The dispersion was stirred using stainless steel half moon shaped paddle stirrer at 2000 rpm for 5min and 10ml of glutaraldehyde saturated toluene (GST) prepared according to the method of patel et al., [9] and introduced into the flask and the stirring was continued. At the end of 30mins, glutaraldehyde (25% v/v aqueous solution) was added and stirring was sustained. The volume of cross linking agent and cross linking time was varied in beginning trial batches from 1-4ml and 1-3 hours respectively. The stirrer speed was also diverse from 1000-4000rpm. The stirring was sustained for a total duration of 90 mins, at the end the hardened microspheres were filtered, washed numerous times with petroleum ether followed by acetone, a 5% solution of sodium metabisulphate and lastly with water. The microspheres thus obtained were dried overnight in an air oven at 60ºC. Microspheres having different cross linking densities were prepared using dissimilar amounts of glutaraldehyde for cross linking. The microspheres were stored in a desiccator.

Optimization of formulation process variables

The consequence of formulation process variables such as stirring time, stirring speed on the particle size was studied. From the consequences obtained, optimum level of those variables was selected and reserved constant in the succeeding evaluations. Dissimilar ACV incorporated microspheres were prepared using effect of chitosan, span-80 quantity and stirring speed. The prepared microspheres were additional evaluated for entrapment efficiency, particle size and *In Vitro* drug release study.

Table1 Effect of polymer concentration

Formulation code	% Polymer Conc.	Particle size (µm)	%Entrapment Efficiency
F1	1	13.11±0.9	69.3±1.7
F2	2	13.69±1.2	76.8±1.9
F3	**3**	**14.28**±1.3	**79.3**±1.9
F4	4	16.57±1.6	78.1±1.8

Table 2 Effect of drug concentration

Formulation code	% Drug Conc.	Particle size (µm)	%Entrapment efficiency
F5	5	13.15±0.8	69.7±1.1
F6	10	13.62±1.1	72.3±1.4
F7	**15**	**13.73±1.3**	**77.1±1.9**
F8	20	16.98±1.6	72.1±2.3

Table 3 Effect of glutaraldehyde concentration

Formulation code	GST conc. (ml)	Particle size (µm)	%Entrapment efficiency
F9	1	12.87±0.6	70.2±1.3
F10	**2**	**13.72±0.8**	**76.2±1.4**
F11	3	10.19±1.2	60.7±1.1
F12	4	10.02±1.1	59.5±0.9

Table 4 Effect of span 80 concentration

Formulation code	Span 80 conc. (ml)	Particle size (µm)	%Entrapment efficiency
F13	0.75	14.32±1.9	71.7±1.2
F14	**1.0**	**13.82±1.6**	**75.8±1.4**
F15	1.25	10.28±1.2	70.1±1.1
F16	1.5	10.05±1.1	69.5±0.9

Table 5 Effect of stirring speed

Formulation code	Stirring speed (rpm)	Particle size (µm)	%Entrapment efficiency
F17	**1000**	**13.51±1.1**	**80.8±2.1**
F18	2000	13.93±1.3	77.5±1.8
F19	3000	9.80±0.8	74.3±1.6
F20	4000	9.23±0.7	73.5±1.3

Table 6 Effect of stirring time

Formulation code	Stirring speed (rpm)	Particle size (µm)	%Entrapment efficiency
F21	**3.0**	**14.51±1.8**	**77.4±1.6**
F22	4.0	13.79±1.6	76.2±1.4
F23	5.0	10.70±1.2	73.8±1.1
F24	6.0	10.12±0.9	72.2±1.0

Characterization of ACV loaded chitosan microspheres

Particle size analysis

The size allocation in terms of average diameter (davg) of microspheres was indomitable using the optical microscopic method. Scanning election microscopy (SEM) was performed to characterize the surface morphology of formed microspheres by using Hitachi S-520 SEM [10].

Entrapment efficiency

50mg of exactly weighed microspheres were crushed in a glass mortar-pestle and the powdered microspheres were suspended in 10ml of pH6.8 phosphate buffer solution. After 24 hrs the solution was filtered and the filtrate was analysed for drug content. The drug entrapment was calculated using the formula [9].

Percentage Drug entrapment = [Weight of drug present in microspheres (practical drug content) / Theoretical weight of drug] X 100.

Shape and surface morphology

Shape and surface morphology of chitosan microspheres was visualized by scanning electron microscopy (SEM), was conducted to describe the surface morphology of chitosan microspheres. The sample were prepared by taking a drop of chitosan microspheres dispersion on a double adhesive tube, which was fixed to an aluminum stub and air dried the stub was then coated with gold. The stub was allowed to air dry thoroughly and samples were analyzed under a scanning electron microscope and photographs were taken at suitable magnification.

Invitro release study

In vitro drug release studied of microspheres were carried out in 100 ml dissolution medium, which was stirred at 100rpm at 37±2°C (USP type II) of using different pH was as at simulated gastric fluid (SGF) of (pH 1.2) simulated intestinal fluid (SIF) of (pH 6.8) PBS (pH 7.4) solution. Cross linked ACV loaded chitosan microspheres bearing drug were suspended in dissolution media (100 ml) at 37±2°C. Sample were withdrawn periodically and compensated with same amount of fresh dissolution media. The samples were analyzed for drug content by measuring absorbance using UV spectrophotometer (Shimadzu 1800) [11].

Stability studies for formulation

Accelerated testing, are the studies intended to intensify the rate of chemical degradation or physical change of a drug substance or drug product by exaggerated storage conditions as part of the formal stability studies. The formulation was taken and accelerated stability study was performed by taking suitable quantity of microspheres. The microspheres were placed in air-tight glass container at 4°C, room temperature and at 45±2°C for 30 day period. At suitable sampling interval the samples were withdrawn and evaluated for various parameters.

RESULTS AND DISCUSSION

ACV was establishing to be white off crystalline powder in appearance, odourless and tasteless. The melting point of ACV (pure drug) was established to be 256-258°C; it matches with the standard (256.5 °C). ACV was freely soluble in ethanol, methanol, 0.1 N HCl, slightly soluble in 0.1 N NaOH, distilled water and soluble in chloroform, phosphate buffer pH 7.4. Recognition of ACV was done by FTIR spectroscopy with respect to marker compound. It was identified from the consequence of IR spectrum as per specification. The calibration curve of ACV was found to be linear in the concentration range of 5-25µg/ml at 253.4nm. The prepared chitosan microspheres were characterized for its shape, surface morphology, particle size, % entrapment efficiency. Shape of chitosan microspheres was established to be spherical with smooth surface Fig. 1. The particle size and % entrapment efficiency of chitosan microspheres enhance from 13.11±0.9µm to 16.57±1.6m and 69.3±1.7% to 79.3±1.9% respectively as the polymer concentration enhance from 1 to 4 which is because of the enhance in the viscosity of polymer solution which leads to enhance in particle size and % entrapment efficiency of microspheres Table 1. The drug concentration was assorted from 5-20 as the concentration of drug was increased the particle size was increased from 13.15±0.8µm to 16.98±1.6µm, with enhance in the % entrapment efficiency from 69.7±1.1% to 77.1±1.9% and on additional increasing the drug concentration, there is no considerable increase in % entrapment efficiency of microspheres was seen Table 2. Span 80 concentration was optimized for chitosan microspheres and it was establish that particle size and % entrapment efficiency was reduce from 14.32±1.9µm to 10.05±1.1µm and 71.7±1.2% to 69.5±0.9% when the span 80 concentration was increased from 0.75% to 1.5% which leads to the formation of more finer particle which is because of stabilization of emulsion droplets avoiding there coalescence Table 4. Similarly for process variables stirring speed and stirring time was optimized. It was observed that on increasing the rpm and time the particle size and % entrapment efficiency was decreased. The particle size was decreased from 13.51±1.1µm to 9.23±0.7µm and 14.51±1.8µm to 10.12±0.9µm, when rpm was varied from 1000 to 25000 and stirring time was varied from 3-6 hrs respectively. Similarly consequence were obtained for % entrapment efficiency, which was decreased from 80.8±2.1% to 73.5±1.3% in case of stirring speed and 77.4±1.6% to 72.2±1.0% for stirring time Table 5,6. *In vitro* drug release from microspheres was indomitable using GIT fluid of different pH according to Souder and Ellenbogan technique. In case of cross linked chitosan microspheres nearly 70-80% of the drug released in initial 4-5hrs Table 7. This situation is best upper part of GIT. As for as treatment of hepatitis B is concerned it is of almost significant to make sure the delivery of drug in intact from in the vicinity of target organ. Cross linked ACV loaded chitosan microspheres were to retard the release of the drug until pH reaches above 6.0 which were then analyzed for *in vitro* drug release by following the same technique as followed above and consequence suggested that ACV loaded chitosan microspheres retards the rate of drug release till they reaches the colon and hence they are suited for the delivery of drug for the treatment of diseases associated with hepatitis B. ACV loaded chitosan microspheres were assessed after storage of the formulation for 30 day at 4±2°C, room temperature and 45±2°C and consequence were compared with that obtain before storage. Consequence of stability studies reveals the particle size of chitosan microspheres gets increased from 15.9.±0.8µm to 16.8±1.1µm when stored at room temperature and at 45±2°C, respectively and decreased from 15.9µm to 15.1±0.9µm when stored at 4±2°C. % entrapment efficiency of chitosan microspheres decreased from 76.2% to 75.7±1.3% at 4±2°C, 75.9±1.5% at room temperature and 73.8±1.2 at 45±2°C Table 8.

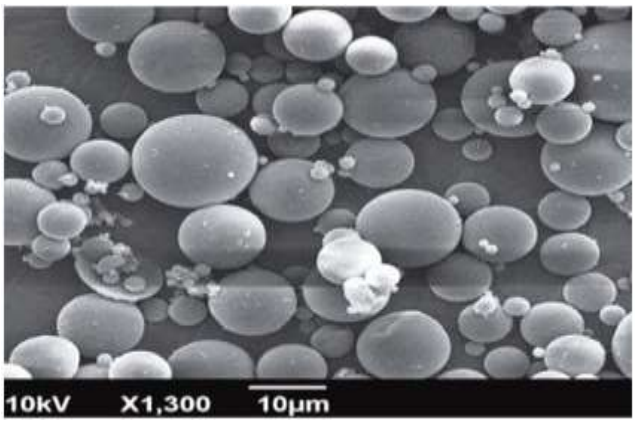

Figure 1 Scanning electron photomicrograph of chitosan microspheres

Table 7 *in vitro* drug release of ACV loaded chitosan microspheres atdifferent pH

S. No.	Timehrs	% drug release chitosan microspheres SGF PH 1.2	% drug release chitosan microspheres SIF PH 6.8	% drug release chitosan microspheres PBS PH 7.4
1	1	35.6±0.9	37.6±0.8	38.6±0.7
2	2	54.7±0.6	56.7±1.1	57.7±1.0
3	3	68.4±0.8	70.4±1.4	71.4±1.2
4	4	74.1±1.2	76.1±1.6	77.1±1.3
5	5	79.3±1.3	81.3±1.9	82.3±1.6
6	6	84.5±2.1	86.5±2.2	87.5±1.9
7	7	88.2±2.6	90.2±2.4	91.2±2.1
8	8	91.2±2.8	93.2±2.5	94.2±2.5

Table 8 Effects of storage on particle size and % entrapment efficiencychitosan microspheres

Parameters	Initial Observation	After 30 day		
		At4°C	At RT	At 45±2°C
Particle size (µm)	15.9	15.1±0.9	15.9±0.8	16.8±1.1
% EE	76.2	75.7±1.3	75.9±1.5	73.8±1.2

CONCLUSION

ACV loaded chitosan microspheres using glutaraldehyde as cross-linking agent by simple emulsion technique could be produced successfully with rapid burst release of 56% and subsequent release of 93% up to 8 hrs. Chitosan being natural biodegradable polymer gives no toxicity when incorporated in formulations. The present ACV loaded microspheres are suggested to be helpful for the improvement of ACV efficacy against hepatitis B virus.

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