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FORMULATION OF CHITOSAN MICROSPHERES COVALENTLY ATTACHED WITH GALLIC ACID FOR DELIVERY IN ACIDIC ENVIRONMENT

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ABSTRACT

Microspheres of chitosan covalently attached with gallic acid were prepared using glutaraldehyde as the crosslinking agent and esterification as the process of attachment of gallic acid to chitosan surface. The formulated micropsheres were characterized for yield, particles size, micromeritic properties (angle of repose, bulk density, tapped density, Hausner's ratio, Carr's index), and *in vitro* drug release in various pH solutions (pH 1, 3, 4.5 and 6.5). The mean particle size of chitosan microspheres ranged from 46.147 ± 24.36 to 60.065 ± 21.72 µm while the size increased on attachment of gallic acid to be ranging from 57.867 ± 27.50 to 71.785 ± 17.07 µm. The percentage yield of different formulations was in the range 64.73 ± 0.6027 to 82.36 ± 0.4509 %. All the formulated microspheres exhibit good flow properties with a value of angle of repose between 26°18' to 29°53'. The Carr's index of all the formulations was found in the range 14.03 to 19.95 % indicating good flow ability. The Hausner's ratio of the formulations was found in the range 1.16 to 1.25. The highest amount of drug was released at pH 3 (79.4%) after 40 min of incubation while the lowest amount of drug was released at pH 6.5 (7.0%).

KEYWORDS: Gallic acid, Chitosan, Covalent linkage, Esterification, Antioxidant.

INTRODUCTION

Antioxidants are chemicals that help stop or limit damage caused by free radicals. Your body uses antioxidants to balance free radicals. This keeps them from causing damage to other cells. Antioxidants can protect and reverse some of the damage. They also boost your immunity. Gallic acid (GA) (3,4,5-trihydroxybenzoic acid) is an endogenous plant polyphenol product (phenolic acid) widely distributed in many species of plants.[1] GA is reported to have memorably cardioprotective activity and receiving considerable attention in medical and nutritional research. A previous study has revealed that GA has beneficial effect on lysosomal enzymes, lipid peroxidation, and reduced glutathione in isoproterenol induced cardiac damage in rats.[2] Gallic acid is known to achieve peak plasma concentration after 1.3 to 1.5 hours of administration and a half life of around 2 hours and is one of the best bioavailable natural antioxidants.[3] The lower half life of the phytoconstituent limits its use as conventional therapy for IHD. Some previous studies to improve the half life have been reported mainly comprising of encapsulation of the drug into polymeric particles of micro or nano size.[4-7] Covalent linkage of gallic acid to chitosan microspheres would be an effective strategy to delivery gallic acid to the acidic environment of the stomach by virtue to mucoadhesive property of chitosan and hydrolytic degradation of the covalent linkage leading to release of gallic acid.

MATERIAL AND METHODS

Chitosan was procured from Himedia; Gallic acid was obtained from CDH;, all other reagents and chemicals were purchased from Oxford Fine Chemicals, Mumbai, India and were of analytical grade.

Preformulation studies

Organoleptic evaluation

The color, odour and taste of the obtained drug sample were observed with the help of the sensory organs.

Solubility (at room temperature)

Solubility was determined in different solvents like water, methanol, ethyl Alcohol, and DMSO.

Loss on Drying (LOD)

LOD was determined on IR moisture balance by heating a weighed amount of drug.

Melting point determination

Melting point was determined by open capillary method and is uncorrected. A small quantity of powder was placed into fusion tube and placed in the melting point apparatus.

Preparation of saturated solution of glutaraldehyde in toluene[8]

Equal quantity of aqueous glutaraldehyde solution and toluene was taken in a separating funnel and shaken for 1 hour to allow the saturation of glutaraldehyde in toluene. Then the aqueous phase and toluene phase was separated. Thus obtained toluene saturated with glutaraldehyde was used to cross-link gelatin microspheres.

Preparation of chitosan microspheres[9]

Accurately weighed quantity of chitosan (Table 1) was dissolved in 10 ml of 1%v/v aqueous solution of acetic acid in a 500 mL beaker. The viscous solution was mixed using a mechanical stirrer at a speed of 1200 rpm. 5.0 mL of distilled water was added and the chitosan solution was mixed for another 1 h. Span 40, Span 80 and cyclohexane were then added to the chitosan solution. The emulsion obtained was heated to 40 °C and mixed further for 1 h. Accurately measured volume of solution containing glutaraldehyde was added dropwise over a period of 15 min. The mixture was kept overnight (for about 10 h) to complete the cross linking. The microspheres were then filtered out, washed with an excess of water, followed by methanol and finally twice with diethyl ether, and dried at 50 °C.

Table 1: Composition of different batches of microspheres.

S. No.	Ingredient	Batch Code				
		F1	F2	F3	F4	F5
1	Chitosan (mg)	200	200	200	200	200
2	Span 40 (mg)	200	200	200	200	200
3	Span 80 (mg)	100	100	100	100	100
4	Glutaradehyde (mL)	2	3	4	5	6
5	Cyclohexane (mL)	100	100	100	100	100

Covalent attachment of gallic acid to chitosan microspheres[10]

Esterification method was used for covalent attachement of gallic acid to the microspheres. Briefly, in a 50 ml flask 100 mg of the crosslinked chitosan microspheres was added to 1 mL of the solution prepared by dissolving 1.5 g of $LiNO_3$ in 10 ml of DMA at 70 °C. 0.117 g (0.932 mmol) of DIC, 6 mg (0.47 mmol) of DMAP and 0.158 g (0.932 mmol) of gallic acid (DIC/GA =1:1 mol:mol) were then added one by one to the $LiNO_3$ solution. The suspension obtained was allowed to stand overnight at room temperature while bubbling with nitrogen and stirring on a magnetic stirrer. The microspheres were then filtered out, washed with an excess of water, methanol and diethyl ether twice, and dried in 50 °C.

Characterization of microparticles[11]

Determination of yield

The dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formulae given below:

$$\% \text{ yield} = \frac{\text{Mass of the dried microspheres obtained} * 100}{\text{Total Weight of drug and polymer}}$$

Determination of particle size of microspheres

The particle size of the microspheres was determined by using an Olympus microscope, employing the calibrated eye piece and stage micrometer method.

Micromeritic properties

Angle of repose was measured using fixed funnel method; the apparent bulk and tapped densities were measured using tapped density testing apparatus.

***In vitro* release of gallic acid**

In vitro release of gallic acid from microparticles was determined by dispersing the accurately weighed gallic acid attached microparticles (10 mg) and in 1 mL of aqueous solutions of pH 1, 3, 4.5 and 6.5 and incubating them at 37°C under gentle shaking at 100 rpm. After 5 min of incubation, the supernatant was withdrawn and assayed for gallic acid release by UV spectrophotometery at 256.2 nm.[12] The percent release at each pH was measured to establish the release mechanism. The process was repeated for up to 40 min.

RESULTS AND DISCUSSION

Physical characterization of the drug

Gallic acid was slightly yellow crystralline powder with a melting point range of 257-262°C. It was odorless and had a LOD value of 0.26%. It was soluble in water, methanol and ethanol.

Particle size of formulations

The particle size of various formulations was determined by calibrated eye piece and stage micrometer. The mean particle size of chitosan microspheres ranged from 46.14 to 60.06 µm while the size increased on attachment of gallic acid to be ranging from 57.86 to 71.85 µm (Table 2).

It was found that the amount of glutaraldehyde affected the size of the particles. Using lower amounts of glutaraldehyde (F1 & F2) the size was higher while in increasing the amounts of glutaraldehyde decreased the size of the chitosan microspheres. The attachment of gallic acid on the surface of the chitosan particles increased the particle size.

Table 2: Particle Size and Percent yield of formulated microspheres.

Formulation	Particle Size of chitosan microsphere(µm)	Particle size of gallic acid attached microsphere (µm)	Percent Yield (%)
F1	60.065 ± 21.72	71.785 ± 17.07	64.73 ± 0.6027
F2	52.007 ± 24.45	66.657 ± 23.93	65.96 ± 0.4041
F3	50.542 ± 23.51	62.265 ± 24.64	81.53 ± 0.4041
F4	48.345 ± 27.34	60.797 ± 27.41	82.26 ± 0.4725
F5	46.147 ± 24.36	57.867 ± 27.50	82.36 ± 0.4509

Percentage yield of microspheres

The various formulations of the prepared microspheres were evaluated for the percentage process yield. The percentage yield varied from 64.73-82.36% (Table 2). It was evident from the yield that the yield of the microspheres was unaffected by increasing the amount of glutaraldehyde beyond a level. Formulations F3-F5 exhibited almost similar yield suggesting that 4 mL of glutaraldehyde solution was sufficient to produce the cross-linking of chitosan particles to yield stable particles.

Micromeritic properties

The angle of repose was found to be in the range of 26°18’ to 29°53’. The bulk density value ranged from 0.321 to 0.386 g/cm^3. The tapped density value of various formulations of the microspheres was found to be in the range from 0.401 to 0.465 g/cm^3 (Table 3).

Table 3: Micromeritic properties of microparticles.

Batch code	Angle of Repose	Bulk Density	Tapped Density	Carr's Index	Hausner's ratio
F1	27°41'	0.321	0.401	19.95	1.25
F2	28°03’	0.348	0.421	17.34	1.21
F3	26°18’	0.356	0.418	14.83	1.17
F4	27°29’	0.379	0.465	18.49	1.23
F5	29°53’	0.386	0.449	14.03	1.16

The micromeritic properties of F3 were found to be optimum and hence it was considered to be the best formulation for studying the release of gallic acid in various pH solutions.

***In vitro* drug release study**

The *in vitro* drug release study of the gallic acid was evaluated in solutions of pH 1, 3, 4.5 and 6.5. The % cumulative release and % log cumulative release was calculated

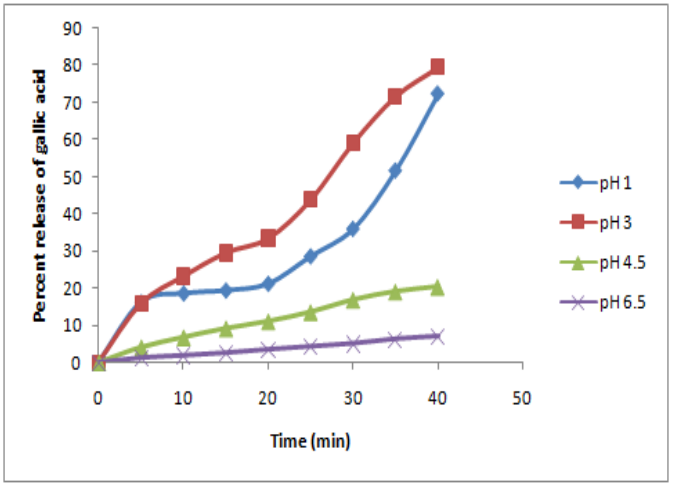

Figure 1: *In vitro* release of gallic acid from F3.

The microspheres of were prepared using chitosan as the biodegradable polymer and glutaraldehyde as the crosslinking agent. The microspheres were prepared by emulsion solvent evaporation method by varying the

concentration of the crosslinking agent (F1-F5). The drug (gallic acid) was covalently attached to the surface of the chitosan microspheres using esterification process.

The microencapsulation process utilized in the investigation produced satisfactory yield of microspheres with free flowing nature. The usage of cylcohexane with span 40 and span 80 was found to be effective in dispersing the aqueous globules containing polymer and resulted in good yield of microspheres. The cross-linking of chitsan microspheres was done by using glutaraldehyde-saturated toluene solution. The particle size of the microspheres was in the range of 500 to 1100 µm. The size of microspheres was found to decrease with the increase in glutaraldehyde concentration suggesting good cross-linking of particles.

The *in vitro* release data from different formulations were studied various pH solutions. It was found that the higher acidic solutions were able to release the drug while increasing the pH values decreased the release of drug. This indicates that the drug release occurs due to the acidic degradation of the ester linkage formed between the gallic acid carboxyl and the chitosan hydroxyl groups. Increasing the pH leads to non cleavage of the ester linkage and hence the drug release decreases.

CONCLUSION

In the present study, microspheres covalently with gallic acid were prepared using emulsion solvent evaporation methods using chitosan as the natural polymer and gluataraldehyde as the crosslinking agent. The results obtained showed that this methodology was able to produce reproducible microspheres and resulted in localized delivery of gallic acid in acidic medium. Hence it could be concluded that the microspheres produced from chitosan, covalently attached to gallic acid is an excellent delivery system that has good release behavior for actively releasing drug in stomach.

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