Research Article

Preparation and characterization of microspheres containing Gallic acid

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Abstract

Objective: The present study aimed at the formulation and evaluation of microspheres loaded with Gallic acid to overcome the issues of poor solubility and bioavailability. **Materials and methods:** Solvent evaporation technique was used to prepare microspheres containing Gallic acid. Formulated microspheres were characterized for its entrapment efficiency, drug loading, *in-vitro* drug release, surface morphology and FTIR spectroscopy. **Results:** The microspheres were spherical in shape with smooth surface. The entrapment efficiency was found to be 84%. The FTIR spectra showed that there was no potential drug interaction between the drug and polymer. **Conclusion**: From the data obtained it can be concluded that the eudragit microspheres could be considered as a potential biodegradable carrier for controlled drug delivery of Gallic acid.

Keywords: Eudragit, Gallic acid, microspheres, optimization

Introduction

Microspheres play a significant role in novel drug delivery system due to their small size and efficient carrier capacity ranges from 1-1000 µm. They have a core of drug and entirely outer layers of polymers as coating material. These spheres are free flowing powder. They mainly contain proteins or synthetic polymers, which are biodegradable in nature. Microspheres have significant role in delivering therapeutic drug to the target site in sustained and controlled release formulation (Manca et al., 2011; Meena et al., 2011). As the name indicates, microspheres are small and spherical particles. Its particle size range from 1 to 1000 µm in diameter, and wall thickness can vary from several microns to as low as 0.1 micron (Mittal et al., 2011; Ramteke et al., 2012).

Microspheres can be prepared from many natural and synthetic substances. The most available microspheres are ceramic microspheres, glass microspheres and polymer microspheres. The two most widely used polymer microspheres are polyethylene and polystyrene microspheres. They are synthesis from polymeric, waxy, or other protective materials such as starches, gums, proteins, fats and waxes (Hire et al., 2014; Tabassi et al., 2003).

Microsphere size will be strongly affecting the rate of drug release. As size decreases, the surface area to volume ratio of the particle increases. Thus, for a given rate of drug diffusion through the microspheres, the rate of flux of drug out of the microspheres, per mass of formulation, will be increase with decreasing particle size (Saralidze et al., 2010; Saravana et al., 2012). In addition, water penetration into smaller particles may, quicker due to the shorter distance from the surface to the center of the particle. Also, while the decrease in surface area with particle size may be lead to decreased rate of erosion of poorly water-permeable polymers like polyanhydrides and other polymers because surface area to volume ratio increases with decreasing particle size, drug release rates (per mass of polymer) will be faster for smaller polyanhydride microspheres (Alagusundaram et al., 2009; Nayeem et al., 2016 ; Sinha et al., 2004).

Gallic acid and its congeners are commonly present in variety of fruits and number of plants. It is a phytoconstituents with broad pharmacological activity (Saraf, 2010). In addition to its natural origin, large numbers of synthesized gallic acid derivatives are also available. It has a widely used in industrial for estimation of phenolic content of analytes. It is also used as a source material for ink, paints and colour developer (Kasture et al., 2009).

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Gallic acid is a trihydroxy benzoic acid, a type of phenolic acid, also known as 3,4,5-trihydroxybenzoic acid, having an antioxidant property. The antioxidant activity can be estimated by procedure reported by Saraf, (2011). Gallic acid and its derivatives have antioxidant activities and neuroprotective effects with free radical scavenging effects. Furthermore, it is used in the treatment of diabetes, ischemic heart diseases, ulcer lymphatic disorders, cancer and other ailments (Shrivastava et al; 2016). Various studies on gallic acid and its derivatives showed that it has potential for combating oxidative damages, cancer manifestations (Nayeemet al., 2016; Gidwani et al., 2013).

The objective of the present study was to develop microspheres of Gallic acid by solvent evaporation technique. The effect of process variables like drug concentration, drug encapsulation efficiency and drug release characteristics were studied.

Material and methods

Materials

All the chemicals and reagents used in the study were of analytical grade and were procured from sigma chemical company and used without further purification inwhich includes ethanol, petroleum ether and light liquid paraffin.

Drugs: Gallic acid was procured from Vigor Pharmaceuticals Pvt. Ltd., Mumbai, Maharashtra (India).

Methods

Preparation of Microspheres

Solvent evaporation method

In this method, the drug and the polymer should be soluble in organic solvent. The solution containing polymer and drug may be dispersed in an aqueous phase to form droplets. Continuous mixing and elevated temperatures may be employed to evaporate the more volatile organic solvents and leave the solid polymer-drug particles suspended in an aqueous medium. The particles are finally filtered from the suspension (Behera et al., 2008; Goli et al., 2012).

The Polymer Eudragit S 100 was dissolved in ethanol to get a clear solution. The drug Gallic acid was added and dissolved in the polymeric aqueous solution. The solutions were poured into 80 ml of liquid paraffin containing 2 % span 80 as an emulsifying agent. The resultant mixture was stirred at 2000 rpm and temperature 38 + 0.5 °C for 5 hrs to allow complete evaporation. Petroleum ether (100 ml) was added drop wise to above solution. Then the drug and polymer was transformed into fine droplet which solidified into rigid microspheres and then collected by filtration and finally dried in vacuum desiccators (Kaurav et al., 2012; Tabassi et al., 2003).

Preformulation study

Detection of Melting Point: Melting point of pure drug Gallic acid and excipients was determined by capillary method (Copolymer fo.; Garud et al., 2012).

Solubility study: Solubility study was done by using different organic solvent. The solubility criteria as per BP are shown in table 1.

Table 1. Solubility as per BP

S. No.	Solubility of Drug	Results
1.	Very soluble	Less than 1 part
2.	Freely soluble	From 1 to 10 parts
3.	Soluble	From 10 to 30 parts
4.	Sparingly soluble	From 30 to 100 parts
5.	Slightly soluble	From 100 to 1000 parts
6.	Very slightly soluble	From 1000 to 10000 parts
7.	Practically soluble	More than 10000 parts

Detection of absorbance maxima by UV method: AUV spectrum of Gallic acid in ethanol was recorded on model UV-1800, UV-Visible spectrophotometer (Shimadzu) between wavelengths 400 to 200nm.

FTIR spectrum: The FTIR spectrum was recorded on model FT-IR 8400S, Fourier Transform Infrared Spectrophotometer (Shimadzu). The pellets was prepared on KBr press using mixture of sample and KBr in about 1:10 ratio. The spectrum was recorded over the wave no. range of 4000 to 400 cm^{-1} (Prasant et al., 2009; Vidyavathi et al., 2009).

Characterizations and evaluation of microspheres

Practical Yield (%) : The practical yield (%) was calculated from the obtained weight of dried microspheres in relation to the sum of the initial weight of starting materials. The practical yield (%) was calculated using the following formula:

$$\text{Practical Yield (\%)} = \frac{\text{Practicle Mass (Microsphere)}}{\text{Therotical Mass(Drug + Polymer)}} \times 100$$

Scanning electron microscopy (SEM): Surface topography and morphology of the microspheres were investigated with a scanning electron microscope. SEM is simple easy method for sample preparation (Nanjwade et al., 2011).

Scanning electron microscopy was used to estimate the surface morphology of the microspheres like their shape and size. In this method, microspheres are mounted directly on the SEM sample slub and coated with gold film under

reduced pressure. Scanning Electron photomicrographs of drug-loaded microspheres are taken. A small amount of microspheres spread on gold stub. Afterwards, the stub containing the sample is placed in the Scanning electron microscopy (SEM). A Scanning electron photomicrograph is taken at an acceleration voltage of 20KV and chamber pressure of 0.6 mm Hg.

Drug loading analysis: Drug loading capacity was calculated according to these following equation:

$$\text{Drug Loading (\%)} = \frac{\text{Wt. of drug in microsphere}}{\text{Wt. of microsphere}} \times 100$$

100 mg of microspheres was taken and extracted with 5ml of ethanol. Shaken well for 15 minutes and then, stand for 10 minutes to centrifuge. Take 1ml of the supernatant liquid and dilute up to 100 ml with distilled water. Measure the absorbance at 276 nm in UV-visible spectrophotometer.

Entrapment/ Encapsulation efficiency: The capture efficiency of the microspheres or the % entrapment can be determined via allowing washed microspheres to lyse. The lysate arethen employed for the estimation of active constituents as per monograph requirement. The % entrapment efficiency is calculated using following equation:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Actual content}}{\text{Theoretical content}} \times 100$$

Angle of repose: Angle of repose was estimated by using funnel method. The accurately weighed microspheres were taken in a funnel and then height of funnel was adjusted in such as way that the tip of funnel just touches the apex of heap of blends. The blends were allowed to flow through funnel freely on to surface. The diameter of powder cone was measured and angle of repose was calculated by using following equation:

$$\tan \Theta = h/r$$

$$\theta = \tan^{-1} h/r$$

Where, **h** –height of pile,

r – Radius of base.

Table 2. Angle of repose and their range

Flow property	Angle of Repose (Degrees)
Excellent	25 –30
Good	31 –35
Fair – aid not needed	36 – 40
Passable – may hang up	41 – 45
Poor – must agitate, vibrate	46 – 55
Very Poor	56 – 65
Very, Very poor	> 66

Optical microscopy: Optical microscopy technique was used to estimate particle size by using optical microscope (Meizer OPTIK). The measurement was done under 450x (10x eye piece and 45x objective) and 100 particles were calculated.

Results and discussion

The microsphere of gallic acid was formulated using solvent evaporation method.

Detection of Melting Point: Melting point of pure drug and excipients was determined by capillary method. Melting point of Gallic acid was found to be 260°C.

Solubility studies: The solubility study of Gallic acid drug formulation wasdetermined in various solvent. Initially 5 mg of drug was weighted and then drop wise 1 ml solvent was added & later on in the same way solvent is increased like 2, 3, 4, 5, 10, 30 & so on. The results are shown in table no. 3.

Table 3. Solubility study of Gallic acid

S. No	Solvent used	Results of Gallic acid
1.	Distilled Water	Slightly soluble
2.	Methanol	Freely Soluble
3.	Ethyl alcohol	Soluble
4.	Acetone	Freely Soluble
5.	Chloroform	Sparingly soluble
6.	Benzene	Slightly soluble
7.	Acetic acid	Soluble

pH of the Gallic acid: pH was determined by the pH meter and was found to be 7.2.

Preparation of calibration curve of pure drug: The calibration curve was plotted by taking different concentration of drug on x-axis and absorbance on y-axis and is shown in figure of Gallic acid. The drug (Gallic acid) obeys Beer's law in the concentration range of 5-30 µg/ml with coefficient of correlation (R^2) = 0.996. The calibration curve was determined at lambda max 270 nm.

Table 4. Concentration and absorbance of Gallic acid

S. No.	Concentration (µg/ml)	Absorbance
1	05	0.061
2	10	0.116
3	15	0.178
4	20	0.241
5	25	0.316
6	30	0.389

FTIR Study: The samples were crushed with KBr and form pellets. For estimation of functional groups of a drug, Infrared spectroscopy is the most suitable analytical techniques. Ratio of drug and KBr 1:10. IR spectrum has two distinct regions such as functional group region (4000-1000 cm^{-1}) and fingerprint region (< 1000 cm^{-1}).

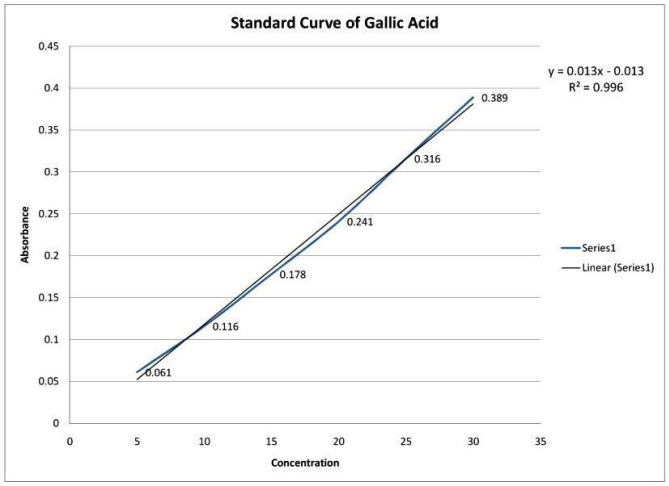

Figure 1. Calibration Curve of Gallic Acid

Evaluation of Gallic acid microspheres

The evaluation parameters of Gallic acid microsphere such as Practical Yield (%), Drug Loading, Encapsulation efficiency, Angle of repose were estimated. Different formulation of drug and polymers, F1, F2, F3, F4 and F5 showed entrapment efficiency as55.96, 62.35, 65.15, 70.63 and 76.54 respectively. This demonstrates that an increase in the amount of polymers results in an increase in entrapment efficiency. Results showed that the concentration of polymer also significantly affects the entrapment efficiency.

Table 5. FTIR study of Gallic acid

S. No.	Functional groups	Values (cm^{-1})
1.	C-H	3028.24
2.	S-H	2557.61
3.	N-H	1570.45
4.	C=N	1770.65
5.	C-O	1375
6.	C=C	1541.12
7.	C-H	3005.10

Scanning electron microscopy (SEM)

The shape and surface characteristics of the drug loaded microspheres was evaluated by scanning electron microscopy (SEM).

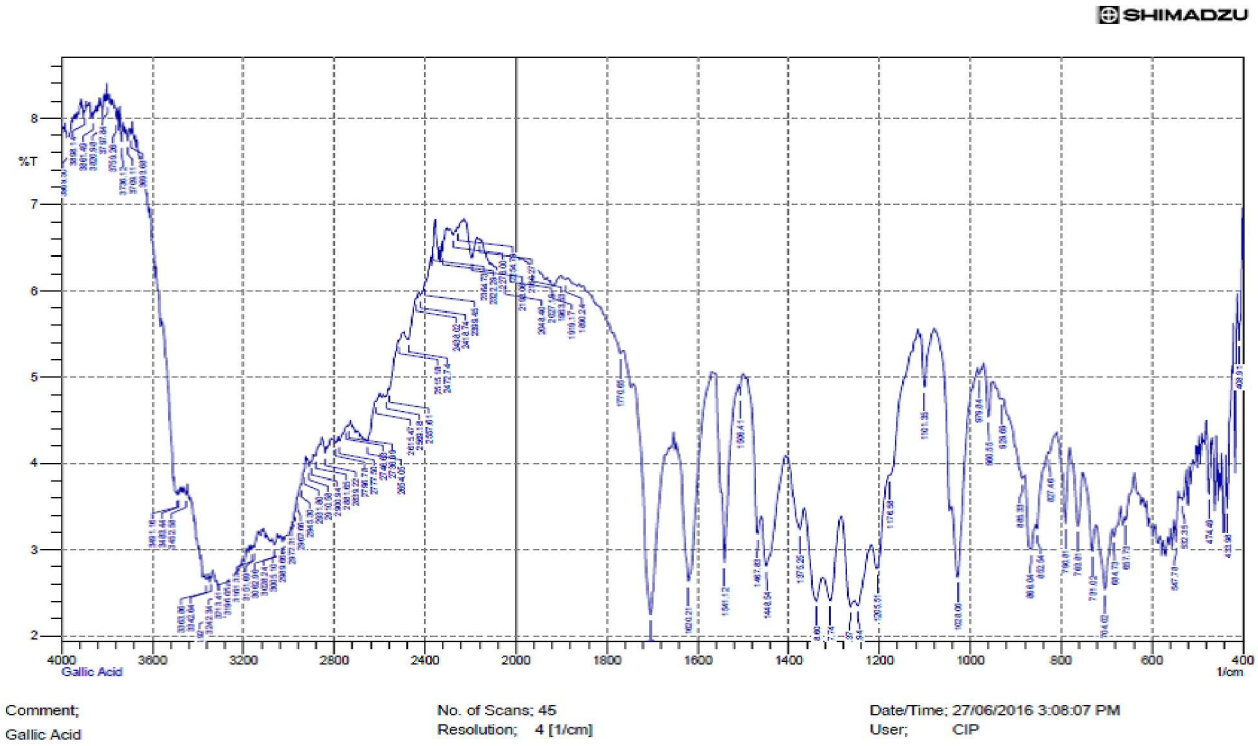

Figure 2. FTIR study of Gallic acid

Table 6. Formulation of microspheres

S. No.	Formulation Code	Drug polymer ratio	Drug content (%)	Entrapment efficiency (%)	Percentage Yield (%)	Mean particle size (µm)	Angle of repose
1	F1	1:1	30.55	55.96	78.56	31.65	31.06
2	F2	1:2	33.48	62.35	79.69	34.95	32.15
3	F3	1:3	36.85	65.15	80.62	37.62	32.65
4	F4	1:4	40.35	70.63	81.63	39.15	33.45
5	F5	1:5	41.62	76.54	83.45	40.15	35.15

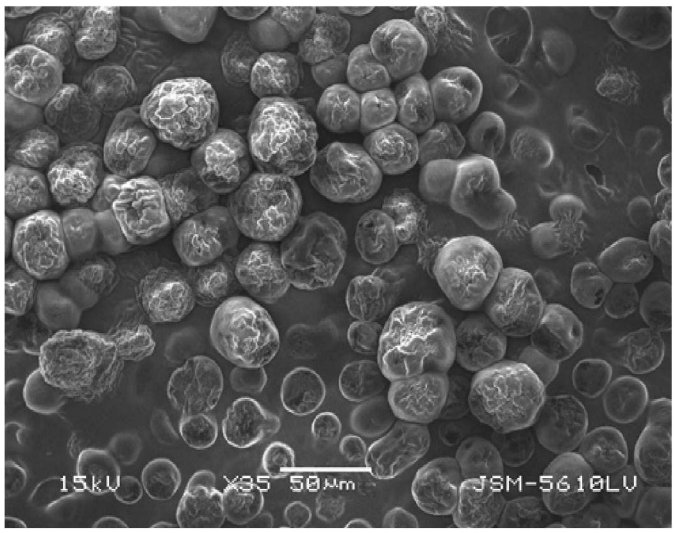

Figure 3. Scanning electron micrograph of Gallic acid microsphere

Optical microscopy

To determine particle size of microsphere optical microscope were used. The measurement was done under 450x (10x eye piece and 45x objective) and100 particles were calculated.

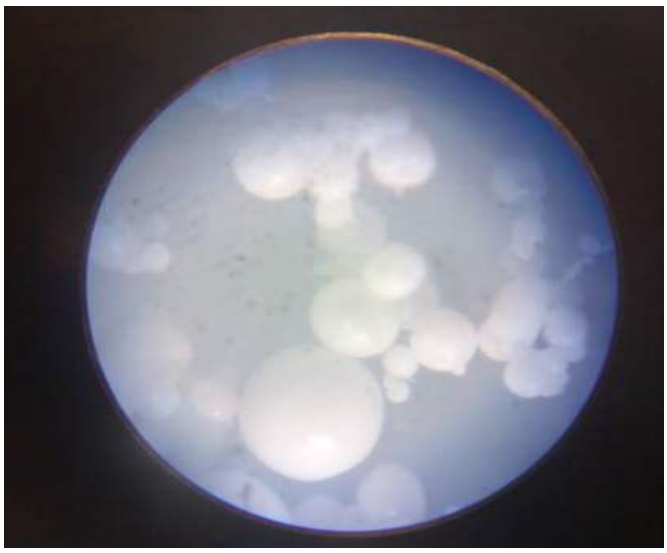

Figure 4. Microscope picture of microsphere

Conclusion

Based on the above research, microspheres loaded with Gallic acid were prepared and characterized. This led to overcome the limitation of poor aqueous solubility and bioavailability. Formulated microspheres were characterized for its entrapment efficiency, drug loading, *in-vitro* drug release, surface morphology and FTIR spectroscopy. The microspheres were spherical with smooth surface. In future in-vivo animal study and in-vitro drug release study will be performed to achieve better result. On this basis, it can be concluded that the eudragit polymer is the potent and biodegradable carrier to achieve sustained/controlled drug delivery of Gallic acid.

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