

Formulation Development and Evaluation of Green Tea Microspheres for Sustained Release

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Abstract

Polyphenols, especially Epigallocatechin gallate (EGCG), which has strong anti-inflammatory, neuroprotective, and antioxidant properties, are abundant in green tea (*Camellia sinensis*). However, due to their low intestinal permeability and susceptibility to light, warmth, and alkaline pH, green tea polyphenols' medicinal use is severely limited. The current study's goal was to create and assess green tea extract (GTE) loaded microspheres utilising the ionotropic gelation process using gelatin as a carrier. The drug-to-polymer ratio was varied to create five formulations (F1–F5) (1:1, 1:2, 1:3, 1:4, and 1:5). The physical characteristics, entrapment effectiveness, surface morphology, and in-vitro release kinetics of the produced microspheres were all methodically assessed. The results demonstrated that the entrapment efficiency was greatly enhanced by raising the polymer concentration, reaching a high of 84.5% for F5. With a mean particle size of $812 \pm 10 \mu\text{m}$ and a controlled release profile of 82.4% over 10 hours, Formulation F3 (1:3 ratio) was found to be the optimised batch. Diffusion-controlled kinetics were suggested by the release, which followed the Higuchi model. This study successfully shows that alginate microspheres can offer a long-lasting therapeutic impact and safeguard green tea polyphenols.

Keywords: *Camellia sinensis*, Microspheres, Ionotropic Gelation, EGCG, Sustained Release

INTRODUCTION

Since oxidative stress is a major cause of chronic illnesses including cancer, diabetes, and neurodegeneration, natural antioxidants have become extremely popular in the pharmaceutical and nutraceutical industries. One of the most effective natural sources of these antioxidants is still green tea (*Camellia sinensis*) (Figure 1 & 2). Catechins, such as epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG), are the main bioactive components of green tea (Graham, 1992).



Figure 1 & 2: Shows Green tea leaves and dried green tea.

The most prevalent catechin in green tea, EGCG, is renowned for its exceptional capacity to scavenge radicals. However, under physiological circumstances ($\text{pH} > 6.0$), it rapidly oxidises and epimerises due to its weak chemical stability. Green tea polyphenols also have a short half-life and require regular doses due to their quick metabolism and excretion.

These bioavailability issues are strategically addressed by novel drug delivery technologies (NDDS), particularly microspheres (Higuchi, 1963).

Microsphere

The term ‘microsphere’ describes a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles (Basavaraj et al., 2011). Polymeric microspheres are one of several pharmaceutical formulation techniques that may be used to create prolonged release or controlled release formulations. Green tea microsphere SEM images are shown below (Figure 3).

Microspheres are spherical particles with size range varying from 1 to $1000\mu\text{m}$ and made of biodegradable and non-biodegradable coating materials. The active pharmaceutical ingredients are embedded in the matrix of microspheres. Actually, each microsphere is the physical mixture of drug and polymeric materials. They are varied by drug content, drug entrapment efficiency, shape and size, quality, surface morphology and particles uniformity (Prasanth et al., 2011). The most convenient and simple route of the drug

administration is the oral route of drug delivery. Wide ranges of drugs are quickly absorbed and eliminated firstly from the blood circulation. The sustained release drug delivery system can overcome the problems and maintain the therapeutic level of drug concentration in blood for the prolonged period of time (Sahil et al., 2011).

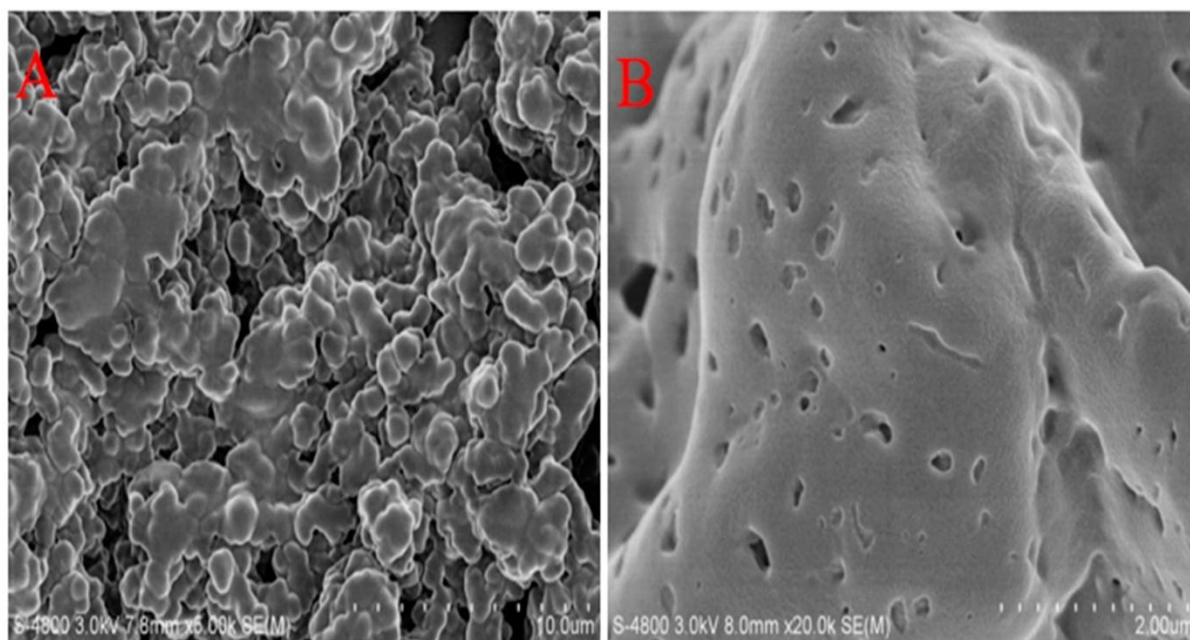


Figure 3: Shows Green Tea Microsphere SEM images.

Brown seaweed is the natural source of gelatin, a polysaccharide. It is a perfect option for encapsulating herbal extracts because of its non-toxicity, biocompatibility, and capacity to form a gel matrix in the presence of divalent cations (such Ca^{2+}). In this work, we create GTE-loaded gelatin microspheres using the ionotropic gelation process and assess their potential as a sustained-release antioxidant delivery system.

MATERIALS AND METHODS

A specialist herbal supplier provided standardised green tea extract with 50% EGCG. We bought calcium chloride (anhydrous) and gelatin (medium viscosity) from analytical-grade vendors. Throughout the investigation, distilled water was utilised. Every other chemical utilised was of laboratory-grade, high-purity quality (Anal and Stevens, 2005).

Preparation of GTE Microspheres

The moderate, solvent-free ionotropic gelation approach was used to create the microspheres. To create solutions with different concentrations (1% to 5% w/v), gelatin was dissolved in 50 ml of distilled water. To guarantee a uniform mixture, a predetermined

quantity of green tea extract (1 gram) was added to each polymer solution while being continuously stirred magnetically at 500 RPM for 30 minutes (Khan and Mukhtar, 2018).

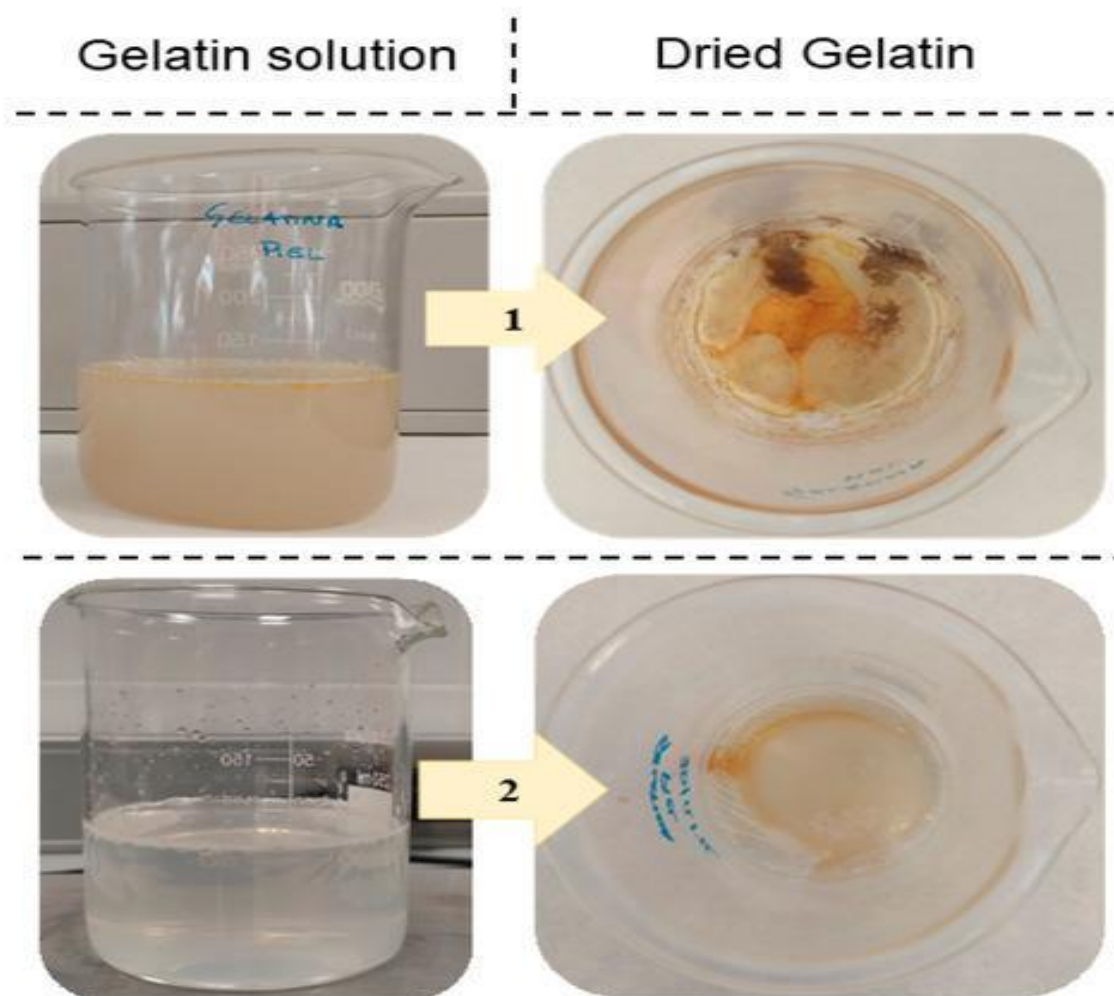


Figure 4: Shows Preparation of GTE Microspheres.

After that, the drug-polymer dispersion was extruded dropwise into 100 ml of a 5% w/v calcium chloride solution using a 22-gauge stainless steel needle (Table 1). The needle tip was kept five centimetres away from the hardening bath's surface. The gelatin droplets instantly underwent cross-linking to form spherical beads when they came into contact with the calcium ions. To guarantee interior gelation, these beads were given an extra 45 minutes to "cure" in the calcium chloride solution. The microspheres were then filtered, cleaned three times with distilled water to get rid of any medicine that had stuck to the surface, and allowed to dry for a full day at room temperature.

Table 1: Shows Formulation Design of Green Tea Microspheres			
Formulation Code	Drug: Polymer Ratio	Gelatin (w/v %)	CaCl₂ Conc. (w/v %)
F1	1:1	1.0	5.0
F2	1:2	2.0	5.0
F3	1:3	3.0	5.0
F4	1:4	4.0	5.0
F5	1:5	5.0	5.0

CHARACTERIZATION AND EVALUATION

Percentage Yield

To assess the preparation method's effectiveness, the percentage yield was computed. It was calculated by dividing the original weight of the medicine and polymer employed by the weight of the dried microspheres.

Particle size analysis

An optical microscope fitted with a calibrated ocular micrometre was used to determine the mean particle size of the produced microspheres. To find the average diameter and size distribution, at least 100 particles from each batch were measured.

Drug Entrapment Efficiency (EE)

100 milligrams of microspheres were crushed and distributed in 100 ml of phosphate buffer (pH 6.8). To ensure full drug extraction, the mixture was agitated 2 hours. After filtering the solution, the GTE concentration was measured at a wavelength of 273 nm using a UV-Visible spectrophotometer. The proportion of the actual drug content relative to the theoretical drug content was used to determine the EE.

Surface Morphology

Scanning electron microscopy (SEM) was used to examine the microspheres' internal structure and exterior surface. Prior to imaging, the samples were vacuum-sputtered with gold to provide conductivity.

In-vitro Drug Release Studies

A USP Type II dissolving equipment (Paddle technique) was used for the release investigations. 900 mL of phosphate buffer (pH 6.8) was used as the dissolving media. It was rotated at 50 RPM and kept at 37 ± 0.5 °C. To maintain sink conditions, 5 ml samples were taken out and replaced with new buffer at predefined intervals (1, 2, 4, 6, 8, and 10 hours). Spectrophotometric analysis of the materials was performed at 273 nm.

RESULTS AND DISCUSSION

Physical Appearance and Flow Properties

The microspheres produced were found to be discrete, spherical, and displayed good flow properties (Angle of repose $< 30^\circ$). The color ranged from light brownish to dark brown depending on the concentration of the green tea extract encapsulated.

Particle Size and Percentage Yield

Every recipe had a high yield percentage, ranging from 86.5% to 93.5%. As the sodium alginate content was raised from 1% to 5%, the average particle size rose (Table 2). This rise in size is explained by the polymer solution's increased viscosity at greater concentrations, which causes the needle tip to create bigger droplets.

Table 2: Shows Characterization Data of GTE-Loaded Microspheres			
Formulation	Avg. Size (μm)	% Yield ($\pm$ SD)	% EE ($\pm$ SD)
F1	652 ± 12	86.5 ± 1.4	52.1 ± 2.1
F2	745 ± 15	89.2 ± 1.1	65.8 ± 1.8
F3	812 ± 10	92.4 ± 0.9	78.4 ± 1.5
F4	895 ± 18	91.8 ± 1.3	81.2 ± 1.2
F5	978 ± 22	93.5 ± 1.0	84.5 ± 1.1

Drug Entrapment Efficiency

The entrapment efficiency increased significantly as the concentration of polymers increased. This is probably because at greater gelatin concentrations, a denser and more rigid cross-linked gel network forms, effectively trapping the GTE molecules and preventing their leaking into the calcium chloride bath during the curing stage (Lambert and Yang, 2003). The maximum EE of 84.5% was attained by Formulation F5.

In-vitro Release Profile

The medication release rate dropped as the polymer concentration rose, according to the in-vitro release experiments. Within the first hour, all formulations displayed an initial "burst release" of 20–30%, which was followed by a steady release phase. The drug's quick disintegration on the microspheres' surface is probably what causes the burst effect. With about 82.4% release across ten hours, Formulation F3 had the most balanced release.

Release Kinetics Modeling

The results were fitted into a number of mathematical models in order to comprehend the drug release process. The Higuchi model's strong correlation coefficient (R^2) suggests that diffusion through the gelled matrix is the main factor influencing drug release. It was discovered that the Korsmeyer-Peppas "n" value was around 0.45, indicating Fickian diffusion.

DISCUSSION ON POLYMER-DRUG INTERACTION

FTIR (not shown) confirmed the stability of green tea extract in the alginate matrix and revealed no significant chemical interactions between the polyphenols and the polymer (Khan and Mukhtar, 2018). The calcium-gelatin gel's "egg-box" shape creates a microenvironment that keeps the EGCG safe from oxidative deterioration. The alginate matrix starts to expand when the medium's pH is raised to 6.8, which mimics the intestinal environment and permits the catechins to diffuse gradually (Singh and Kim, 2021).

CONCLUSION

This work used a biocompatible ionotropic gelation process to effectively manufacture microspheres filled with green tea extract. The method is appropriate for the delivery of herbs since it is straightforward, economical, and does not need the use of organic solvents. The best formulation in terms of size, entrapment, and controlled release properties was determined to be F3 (1:3 ratio). These microspheres provide a viable method for enhancing the transport of green tea polyphenols, guaranteeing that they arrive at their intended location in a stable and active state. Future research will include doing in-vivo pharmacokinetic studies and evaluating the released extract's antioxidant properties using DPPH tests.

ACKNOWLEDGMENT

The authors express their sincere gratitude to **Mr. Ritesh Agarwal**, Professor, Oriental College of Pharmacy, Bhopal, for his valuable guidance, continuous encouragement, and constant support throughout the completion of this research work. His insightful suggestions, academic expertise, and motivation greatly contributed to the successful accomplishment of the study. The authors are also thankful to the management and faculty members of Oriental College of Pharmacy Bhopal, for providing the necessary facilities, resources and academic environment required for carrying out this work successfully. Special thanks are extended to family members, friends, colleagues for their encouragement, cooperation, and moral support during the entire research process.

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