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Formulation and Characterization of Kaempferol Nanoparticles

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Abstract
Nanoparticles of Kaempferol were prepared using varying polymer drug ratio with PLGA (50:50) as the polymer, using solvent displacement method. The prepared nanoparticles were screened for the various studies like size and shape determination using light scattering and SEM. The *in-vitro* antioxidant profiles of the prepared nanoparticles were studied and compared with free Kaempferol using DPPH method. The nanoparticles of uniform size were prepared, the % EE of 20:1 polymer drug ratio was found to be maximum and the free radical scavenging activity of Kaempferol trapped in nanoparticles was even increased when compared with its free form.

Keywords: Nanoparticles, Kaempferol, PLGA & antioxidant.

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1. INTRODUCTION

Kaempferol is a natural flavonoltype of flavonoid, that has been isolated from tea, broccoli, Delphinium, Witch-hazel, grapefruit, cabbage, kale, beans, endive, leek, tomato, strawberries, grapes, brussels sprouts, apples, Kaempferiagalanga, Opuntiaficus-indica var. saboten and other plant sources. It is a yellow crystalline solid with a melting point of 276-278 °C.1,2 Kaempferol and its glucoside can be isolated from the methanolic extract of fronds of the fern Phegopteris connectilis.3 Some epidemiological studies have found a positive association between the consumption of foods containing kaempferol and a reduced risk of developing several disorders such as cancer and cardiovascular diseases. Numerous preclinical studies have shown kaempferol and some glycosides of kaempferol have a wide range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, neuroprotective, antidiabetic, antiosteoporotic, anxiolytic, analgesic, and antiallergic activities. Many glycosides of kaempferol, such as kaempferitrin and astragalin, have been isolated as natural products from plants. Kaempferol consumption in tea and broccoli has been associated with reduced risk of heart disease.4 Antidepressant properties have been reported in tests on animals.5 An eight-year study found the consumption of three flavonols (kaempferol, quercetin, and myricetin) correlated with a lower risk of pancreatic cancer among current smokers, but not non-smokers and ex-smokers.6Kaempferol consumption is also correlated with a reduced lung cancer incidence.7 Kaempferol may be a potent prophylactic against NOX-mediated neurodegeneration.8 Kaempferol has been found to inhibit the enzyme fatty acid amide hydrolase (FAAH).9,10

Controlled delivery systems, such as nanoparticles, have shown their potential to protect, control the release, and increase the action of different bioactive compounds.11,12 Therefore, the aim of this study was to encapsulate kaempferol within polymeric nanoparticles, using the biocompatible copolymer poly (D, L lactide-co-glycolide) (PLGA) and characterize their physicochemical and antioxidant properties. One of the most important aspects of this study is the determination of the encapsulation efficiency and in-vitro release profiles.

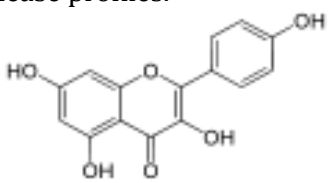


Figure 1: Molecular structure of Kaempferol

2. MATERIALS AND METHODS

2.1. Materials

Kaempferol, Polyvinyl alcohol (PVA), 50 : 50 Poly (D, L-lactide-co-glycolide) (PLGA), acetone, ethanol, Phosphate Buffer, DPPH, methanol.

2.2. Methods

2.2.1. Preparation of Polymeric Nanoparticles

PLGA nanoparticles were prepared by the solvent displacement method with minor modifications using a previously described protocol.[13,14] PLGA (200 mg) was dissolved in 20 ml of acetone under magnetic stirring for 30 min at 25°C. The resulting PLGA solution was then slowly added to a 5% PVA aqueous solution (40 ml) using a syringe. The solution was then homogenized using a high shear mixer at 19,000 rpm for 5 min. The organic solvent and water were evaporated for 30 min under vacuum using a rotary evaporator. The nanoparticles formed were washed with distilled water and ultra-centrifuged at 20,000 rpm for 20 min at 5°C. The pellet was resuspended in distilled water for 24 h and then stored for 12 h at −20°C in a freezer. The frozen nanoparticle dispersion was freeze dried at −70°C (10−3 Torr) for 48 h using a commercial freeze drier. The lyophilized nanoparticle dispersions were stored in desiccators containing dehydrating salts at 25°C until used. To prepare kaempferol-loaded PLGA nanoparticles, separately, kaempferol (10 mg, 20mg, 30mg and 40mg) was dissolved into the PLGA-acetone solution prior to nanoparticle synthesis to prepare a solution of 5:1(Form. A), 10:1(Form. B), 15:1(Form. C) and 20:1(Form. D) polymer:drug ratio and nanoparticles with no kaempferol were also prepared(Form. E). Two independent batches of PLGA nanoparticles were prepared with or without kaempferol for physicochemical and antioxidant characterizations.

2.2.2. Characterization

Particle Size and Charge Measurements

The mean particle diameter of the nanoparticles was determined by dynamic light scattering Counter. The samples were diluted with distilled water to ensure that the number of particles counted per second was within the range of the instrument's sensitivity. Measurements were made at an angle of 90° for 180 s at 25°C. The electrical charge on the nanoparticles was measured by particle electrophoresis after they had been diluted in deionized water to avoid multiple scattering effects and then placed in a folded capillary cell (25°C). Measurements were made in triplicate, and the results are shown as mean ± standard error.

Morphology

SEM studies of the prepared nanoparticles (Form.D) and only PLGA nanoparticles (Form.E) were performed.

Determination of Percentage Drug Entrapment Efficiency (%EE)

Drug content was determined by centrifuging the redispersed nanoparticle suspension at 15000 rpm for 40 min. at 25^{0}C to separate the nanoparticles from the free drug. The supernatant was collected and the concentration of kaempferol in it was determined by taking the absorption.

The encapsulation efficiency of the kaempferol was determined using as follows:

EE (%) = Amt. of Kaempferol in entrapment − amount of Kaempferol in supernatant x 100

Total amount of Kaempferol in formulation

2.2.3. Drug *In-Vitro* Release from Nanoparticles

Drug *in-vitro* release tests were performed using the described electrochemical protocol.[15] Kaempferol-loaded PLGA nanoparticles (5 mg) were added into a Phosphate Buffer, pH adjusted to 7.4 and were magnetically stirred for 32 h. The amount of kaempferol released from the nanoparticles was monitored at 30, 60, 90, 120, 150, 180 and 210 h.

2.2.4. Antioxidant Capacity of Free/Encapsulated kaempferol

Free Radical Scavenging Activity Assay [16]

The antioxidant activity of free Kaempferol and Kaempferol loaded Nanoparticles (Form.D) was determined using the samples in the same concentration in the presence of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and compared with that of Ascorbic acid taken as standard. All the samples were prepared and analyzed in triplicate.

The assay is based on the reduction of DPPH. Because of its odd electron, DPPH gives strong absorption maxima at 517 nm (purple color) by visible spectroscopy. As the odd electron of the radical becomes paired off in the presence of a hydrogen donor, i.e., a free radical scavenging antioxidant, the absorption intensity is decreased, and the resulting decolourization is stochiometric with respect to the number of the electrons captured.

DPPH solution (0.004% w/v) was prepared in 95% methanol. Sample was mixed with 95% methanol to prepare the stock solution (5 mg/ml). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and synthesised Kaempferol nanoparticles were added in serial dilutions (20 µg to 120 µg) to every test tube so that the final volume was 3 ml and after 10 min, the absorbance was read at 517 nm using a spectrophotometer (Labmed double beam spectrophotometer). Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration (5 mg/ml). Control sample was prepared containing the same volume. 95% methanol served as blank. % scavenging of the DPPH free radical was measured. IC_{50} values were obtained using the graph between concentration and percentage inhibition.

$$\% \text{ Inhibition of DPPH} = \frac{A-B}{A} \times 100$$

A = optical density of Blank; B = optical density of Sample

Half maximal inhibitory concentration (IC_{50}) (average ±SD of four assays) for the nanoparticles and free kaempferol was calculated.

3. RESULTS

3.1 Morphological and Electrical Properties of Polymeric Nanoparticles

The morphological and electrical properties of the prepared nanoparticles effect its functionlities.[17,18]Hence nanoparticle size, appearance and zeta potential were studied. The size and zeta potential values are shown in Table.1 and SEM figures of Form.D&Form.E are shown in Fig.2&3.

Nanoparticles with mean diameters from 245 to 500 nm were obtained. The size of the nanoparticles is effected by many factors like properties of polymer, solvency, viscosity etc.[19, 20] The electrical charge of the free and loaded kaempferol polymeric nanoparticles was negative at pH 7, an observation that can be attributed to the presence of ionized carboxyl groups on the PLGA matrix.[21] Differences in ζ values were also observed between kaempferol free-nanoparticles and kaempferol-loaded PLGA nanoparticles (−33.2 mV&−46.6 mV resp.). These results indicate that the presence of each specific kaempferol alter the electrical charge of the polymeric nanoparticles.

Polymer:drug ratio	Particle size (nm)	Zeta potential(mV)
Kaempferol free NP	55.2±2.5	−28.7±1.2
5:1	96·05±10·8	−33.5±5·1
10:1	163·7±15·1	−47·9±8·6
15:1	178·5±25·4	−48·5±9·5
20:1	195.8±20.1	−68.9±12.8

Table 1: Characterization of Nanoparticle Matrix Properties
Values are expressed as Mean±SD

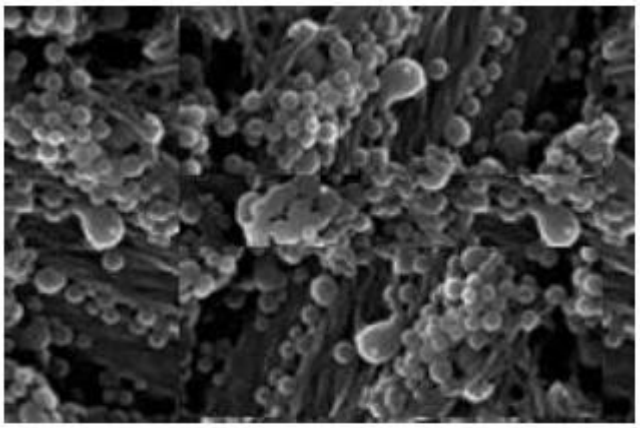


Figure 2: SEM of PLGA nanoparticles

1.

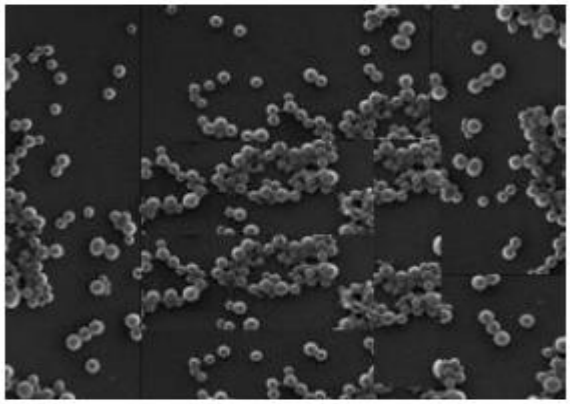
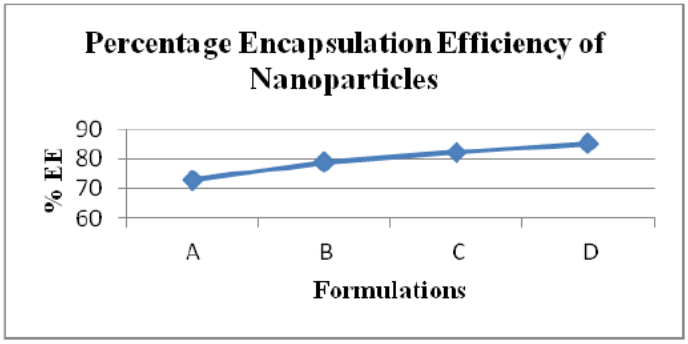


Figure 3: SEM of kaempferol-loaded PLGA nanoparticles

3.2. Encapsulation Properties of Polymeric Nanoparticles

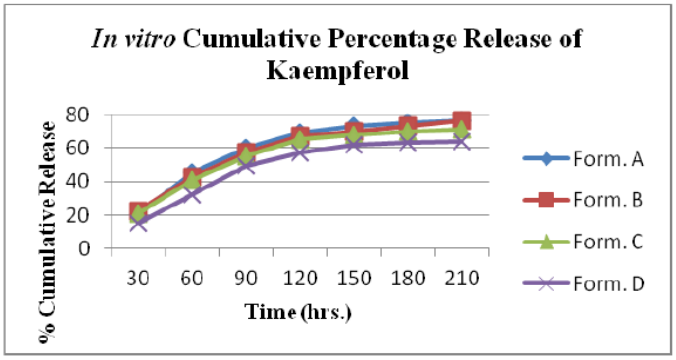
The encapsulation efficiency (EE) of kaempferol within the PLGA nanoparticles was around 73%, 79%, 82% and 85% for Formulations A, B, C and D respectively. These results suggest 20:1 polymer drug ratio to be optimum for the entrapment of kaempferol in PLGA matrix.



Graph 1

3.3 Kaempferol Release from Nanoparticles

In this section, the release of kaempferol from PLGA nanoparticles as a function of time was determined.



Graph 2

3.4.Antioxidant Capacity(DPPH study)

Test compound	IC_{50} after10 min
Control	--
Kaempferol	117±7.47*
Kaemferol NP	75±4.23*
Ascorbic Acid (Std.)	22±1.5

Table 2: Antioxidant Profile Study

Values are expressed as Mean±SD $p<0.05$, values are significant when compared with standard

4. DISCUSSION

PLGA encapsulated nanoparticles of kaempferol were prepared. The %EE of 20:1 polymer drug ratio was found to be 85% (maximum) as shown in Graph. 1 and hence formulation D was further used for antioxidant studies. The particle size studies show the uniform nanoparticles (Fig. 2 & 3) from range of 55 to 196 nm size as shown in Table 1. The percentage cumulative drug release profile suggests that all the nanoparticles showed an initial fast and later a more steady release of drug, but the release profile of formulation D has found to be most steady amongst all the formulations as shown in Graph 2. The antioxidant study shows the improved IC_{50} of kaempferol when loaded in PLGA matrix, when compared with free kaempferol as shown in Table 2.

5. CONCLUSION

Kaempferol(3, 4′, 5, 7-tetrahydroxyflavone) is an important flavonoid distributed widely. It has many pharmacological properties including cancer preventive agent by virtue of its antioxidant activity. The extensive first pass hepatic metabolism by glucuronidationlimits its bioavailability at ~ 2%. The encapsulation of the flavonoid into the matrix of polymer PEG, in various drug polymer ratios, gives it a more steady release, but the 20 : 1 polymer drug ratio has given the most satisfactory results. The free radical scavenging activity of the flavonoid has augmented after encapsulation. This may in turn enhance its pharmacological activities like that of anticancer. The effect of the action of encapsulation on the cancer cell apoptosis shall be an interesting subject to study.

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