Biodegradable Poly (Lactic-co-Glycolic Acid)–Polyethylene Glycol Nanocapsules: An Efficient Carrier for Improved Solubility, Bioavailability, and Anticancer Property of Lutein

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ABSTRACT: Lutein bioavailability is limited because of its poor aqueous solubility. In this study, lutein–poly (lactic-co-glycolic acid) (PLGA)–polyethylene glycol (PEG) nanocapsules were prepared to improve the solubility, bioavailability, and anticancer property of lutein. The scanning electron microscopy and dynamic light scattering examination revealed that the nanocapsules are smooth and spherical with size ranging from 80 to 500 nm (mean = 200 nm). *In vitro* lutein release profile from nanocapsules showed controlled sustainable release (66%) up to 72 h. Aqueous solubility of lutein nanocapsules was much higher by 735-fold than the lutein. Fourier transform infrared spectroscopy analyses showed no chemical interaction among PLGA, PEG, and lutein, indicating possible weak intermolecular forces like hydrogen bonds. X-ray diffraction revealed lutein is distributed in a disordered amorphous state in nanocapsules. Postprandial plasma kinetics (area under the curve) of an oral dose of lutein from nanocapsules was higher by 5.4-fold compared with that of micellar lutein (control). The antiproliferative effect of lutein from nanocapsules (IC_{50} value, 10.9 μM) was higher (43.6%) than the lutein (IC_{50} value, 25 μM). Results suggest that PLGA–PEG nanocapsule is an efficient carrier for enhancing hydrophilicity, bioavailability, and anticancer property of lipophilic molecules such as lutein. © 2015 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 104:2085–2093, 2015

Keywords: bioavailability; biodegradable polymers; antioxidants; nanocapsules; pharmacokinetics; poly(lactic/glycolic) acid (PLGA or PLA); polymeric drug carrier; X-ray powder diffractometry; poorly water-soluble drugs

INTRODUCTION

Oxidative stress caused by free radicals is known to be the major cause factor for many degenerative diseases, such as cancer, diabetes, cardiovascular diseases, autoimmune diseases, neurodegenerative disorders, and age-related macular degeneration (AMD).[1,2] In this context, natural antioxidant carotenoids found to play an important role in the prevention of these diseases owing to their therapeutic properties. Lutein, a xanthophyll carotenoid, selectively accumulates in the macula of retina and guards photoreceptors from pro-oxidants generated by actinic blue light, and endogenous proxidants thus suppress the progression of cataract and AMD.[3] In addition, lutein is also reported to have specific biological functions such as enhancing the immune response,[4] decreasing breast cancer development,[5] and atherosclerosis.[6] The effectiveness of lutein in ameliorating disease depends on its bioavailability. Nevertheless, intestinal lutein absorption is limited (10%–15%) because of high hydrophobicity of its C-40 isoprenoid structure, which reduces its solubility and permeability across the enterocytes during the intestinal absorption process.[7] Further, humans cannot synthesize lutein *de novo*, and lutein uptake is dependent on the dietary intake (fruits, vegetables, and synthetic supplements). Bioavailability of lutein is also limited because of its complexity with food matrix.[8] The intestinal absorption of lutein from food is dependent on various dietary components such as fat, dietary fiber, interaction of other carotenoids, and other micronutrients.[8] Hence, in order to deliver lutein intact, nanoencapsulation has been advocated in this study to improve not only the solubility, stability, but also the biological availability of lutein so as to achieve maximum efficacy.

Nanocapsules have been one of the prominent delivery systems to improve the poorly bioavailable lutein.[9] In this study, poly (lactic-co-glycolic acid) (PLGA)–polyethylene glycol (PEG) was used as a carrier polymer for nanoencapsulation of lutein, which is considered safe by US FDA and it is GRAS certified.[10,11] PLGA–PEG has also been used as a competent nutraceutical and drug delivery system in food and pharma industries.[12] Moreover, nanoencapsulation helps in improving the bioavailability and efficacy of poorly water-soluble drugs and nutraceuticals such as lutein,[9] curcumin,[12] paclitaxel,[13] and estradiol.[14] The ability of nanoparticles to deliver antioxidants directly into tumor cells is known.[1] These studies suggest that antioxidants encapsulated with PLGA–PEG can effectively enhance the bioavailability and bioactivity. It is evident from the literature that no studies are available on lutein–PLGA–PEG nanocapsules for food and pharmaceutical application.

The present study hypothesized that nanoencapsulation of lutein with PLGA–PEG will improve the lutein stability by

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protecting from oxidation and improve its aqueous solubility, biological availability, and bioactivity. Hence, the aim of the study was to prepare lutein–PLGA–PEG nanocapsules to improve hydrophilicity, bioavailability, and anticancer property. To achieve this objective, scanning electron microscopy (SEM), atomic force microscopy (AFM), and photon correlation spectroscopy were used for the characterization of nanocapsules. Fourier transform infrared (FT-IR) techniques were used to find out the interaction among lutein, PLGA, and PEG. X-ray diffraction (XRD) was used to study the physical (crystalline or amorphous) nature of lutein in nanocapsules. The lutein bioavailability was analyzed in lutein-devoid mice model. The antiproliferative activity of lutein and lutein–PLGA–PEG nanocapsules was examined in Hep G2 cells. Findings from the present study demonstrate the application of PLGA–PEG as a lutein carrier for improved bioavailability and anticancer property.

MATERIALS AND METHODS

Materials

The standard lutein (99%), PLGA with lactide–glycolide molar ratio of 50:50, (Resomer RG 50:50 H; Mw 40–75 kDa, inherent viscosity 0.45–0.6 dL/g), PEG (Mw 10 kDa), and polyvinyl alcohol (PVA; 31 kDa, 88% hydrolyzed), butylated hydroxyl toluene (BHT), pepsin (porcine), bile extract (porcine), and pancreatin (porcine) were purchased from Sigma–Aldrich (St. Louis, Missouri). Sodium sulfate, potassium hydroxide, ammonium acetate, and analytical and HPLC grade solvents were purchased from Sisco Research Laboratories (Mumbai, Maharashtra, India). Lutein was extracted from marigold flower petals collected from local flower market (Mysore, Karnataka, India).

Extraction and Purification of Lutein from Marigold Petals

Lutein was extracted and purified from fresh marigold (*Tagetes erecta)* petals as per Lakshminarayana et al.[15] with slight modification. In brief, lutein was repeatedly extracted by cold acetone with 0.1% BHT and filtered over anhydrous sodium sulfate (20 g). The pooled extract was saponified with 30% methanolic KOH for 3 h in the dark and were extracted three times with 50 mL hexane, followed by washing with deionized water. The evaporated hexane fraction was purified by activated silica column (60–120 mesh) by methanol–dichloromethane (DCM; 1:1, v/v). The purity (96 ± 2%) of lutein was confirmed by HPLC and LC–MS (Liquid chromatography-Mass spectrometry) with authentic lutein standard.

HPLC and LC–MS Analysis

Lutein from lutein–PLGA–PEG nanocapsules, plasma and tissue samples (liver, kidney, and eye), and marigold petal extracts were analyzed by HPLC. Lutein was separated on a SGE C-18 column (250 × 4.6 mm^2; SGE Company, Mumbai, Maharashtra, India) by mobile phase (acetonitrile/methanol/DCM; 3:1:1, v/v/v) containing ammonium acetate (0.1%). The sample (20 μL) was injected to HPLC system (LC-10 Avp, Shimadzu, Kyoto, Japan) equipped with PDA detector (SPD-M20A; Shimadzu) at a flow rate of 1 mL/min and was monitored at 444 nm. Lutein was further confirmed by LC–MS as per Lakshminarayana et al.[15] Lutein-positive ions were recorded on LC-Q mass spectrometer (Waters 2996 modular HPLC system, Hertfordshire, UK) equipped with atmospheric pressure chemical ionization module ($APCI^+$) source and the probe was heated at 130°C and 500°C, respectively. The $(M+H)^+$ ion signals were recorded and confirmed with respective standard. The LC–MS conditions such as high-voltage lens, corona, cone voltage, sheath, and drying (N_2) gas were optimized at 0.5 and 5 KV, 30 V, and 100 and 300 L/h, respectively.

Preparation of Lutein–PLGA–PEG Nanocapsules

Lutein–PLGA–PEG nanocapsules were prepared by single emulsion sonication-solvent evaporation technique as described by Khalil et al.[16] with slight modification. Briefly, PLGA (10 mg/mL), lutein (2 mg/mL), and PEG (4 mg/mL) were dissolved in 2.5 mL DCM. The nanoemulsion of lutein was prepared by adding organic phase drop by drop to an aqueous phase containing a surfactant (0.5%–2%), PVA (15 mL), which is broken down into nanodroplets by sonication (PCI Pvt. Ltd., Mumbai, India; 50 w) for 10 min. The solvent was evaporated using a magnetic stirrer at 600 rpm to form PLGA–PEG nanocapsules entrapped with lutein as colloidal suspension in water. The suspension was centrifuged at 12,000*g* for 1 h to pellet down the nanocapsules. The pellet was washed three times with distilled water to remove surfactant, lyophilized (VirTis, New York, USA) and stored at −80°C. All the preparation processes were carried out under a dim yellow light so as to prevent oxidation of lutein by light exposure. Henceforth, lutein–PLGA–PEG nanocapsules are referred as lutein nanocapsules.

SEM and AFM Analysis

The surface morphology of the lutein nanocapsules was analyzed by SEM LEC-435 VP (LEO Electron Microscopy Ltd., Cambridge, UK). A drop of sample was placed on a carbon strip or cover slips over an aluminum stage and coated with gold and observed. Lutein nanocapsules morphology was further characterized by AFM (Nanosurf AG, Liestal, Switzerland) equipped with Nanosurf Easyscan-2 software. In brief, 100 μL of the sample was dried on a glass slide and analyzed.

Particle Size Analysis

Nanocapsules size and surface charge were analyzed by dynamic light scattering (DLS) spectroscopy using zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). Each sample was measured ($n = 3$) and the average diameter was calculated.

Encapsulation Efficiency

To determine the lutein encapsulation efficiency (EE, %), lutein from nanocapsules were extracted, analyzed by HPLC, and quantified with authentic lutein standard.[15] Lutein EE was calculated by the following equation:

$$\text{Encapsualtion efficiency of lutein (EE\%)} = \frac{\text{concentration of lutein entrapped in nanocapsules}}{\text{concentration of lutein used for nanaocapsulation}} \times 100$$

Solubility and Stability Study

To find out the solubility of lutein, lutein nanocapsules (2 mg) were dispersed in 10 mL of distilled water separately and incubated in a water bath shaker (Orbitek, Chennai, India) at 100 rpm at 37°C for 24 h. Samples were filtered through a