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Antioxidant, Antimicrobial and Physicochemical Properties of Turmeric Extract-Loaded Nanostructured Lipid Carrier (NLC)

Nayyer Karimi[a,b], Babak Ghanbarzadeh[a], Hamed Hamishehkar[b], Bahareh Mehramuz[c], Hossein Samadi Kafil[d,*]

[a] Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, P.O. Box 51666-16471, Tabriz, Iran
[b] Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[c] Connective tissue Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[d] Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

The objective of this research was to encapsulate turmeric extract as a water-insoluble bioactive material by nanostructured lipid carrier (NLC). Turmeric extract loaded NLC (T-NLC) was produced by high shear homogenization method. The average size of T-NLC was 112.4 nm and it showed good physical stability, and a sustained release pattern. Higuchi model fit *in vitro* release of turmeric extract from NLC the best. The antioxidant activity of T-NLC was significantly higher than free turmeric extract. The results of microbiological tests by agar-dilution method showed that NLC had higher antimicrobial activity than free turmeric extract against all examined gram negative bacilli microorganisms. The results indicate that T-NLC can be used in food products with high functional effects.

Turmeric is an indigenous herb in Southern Asia and it is well recognized with therapeutic properties including anti-cancer properties, healing of wounds and reduction of blood sugar [1]. Curcumin, the natural diphenolic compound and bioactive ingredient of *Curcuma longa*, is responsible to its yellow color and shows the most anti-cancer, antioxidant, antimicrobial and healing properties, as well as anti-inflammatory, anticancer, antitumor and neurodegenerative diseases-treating activities [2]. However, two other curcuminoids also hold the same properties. Curcumin dye acts as a factor to eliminate free radicals and converts them into materials with less activity. Curcuminoids because of their lipophilic structure, like many of nutraceutical compounds, show less bioactivity than optimal state in water solution and their use is limited. In addition, encapsulation is used to protect bioactive material and prevent the declining of its beneficial effects. In addition, this technology increases the stability, water solubility and bioavailability of lipophilic substances [3]. A number of strategies have been proposed to develop suitable delivery systems for curcumin or other similar neutriciticals, *e.g.*, powder particles, o/w emulsions, molecular complexes, and liposomes. In addition, recently the use of nanotechnology to improve stability and solubility of nutraceuticals has become one of the most useful research areas in the food and drug industries [4]. Lipid nanoparticles have been proposed as alternative carriers to well-known liposomes and polymeric nanoparticles in order to overcome some of their common problems such as short shelf life, poor stability, low encapsulation efficacy, cell interactions or adsorption and membrane transfer [5]. Generally, encapsulation is done for various reasons: i) to deliver the bioactive components at the suitable gastrointestinal target with controlled release, ii) to ensure the stability of the compounds in the gastrointestinal system and functional food systems, iii) to progress nutraceuticals absorption at the intestinal site [6] and iv) to hide the unpleasant characteristics of core material in food products.

Nanostructured lipid carrier (NLC) is able to enhance encapsulation efficiency, physicochemical stability, bioavailability and controlled release of functional hydrophobic ingredients in food products. In addition, it immobilizes the bioactive compound in the solid particle matrix and protects the incorporated material from degradation. The lipid system acts as a physical barrier, that may protect encapsulated sensitive bioactive component from unpleasant factors in aqueous phase. These carriers have advantages in comparison to other carriers among which the most important are: i) colloidal stability against gravitational separation due to the higher density of solid lipids; ii) higher chemical stability due to the higher stability of solid lipid; iii) the possibility of sterilization; iv) not using of organic solvents in its production; v) the high encapsulation efficiency. Therefore, NLC as a new delivery system is in entering stage of food production field. The incorporation of these

* Corresponding author.
E-mail address: Kafilhs@tbzmed.ac.ir (H.S. Kafil).

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bioactive materials into lipid carriers, increases their stability and bioavailability and improves the performance of curcuminoids in the body. NLC is composed of solid and liquid lipids, surface active agents and water, in which a liquid lipid core is surrounded by solid lipid matrix. NLC offers a useful tool for handling and transmission of lipophilic biomaterials and other fat-like substances in food or water-based transparent drinks [7].

The main objective of this study was to produce T-NLC for investigating of its antioxidant, antimicrobial and physical characteristics. The antimicrobial activity of T-NLC compared to free turmeric extract by macro-broth-dilution and agar-dilution methods, and the antioxidant properties were studied by DPPH assay. In addition, the release kinetic of T-NLC and free turmeric extract from dialysis bag fitted in four models.

Campritol 888-ATO (glyceryl behenat) was kindly donated from Gattefosse (Saint Periest Cedex, France). Miglyol 812 (caprilic/capric triglycerids) was provided from Sasol (Witten, Germany). Nonionic surfactant poloxamer 407 and DPPH powder were provided from Sigma-Aldrich (Steinheim, Germany). Turmeric extract was provided from Magnolia flavor and fragrance Co. (Iran, Saveh).

T-NLC was prepared by high shear rate homogenization method in which, cavitation, high shear forces and particle collision reduced the particle size after leaving the homogenizing gap [7]. For this purpose, 0.207 ml of turmeric extract was dissolved in 50 mg liquid oil (Miglyol) and the mixture was added into 500 mg melted solid lipid (Campritol). Then, the hot aqueous surfactant solution was prepared by dissolving 500 mg of poloxamer in 25 ml distillated water and it was added gradually into the lipid phase (at the same temperature as melted lipids), under homogenization (Silent crusher M, Heidolph, Nuremberg, Germany) at 20,000 rpm for 45 min. The produced hot o/w nanoemulsion was cold down in the ambient temperature (25 °C) resulting in the lipid phase recrystallization, and finally the T-NLC was formed.

T-NLC samples was diluted with distilled water (20 times) and the mean particle size, PDI and zeta potential of T-NLC were measured by DLS method at 25 °C using Nano-ZS90 system (Malvern Instruments Ltd., UK) with a measurement angle of 90° in three replications.

Encapsulation efficiency (EE) of turmeric extract into the NLC was determined by an indirect method. A volume of 0.5 ml of NLC sample was diluted with 3.5 ml of ethanol 50%. The samples were incorporated to Amicon filter and centrifuged at 4000 rpm for 10 min. The amount of free turmeric in the supernatant was determined spectrophotometrically at λ_{max} = 424 nm using Ultraviolet-visible spectrophotometer (Ultraspec2000, UK). The EE% was calculated using the following equation (Eq. (1)).

$$EE\% = \frac{W_i - W_f}{W_i} \quad (1)$$

where "w_i" is the mass of initial added turmeric extract and "w_f" is the mass of free turmeric extract detected in the supernatant after centrifugation of T-NLC.

The physical stability of T-NLC system was monitored for 40 days at room temperature. The particle size, PDI and zeta potential were determined at first and fortieth days by DLS method at 25 °C using Nano-ZS90 system (Malvern Instruments Ltd., UK) with a measurement angle of 90° in three replications.

In order to certify the encapsulation of turmeric extract in the nanocarrier and the interaction between the turmeric extract and NLC excipients, FTIR analysis was carried out using a Fourier transform infrared spectrometer (Tensor 27, Bruker). Free turmeric extract, blank NLC and T-NLC were analyzed by the potassium bromide disk method over the scanning wavelength range of 4000–500 cm^{-1}.

The dry powder was sprinkled on a double-sided carbon tape and placed on a brass stub. The surface powder was coated with a thin layer of gold in auto fine coater (SEM-TESCAN MIRA3-FEG). Then, it was placed in the sample chamber of SEM and the morphology of the complex was observed.

The crystallographic structural analysis was carried out by X-ray diffractometer (Siemens, Germany) applying Cu Kα (λ = 1.5406 Å). The samples (turmeric extract, T-NLC and blank NLC) were scanned over a 2θ of 10–60° at a scan rate of 0.05°/s.

The *in vitro* release behavior of turmeric-NLC were investigated in the 8 ml of phosphate buffered saline (1 × PBS, pH = 7) and ethanol (80:20, v/v) using a dialysis method [8] and intestinal fluid dissolution media simulated containing lipase 0.4 mg/ml, bile salts 0.7 mg/ml, pancreatin 0.5 mg/ml and calcium chloride solution 750 mM. The release of turmeric extract from NLC was determined at different time intervals, in 37 °C and under constant stirring (50 rpm). Quantification of the released turmeric from the NLC was monitored using Ultraviolet-visible spectrophotometer at λ_{max} = 424 nm. The release medium was replaced with freshly prepared PBS for continual studies. Volume of release medium was maintained constant throughout the *in vitro* release study. The turmeric extract release data were kinetically determined by zero-order, first-order, Higuchi, and Rigter–Peppas models [9] (Eqs. (2)–(5)), respectively:

$$C = Kt \quad (2)$$

$$C = [1 - \exp(-Kt)] \times 100 \quad (3)$$

$$C = Kt^{0.5} \quad (4)$$

$$C = Kt^{n} \quad (5)$$

where, C is turmeric extract concentration (%) at time t, K is kinetic constant and n is release exponent.

For investigating of antioxidant activity of T-NLC and free turmeric extract, at first DPPH chloroform solution was prepared (0.2 mM). The serial dilution of free turmeric extract and T-NLC were prepared in chloroform. Final concentrations of turmeric extract ranged from 62.5 to 1000 μg/ml. Then the solutions were mixed in the same volume of DPPH chloroform solution, and incubated at room temperature for 30 min in dark. The mixture without turmeric extract was used as a control sample and provided maximum absorbance. The absorbance of the reaction solution was measured by Ultraviolet-visible spectrophotometer at 517 nm. The percentage of DPPH free radical scavenging ability was calculated using Eq. (6).

$$\%\text{Reduction} = \frac{A0 - AA}{A0} \times 100 \quad (6)$$

where "AA" is the absorbance of samples at λ = 517 nm, 30 min after incubation in the presence of antioxidant and "A0" is the absorbance of control at λ = 517 nm, after a period of 30 min. All of the experimental procedures were repeated three times and reported as mean ± SD (standard deviation).

For MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) determination, 0.5 McFarland standard of bacterial suspension (10^8 cfu/ml) were prepared in sterile tubes containing saline. The primary stock solution with a concentration of 20 mg/ml medium was prepared using MHB. For a more detailed study of MIC, T-NLC and free turmeric extract dilutions were obtained by serial dilution of the primary stock solution as follows:

(20, 18.75, 9.37, 4.69, 2.34, 1.17, 0.58, 0.29, 0.15, 0.073, 0.036 mg/ml). Next, the same volume concentration of prepared bacterial suspension was added to them, and the samples were incubated for 24 h at 37 °C. After incubation, each sample was re-cultured on Muller Hinton agar (MHA), and incubated for 24 h at 37 °C. Finally, the growth or lack of growth of bacteria in different concentrations of extracts and NLC was observed and MIC and MBC were determined.

Different concentrations of free turmeric extract and T-NLC (45, 40, 30, 20, 15, 10, 7.5, 5, 2.5 and 1.25 mg/ml) were prepared and transferred to the agar medium, since agar was tightened [10]. Then, each bacterium was spot planted using 3 μl of 0.5 McFarland solution of bacteria. These plates were incubated for 24 h at 37 °C and the growth or lack of growth of bacteria was investigated [11]. In this method, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas*