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FULL PAPER

Microencapsulation of herbal bioactive drug by *Chlorella Vulgaris* microalgae as a nano-formulation for drug delivery to cells

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Curcumin (CUR) acts as a strong protector against various diseases, including HIV, cardiovascular infection, cancer, and neurological and skin diseases. CUR, a polyphenols with pharmacological function, was successfully encapsulated in algae (Alg) cell (*Chlorella Vulgaris*) as confirmed by fluorescence microscopy, thermogravimetric analysis (TGA), and Fourier transform-infrared spectroscopy (FTIR). The effects of molar ratio, salutation, loading capacity, drug release rate, and selective toxicity were investigated in this study. After obtaining *C. Vulgaris* with entrapped CUR, this mixture was centrifuged and re-suspended in 10 mL of water along with the ultra-sonication. This step was carried out twice to remove methanol. Finally, the CUR-loaded *C. Vulgaris* was prepared to perform further experiments to determine the role of this algal species as a carrier. Thermal gravimetric analysis (TGA) showed that 83% of Chlorella microalga and 64% of CUR were destroyed at 600 °C. DPPH was used to evaluate CUR, which was more than 85% pure CUR. Fourier transform infrared spectroscopy (FTIR) spectral data were derived from all samples, including the control *C. Vulgaris*, CUR, and CUR-loaded *C. Vulgaris* using a Perkin-Elmer Lambda 30 UV/VIS spectrophotometer (AH and Aysel 2003) in the 200-400 nm UV region. Then, the FTIR spectrums of the items mentioned above were determined using a Shimadzu IR-470 plus device and were plotted. This study provides an overview of an effective nano-formulation of CUR for a targeted treatment option for various human diseases. In conclusion, the data proved that the *C. Vulgaris* cell could be used as a new stable carrier for CUR.

KEYWORDS

Microencapsulation; curcumin; bioactive; *Chlorella Vulgaris*; microalga; drug delivery.

Introduction

Microencapsulation is a process by which tiny particles, such as antioxidants, enzymes, and many other substances, are surrounded by substances known as carriers and is currently used in the pharmaceutical industry [1]. The active ingredients are protected against decomposition by oxygen, temperature, light, or other destructive agents [2]. This increases their stability and allows the active substance to be released in a controlled and stable manner. However, the current carriers have costly manufacturing procedures as well as low biocompatibility and biodegradability [3]. Therefore, there is a high demand for a biocompatible, biodegradable, inexpensive, and affordable carrier [4].

Chlorella Vulgaris is a microalga used in the pharmaceutical industry. The advantages of this microalga as a carrier include higher biocompatibility and biodegradability, cost-effectiveness, availability, and antioxidant and antimicrobial properties [5].

The microalgal cell wall is composed of polysaccharides and glycoproteins. While strengthening the cells, the cell wall further acts as a permeable barrier. The alga contains a unique combination of the *chlorella* growth factor (CGF), appearing to be the core of *Chlorella* [6]. It contains all of the elements in the nucleus of a *chlorella* cell (peptides, proteins, nucleic acids, DNA and RNA, polysaccharides, beta-glucans, sulfur, and manganese). In addition, CGF is produced at the peak of photosynthesis and accelerates cell growth, by which a single cell turns into four cells within 20-24 hours [7].

A growing number of studies revealed the beneficial therapeutic impacts of CGF on treating chronic gastrointestinal ulcers and chronic gastritis [8]. It has also been shown that CGF can stimulate cell growth in patients unable to repair injured cells. CGF and nucleic acids are the most important compounds in *Chlorella* [9]. They can increase the body's energy level and improve the repair of organs, glands, and tissues [10].

Polyphenolic compounds are among the most abundant plant metabolites and a major part of human and animal diets. Besides, it has been shown that polyphenols with antioxidant properties effectively prevent and heal cancer and prevent degenerative brain diseases such as Alzheimer's in mice and humans [11]. Using plant polyphenols effectively treats cancer, Alzheimer's disease, microbial diseases, diabetes, rheumatoid arthritis, atherosclerosis, autoimmune diseases, and digestive disorders [12].

Studies have shown that blood plasma and tissue levels reveal Cur as poorly absorbed in the gut, independently of the route of administration, due to the intestinal and hepatic metabolism as well as rapid elimination, hence restricting its bioavailability [13]. A technique often used to overcome the above mentioned problems is microencapsulation. It has been already encapsulated in gelatin cyclodextrins [14].

The other considerable limitations include low absorption and availability due to the poor stability, low solubility in water, inactive transfer system to the gastrointestinal tract epithelial cells, and an active transfer system for excretion through epithelial cells into the gastrointestinal tract space [15].

Curcumin (CUR) is one of the major constituents of turmeric and forms the polyphenolic part of the formula. [1,7-bis (4-hydroxy-3-methoxypHenyl)-1,6-heptadiene-3,5-dione], a fluorescent, and hydrophobic molecule that can enter the cell membranes very quickly. Strong evidence has confirmed the antitumor effect of CUR and its function on tumor cells [16]. Moreover, its anti-inflammatory, antioxidant, anti-angiogenic, and anti-proliferation activities have been proven in the cancer cells. CUR can inhibit carcinogenicity in breast, ovary, colon, liver, leukemia, pancreas, and prostate cancers. CUR has a protein Kinase-C inhibitory role, by which it modulates growth factor signaling and cytotoxic activity in various human cancer cell lines [17].

Besides, CUR inhibits both the cell cycle and the NF-κB signaling pathway and plays a role in inducing apoptosis in cancer cells. Although various carriers and systems, such as liposomes, polylactic glycolic acid, microspheres, etc. have been used as encapsulating agents, they have many limitations such as a high cost of production, toxicity, low stability, etc. [18]. Therefore, having an accessible, low-cost agent with low toxicity can improve the outcome of delivery [19].

Given these problems, the goal of this study was to determine the potential of an encapsulating and cost-effective substance, called CUR, with high biocompatibility, availability, and low toxicity in a microalga.

Materials and methods

Materials

Curcumin (CUR) was purchased from Merck. DPPH was obtained from Sigma. Fresh *Chlorella vulgaris* was cultured before using. The deionized-water was used to prepare solutions. All used chemicals were of analytical grade and were used as received without further purification. The *Chlorella vulgaris* was grown in sterile BG-11 liquid medium. Alg was inoculated at 10% (inoculation media) in 500 mL. Erlenmeyer flasks containing 100 mL liquid medium. The culture flasks were incubated under stationary condition at 25±2 °C, with 100 rpm rotation speed. Cells were harvested by centrifugation (8000 rpm, 10 min), and then they were freeze dried.

Methods

Preparation of CUR-loaded C. Vulgaris

To prepare CUR-loaded *C. Vulgaris*, 5 mg of CUR was dissolved in 5 mL of methanol, which was then added dropwise to the prepared amount of the alga with constant stirring. After obtaining *C. Vulgaris* with entrapped CUR, this mixture was centrifuged and re-suspended in 10 mL of water along with the ultra-sonication. This step was carried out twice to remove methanol. Finally, the CUR-loaded *C. Vulgaris* was prepared to perform further experiments to determine the role of this algal species as a carrier.

FTIR

Fourier transform infrared spectroscopy (FTIR) spectral data were derived from all samples, including the control *C. Vulgaris*, CUR, and CUR-loaded *C. Vulgaris* using a Perkin-Elmer Lambda 30 UV/VIS spectrophotometer (AH and Aysel 2003) in the 200-400 nm UV region. Then, the FTIR spectrums of the items mentioned above were determined using a Shimadzu IR-470 plus device and were plotted and scanned as intensity vs. wavenumber in the resolution of 00-4000 cm^{-1} range, respectively [20].

Thermal gravimetric analysis (TGA)

The TGA was made by a thermal analyzer apparatus (Shimadzu DTGA 60H). In brief, the weight of samples (4 to about 7) was calculated by a platinum pan. Measurements were done using a dynamic nitrogen atmosphere consisting of an ambient system at 900 °C with a heating rate of 10 °C per minute and a 30 mL per minute flow rate. Coats Redren and Horowitz Metzger methods were performed to determine the TGA curves of the initial step of drug activation decomposition of energy.

Determining samples' fluorescence

To do this, the prepared samples, including *C. Vulgaris*, CUR, and CUR-loaded *C. Vulgaris*, were positioned under a fluorescent microscope to evaluate the fluorescence emission by samples.

Antioxidant assay (DPPH)

The antioxidant activity of each sample was determined using a free radical scavenging assay named 1-Diphenyl-2- picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, Missouri, USA) with a minor modification.

The procedure was carried out based on the manufacturer's protocol and the mentioned reference conducted in Drug Applied Research Center, Tabriz University of Medical Sciences, Iran [21]. Hydrogen atoms or electrons donation ability of encapsulated Cur was measured by their bleaching capability of purple colored DPPH methanol solution. The reaction mixture was comprised of 0.3 mL of DPPH in methanol solution (0.1 mM equivalent 39.4 mg L^{-1}), 0.5 mL (100 lg Cur mL^{-1}) of each kind of microcapsule (storage under sunlight for 2 weeks, 1 and 2 months)

and it was kept in the dark for 30 min at room temperature, which was enough time to ensure that reaction reaches the steady state. The absorbance was measured by Thermo UV/VIS Spectrophotometer at 517 nm. Decreasing amplitude of signal at the selected wavelength confirmed the radical scavenging activity. The antioxidant activity of Cur stored under dark condition (sealed bottle) as standard reference was assayed. Methanol was used as blank and the measurement of solutions without sample were used as the control.

The inhibition of DPPH radicals by the samples was calculated as follows:

DPPH inhibition% = $[(A_{control} - A_{sample})/A_{control}]\times100$

Where $A_{control}$ is the absorbance spectrum Without Cur and A_{sample} is the absorbance of Cur microcapsule.

Results

FTIR analysis of microalgae

Each of these peaks represents a specific feature of a group. The 1649 cm^{-1} band is related to the tensile vibration of the C=O bond of amide 1 protein, while the 1543 cm^{-1} band corresponds to the flexural C-N bond and the tensile vibration of the amide two protein N-H bond. The 2928 cm^{-1} band is associated with the C-H2 flexural bonds of the lipid-carbohydrate functional group, while the 1737 cm^{-1} band belongs to the tensile vibration of the side chain of the C=O bond lipid carbonyl ester. The bands 1149, 1082, and 1036 cm^{-1} belong to the C-O-C group of carbohydrates.

FTIR analysis of CUR

The FTIR analysis of CUR shows that the 3501 and 1622 cm^{-1} bands are related to the tensile vibration of O-H and C=O bonds of the phenolic group, respectively, and the 1600 cm^{-1} band corresponds to the tensile vibration of the benzene group. The 1504 cm^{-1} band corresponds to a combination of C=O and C=C bonds. Besides, the 1435 cm^{-1} band belongs to the flexural vibration of the C-H bond, the 1279 cm^{-1} band is related to the tensile vibration of the phenolic C-O bond functional group, the 1024 cm^{-1} band corresponds to the methoxy C-O bond functional group, and the 966 cm^{-1} band belongs to the aromatic C-H bond functional group.

FTIR analysis of microalga-CUR microcapsules

The results of this section revealed that the IR spectrum of microcapsules was similar to that of the microalga, indicating that CUR is encapsulated inside the alga. Figure 1 shows The disappearance of the 3501 cm^{-1} CUR bands in the IR spectrum of the microcapsule indicates the interaction of the phenolic O-H group with the microalgal cell, which is mainly via hydrogen bonding. The disappearance of the 1600 and 966 cm^{-1} bands illustrates the interaction of the aromatic group of CUR with the microalgal cell. Likewise, 1149, 1082, and 1036 cm^{-1} of the alga change to 1133, 1059, and 1022 cm^{-1}, respectively. These results indicate the CUR interaction with the algal polysaccharide carbohydrate. Besides, the change of the algal 1543 cm^{-1} to the 1515 cm^{-1} group indicates the interaction of CUR with the algal protein.

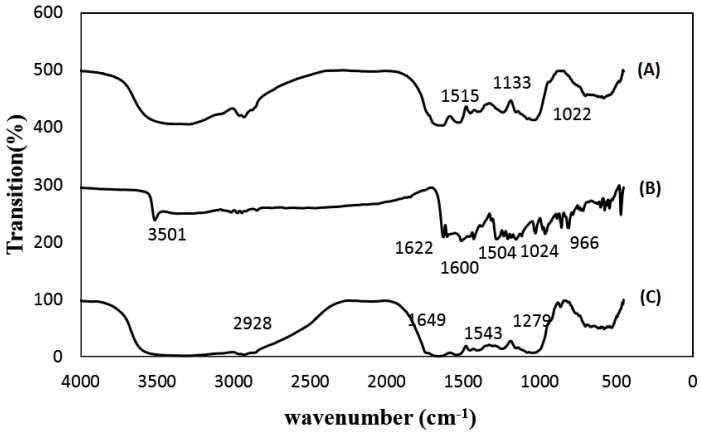

FIGURE 1 FTIR spectrums of CUR-microalga (A), CUR (B), and microalga (C)

TGA thermal analysis

As can be seen, 10% of the microalga loses their weight at 35-120 °C, which is due to the evaporation of algal water. Increasing the temperature to 230 °C causes the microalga to dry completely. Figure 2 shows the thermal analysis of CUR, microalga, and microalga-CUR microcapsules. It is observed that 83% of *Chlorella* microalga and 64% of CUR are destroyed at 600 °C. Unlike *Chlorella* microalga, the CUR-microalga microcapsule improved the microcapsule temperature stability due to the CUR presence.

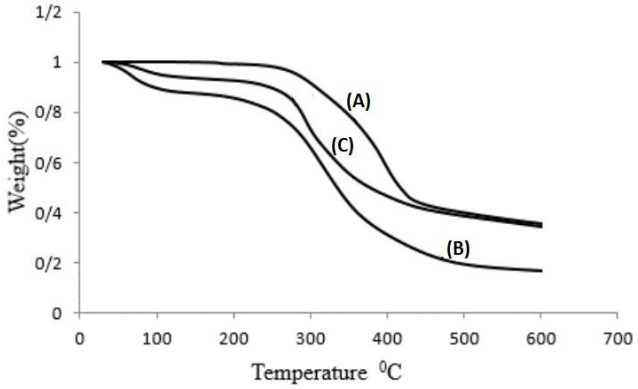

FIGURE 2 Thermal analysis of CUR (A), microalga (B), and microalga-CUR microcapsules (C)

Fluorescence microscopy analysis

To this end, some of the samples were initially placed on a slide covered by a coverslip, and then the sample was examined under a fluorescence microscope. As depicted in Figure 3D, the fluorescence emission is due to the CUR presence in the microalga. In Figure 3C, however, no fluorescence emission is seen due to the CUR absence, indicating that the microalga has no fluorescent properties.

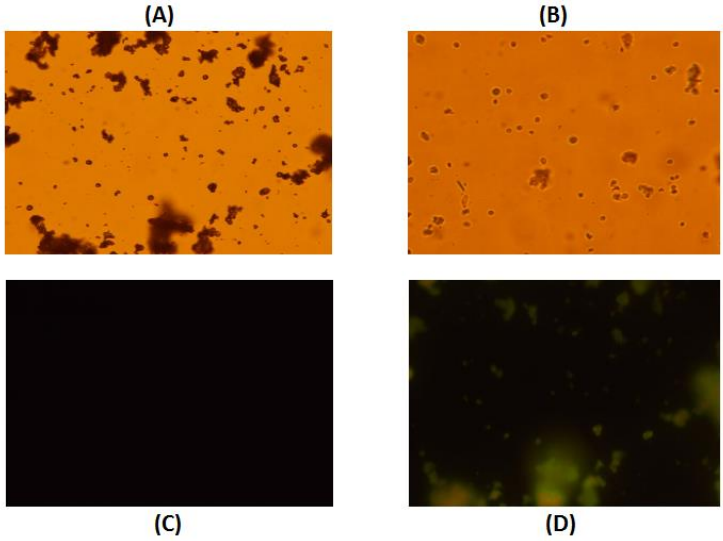

FIGURE 3 The optical photos of microalga and microalga-CUR microcapsules (A, B), respectively, and microalga and microalga-CUR fluorescence photos (C, D), respectively

Assessment of antioxidant activity

CUR has a strong antioxidant activity, and DPPH was used to evaluate microalgal-coated CUR at different times. Figure 4 shows that more than 85% of pure CUR was active, while the CUR plus freshly prepared microalga had an activity of more than 70%, the activities of CUR-microalga exposed to the light for two weeks, one month, and two months were 54, 55, and 54%, respectively. Since the results were not statistically different, it can be concluded that the microalga plays an important role in protecting the antioxidant activity of CUR *via* harnessing the destabilizing agents.

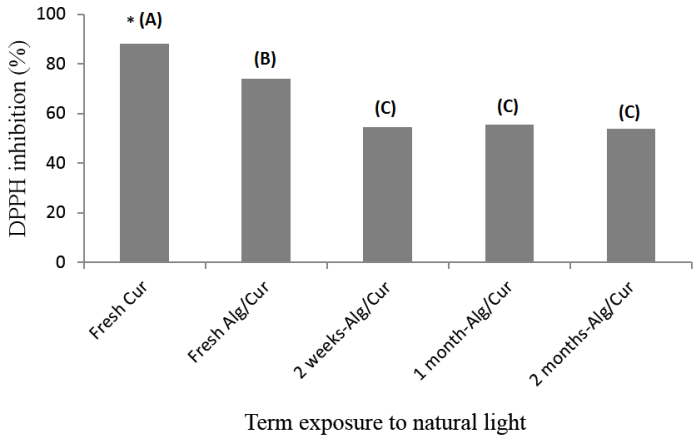

FIGURE 4 (Cur) curcumin, (Alg/Cur) curcumin-microalga

Treatments with common letters have no significant differences at the level of 5% ($p<0.05$). Mean comparisons were performed by Duncan's method at 5% level.

Discussion

CUR has unique biological and medicinal properties. However, the instability and low solubility of CUR are its major limitations. A promising approach to tackle this problem is to make use of pharmaceutical carriers [22]. The use of nanoparticles has improved its solubility and delivery. Currently, most drugs are absorbed systemically through the action site. In this regard, if a sufficient amount of the drug enters the bloodstream, it can reach the

action site and exert its healing properties. Therefore, providing methods that can solve the solubility of water-soluble drugs is beneficial to increase and protect the drug until it reaches the desired place in the body. The results of various studies have shown a lower percentage of CUR-loading capability in algae. The results of our study revealed that the loading capability of CUR in the alga was improved by approximately 50%, which was a higher percentage than those of the previous similar studies.

Sabitha Mangalathillam *et al.* reported that the amount of CUR loaded within the chitin nano gel was 20% [23]. Similarly, Lei Liu *et al.* mentioned that the amount of CUR loaded in the polymer micelle was 14.84% [24]. CUR is an appealing encapsulating and carrier agent because of its cost-effectiveness and availability over the other encapsulating agents.

Conclusion

Curcumin (CUR) has a wide range of biological activities. However, photodegradation, short half-life, and low bioavailability have limited its clinical application. CUR acts as a significant protector against various diseases, including HIV, cardiovascular infection, cancer, and neurological and skin diseases. The aim of this study was to use curcumin loading by *Chlorella vulgaris* microalgae as a drug delivery system. The observations illustrated that the interactions of CUR with the algal cell wall (Cellulose Microfibrillar) could protect CUR from the photodegradation effect of light. A significant number of nano-formulations can be converted into pharmaceutical use after completing clinical trials in humans. In conclusion, the data proved that the *C. Vulgaris* cell could be used as a new stable carrier for CUR.

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Conflict of interest

The authors declare no conflict of interest in publishing this paper. This study was not supported by any grant money from a pharmaceutical company or for-profit organization.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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