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Research Article

INCREASING THE WATER SOLUBILITY OF CURCUMIN, BY ALGINATE IN ULTRASOUND MEDIUM

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ABSTRACT

Curcumin is a natural bioactive material which has very low water solubility. In this work, solubility of curcumin was increased with a natural edible linear polysaccharide, alginate, in ultrasound medium at 25°C. Curcumin solubility in alginate-water solution (2% by weight) was increased up to 1.525×10^{-3} M at pH 7 and up to 0.317×10^{-3} M at pH 2.4. The critical aggregate concentration of alginate for curcumin-alginate complexes, were determined as 1 % (by weight). The sizes and surface charges of curcumin-alginate complexes were measured. Nano sized complexes with a uniform size distribution were obtained. Antioxidant capacities of the complexes were determined by 2, 2-Diphenyl-1-picrylhydrazyl and Folin- Ciocalteu methods. For both methods, the antioxidant capacities of complexes were higher than that of curcumin itself. Complexes were stable at all dilutions with water and can be considered as candidate materials for food and drug preparations.
Keywords: Curcumin, solubility, sodium alginate, ultrasound, antioxidant capacity.

1. INTRODUCTION

Curcumin is a natural compound, originally used in India as a food supplement and medicinal agent. Curcumin was reported to have chemo-preventive, anti-inflammatory, antioxidant and antitumor properties, however, the low water solubility has been the major problem for its applications [1-3].

In the literature, the highest curcumin solubility in water (phosphate buffer) determined by high performance liquid chromatography was 3×10^{-8} M [4,5]. For this reason, increasing its water solubility is an important research topic. Increasing the solubility by using cyclodextrin was preferred by many groups [6-11]. Other procedures which have improved the water solubility of curcumin include, solid dispersion techniques in polyvinylpyrrolidone [12] and cellulose matrixes

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[13]. Modasiya et al. have used solid dispersion technique and polyethylene glycol 4000 and 6000 to increase the water solubility by triturating curcumin and polymer [14]. PEG 200, 600 and 2000 were used in ultrasond medium and water soluble PEG-curcumin complexes were prepared by Catalgil -Giz et al. [15]. In the work of Song et al. curcumin was solubilized by food emulsifiers and deposited in alginate beads [16]. Other techniques for improving its solubility include using soyprotein [17], hyaluronic acid [18] and micellization of curcumin in cationic, anionic and non-ionic surfactant solutions [19]. Conjugation of curcumin onto alginate beads requires chemical synthesis [20]. Alginate and natural polymers were used in solubilization of curcumin but ethanol was used in preparation[21].

Alginic acid is a linear polysaccharide copolymer with of D-mannuronic acid and L-guluronic acid residues, covalently linked together in different sequences or blocks. Sodium alginate, is the sodium salt of alginic acid and widely used in drug and food applications due to its biocompatibility, non-toxicity, gel and film forming properties, and biodegradability [22, 23].

Here, alginate was chosen to provide a suitable medium to increase the solubility of curcumin in water and ultrasonic processing was applied in order to increase the interaction of curcumin and alginate. When polymer solutions are subjected to high-intensity ultrasound, formation, growth, and collapse of cavitation bubbles creates local high temperature and pressure effects and breaks polymer chains and this process lowers the mean chain length [24-27]. An advantageous side effect is that elimination of the long chains, improves the homogeneity of the chain length distribution. Viscosity suppresses the chain scission therefore dilute solutions are more suitable for ultrasonic chain scission. Here very low alginate concentrations were preferred in order to keep the ultrasonic efficiency high.

In this work, curcumin-alginate complexes were prepared in ultrasound medium at pH: 7 and pH: 2.4. Here alginate provided the necessary organic environment by wrapping around the curcumin molecules. Ultrasound was applied for chain scission, homogenization and increasing the efficiency of curcumin-alginate interaction. Stable curcumin solutions was obtained in alginate-water medium. Critical aggregate concentration (CAC) of alginate for complex preparation was determined. Sizes and zeta potentials of complexes were measured. Antioxidant capacities were determined by 2,2-Diphenyl-1-picrylhydrazyl free radical scavenging method and Folin-Ciocalteu methods.

2. EXPERIMENTAL

2.1. Materials and Methods

Curcumin and acetic acid were from Merck, alginate was from Sigma Aldrich (alginic acid sodium salt, low viscosity, 100-300 cP, 2% w/w). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was from Sigma (Steinheim, Germany). Folin-Ciocalteu's reagent was from Fluka (Buchs, Switzerland). All other chemicals were analytical grade and all solutions were freshly prepared.

FTIR analyses were performed using a Perkin Elmer FT-IR 100 Spectrometer in the range of 600-4000 cm^{-1} . A Shimadzu 1800 double beam spectrophotometer was used for UV measurements using a 10 mm path quartz cell. Samples were sonicated in a Bandelin Sonorex RK ultrasonic bath (80 watt- 35 kHz) and centrifuged in Hettich EBA 20 Mikro Centrifuge. Viscosity of alginate solutions were measured using a Brookfield DVIII + type low-shear viscometer. Measurements were carried out in duplicate. Size and zeta potential measurements of the complexes were performed with a Malvern Nanosizer and Zetasizer 2000.

2.2. Curcumin solubilization

0.05, 0.1, 0.5, 1 and 2 % (w/w, in water) alginate solutions (10 g) were prepared and 20 mg of curcumin was added to each of them. Samples were sonicated in an ultrasound bath for 2 hours at

25ºC. After the sonication, samples were centrifuged for 1 hour at 6000rpm and the dissolved part was decanted. The amount of dissolved curcumin was determined by UV spectroscopy, at 430nm. Samples were prepared at pH=2.4 and pH=7. Neither alcohol nor any other organic solvent were used during preparations.

2.3. Antioxidant capacity determination

2.3.1. DPPH free radical scavenging assay

The free radical-scavenging activity of curcumin in methanol and curcumin-alginate complexes were determined by the DPPH method [28]. 20 mg/l DPPH-methanol solution was prepared and 1.980 mL of DPPH solution was added to 0.020 mL of sample. The solution was mixed for thirty minutes and absorbance of the solution at 515 nm was measured. Inhibition of free radical DPPH in percent (%) was calculated from Eq 1, here As and Ac are the the absorbances of sample and control respectively.

$$\text{Percentage inhibition (\%)} = [(Ac-As)/Ac] \times 100 \quad (1)$$

Experiments were repeated twice.

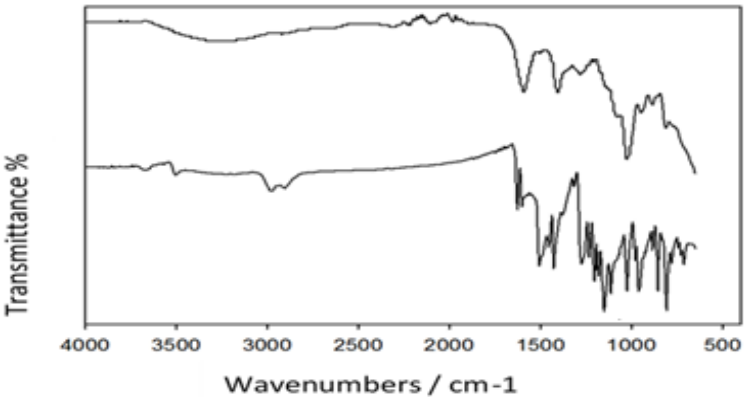

Figure 1. The FTIR spectrum of curcumin-alginate complex and pure curcumin are shown. Curcumin-alginate complex is the one at the top and pure curcumin is at the bottom.

Table 1. Viscosities (mPa.s), (mili pascal.second) of curcumin loaded alginate solutions depending on time and alginate concentrations.

Sonic. Time	Alg. conc. 0.05%	Alg. conc. 0.1%	Alg. conc. 0.5%	Alg. conc. 1%
0 min	2.5720	3.2830	8.133	19.571
30 min	2.2480	2.3020	7.2450	18.831
60 min	1.8070	1.8890	5.4370	19.060
120 min	1.5870	1.7700	4.4870	19.119

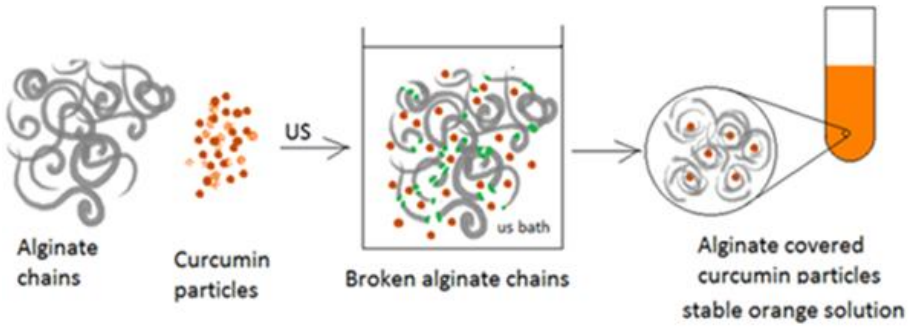

Figure 2. Shematic representation of curcumin solubilization by alginate in ultrasound medium.

Table 2. Amounts of curcumin solubilized in alginate-curcumin complexes at pH=7 and pH=2.4 (sonicated for two hours).

% alg	pH 7			pH 2.4		
	C_{curc} mg/ml	C_{curc}/C_{alg} mg/mg	C_{curc} M.10^4	C_{curc} mg/ml	C_{curc}/C_{alg} mg/mg	C_{curc} M.10^4
0.05	0.126 ± 0.052	0.252	3.42	----	----	
0.1	0.091± 0.0015	0.091	2.47	0.059 ± 0.007	0.059	1.60
0.5	0.171 ± 0.002	0.034	4.64	0.163 ± 0.003	0.032	4.42
1	0.189 ± 0.046	0.019	5.13	0.129 ± 0.006	0.012	3.50
2	0.562 ± 0.070	0.028	15.25	0.117 ± 0.013	0.006	3.17

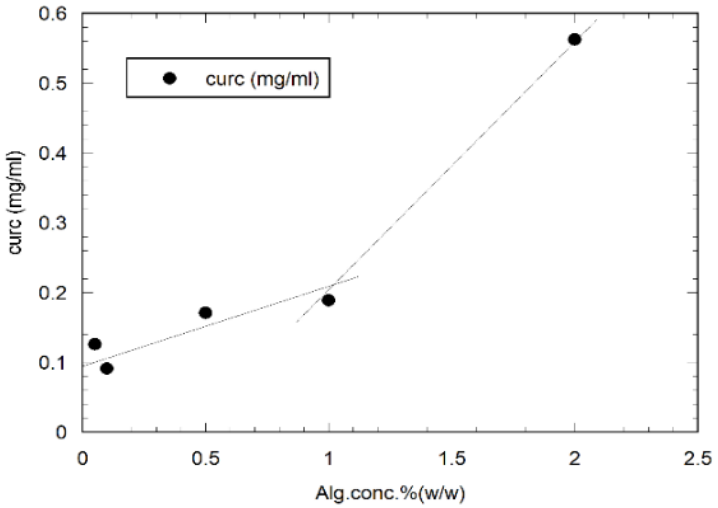

Figure 3. Solubilized curcumin concentration versus alginate concentration for critical aggregate concentration determination.

Table 3. Zeta potential,diameter, abundance and distribution width of the curcumin-alginate complexes.

% alg	**0.05**	**0.1**	**0.5**	**1.0**	**1.5**	**2**
zeta potential (mV)	-60.9± 8.9	-64.2±3.3	-63.5±4.6	-65.1± 2.5	-71.7±4.6	-68.6±3.8
diameter (nm)	74.45±1.68	---	60.21±15.0	64.06±4.03	---	---
% abund.	97.65±0.75	----	97.25±1.85	99.55±1.05	---	---
distrib. width	11.95±0.01	----	14.38±2.98	10.68±0.97	---	---

2.3.2. Folin-Ciocalteu method (Determination of Total Phenolics)

The total phenolic content of curcumin-alginate complexes were determined by using the Folin-Ciocalteu method [29]. 1 mg of curcumin was dissolved in 1 ml methanol and standard curcumin solution was prepared.

Folin reagent was diluted 1:10 with water. 0.3 ml of the complex and standard curcumin solution were mixed with 1.5 ml of dilute Folin–Ciocalteu's reagent and 1.2 ml of sodium carbonate solution (7.5% w/v) was added to the mixture. The mixtures were kept for 10 minutes at room temperature until a stable colour was obtained. The absorbances of the samples were measured at 760 nm. Results were expressed as mg gallic acid equivalents (GAE) in 1ml curcumin-alginate complex and standard curcumin. The calibration equation for gallic acid was equation (2).

$y = 0.627x-0.0475$ ($R^2= 0.999$). (2)

Experiments were repeated twice.

Table 4. Antioxidant capacities of complexes. DPPH radical scavenging activity (inhibition %) column 2 and total phenolic content (mg GAE/ mL solution) of alginate, curcumin and curcumin-alginate complexes are shown in columns 3, 4, 5.

% alginate in complex solution	**curcumin-alginate complexDPPH Inhibition % (ml solution)**	**pure alginate** mg GAE/ml	**pure curcumin** mg GAE/ml	**curcumin-alginate complex** mg GAE/ml
0.1	74.77±5.23	0.053 ± 0.006	0.019± 0.007	0.550 ± 0.020
0.5	28.89±2.36	0.041 ± 0.009	0.015 ± 0.003	0.186 ± 0.043
Standard Curcumin	7.56±1.83			

2.4. FTIR spectrum of complexes

FTIR spectrum of curcumin reported by Kolev et al [30]. In Figure 1 the FTIR spectrum of curcumin-alginate complex is the one at the top and pure curcumin is at the bottom. In the pure curcumin spectrum, 1626cm^{-1} band is due to C= C and C= O groups. Another band at 1601 cm^{-1} is attributed to the symmetric stretching vibrations in aromatic ring. Aromatic C=C bands of curcumin which are at 1506.06, 1455.45, 1426.95 cm^{-1} and C-O-C peak is at 1023cm^{-1}.

FTIR spectrum of curcumin-alginate complex is at the top and similar curcumin bands were observed. Symmetric aromatic ring stretching vibrations of C=C band was shown in 1590cm^{-1}. Aromatic C=C bands of curcumin can be seen at 1500, 1430 and 1404.55 cm^{-1} in the complex's

spectrum and C-O-C peak which belongs to the C-O-CH_3 group of curcumin is shown at 1020 cm^{-1}. So that curcumin–alginate complex has similar bands as pure curcumin has. The broad band at 3500 cm^{-1} is due to the hydroxide groups in the alginate.

3. RESULTS AND DISCUSSION

Viscosities of sonicated curcumin-containing slurries of 0.05%, 0.1%, 0.5% and 1% alginate are shown as functions of sonication time in Table 1. The viscosities of 0.05, 0.1 and 0.5 % solutions decreased during sonication, indicating that long alginate chains were broken and shorter alginate fragments appeared. The viscosity of the 1 % solution was not significantly affected. We ascribe this behaviour to the suppression of cavitation and hence, chain scission by the high viscosity of the concentrated solutions. During chain scission, transiently charged alginate fragments attract and cover the curcumin particles. Schematic representation of solubilization is shown in Figure 2.

Curcumin–alginate complexes were prepared at pH=7 and pH=2.4 to investigate the effects of pH and alginate concentration on curcumin solubility. Amounts of curcumin solubilized in complexes at pH=7 and pH=2.4 are given in Table 2. As can be seen from the table, solutions with higher alginate concentration carried more curcumin. Curcumin solubilized per unit amount of alginate, however, decreased with increasing alginate concentration. This is expected, since the effect of sonication decreases with increasing alginate concentration as shown in Table 1. There are more broken alginate fragments in dilute solutions so that they carry more curcumin. Curcumin solubility was found to be higher at pH=7 than at pH=2.4 at all alginate concentrations. In the size, zeta potential and antioxidant measurements, curcumin-alginate complexes at pH=7 were used. In the Figure 3, alginate concentration versus curcumin amount in the complexes were plotted and can be seen that curcumin concentrations in the complexes were very low below 1% alginate and increased steeply above that concentration. This indicates that CAC is around 1% alginate for curcumin-alginate complexes.

Zeta potential and size measurements of the complexes were shown in Table 3. Zeta potentials of the sonicated complexes were negative due to ionized sodium alginate fragments in water and voltage changed slowly from about -60 mV to -68 mV with increasing alginate concentration. The sizes of curcumin-alginate complexes were around 60-70nm and the widths of the distributions containing 97 – 99 % of the solubilized material were 10-11 nm. This shows that a nearly uniform nano-size distribution was obtained as expected from sonication.

Antioxidant capacities of complexes determined as DPPH radical scavenging activity (inhibition %) were shown in Table 4, column 2. Total phenolic content (results of Folin-Ciocalteu method) (mg GAE/ mL solution) of alginate, curcumin and curcumin-alginate complexes were shown in columns 3, 4, 5. Antioxidant capacities of the complexes were found to be higher than the antioxidant capacity of curcumin itself. In the case of Folin method, pure curcumin and pure alginate have some antioxidant capacity value (mg GAE/ml) however antioxidant capacity of the curcumin-alginate complex (mg GAE/ml) was much higher than both of these as shown in the last column. The dilute solution (0.1% alginate) had the higher antioxidant capacity due to fewer entanglements and the phenolic groups are easily assessable in more dilute solution.

4. CONCLUSION

Sonicated alginate chains covered and solubilized curcumin in biocompatible and edible way. 1 mL alginate curcumin solutions carried 0.5-0.6 mg curcumin at pH=7 and 0.1-0.2mg curcumin at pH=2.4. Complexes have around -65 mV zeta potential and nearly uniform size distribution. Antioxidant capacities of the complexes were higher than the curcumin itself in both of the methods tested. Complexes were stable at all dilutions with water. Considering their high

antioxidant capacities and uniform size distribution the method is promising for the development of solubilised curcumin for use in medicinal applications, in juices, drinks, or food supplements.

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