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Solubility and stability of curcumin in solutions containing alginate and other viscosity modifying macromolecules

Studies of curcumin and curcuminoids. XXX

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Received August 1, 2005, accepted November 21, 2005

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Pharmazie 61: 696–700 (2006)

The solubility, chemical- and photochemical stability of curcumin in aqueous solutions containing alginate, gelatin or other viscosity modifying macromolecules have been investigated in order to obtain an alternative to the use of surfactants or cyclodextrins. The solubility of curcumin in aqueous solution at pH 5 increased by a factor $\geq 10^4$ in the presence of 0.5% (w/v) alginate (various qualities) or gelatin compared to plain buffer, while propylene glycol alginate ester, cesapectin and sodium carboxymethyl cellulose did not have a similar solubilizing effect. The solubilization was slightly influenced by pH, ionic strength and type and concentration of buffer salts. The macromolecules do, however, not stabilize towards hydrolytic- or photolytic degradation of curcumin.

1. Introduction

Curcumin is widely used as a coloring agent in food, drugs and cosmetics. However, the most intriguing aspect of this compound is that it has a therapeutical potential due to its known biological activity including anti-oxidant and cytotoxic properties (Bruzell et al. 2005; Karunagaran et al. 2005; Miguel et al. 2002). Unfortunately, curcumin has a very low aqueous solubility at acidic or neutral pH which is limiting its use. At pH values above neutral dissociation takes place and the solubility increases, but the compound then undergoes a rapid hydrolytic degradation (Tønnesen and Karlsen 1985a, b). One challenge is therefore to make a physiologically compatible preparation with acceptable curcumin solubility and stability. Complex formation with cyclodextrins and micelles have been reported (Baglole et al. 2005; Iwunze 2003, 2004; Qi et al. 2003; Szente et al. 1998; Tang et al. 2002; Tønnesen 2002; Tønnesen et al. 2002). In cyclodextrin solutions the solubility at pH 5 is increased by a factor of at least 10^4 and a 50–500-fold increase in hydrolytic stability can be otained above pH 7. Under the same conditions in media containing micelles the solubility increases by approximately 10^5 while nearly an 1800-fold increase in hydrolytic stability has been observed (Tønnesen 2002; Tønnesen et al. 2002). The photostability of curcumin was, however, decreased in the presence of both micelles and cyclodextrins.

In certain formulations it is required to avoid surfactants and other excipients that can influence the phase equilibrium within the preparation or, as in the case of curcumin, lead to a photolabile product. It was therefore of interest to study the possibility of increasing curcumin solubility and stability by alternative methods that could be easily applicable to pharmaceutical or alimental preparations. Viscosity modifying excipients (e.g.macromolecules) can be found in many types of products, and the question was to which extent their presence also would modify the solubility and stability of curcumin. Attempts to prepare water-soluble curcumin by complex formation with macromolecules have previously been reported (Schranz 1983, 1984; Todd 1991). The complex formation in these studies are, however, carried out under conditions that are of little relevance to drugs or food products (i.e. under strongly acidic or alkaline conditions). In the present work we have applied a simple *in situ* method for the preparation of viscous curcumin containing solutions under pharmaceutically acceptable conditions.

2. Investigations and results

2.1. Curcumin solubility in samples containing alginate

The amount of curcumin dissolved in the viscous solution was determined spectrophotometrically at 420 nm and 470 nm. Precipitation of a gel was observed in all samples below pH 8 at individual curcumin concentrations depending on the experimental conditions. The gel formed a separate layer at the bottom of the test tube, leaving a clear, yellow supernatant. In each sample there was a linear increase in absorbance up to the level where precipitation occurred. At this point the absorbance showed an abrupt decrease. The curcumin solubility was taken as the maximum amount added to the system before precipitation occurred, i.e. before a drop in absorbance was detected. The amount of stock solution added was at 50 µl intervals (e.g.

50 µl, 100 µl etc.) and the experiments were performed in triplicate. The maximum curcumin concentration that theoretically could be obtained in the final preparations under the present experimental conditions was 2.5×10^{-4} M due to the saturation of curcumin in the stock solution and a maximum amount of 5% alcohol in the samples containing macromolecules. The alginate quality "Unspecified RB" from the leaves of *Laminaria* hyperborea and the propylene glycol alginate ester (Protanal SD-LB) formed a precipitate at low curcumin concentration (5×10^{-5} M at pH = 5) and were therefore not further investigated.

The amount of curcumin dissolved in the alginate samples was nearly independent of pH in the interval 3.4–7, but increased slightly at pH 8 (Table 1). There was only a minor difference between the alginate qualities, and therefore only one sample (LF 60/10LS) was selected in most of the further studies. The curcumin solubility at pH 5 and 8 decreased slightly with an increase in ionic strength (Table 2). The effect was most pronounced at pH = 5. One exception was unspecified Durvillea at pH = 8 where an increase in solubility was observed at higher ionic strengths. At pH = 5 an increase was observed with an increase in phosphate (i.e. buffer salt) concentration, going from a curcumin solubility of 7.5×10^{-5} M at 0.05 M phosphate to 1.75×10^{-4} M at 0.15 M phosphate. The solubility of curcumin in alginate LF 60/10LS at pH 5 and ionic strength 0.085 was slightly higher in acetate buffer compared to phosphate buffer (1.75×10^{-4} M compared to 1.25×10^{-4} M). Under equal conditions citrate buffer did result in an immediate precipitation. The solubility of curcumin at pH 5 was not influenced by a change in organic solvent; i.e. 5% ethanol was substituted with 5% PEG.

2.2. Curcumin solubility in samples containing other macromolecules

The studies were carried out in 0.05 M phosphate buffer at pH = 5 (µ = 0.085). Curcumin formed a precipitate in the presence of Cesapectin LM-32, Cesapectin HM SS and sodium carboxymethyl cellulose (Frimulsion CM-7) at concentrations of 2.5×10^{-5} M, 5×10^{-5} M and 2.5×10^{-5} M respectively and these polymers were therefore not further investigated. The gelatin samples appeared opaque and were centrifuged before the spectrophotometric measurement. Saturation of the supernatant with respect to curcumin was not obtained at pH 5. The lack of saturation was also observed for gelatin samples prepared in water with curcumin dissolved in PEG.

2.3. Chemical stability of curcumin

The chemical stability of curcumin in samples containing alginate (LF 60/10LS) (n = 6), gelatin (n = 6) or EtOH/buffer (5 : 95) (n = 3) at pH 5 and 8 was evaluated as a change in absorbance at 420 nm.

2.3.1. Samples at pH 5

In the presence of alginate the absorbance at 420 nm remained constant after 5 h in the dark (with the exception of one sample which showed a 17% decrease in absorbance), while a 15–80% decrease in absorbance accompanied by a change in absorption spectrum (λ_{max} changed from 417 to 412 nm) was observed after 20 h. The absorbance also remained constant for 5 h in the presence of gelatin. After 20 h a 3–11% decrease was observed, while a 17% decrease (n = 2) was observed after 44 h. The decrease was accompanied by a change in λ_{max} from 410 to 403 nm. The samples prepared in ethanol/buffer showed an immediate precipitation of curcumin and were not further evaluated.

2.3.2. Samples at pH 8

The samples containing alginate (LF 60/10LS) or gelatin immediately turned orange-red by addition of curcumin. Within a few minutes however, the color has changed into yellow with an absorption maximum at 368 nm. This color steadily faded showing a decrease in absorbance of 14% and 10% in the presence of alginate and gelatin respectively over the first 2 h; the first measurement made after 1 h agitation (see Experimental). Samples containing 5% ethanol in buffer turned deeply red by addition of curcumin. An 80% decrease in absorbance at 420 nm was observed during the first hour, followed by an 18% decrease over the next 2 h, i.e. the time-interval comparable to the samples containing macromolecules.

Table 1: Solubility of curcumin in viscous solutions of alginate as a function of pH and type of alginate

pH	LF60/10LS	LF60/10	Unsp. durv.	Unsp. RB	Alg. ester
3.4	5×10^{-5} M	1×10^{-4} M	1.25×10^{-4} M		
5.0	1.25×10^{-4} M	1.25×10^{-4} M	1.75×10^{-4} M	1×10^{-5} M	1×10^{-5} M
6.0	1.25×10^{-4} M	1×10^{-4} M	2×10^{-4} M		
7.0	5×10^{-5} M	1.25×10^{-4} M	1.25×10^{-4} M		
8.0	$>2.5 \times 10^{-4}$ M	2.25×10^{-4} M	1.75×10^{-4} M		

Ionic strength µ = 0.085, 0.05 M phosphate buffer; Unsp. RB = unspecified RB (from the leaves of Laminaria hyperborea); Unsp. durv. = unspecified Durvillea; Alg. ester = propylene glycol alginate ester

Table 2: Solubility of curcumin in viscous solutions of alginate as a function of ionic strength, pH and alginate type

Ionic strength (µ)	pH = 5			pH = 8		
	LF10/60LS	LF10/60	Unsp. durv.	LF10/60LS	LF10/60	Unsp. durv.
0.085	1.25×10^{-4} M	1.25×10^{-4} M	1.75×10^{-4} M	$>2.5 \times 10^{-4}$ M	2.25×10^{-4} M	1.75×10^{-4} M
0.17	7.5×10^{-5} M	1×10^{-4} M	1.25×10^{-4} M	$>2.5 \times 10^{-4}$ M	$>2.5 \times 10^{-4}$ M	2.5×10^{-4} M
0.30	5×10^{-5} M	7.5×10^{-5} M	7.5×10^{-5} M	2.25×10^{-4} M	2×10^{-4} M	$>2.5 \times 10^{-4}$ M

0.05 M phosphate buffer; Unsp. durv. = unspecified Durvillea

2.4. Photochemical stability

A plot of the absorbance at 420 nm against exposure time resulted in a straight line ($r > 0.99$) for all the samples. The slope of the line was determined to 0.03, 0.035 and 0.013 for solutions of curcumin in alginate (LF 60/10LS), gelatin and ethanol respectively. A scan of the samples revealed an isosbestic point at 354 nm and 351 nm in the presence of alginate and gelatin respectively, while no such point could be detected in the ethanolic solution.

2.5. Dilution effects

Samples of curcumin in the presence of alginate (LF 60/10LS) or gelatin at pH 5 were diluted with buffer in order to determine the "robustness" of the curcumin solubilization. Up to a 25-fold dilution was performed and the absorbance at 420 nm was determined immediately and after 30 min. There was a linear decrease in absorption vs dilution ($r > 0.99$) and no sign of precipitation was observed after 30 min.

3. Discussion

Alginates are natural polysaccharide polymers isolated from brown seaweed (Phaeophyceae). Alginic acid is a linear polymer consisting of L-guluronic acid (G) and D-mannuronic acid (M) residues that are arranged in the polymer chain in blocks. Alginates from different sources vary in their proportions of blocks (Tønnesen and Karlsen 2002). The alginates used in the present study show a variation in the ratio between G and M; ranging from a G/M ratio 0.32/0.68 to 0.71/0.29. The alginates containing the highest fractions of guluronic acid possess the highest ability to form ionotropic gels as the reactivity with divalent ions and thereby the gelling capacity is a direct function of the average length of the G-blocks. The pK_a values for mannuronic and guluronic acid are 3.38 and 3.65, respectively. Standard alginates will precipitate or form gels in acidic conditions, i.e. at pH below 3 (Rowe et al. 2003). The M-blocks are more rapidly hydrated than the G-blocks under acidic conditions (FMC Biopolymers, personal communication). Sodium alginate is incompatible with ethanol in concentrations above 5%. Low concentrations of electrolytes will cause an increase in viscosity. At high electrolyte concentrations (e.g. >4% NaCl, $\mu > 0.68$) alginate is salted out (Rowe et al. 2003). To increase the stability of alginates to acid the propylene glycol ester can be formed. This derivative forms a viscous, colloidal solution at pH 3. It is also soluble in solutions containing up to 60% ethanol. In alkaline solution the ester will be rapidly hydrolysed (Rowe et al. 2003).

Gelatin consists of purified protein fractions obtained by partial hydrolysis of animal collagen. In the present study type A gelatin (i.e. obtained from acid hydrolysis) was applied. Being an amphoteric material it will react with both acids and bases. It further exhibits the chemical characteristics of proteins. Gelatin swells in water and will react with electrolytes (Rowe et al. 2003). We have previously observed that curcumin has a strong affinity for amines and positively charged amino acids (unpublished results). pH 5 is below the isoelectric point for type A gelatin (i.e. pH 7–9) and the macromolecule is therefore positively charged under acidic conditions. In spite the fact that curcumin is unionized at pH 5 it appears to have a higher affinity for the positively charged gelatin than for the negatively charged alginate, but the 3D structure of the macromolecules should also be taken into account. The charge of both gelatin and curcumin will change as the pH of the medium increases to above 7, changing the interactions between the molecules.

Quantitation of curcumin in plain buffer at pH 5 was not possible because the saturation concentration was below the detection limit of the analytical system. Like in previous studies we therefore selected the analytical detection limit of a suitable HPLC method as the highest possible value for the curcumin solubility in buffer (i.e. 3×10^{-8} M) (Tønnesen 2002; Tønnesen et al. 2002). Compared to this value the solubility of curcumin in aqueous solution at pH 5 increased by a factor of $\geq 10^4$ in the presence of 0.5% (w/v) alginate or gelatin combined with 5% ethanol or PEG. For practical reasons the concentration of curcumin could not exceed 2.5×10^{-4} M in the final preparation containing macromolecules and therefore some of the samples never came to saturation. The increase in solubility is within the same range as obtained by cyclodextrins or micelles (Tønnesen et al. 2002; Tønnesen 2002). The samples did withstand a 25-fold dilution, i.e. no precipitation could be observed after 30 min. For samples containing alginate a slight increase in curcumin solubility was observed with an increase in pH. This can probably be ascribed to an increase in both alginate and curcumin solubility over the actual pH interval. The slight decrease in solubility observed with an increase in ionic strength might be explained by a decrease in alginate solubility, although the ionic strength is well below the limit for alginate to be salted out. However, any change of ionic strength has a profound effect on polymer behaviour like polymer chain extension, and it was previously reported that less than 0.1 M salt is necessary to limit the solubility of alginate (Draget 2000). Polyphosphates are observed to have a destabilizing effect on alginates (FMC Biopolymers, personal communication) and to have a modulating (solubilizing) effect on gel formation (Draget 2000). The overall effect would be an increase in the concentration of solubilized alginate or alginate fragments that might explain the observed increase in curcumin solubility at higher concentrations of phosphate buffer. It is not clear why citrate caused an immediate precipitation of curcumin under equal conditions. Citrate buffer is routinely used in our lab for the quantitiative analysis of curcumin by HPLC and incompatibilities have not been observed previously. Citrate is further reported to have a modulating effect on alginate gel formation similar to phosphate (Draget 2000).

Curcumin is uncharged at pH < 7–8. The interaction between alginate and curcumin in acidic medium apparently leads to the formation of a gel above a certain curcumin concentration. The process seems to be independent of the alginate type, although the curcumin concentration where it occurs varies between the alginates. Gel formation is not observed at pH 8 where curcumin is partly ionized. Alginate usually contains protein residues that could influence the interaction with curcumin. The nitrogen content of the alginates used in the present study was in the range 0.077–0.096%w/w, indicating a low protein fraction (FMC Biopolymers, personal communication). The protein content is therefore unlikely to have a major effect on the results. The complex formation between curcumin and alginate or any of the other polymers investigated in the present work is likely to be induced by electrostatic interactions and/or intermolecular H-bond formation that can influence the hydratization process. Curcumin is also a

well known complexing agent and could possibly bind to the polymers through divalent ions (Uppstrøm and Østling 1976; Arrieta et al. 1988).

The chemical stability of curcumin at pH 5 in the presence of alginate and gelatin seems to be limited to a few hours. Gelatin has apparently a slightly better stabilizing effect on curcumin than alginate under the present conditions. The macromolecules seem to have a neglectable effect on the stability at pH 8 in the time interval 2 h after the initial swelling compared to plain buffer. These results are very different from the observations previously made in the presence of cyclodextrins or micelles (Tønnesen et al. 2002; Tønnesen 2002) and indicate that the formation of an inclusion complex is needed in order to stabilize the curcumin molecule towards hydrolysis.

The slope of the plots of the absorbance *vs* exposure time indicates that the photolytic degradation of curcumin is more rapid in aqueous solution in the presence of gelatin or alginate than in a hydrogen-bonding organic solvent like ethanol. This is consistent with the observations made in cyclodetrins and micellar solutions (Tønnesen et al. 2002; Tønnesen 2002). Destabilization of the excited state seems to occur when the non-bonding electrons on the oxygen atom of the OH-group in curcumin become engaged in intermolecular hydrogen bonding instead of being given to the ring (Tønnesen et al. 1995). In general, this would lead to an increase in destabilization of the excited state by an increase in hydrogen-bonding capacity of the medium. Thus destabilization in the presence of alginate and gelatin could be explained by intermolecular H-bond formation.

Sodium alginates and type A gelatin would have a potential as solubilizing agents for curcumin, for instance in food products. However, unlike inclusion complexes (e.g. cyclodextrins) these macromolecules offer little protection of curcumin towards hydrolysis. The solutions are also photolytically unstable. The properties of a formulation containing macromolecules will depend on the preparation method. In the present work we have applied a simple *in situ* method for the preparation of the samples. The content of the macromolecule (% w/v) has been kept constant, leading to a difference in molar concentration and viscosity. In the case of gelatin, the properties of the macromolecule are likely to change as the isoelectric point is reached at pH > 7. The preparations could possibly be optimized by use of more sophisticated preparation methods (e.g. coaservation). The formation of an inclusion complex might however, be required in order to improve the hydrolytic stability of curcumin while other efforts are needed to reduce photodegradation. The importance of interactions between curcumin and pharmaceutically relevant macromolecules, the influence of preparation method and presence of other excipients in the formulation of aqueous, viscous preparations are now under further investigation in our laboratory.

4. Experimental

4.1. Materials

The macromolecules were generously provided by FMC Biopolymers, Drammen, Norway, and were as follows: alginate LF10/60 LS (batch 912912, F(G) 0.43, pH 6–7), LF 10/60 (batch 911749, F(G) 0.71, pH 6.5), unspecified RB (batch 333629-A, F(G) 0.49, pH 6.9) and unspecified Durvillea (batch 645034, F(G) 0.32, pH 6.7), alginate ester (Protanal SD-LB), pectin (Cesapectin HM SS, pH 2.8–3.2 and Cesapectin LM-32, pH 3.0–4.5), sodium carboxymethyl cellulose (Frimulsion CM-7, pH 6.5–8.5) and gelatin (Sigma G2625, pH 3.8–5.5, M_w 40–50000). The pH values refer to 1% solution. Curcumin was synthesized according to the method of Pabon (1964). The buffers were prepared in destilled water from NaH_2PO_4, Na_2HPO_4 (pH = 3.4–8), citric acid (pH = 5) or acetate (pH = 5). The ionic strength of the buffer systems was adjusted by addition of sodium chloride. The buffer salts and sodium chloride were of analytical grade and provided by Merck. Polyethylene glycol (PEG) was provided by Norsk Medisinaldepot, Oslo (Norway).

4.2. Preparation of samples

A stock solution of curcumin (5×10^{-3} M) was prepared in ethanol. A selected volume of the curcumin stock solution (up to 500 µl) was added to 50 mg of the polymer. In the chemical and photochemical stability studies and studies of dilution effects, the amount of stock solution was kept constant at 100 µl. Ethanol was then added to give a total volume of 500 µl ethanol (5%) in the sample. In some samples the stock solution was prepared in polyethylene glycol (PEG) and PEG was used instead of ethanol in the final prearations. The samples were further dispersed in 9.5 ml of the selected buffer system and continuously agitated at 200 rpm (Bühler agitating system) for 60 min followed by 30 min without agitation prior to the spectrophotometric detection. The samples were protected from light throughout the preparation. The absorbance was measured at 420 nm and 470 nm (Shimazdu UV-2101 PC Scanning Spectrophotometer). As reference an identically prepared polymer sample without curcumin was used. Samples containing gelatin were centrifuged ($1300 \times$ g, MSE Scientific Instruments) prior to the spectrophotometric detection. The samples were measured immediately after preparation. All experiments were carried out in triplicate unless other is stated.

4.3. Irradiation

Irradiation was performed in a SUNTEST CPS+ (Atlas, Germany). The light source was a xenon lamp (1.5 kW) equipped with a glass filter, transmitting light corresponding to exposure behind window-glass (cut-off approximately 310 nm). The cabinet was equipped with a SunCool™ device (Atlas) which maintains a constant chamber temperature (30 °C). The intensity was determined to 250 W/m^2 (±2.8%) by using a XenoCal Sensor (Atlas). A 3 ml sample prepared as described above (pH 5) was filled in each of 3 quarts cuvettes and the samples were exposed for intervals of 2 min up to a total exposure time of 12 min. The change in curcumin concentration with exposure time was measured as a change in absorbance at 420 nm. As reference was used curcumin in ethanol adjusted to the same initial absorbance at 420 nm (Abs = 1.4) as the aqueous preparations.

Acknowledgement: The author is grateful to FMC Biopolymers, Drammen, Norway, for generously providing samples of the macromolecules applied in this study, and for providing useful technical information on the substances.

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