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Solubility, chemical and photochemical stability of curcumin in surfactant solutions

Studies of curcumin and curcuminoids, XXVIII.

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The natural compound curcumin was incorporated into various micellar systems in order to improve the water solubility and the hydrolytic and photochemical stability. The presence of micellar structures resulted in an increase in water solubility at pH 5 by a factor of at least 10^5. The hydrolytic stability of curcumin under alkaline conditions was strongly improved by incorporation into micelles while the photodecomposition rate was increased compared to curcumin in hydrogen bonding organic solvents or aqueous solutions. The ability of curcumin to act as a photosensitizer was dependent on the type of micelles and pH of the medium.

1. Introduction

Curcumin is a molecule of considerable interest as a consequence of its known biological activity [1]. The substance which is the main constituent of the spice turmeric, is also widely used as a coloring agent in food, drugs and cosmetics. It is known that curcumin has a stabilizing effect on certain photolabile drugs in solution and in topical preparations [2, 3]. Curcumin can also be applied as a formulation aid to protect light-sensitive drugs in soft gelatine capsules [4]. Although the interest in curcumin as a pharmaceutical excipient and as a potential drug or drug model is steadily increasing, the use of this compound is limited by its low water solubility and poor stability in solution. Curcumin is practically insoluble in water at acidic or neutral pH. However, the compound is soluble in alkali. The pK_a values for the dissociation of the three acid protons in curcumin have previously been determined to 7.8, 8.5 and 9.0 respectively [5]. At a pH above neutral the compound undergoes rapid hydrolytic degradation [5, 6]. In organic solvent curcumin decomposes under exposure to light [7]. Attempts to prepare water-soluble curcumin by complex formation or interaction with various macromolecules (e.g. gelatine, polysaccharides) have been reported [8–11]. In these studies complex formation with macromolecules was carried out under alkaline conditions, i.e. in a medium where curcumin is decomposed within minutes. The rapid initial degradation of curcumin clearly complicates the production process. It is therefore of interest to study the possibility of forming a water soluble curcumin at a pH where the hydrolysis is at a minimum and to investigate the stability of curcumin in such a complex. We have recently investigated the influence of complex formation with cyclodextrins on the solubility and stability of curcumin [12]. The present paper focusses on the solubility and reacticity of curcumin in surfactant solutions. The surfactants employed had neutral, anionic or cationic headgroups.

2. Investigations, results and discussion

The structure of curcumin and the surfactants investigated in this work are shown in the Fig. Commercially available curcumin is isolated from the plant *Curcuma longa* L. The "pure curcumin" on the market consists of a mixture of three naturally occurring curcuminoids; curcumin, demethoxy- and bisdemethoxycurcumin, with curcumin as the main constituent. In the present work curcumin has been synthesized according to the method of Pabon [13] in order to avoid interference from demethoxy- and bisdemethoxycurcumin. The surfactants investigated in this study were the neutral Triton X-100 (TX-100), the cationic tetradecyltrimethylammonium bromide (TTAB) and cetylpyridinium bromide (CPB) and the anionic sodium dodecyl sulfate (SDS).

2.1. Substrate binding constants and maximum solubilizing power

The amount of substrate solubilized within micelles can be calculated from the relative solubilities of the substrate

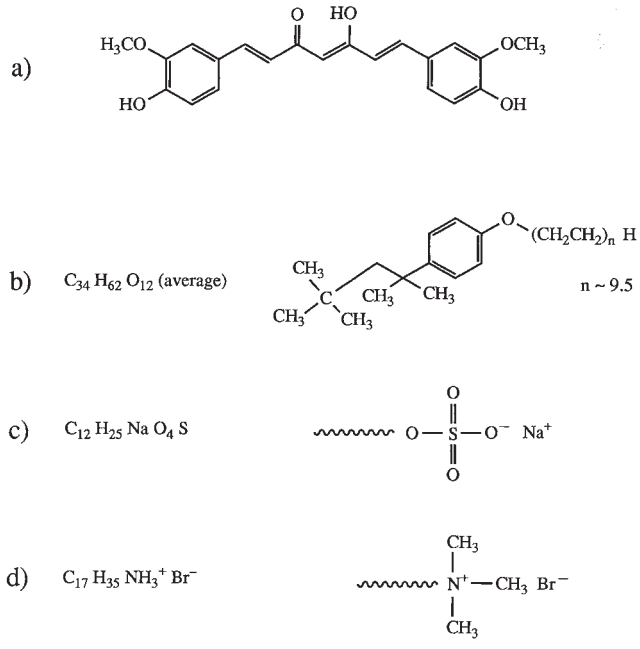

Fig.: Structure of curcumin and the surfactant molecules investigated in this work. a) Curcumin; b) Triton-X 100 (TX-100); c) Sodium dodecyl sulfate (SDS); d) Tetradecyltrimethylammonium bromide (TTAB); e) Cetylpyridinium bromide (CPB)

in surfactant solution and in the aqueous medium by assuming that the increase in solubility is caused wholly by incorporation into the micelles [14]. The value of the substrate binding constant (K_s) can be obtained from the following equation:

$$K_s = N \times S_m/(1 - S_m)\,(C_D - CMC) \qquad (1)$$

where K_s is the substrate binding constant, N is the aggregation number, S_m is the concentration of micellar bound substrate, C_D is the total surfactant concentration and CMC is the critical micellar concentration. The concentration of surfactant employed in the study of binding constants in the present work was 4% above the reported CMC values and thus it can be assumed that most of the surfactant is in the micellar phase. At pH 5 the substrate binding constants (K_s) were determined to 2.2×10^3, 0.166×10^3 and 0.035×10^3 for TX-100, TTAB and SDS, respectively. Curcumin therefore showed the strongest binding to the neutral micelles (TX-100). The observed value of K_s in TX-100 at pH 5 is in the same range as the value reported previously for curcumin in TX-100 at pH 7 [15]. The affinity for the cationic TTAB was nearly 5 fold the affinity for the anionic SDS. This is different from what is observed in cyclodextrin solutions, where interactions with an anionic cyclodextrin seemed to be preferred compared to the cationic derivative [12]. This emphasizes the importance of other factors than charge to the solubilization process. Curcumin in the neutral form is strongly lipophilic and is likely to penetrate deeper into the neutral micelles. In general, non-ionic surfactants form larger micelles than do their ionic counterparts and they are also highly hydrated. The micellar aggregation number is 143, 81 and 60 for TX-100, TTAB and SDS, respectively. The aggregation number and size of ionic micelles will, however, be affected by the addition of electrolytes. The binding constant is also greatly influenced by the length of the surfactant alkyl group, i.e. it increases with an increase in chain length [14]. The alkyl chain of the cationic and anionic surfactants used in the present study is, however, nearly identical and the difference in binding constants between the two must be due to other effects. Curcumin is previously demonstrated to have strong affinity for amines, e.g. amino acids (unpublished results). A specific reaction between curcumin and the amino groups in TTAB may partly explain the difference in affinity between the ionic surfactants. A linear increase in curcumin solubility was observed as a function of an increase in surfactant concentration above CMC ($reg > 0.996$). The maximum amount of solubilisate that can be incorporated into the various micellar systems was determined by adding an excess amount of curcumin to an aqueous buffer solution (pH 5) containing various amounts of different surfactants. When equilibrium conditions were established the concentration of curcumin in the aqueous phase was detected. The ratio between the moles of dissolved curcumin per mole of micelles was calculated assuming that the aggregation number for each surfactant was constant over the actual concentration range. The data are presented in Table 1. The slight decrease in the ratio observed with an increase in surfactant concentration may reflect a variation in aggregation number or a change in structure over the actual concentration range. The highest concentration of curcumin dissolved (2×10^{-3} M or $\approx$740 μg/ml) was obtained in a TX-100 solution at a surfactant concentration ten times CMC. Solutions with a higher concentration of surfactant were not investigated. 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. The analytical detection limit was used as the highest possible value for the curcumin solubility (S_0) (i.e. $S_0 = 3 \times 10^{-8}$ M or 11 ng/ml). The minimum increase in curcumin solubility was therefore by a factor $\approx 10^5$. This is approximately 10 times more than what was detected in cyclodextrin solutions [12].

Table 1: Moles of dissolved curcumin per mole of micelles as a function of surfactant concentration in saturated curcumin solutions

Surfactant concentration	TX-100	SDS	TTAB
CMC	117 ± 7	1.5 ± 0.1	14.7 ± 0.2
2.5 × CMC	89 ± 5	0.89 ± 0.01	10.9 ± 0.9
5.0 × CMC	89 ± 5	0.81 ± 0.05	8.7 ± 0.6
7.5 × CMC	89 ± 5	0.73 ± 0.04	9.3 ± 0.7
10 × CMC	89 ± 0.5	0.80 ± 0.02	8.2 ± 0.2

average ± min/max, n = 3

2.2. Hydrolytic stability of curcumin

As discussed above curcumin is rapidly hydrolysed with a half-life in the range of a few minutes in buffer at pH above neutral [5]. The main decomposition products have previously been identified to feruloyl methane, ferulic acid and vanillin, the latter being a secondary degradation product formed by hydrolysis of feruolyl methane [6]. The formation of coloured condensation products was also observed. The influence of various surfactants at a concentration equal to CMC on the hydrolytic degradation of curcumin in buffer at pH 8 and pH 5 was studied. The effect of both charged and neutral micelles was investigated. When no surfactant was present the pseudo first order rate constant (k_o) was $6.9 \pm 1.1\ h^{-1}$ at pH 8. Nearly an 1800 fold increase in stability could be obtained by addition of TX-100 or SDS to the solution (Table 2). The hydrolysis is probably inhibited due to the relatively nonpolar character of the micellar interior compared to that of water. Anionic micelles further tend to inhibit reactions of neutral substrates with anions like hydroxyl [14]. The cationic micelles (e.g. TTAB and CPB) showed a less stabilizing effect than the anionic and neutral counterparts. Cationic micelles are reported to increase the rate of basic hydrolysis reactions due to electrostatic attraction of the OH^--ion [14]. The colour of curcumin in aqueous media changes as a function of a change in dissociation form. In the neutral form (i.e. at pH below 7) the colour is yellow while turning into deep-red as deprotonation takes place. Hence the colour of the samples at pH 8 in the abscence of mi-

Table 2: Observed first order rate constants for the degradation of curcumin in micellar systems containing phosphate buffer at pH 8 and pH 5 (n = 3)

Surfactant	k_{obs} (h^{-1}) (pH 8)	$t_{1/2}$ (h) (pH 8)	k_{obs} (h^{-1}) (pH 5)	$t_{1/2}$ (h) (pH 5)
TX-100	0.0039 ± 0.0002	178	0.00044	1584
SDS	0.0040 ± 0.0001	173	*	*
TTAB	0.0115 ± 0.0004	60	0.00026	2665
CPB	0.0081 ± 0.0007	86	0.00082	845
Buffer	6.9 ± 1.1	0.10	**	

*) No change in concentration was observed after 1.5 month
**) An accurate measurement of the degradation rate in plain buffer at pH 5 is difficult to obtain due to the low solubility of curcumin in the reaction medium

celles is deep-red. The samples containing neutral or anionic micelles remain yellow at pH 8 indicating a change in the curcumin pK_a value. The presence of cationic micelles do, however, not prevent the samples from turning red or orange, i.e. the pK_a value seems to be little affected and the substrate is in the deprotonated form. It has previously been demonstrated that curcumin in an aqueous cetyltrimethylammonium bromide (CTAB) system was predominantly located on the outer surface of the micelles, in the anionic form, and associated with the tertiary ammonium head groups of the CTAB components [16]. If the same assumption is valid for curcumin in TTAB and CPB these micellar systems will obviously offer less protection against hydrolysis than the systems where curcumin occupies the hydrophobic region of the structure. The micellar systems increased the hydrolytic half-life at pH 8 by approximately a factor 10 compared to the cyclodextrin systems investigated previously [12].

An accurate measurement of the degradation rate is difficult to obtain in plain buffer at lower pH (e.g. pH 5) due to the low solubility of curcumin in the reaction medium. The degradation could however be studied in the presence of micelles. At pH 5 all the samples containing surfactants remained yellow, indicating that curcumin exists in the neutral, highly lipophilic form. In this case both SDS and TTAB offer better protection against hydrolysis than TX-100, while CBP had the least stabilizing effect (Table 2). In the SDS samples no change in curcumin concentration was observed after 1.5 months. Micelles excert a medium effect that can change the reactivity of the substrate due to changes in for instance microviscosity, polarity and molecular orientation [14]. A small change in micellar structure can markedly affect the substrate reactivity. An increase in head group bulk would generally result in higher reactivity [14]. This may partly explain the observed differences in this study at pH 5.

2.3. Photostability of curcumin in micellar systems

Curcumin in organic solvents is rapidly decomposed when exposed to light. A number of photolysis products have previously been identified [7]. The degradation mechanism is discussed elsewhere [17]. In the present study the influence of various micellar systems on curcumin photostability was investigated as described under Experimental. The micellar concentration and the curcumin concentration were kept constant at CMC and 5.4×10^{-5} M. The degradation rate was compared to the photochemical degradation of curcumin in various organic solvents and in an aqueous system (ethanol/phosphate buffer pH 5 (40 : 60)) to mimic a series of microenvironments that could be present in the micelles (polar, nonpolar, hydrogen bonding, non-hydrogen bonding). The degradation in pure water or plain buffer at pH 5 could not be studied due to the low curcumin solubility in these solvents. The variation in the overlap integral between the emission spectrum of the irradiation source and the absorption spectrum of the sample was taken into account in the calculation of the rate constants. The results are presented in Table 3.

Solubilization of curcumin in micelles at pH 5 had a destabilizing effect on the molecule with respect to photodecomposition compared to that of the "free" molecule in a hydrogen-bonding solution (e.g. alcohols, ethanol/buffer). The neutral micelles had by far the most destabilizing effect. The degradation rate is close to curcumin in aprotic solvents ($CHCl_3$, ACN). Among the micellar systems at pH 5 the lowest degradation rate was observed in the SDS-solution (i.e. negatively charged micelles). The neutral micelles maintained the destabilizing effect when the pH was increased to pH 8. Again the ionic micelles showed a less destabilizing effect. In the alkaline medium CPB provided the best protection against photolysis of curcumin. The degradation rate of curcumin in the positively charged micellar systems could be compared to the degradation rate in the more viscous and less polar alcohols (i.e. isopropanol and ethylene glycol). It has previously been demonstrated that for curcumin in solution the excited state of the molecule will be stabilized if the unpaired electrons of the phenolic OH are given to the aromatic ring, i.e. acting as a charge-transfer donor to the excited state [18]. Destabilization of the excited state will occur when the non-bonding electrons on the oxygen atom of the OH-group become enganged in intermolecular hydrogen bonding. In general, this would lead to an increase in destabilization of the excited state by an increase in hydrogen bonding donor capacity of the solvent. The micellar systems do, however, represent a novel environment for the molecule that can lead to a change in reactivity. The photochemical and photophysical properties of curcumin in the presence of micelles are now under further investigation.

Table 3: Observed first order rate constants for the photochemical degradation of curcumin in various systems (n = 3)

Medium	k_{obs} (min^{-1})	$t_{1/2}$ (min)
TX-100 pH 5	0.157 ± 0.005	4.4
TX-100 pH 8	0.157 ± 0.030	4.4
SDS pH 5	0.027 ± 0.003	25.3
SDS pH 8	0.043 ± 0.002	16.1
TTAB pH 5	0.040 ± 0.003	17.5
TTAB pH 8	0.028 ± 0.001	25.2
CPB pH 5	0.042 ± 0.005	16.4
CPB pH 8	0.021 ± 0.002	33.0
Chloroform	0.145 ± 0.015	4.8
Acetonitrile	0.136 ± 0.001	5.1
Ethylene glycol	0.018 ± 0.002	29.4
Isopropanol	0.025 ± 0.004	27.7
Ethanol	0.013 ± 0.002	55.4
Methanol	0.007 ± 0.0009	99.0
Ethanol/buffer (pH 5)	0.013 ± 0.0004	52.5

(average ± min/max)

2.4. Photosensitization by curcumin in surfactant solutions

Many drug molecules or excipients are able to photosensitize oxidation reactions in a pharmaceutical product. This ability is dependent on the medium, e.g. polarity. An increase in free radical reactivity of common drugs has previously been demonstrated in micellar systems [19]. Such a possible increase in free radical reactivity as a consequence of solubilization should be taken into account in the formulation process. In organic solvents curcumin is known to act as a photosensitizer of singlet oxygen, superoxide and free radicals [7, 16, 20]. This ability can have a destabilizing effect on a curcumin-containing product. On the other hand, light-induced oxidation can be applied in systems with biological destructive behaviour; e.g. in the killing of bacteria [21]. Free radical activity is demonstrated in curcumin-micellar systems during pulse radiolysis [16] while the formation of singlet oxygen seems to be low in organized media [20]. In order to obtain further

Table 4: Rates of photooxidation in various micellar systems induced by curcumin in the presence of the subtrate 2,5-dimethylfuran

Medium	k_{obs} (mg × ml^{-1} × min^{-1})
TX-100 pH 5	0.089 ± 0.009
TX-100 pH 8	0.067 ± 0.005
SDS pH 5	0.030 ± 0.003
SDS pH 8	0.019 ± 0.002
TTAB pH 5	0.017 ± 0.001
TTAB pH 8	*)
CPB pH 5	0.005 ± 0.001
CPB pH 8	*)

k_{obs} = rate of oxygen uptake (average ± min/max, n = 3)
*) Less than 2% change in oxygen concentration after irradiation for 60 minutes

information on the photosensitizing potential of curcumin in micellar systems the rate of photooxidation of a substrate dissolved in the medium was followed in terms of oxygen uptake measured with an oxygen electrode. The results are presented in Table 4. The amount of curcumin decomposed during irradiation was less than 5% as determined by HPLC. The light-induced oxidation of the substrate 2,5-dimethylfuran was most pronounced when curcumin was solubilized in TX-100 at pH 5. At this pH the reactivity of curcumin solubilized in the ionic micellar solutions was in the order SDS > TTAB > CPB. In alkaline medium (pH 8) photooxidation was hardly detectable in solutions containing TTAB and CPB. Again the sensitizing capacity was larger in neutral micelles than in the negatively charged system. These results demonstrate that the type of micelles strongly influences the photosensitizing potential of solubilized curcumin. This should be taken into account in the formulation of curcumin-containing aqueous preparations.

2.5. Conclusions

Solubilization of curcumin in micelles can take place under slightly acidic conditions (pH 5) at room temperature. The solubilization leads to an increase in the water solubility of curcumin by a factor $\sim 10^5$. Curcumin had the highest affinity for the neutral micelles (TX-100) followed by the cationic micelles (TTAB). The least affinity was observed for the anionic SDS micelles. The hydrolytic stability of curcumin under alkaline conditions was dramatically improved by incorporation in micellar structures. Nearly an 1800 fold increase in stability could be obtained by addition of TX-100 or SDS to the solution while the cationic micelles (e.g. TTAB and CPB) showed a less stabilizing effect. On the other hand, a decrease in photostability was observed by complex formation with micelles compared to solutions of curcumin in hydrogen bonding organic solvents or aqueous media. The neutral micelles had by far the most destabilizing effect. The degradation rate corresponds to curcumin in aprotic solvents ($CHCl_3$, ACN). The photolytic degradation rate of curcumin in charged micellar systems at pH 8 could be compared to the degradation rate in the more viscous and less polar alcohols (i.e. isopropanol and ethylene glycol). The ability of solubilized curcumin to induce photosensitized oxidation was most pronounced in neutral micelles. The mechanism of interaction between curcumin and micelles are under further investigation as this is of great importance for the development of a water soluble and stable curcumin product and for the application of curcumin in aqueous preparations.

3. Experimental

3.1. Materials

The following surfactants were used in this study: Tetradecyltrimethylammonium bromide (TTAB) (Fluka), Triton X-100 (TX-100) (Sigma), Sodium dodecyl sulfate (SDS) (Sigma) and Cetylpyridinium bromide (CPB) (Sigma). Pure curcumin was synthesized using the procedure given by Pabon [13]. All other chemicals were commercially available substances of reagent or analytical grade. The buffers were prepared in total concentration of 0.05 M from potassium dihydrogen phosphate and disodium hydrogen phosphate. The ionic strength was adjusted to $\mu = 0.085$ by addition of sodium chloride. The concentration of curcumin was measured by reversed phase HPLC. The separation was performed on a 15 cm × 3.9 mm Nova Pak® C_{18} column (Waters, Milford, MA, USA). The mobile phase was composed of 0.5% citric acid adjused to pH 3 with KOH and acetonitrile (60 : 40). A flow rate of 1.2 ml min^{-1} was used. Curcumin was detected at 350 nm. The retention time of curcumin was ~9 mins.
The chromatographic system consisted of a Shimadzu LC-9A pump, a Shimadzu SP D-10A UV-VIS detector, a Shimadzu SIL-10 DV auto sampler and a Shimadzu C-R3A integrator.

3.2. Methods

Solubilities were determined by adding an excess amount of curcumin to an aqueous buffer solution (pH 5) containing various amounts of different kinds of surfactants. The suspension formed was equilibrated under continuous agitation for 18 h at ambient temperature (21 °C) and then filtered through a Acrodisc® 0.2 µm filter (Gelman Sciences, USA) to form a clear micellar solution. An aliquot of the filtrate was diluted with the HPLC mobile phase before quantitation of curcumin. For hydrolytic degradation studies a stock solution of curcumin (2.7×10^{-3} M) was prepared in methanol. A volume of 0.2 ml of the stock solution was added to the micellar solutions to make a final volume of 10 ml (5.4×10^{-5} M curcumin). Samples were prepared in phosphate buffer at pH 5 and pH 8, $\mu = 0.085$. The samples were kept in the dark at 30 ± 0.1 °C. The changes of curcumin concentration with time was monitored by HPLC. The observed first-order rate constants (k_{obs}) for the degradation was obtained from linear regression analysis of the logarithm of the curcumin concentration plotted against time. For photolytic degradation studies a volume of the stock solution was added to the micellar solutions or organic solvents to make a final concentration 5.4×10^{-5} M curcumin. The samples were irradiated in a SUNTEST CPS (Heraeus GmbH, Hanau, Germany). The light source was a xenon lamp (1.8 kW) equiped with a glass filter transmitting light corresponding to exposure behind window glass (cut-off ~310 nm). The light intensity was measured to 1.4×10^5 lux and 18.6 W/m^2 in the visible and UV range respectively using a lux meter in combination with a UV-filter radiometer (Hagner EC1 Digital luxmeter, Hagner EC1 UV-A). The samples were exposed in a quartz cuvette under continous stirring. The changes of curcumin concentration with exposure time was monitored by HPLC. The observed first-order rate constants (k_{obs}) for the degradation were obtained from linear regression analysis of the logarithm of the curcumin concentration plotted against time. The photodegradation and photooxidation rate constants are directly proportional to the overlap integral between the emission spectrum of the irradiation source and absorption spectrum of the sample for the actual wavelength range. The calculated rate constants were therefore corrected for the difference in absorbtivity (i.e area under the absorption curve) between the various samples. The absorbtivity was measured on a Shimadzu UV-2101 PC UV-VIS scanning spectrophotometer. For photosensitized oxidation experiments an aliquot (1.4 ml) of the curcumin stock solution was added to 250 ml of air saturated buffered surfactant solution containing 50 µl of 2,5-dimethylfuran (DMF) (Sigma). The DMF was purified by destillation. Irradiations were performed at 20 °C using an irradiation source supplied by Applied Photophysics Ltd., (Surrey, UK), consisting of a monochromator f 3.4 and 900 W xenon arc lamp, operated with a bandwidth of 20 nm at the irradiation wavelength (421 nm). The samples were exposed under continous stirring. The rate of photooxidation was followed in terms of oxygen uptake measured with an oxygen electrode (model Oxi 340, WTW, Weilheim, Germany). The calculated rate constants were corrected for the difference in absorbtivity (i.e. absorbance at 421 nm) between the various samples.

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