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Studies on curcumin and curcuminoids XXXI. Symmetric and asymmetric curcuminoids: Stability, activity and complexation with cyclodextrin

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Abstract

A series of curcuminoids, including curcumin, were studied with the main focus on their solubility, phase-distribution, hydrolytic stability and photochemical stability in cyclodextrin (CD) solutions. Their radical scavenging properties were also briefly studied. All the investigated derivatives were more stable towards hydrolytic degradation in CD solutions than curcumin, and the general order of the stabilising effect was HPβCD > MβCD ≫ HPγCD. In contrast, the photochemical studies showed that curcumin is generally more stable than its derivatives. Solubility and phase-distribution studies showed that curcuminoids with side groups on the phenyl moiety have higher affinity for the HPγCD than for the βCDs and that the relative affinity of the larger HPγCD cavity increases with the curcuminoid molecule size. The radical scavenging studies showed that curcumin is more active than the derivatives investigated and that the free phenolic hydroxyl group may be essential for the scavenging properties. This study also indicates that the two halves of the symmetric curcumin molecule act as two separate units and scavenge one radical each.

Keywords: Curcumin; Curcumin derivatives; Cyclodextrin; Hydrolysis; Photostability; Radical scavenging

1. Introduction

Curcumin is a naturally occurring compound found in the plant *Curcuma longa* L. It has been widely used as a yellow pigment to colour food, drugs and cosmetics, and it is also interesting from a pharmaceutical point of view because of its potential use as a drug or model substance for treatment of various diseases. The most interesting effects are probably its potential use against cancer (Banerji et al., 2004; Syng-ai et al., 2004), HIV-infections (Sui et al., 1993; Mazumder et al., 1995), cystic fibrosis (Egan et al., 2004), and as an immunomodulating agent (Gao et al., 2004; Chueh et al., 2003). The main drawbacks for clinical applications of curcumin are its low solubility in water at acidic and physiological pH, and its rapid hydrolysis under alkaline conditions (Tønnesen and Karlsen, 1985a). It is also very susceptible to photochemical degradation (Tønnesen et al., 1986). These problems can be addressed by incorporation of curcumin into micelles or complexation with cyclodextrins in aqueous solutions (Tønnesen, 2002; Tønnesen et al., 2002).

Cyclodextrins (CDs) are cyclic oligosaccharides with a hydrophilic outer surface and lipophilic central cavity. Hydrophilic drug/cyclodextrin complexes are formed by inclusion of lipophilic drug or lipophilic drug moiety, in the central cyclodextrin cavity. Cyclodextrins are therefore frequently used as solubilizing and stabilizing agents in pharmaceutical preparations (Loftsson et al., 2005). Commonly used cyclodextrins are β-CD (composed of seven α-1-4 linked glucopyranose units), γ-CD (eight units) and their derivatives, such as hydroxypropyl-β-CD (HPβCD), 2-*O*-methyl β-CD (MβCD) and hydroxypropyl-γ-CD (HPγCD). The lipophilic cavity protects lipophilic guest molecules from the aqueous environment, while the polar outer surface of the CD molecule provides the solubilizing effect. The polarity inside the cavity is suggested to be similar to that of a 40% solution of ethanol in water (Frömming and Szejtli, 1994). Different cyclodextrins have previously been found to increase the solubility of curcumin with

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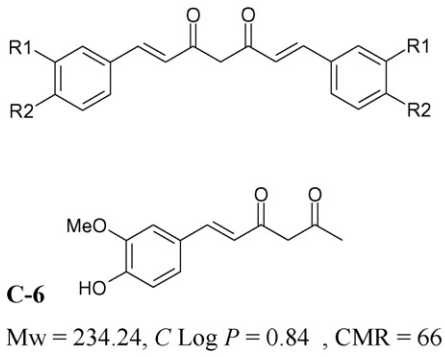

Mw = 234.24, *C* Log *P* = 0.84 , CMR = 66

	R1	R2	Mw [g/mol]	*C* Log *P*	CMR [cm^3/mol]
C-1	-OCH_3	-OCH_3	396.42	3.09	117
C-2	-H	-H	276.32	3.59	88
C-3	-OCH_3	-H	336.37	3.34	102
C-4	-OCH_3	-OH	368.37	2.56	106
C-5	-H	-OH	308.32	2.81	91

Fig. 1. Curcuminoids investigated in the present study and some molecular descriptors. The calculated log *P* (*C* log *P*) and molecular refractivity (CMR) were obtained with the ChemDraw Software (CambridgeSoft Corporation, USA).

a factor of approximately 10^4, and also to dramatically improve the hydrolytic stability (Tønnesen et al., 2002). However, the photochemical stability of curcumin is decreased compared to solutions in organic solvents.

The stoichiometric ratio between curcumin and β-CD in curcumin/β-CD complexes has been studied by Tang et al. (2002), and reported to be 1:2. However, Qi et al. (2003) found the ratio to be 1:1. Both results are based on investigations of the light absorption properties of curcumin in a cyclodextrin solution. Recently, Baglole et al. (2005) have also published a report on this subject, and concluded that a 1:2 complex is formed, based on fluorescence measurements. Phase-solubility diagrams for β-CD derivatives are linear, which is consistent with 1:1 complex formation (Tønnesen et al., 2002). There is a growing body of evidence that cyclodextrin can exert some of their effect by forming non-inclusion complexes and surfactant like molecular aggregates (Loftsson et al., 2004). This type of phenomena may explain the apparent contradictions in previous studies, where different stoichiometries have been found depending on the method used.

Previous studies have focused on the interaction of curcumin with CDs. In the present study we have also investigated the interaction of a series of other curcuminoids with HPβCD, MβCD and HPγCD. The effect of the CD cavity size could also be studied since the HPγCD cavity is larger than the similar sized HPβCD and MβCD cavities.

Curcumin and five other curcuminoids were synthesised. The structure of these molecules and molecular descriptors for lipophilicity (*C* log *P*) and molar bulk (CMR) are shown in Fig. 1. Their hydrolytic and photochemical stability, and their solubility and phase distribution have been investigated in aqueous cyclodextrin solutions. An indication of the biological activity was obtained by measuring their ability to scavenge free radicals (antioxidant activity).

2. Materials and methods

2.1. Materials

Five different symmetrical and one asymmetric curcuminoids were synthesised using the methods of Pabon (1964) and Masuda et al. (2001), respectively (Fig. 1). Their purity and identity was confirmed by ^{1}H-NMR, ^{13}C-NMR (Bruker AC 250 P Spectrometer, 250 MHz), IR-spectroscopy (Avata 370 IF/IR), HPLC (see below for specifications), TLC (Stationary phase: silica gel 60 (Merck), Mobile phase: chloroform:ethanol 25:1) and melting point analysis (Gallenkamp Melting Point apparatus, three parallels of each sample, temperature increase approximately 3 °C per min).

Three different cyclodextrins were used in the study: hydroxypropyl-β-cyclodextrin of molar substitution 0.62 (Kleptose®, Roquette France), 2-*O*-methyl β-cyclodextrin of molar substitution 0.5 (Kleptose® CRYSMEB, Roquette, France) and hydroxypropyl-γ-cyclodextrin of molar substitution 0.6 (Cavasol® W8 HP, Wacker-chemie, Germany). Prior to preparation of CD solutions, the moisture content of the cyclodextrins was measured using a Scaltec SMO 01 (Göttingen, Germany) electronic moisture analyser.

Buffers used were 1% (w/v) citrate buffer pH 5 (citric acid or citric acid monohydrate), 0.7% phosphate buffer pH 5 or pH 8 (potassium dihydrogen phosphate), and 0.5% carbonate buffer pH 10 (sodium hydrogen carbonate). The ionic strength of the buffers was adjusted to $\mu = 0.145$ by addition of NaCl.

2.2. Quantification of the curcuminoids

The curcuminoid concentration was measured using reversed phase HPLC. For practical reasons, two different HPLC units were employed: HPLC system I (used in all studies except the photochemical degradation studies): *Pump*: LDC Analytical ConstaMetric 3200 Solvent Delivery System. *Autosampler*: Merck Hitachi AS-4000 Intelligent Autosampler. *Column*: Phenomex C_{18}, 3.9 mm × 150 mm, 5 μm particle size. *Detector*: Spectra-Physics SP8450 UV/VIS detector. *Software*: Igor Pro, version 4.0.8.0. WaveMetrics Inc. HPLC system II (used for the photochemical degradation studies): *Pump*: Shimadzu Liquid Chromatography LC-9A. *Autosampler*: Shimadzu Auto Injector SIL-9A. *Column*: Waters Nova-Pak® C_{18}, 3.9 mm × 150 mm, 4 μm particle size. *Detector*: Shimadzu UV–Vis Spectrophotometric detector SPD-10A. *Printer*: Shimadzu C-R5A Chromatopac.

In both systems, the mobile phase was a mixture of acetonitrile and 0.5% citric acid adjusted to pH 3 with KOH. The ratio of aqueous phase/organic phase was optimized for each compound, to get acceptable retention times. The detection wavelength selected was the absorption maximum for the individual compounds in pure acetonitrile. In some of the curcumin studies a detection wavelength of 350 nm was applied in order to detect the peaks from possible degradation products.

2.3. Differential scanning calorimetry

Two different batches (new and old) of curcumin were investigated by use of differential scanning calorimetry (DSC) (Mettler Toledo Star^e DSC822^e Module). All samples were heated at a scanning rate of 5 °C/min and 10 °C/min, respectively. Aluminium pans and lids were used for all samples and the analyses were carried out under nitrogen flow. Energy calibration was performed with indium (99.99% purity, melting point 156.6 °C). The melting point was measured as the onset temperature (t_{onset}) of the peak.

2.4. Hydrolytic stability

The hydrolytic stability of the curcuminoids was investigated in buffered 10% (w/v) CD solutions at pH 5, 8 and 10 in citrate, phosphate and carbonate buffers, respectively. The temperature was kept constant at 30 °C. Stock solutions of the curcuminoids were prepared in methanol at a concentration of 2 mg/ml. Hundred μl of this solution was added to 10 ml CD solution. The vials were then stored at 30 °C in the dark, and samples were withdrawn and analysed by HPLC at regular time intervals. The observed first-order rate constant (k_{obs}) was obtained from the linear regression of a plot of the natural logarithm of the peak area versus time.

2.5. Phase-distribution studies

Solutions of the curcuminoids were prepared at a concentration of 1 mg/ml in 1-octanol that had been saturated with water. One ml of the octanol solution was added to 1 ml of a 10% CD solution at pH values ranging from 5 to 10. After mixing the phases, the vials were sealed and allowed to shake for about 1 h. A previous study has shown that 30 min is more than sufficient to achieve equilibrium between the two phases (Másson et al., 2005).

Prolonged shaking was avoided due to stability concerns, especially at high pH values. The concentration in the aqueous phase was then analysed by HPLC, and the observed distribution coefficient (D_{obs}) was calculated as the ratio between the concentration in the octanol phase and the aqueous phase.

2.6. Solubility studies

The solubility of the curcuminoids in CD solutions was examined by adding excess of the curcuminoid to vials containing 10% (w/v) CD solutions at pH 5. The vials were sealed, and shaken for 1 week. The solution was filtered through a 0.45 μm filter (Spartan 13/0.45 form Schleicher & Schull) to remove all solid material and the concentration of dissolved curcuminoid was determined by HPLC. The experiments were carried out in triplicate unless other is stated, and the samples were protected from light.

2.7. Photochemical stability

The photochemical stability of the curcuminoids was determined in three different solvent systems: (1) pure methanol (MeOH), (2) 40% (v/v) aqueous citrate buffer pH 5, 60% MeOH, (3) 10% HPβCD in citrate buffer pH 5. Stock solutions of the curcuminoids were prepared in MeOH at a concentration of 1.0×10^{-3} M. The curcuminoid solutions were then diluted a 100 times by adding 250 μl of this solution to 25 ml of the desired solvent system, to give a final concentration of 1.0×10^{-5} M of the curcuminoid. The MeOH concentration in the aqueous HPβCD solution was 1% (v/v). The solutions were then irradiated in a Suntest CPS+ (Atlas, Germany) at medium intensity (550 W/m^2). The radiation 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^{TM} device (Atlas, Germany), which maintains a constant chamber temperature (30 °C). The intensity was determined by using a XenoCal Sensor (Atlas, Germany). A 3 ml sample prepared as described above was filled in each of three quartz cuvettes and the samples were exposed for selected time intervals. Samples were then withdrawn and diluted 1:1 with the HPLC mobile phase prior to quantification. The natural logarithm of the curcuminoid concentration was plotted against exposure time, and linear regression analysis was used to obtain the observed first order rate constant for the photodegradation reaction. All the experiments were carried out in triplicate.

2.8. Radical scavenging properties

To study the antioxidant effect of the curcuminoids, their radical scavenging properties were determined by their ability to scavenge the stable free 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^\bullet$). The procedure was adopted from Venkatesan and Rao (2000), except that pure methanol solutions were used, without any aqueous buffer. Fifty or 500 μl (for the less active compound) of curcuminoid solution was mixed with 100 μM DPPH$^\bullet$ solution to obtain a total volume of 3 ml. The solution was incubated for 30 min to obtain equilibrium, and the absorbance measured at 517 nm. The scavenging was calculated from the following equation:

$$\%\text{Radical scavenging} = \left[\frac{A_0 - A_t}{A_0}\right] \times 100\% \quad (1)$$

where A_0 was the absorbance of DPPH$^\bullet$ solution in the absence of test compound and A_t the absorbance of DPPH$^\bullet$ solution after incubation with test compound.

Table 1
Hydrolytic stability of curcuminoids at 30 °C, reported as half-life (h)

	pH 5	pH 8	pH 10		
	HPβCD	HPβCD	HPβCD	MβCD	HPγCD
C-1	>100	>100	27.6	14.3	2.6
C-2	>100	>100	>100	>100	–[a]
C-3	>100	>100	>100	>100	2.6
C-4 (curcumin)	>100	10.5	4.8	3.8	2.2
C-5	>100	>100	20.6	32.9	7.1
C-6	>100	85	12.6	7.57	0.48

[a] C-2 was not sufficiently soluble in aqueous HPγCD for determination of half-life.

3. Results and discussion

3.1. Hydrolytic stability

In the present study, the hydrolytic stability of the curcuminoids was examined in 10% (w/v) CD solutions at pH 5, 8 and 10. The results are presented as half-life ($t_{1/2}$, h) in Table 1, according to first order kinetics. As can be seen from the results, all the tested substances were reasonably stable at pH 5 in 10% (w/v) HPβCD, with an observed half life > 100 h. Previously, it has been shown that curcumin has low degradation rate at pH 5 and that the hydrolytic stability is improved in the presence of cyclodextrins (Tønnesen and Karlsen, 1985a; Tønnesen et al., 2002). Some degradation could be observed for curcumin at pH 8 in HPβCD solutions. The half-life of the other curcminoids was more than 100 h, with the exception of the asymmetric C-6, which is, like curcumin, *p*-OH, *m*-MeO substituted on the phenyl moiety. At pH 10, the stability was tested in solutions containing three different cyclodextrins. The half-life was slightly less in MβCD than in HPβCD solutions, with the exception of C-5. The stability was significantly reduced in the larger HPγCD cavity.

The degradation rate depends on the degree of protection by the different cyclodextrins, the curcuminoid structure, and the pH of the solution. The hydrogen atoms of the phenolic hydroxyl (OH) groups the in curcumin structure are intramolecularly H-bonded to the adjacent methoxy groups, allowing the oxygen atoms of the phenolic OH-groups to participate in hydrogen bond formation with the solvent or cyclodextrin as a hydrogen bond acceptor (Tønnesen et al., 1995) (Fig. 2, Route I) Curcumin exists in an equilibrium between the diketo- and keto-enol forms; the keto-enol form is strongly favoured by intramolecular H-bonding (Fig. 2). The keto-enol moiety can theoretically also be involved in intermolecular hydrogen bonding. It can be postulated that the hydrolytic degradation starts with an attack from the nucleophilic OH^- ion on the carbonyl carbon in the keto-enol moiety (Fig. 2, Route I). The main hydrolytic degradation products have previously been identified as ferulic acid and feruloyl methane (Tønnesen and Karlsen, 1985b). The two curcuminoids, C-2 and C-3, lacking the –O–R group (OH or O–CH_3) in *para*-position were most stable towards hydrolysis. The difference in the electron structure compared to the *para*-substituted curcuminoids can be the reason for their relative resistance towards hydrolysis.

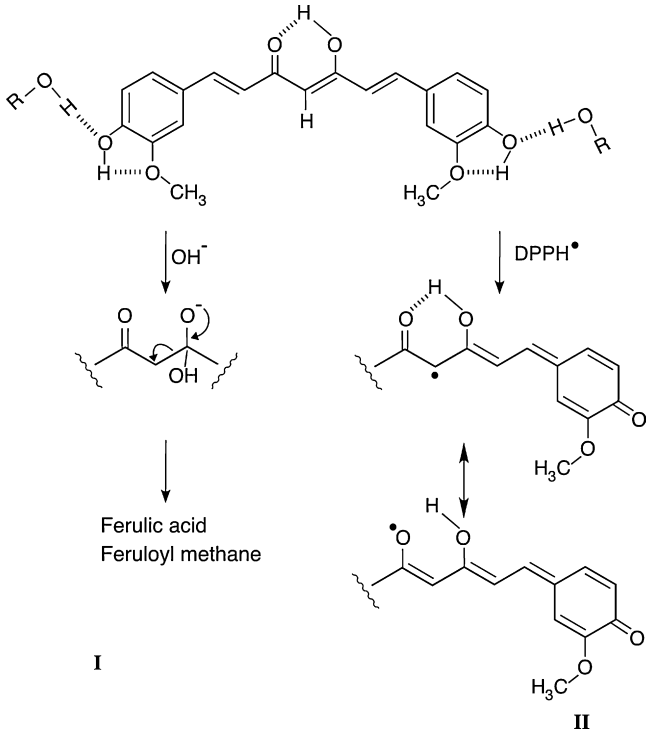

Fig. 2. Postulated inter- and intramolecular binding in curcumin of importance for the overall reactivity of the molecule. *Route I*: hydrolytic degradation of curcumin. *Route II*: formation of a neutral stabilized curcumin radical.

3.2. Phase distribution studies

The observed distribution coefficients (D_{obs}) for the curcuminoids in a two-phase system of octanol and an aqueous CD solution (pH 5 or 10) are summarised in Table 2. Clearly, ionisation of the compounds at higher pH values leads to a higher affinity for the aqueous phase, and hence, a lower distribution coefficient. C-2 had too low aqueous solubility under most conditions to be quantified, and C-3 was investigated only in HPβCD. In general, D_{obs} was higher with MβCD than with HPβCD. Curcuminoids with side groups on the phenyl moiety appear to have higher affinity for HPγCD and in this case the D_{obs} was lowest for this CD. The difference in the distribution coefficient between the βCDs and HPγCD is largest for C-1 and C-4, which have the bulkiest side groups on the phenyl moiety.

Table 2
The observed distribution coefficients (D_{obs}) for curcuminoids in various aqueous CD solutions

	HPβCD		HPγCD		MβCD	
	pH 5	pH 10	pH 5	pH 10	pH 5	pH 10
C-1	<DL	296	5.0	6.3	<DL	335
C-2	<DL	591	<DL	<DL	<DL	<DL
C-3	<DL	560	N.I.	N.I.	N.I.	N.I.
C-4	344	6.2	5.5	1.3	394	6.0
C-5	295	1.6	54	1.0	483	2.2
C-6	55	4.9	20.5	2.8	62	4.8

<DL: quantity in aqueous phase below limit of detection (very high D_{obs}). N.I.: not investigated.

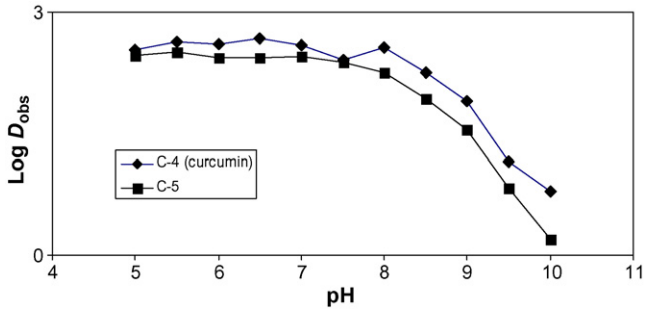

Fig. 3. Log D_{obs} values for C-4 and C-5 as a function of pH ($n = 1$).

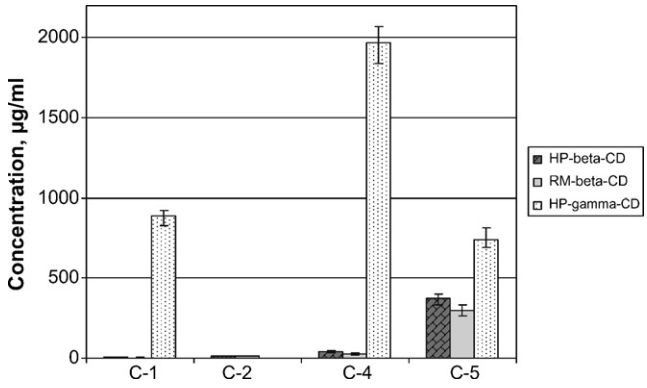

Fig. 4. Solubility of the curcuminoids in HPβCD, RMβCD and HPγCD ($n = 3$).

The distribution coefficients at a pH interval ranging from 5 to 10 were determined in aqueous HPβCD buffer solutions, and the log D_{obs} values were calculated and plotted against pH. The pK_a values for the dissociation of the three acidic protons in curcumin in plain buffer have previously been determined to 7.8, 8.5 and 9.0, respectively (Tønnesen and Karlsen, 1985a). In theory, the pK_a values of the compounds complexed with cyclodextrin can be determinated from the inflection point of the curve. The results for C-4 (curcumin) and C-5 are shown in Fig. 3. As can be seen from this figure, the pH range from 5 to 10 is not extensive enough to draw any conclusions about the pK_a value of the curcuminoids in the complexed form. The results indicate, however, that the ionisation apparently is quite similar for curcumin and its natural occurring derivative C-5. They both seem to be unionised up to pH around 8, where they start to ionize, and their distribution coefficient decreases as they get more hydrophilic. For compounds C-l and C-2 the concentrations at most pH values (i.e., pH < 9.5 and 9.0, respectively) were below the detection limit of the HPLC system. Compounds C-3 and C-6 were only investigated at pH 5 and 10.

3.3. Solubility studies

The solubility of the curcuminoids in the different CD solutions is presented in Table 3. HPβCD is the only CD in which the solubility of all the six curcuminoids was investigated in this experiment. The solubility in MβCD solutions is slightly less than in HPβCD solutions. The solubility is lowest for C-2 and C-1, which are the smallest and largest symmetrical molecules, respectively. The solubility is clearly highest for C-6; in this case, it is more than 1 mg/ml. However, since the structure of this compound is unsymmetrical and very different from the curcuminoid structures, it is difficult to compare this result with results obtained for other substances. In general the lipophilicity ($C \log P$) is not correlated with the solubility in cyclodextrin solutions.

Only for four of these compounds, the solubility was determined in all of the three CDs, and the results are presented in Fig. 4. The solubility is clearly highest in HPγCD for all these compounds, except C-2. The difference between the solubility in HPγCD and HPβCD solutions is illustrated by the log(HPγCD/HPβCD) values in Table 4. The rank order for these values is C-1 > C-4 > C-5 > C-2. The same rank of order is obtained for the molar bulk of these molecules. The CMR values are 117, 106, 102 and 91 cm^3/mol for C-1, C-4, C-5 and C-2, respectively These results are also consistent with the phase-distribution investigations. These observations suggest that the bulkier moieties fit better into the larger γCD cavity than into the smaller βCD cavity.

To our knowledge this is the first investigation of the solubility of curcuminoids C-1, C-2, C-3, C-5 and C-6. In contrast, some investigations have been done on the solubility of curcumin (C-4) in CD solutions. Baglole et al. (2005) has reported that solubility of curcumin is 5.2×10^{-5} M and 1.4×10^{-4} M in 10 mM HPβCD and HPγCD solutions, respectively. The pH or the buffer used was not reported, and therefore, it is difficult to compare these results to our current results. In our previous study (Tønnesen et al., 2002), the solubility of curcumin in 11% (w/v) HPβCD, phosphate buffer pH 5.0, was found to be 0.122 mM, which is consistent with the result in the present work. The solubility in HPγCD solution was found to be 0.38 mM, a value in which is much less than in the present study. The solubility

Table 3
Molar solubility of curcuminoids in three different 10% (w/v) CD solutions at pH 5 ($n = 3$, average, min–max)

	HPβCD	MβCD	HPγCD	log(HPγCD/HPβCD)
C-l	1.51×10^{-5} M (1.29–1.71)	8.18×10^{-6} M (6.72–10.80)	2.24×10^{-3} M (2.09–2.29)	2.17
C-2	4.97×10^{-5} M (4.61–5.02)	4.31×10^{-5} M (4.22–4.38)	N.S.[a]	<0
C-3	1.64×10^{-4} M (1.47–1.76)	N.I.[b]	N.I.[b]	–
C-4	1.16×10^{-4} M (0.90–1.35)	8.08×10^{-5} M (6.03–9.27)	5.35×10^{-3} M (4.99–5.63)	1.66
C-5	1.22×10^{-3} M (1.08–1.30)	9.63×10^{-4} M (8.58–10.80)	2.39×10^{-3} M (2.25–2.65)	0.29
C-6	4.54×10^{-3} M (4.51–4.58)	N.I.[b]	N.I.[b]	–

[a] N.S.: not soluble (i.e., solubility below the detection limit).
[b] N.I.: not investigated.

Table 4
Photochemical stability of different curcuminoids, reported as half-life (min) when exposed to irradiation at 550 W/m^2 ($n = 3$, average, min–max)

	MeOH	Citrate buffer/MeOH (40:60)	10% HPβCD
C-1	14.90 (13.90–15.40)	4.95 (4.76–5.25)	10.80 (10.30–11.50)
C-2	10.10 (9.65–10.50)	3.46 (3.39–3.65)	9.26 (8.86–9.59)
C-3	8.03 (7.32–8.70)	3.32 (3.21–3.49)	5.96 (5.56–6.32)
C-4	26.20 (24.50–27.70)	12.80 (11.60–13.90)	7.80 (7.40–8.15)
C-5	6.06 (5.92–6.14)	4.43 (4.31–4.61)	2.74 (2.61–2.82)
C-6[a]	1700	74	40

[a] The data for C-6 are rough estimates, see text for details.

in 11% (w/v) randomly methylated-β-CD solution was found to be 0.81 mM. Some material was left from the previous study and these results could therefore be reconfirmed. Differences between these two studies are the crystal form of curcumin, and the type and ionic strength of the buffer system. In the present work, a citrate buffer was used because it has better buffer capacity at pH 5, than the phosphate buffer that was used in the previous study. Differential scanning calorimetry also indicates that the batches have different solid characteristics as illustrated by a difference in melting point, i.e. 179.6 °C and 181.5 °C for the old and new curcumin batch, respectively. The new curcumin batch showed a higher solubility in all the cyclodextrin samples. Various parameters that may influence curcumin-CD solubility including different crystal modifications are now under further investigation in our laboratory.

3.4. Photochemical stability

The photochemical half-lives are presented in Table 4. Compound C-6 was considerably more stable than the other investigated curcuminoids and the reported half-lives for this substance are only estimates based on the initial degradation.

It is apparent that all the examined curcuminoids are less stable than curcumin itself, at least in pure methanol and the combined buffer/MeOH solution. In both these solutions, the half-life of curcumin is approximately doubled compared to the second most stable curcuminoid, which in both cases is C-1. It has previously been shown that the photostability of curcumin is lowered in a cyclodextrin solution compared to organic solvent systems (Tønnesen et al., 2002). This is consistent with the present results, and that also seems to be true for the naturally occurring C-5. However, the half-life of C-4 and C-5 in the combined buffer/MeOH solution has an intermediate value rather than a value close to that of the pure methanolic solution. This implies that it is not the cyclodextrin alone that reduces the stability of these compounds. It seems that increased stability depends either on the presence of an organic solvent or the absence of water. However, for all the other investigated curcuminoids, the stability is actually higher in the CD solution than in the aqueous buffer/methanolic solution. In fact, for C-2 the stability is practically the same in methanol as it is in HPβCD, while the stability is dramatically lower in buffer/MeOH. The same trend is seen for C-1 and C-3. The photoreactivity of various curcuminoids is now under further investigation in our laboratory.

3.5. Radical scavenging

The curcumin concentration required to give 50% scavenging was found from concentration versus scavenging activity plots. The results are presented in Table 5. Curcumin C-4 and C-6 had the highest activity. In this case, the reaction was quantitative and almost all of the C-4 and C-6 added was reacted. The stoichiometry of the curcumionid: DPPH$^\bullet$ (90% purity) is 1–4 in the case of C-4 and 1–2 in case of C-6. This indicates that the two parts of the symmetric curcumin molecule might act as two separate units under the experimental conditions. The DPPH$^\bullet$ assay is commonly used to measure radical scavenging activity. What is normally reported is the amount of compound required to obtain 50% scavenging of the DPPH$^\bullet$ radical. This concentration is then assumed to be nearly equal to equilibrium concentration of unreacted scavenger, as is the case with C-5. In the case of C-4 and C-6 the equilibrium concentration of the unreacted scavenger must be much less than what is reported concentration in Table 5. The DPPH$^\bullet$ assay therefore gives a low estimate of the true difference in radical scavenging activity.

Table 5
Radical scavenging activity of curcuminoids ($n = 3$, average, min–max)

	Concentration needed for 50% scavenging of 100 μM DPPH$^\bullet$[a]		Comments
	Concentration (w/v)	Molar concentration	
C-1	>7 mg/ml	>18 mM	Approximate results
C-2	>5 mg/ml	>17 mM	Approximate results
C-3		Results seemed to be similar to C-2[b]	
C-4	8.03 μg/ml (7.98–8.07)	21.80 μM (21.66–21.91)	Quantitative reaction
C-5	162 μg/ml (159–165)	526 μM (515–535)	
C-6	11.10 μg/ml (10.70–11.90)	47.60 μM (46.30–50.80)	Quantitative reaction

[a] The manufacturer reports 90% purity. The true concentration can therefore be close to 90 μM.
[b] Data not sufficient to make an exact calculation of concentration.

Our results are consistent with results reported by Barclay et al. (2000). Venkatesan and Rao (2000) did an experiment similar to the one described in our study, and concluded that the phenolic group is important for the activity. This was based on the fact that the activity of the curcumin derivatives remained close to curcumin as long as the phenolic group was present. Other publications also support this conclusion (Sun et al., 2002; Priyadarsini et al., 2003). That result was partly reproduced here, although this experiment showed that the presence of an OH group is not the only important factor. This is obvious since the compound C-5 showed more than a 20-fold decrease in activity compared to curcumin (C-4) in spite of the presence of phenolic groups. The experimental conditions in the present study were however, somewhat different from the experiment by Venkatesan and Rao (2000), where a solution containing an aqueous buffer at pH 7.4 was used for the experiment. This can probably explain why Venkatesan and Rao observed more similarities between the two phenolic compounds. The hydrogen binding ability of water is different from methanol and this will affect the hydrogen bonding to the aromatic substituents. Further, the compounds are likely to be approaching the pK_a value at pH 7.4, and this will influence their anti-oxidizing properties. The reaction between curcumin and DPPH$^\bullet$ has been thoroughly discussed by Litwinienko and Ingold (2004). Their study emphasizes the importance of the presence of both the keto-enol structure and the phenol group in the *para*-position for the antioxidant properties of curcumin. They conclude that in solvents that support ionization (e.g., water and methanol), curcumin reacts with electrophilic radicals initially at the ionized keto-enol moiety followed by a loss of a phenolic proton. A neutral, stabilized radical is then formed (Fig. 2, Route II). Our results are consistent with their hypothesis. The non-phenolic compounds C-l and C-2 have reduced their activity by a factor of at least 10^3 compared to curcumin (C-4). The results for C-l and C-2 in Table 5 are however, based on scavenging of DPPH$^\bullet$ solutions with a concentration lower than 100 μM, and the SC_{50} value is extrapolated from values in the range 20–40% scavenging. Due to a small amount of available sample, C-3 was measured only at a concentration of approximately 15 μg/ml which resulted in a DPPH$^\bullet$ scavenging of about 1.5%. This is close to C-2, which gave 1.2% scavenging in the same concentration range. However, the data are not sufficient to estimate the exact SC_{50} value of C-3.

4. Conclusion

Generally, the solubilizing ability of the cyclodextrins increased in the order MβCD < HPβCD ≪ HPγCD, with some variation depending on the curcuminoid structure. Curcuminoid molecules with bulky side groups on the phenyl moiety seemed to fit better into the HPγCD cavity than into the cavities of MβCD and HPβCD. While all the investigated derivatives were found to be more resistant towards hydrolysis than curcumin, they all seem to be equally or more susceptible to photochemical degradation, although this depends on the medium. The results from the radical scavenging assay showed that all the synthetic derivatives are less active than curcumin, and it seems like each half of the symmetric curcumin molecule can scavenge radicals independently. Both the phenolic group and the keto-enol moiety seem to be important for the activity.

The investigated derivatives of curcumin all seem to have the advantage of higher hydrolytic stability than curcumin itself, but two obvious limitations of the investigated derivatives compared to curcumin are their lack of photochemical stability and reduced anti-oxidant potential.

The solid characteristics of the curcumin sample and the buffer system used seem to have a significant effect on the saturation concentration obtained in the CD solutions.

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