

Synthesis and characterization of glucosyl-curcuminoids as Fe^{3+} suppliers in the treatment of iron deficiency

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Abstract The Fe^{3+} chelating ability of some curcumin glucosyl derivatives (Glc-H; Glc-OH; Glc-OCH₃) is tested by means of UV and NMR study. The pK_a values of the ligands and the overall stability constants of Fe^{3+} and Ga^{3+} complexes are evaluated from UV spectra. The only metal binding site of the ligand is the β -diketo moiety in the keto-enolic form; the glucosyl moiety does not interact with metal ion but it contributes to the stability of metal/ligand 1:2 complexes by means of hydrophilic interactions. These glucosyl derivatives are able to bind Fe^{3+} in a wide pH range, forming complex species thermodynamically more stable than those of other ligands commonly used in the treatment of iron deficiency. In addition they demonstrate to have a poor affinity for competitive biological metal ions such as Ca^{2+} . All ligands and their iron complexes have a good lypophilicity ($\log P > -0.7$) suggesting an efficient gastrointestinal absorption in view of their possible

use as iron supplements in oral therapy. The ligand molecules are also tested for their antioxidant properties in “ex vivo” biological system.

Keywords Curcuminoidic compounds · Metal complexes · β -Diketo ligands

Introduction

Iron deficiency anemia (IDA) is an extremely disseminate trouble involving about 1/3 of the global population (ACC/SCN 1992). The most widespread route of iron delivery in iron deficient patients is oral, it is simple and effective, its side effects are minor and not dangerous. Oral iron supplementation, typically with ferrous salts, is associate with high incidence of gastrointestinal side effects (Brise and Hallberg 1962). In patients with pathologic conditions of the upper gastrointestinal tract the side effects are intolerable (Solvell and Harwerth 1970). The symptoms, which are nausea, epigastric discomfort, diarrhea and constipation, vary proportionally to the concentration of ionizable iron in the upper gastrointestinal tract and can be reduced by taking iron with food or using chelated forms. Ferrous iron preparations have more side-effects than ferric preparations, probably due to the initiation and propagation of potentially damaging hydroxyl free radicals at the gastrointestinal mucosa (Silvka et al. 1986; Burkitt and Mason 1991). However, iron is poorly absorbed from ferric preparations,

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because ferric salts are rapidly converted to low soluble and poorly absorbed iron hydroxide polymers when passing from the acidic environment of the stomach to the more neutral pH of the duodenum. A stable complexed ferric preparation hinders the precipitation of iron hydroxide in neutral and mild alkaline pH and, in the meantime, may avoid the compliance related to ferrous suppliers. An alternative to oral iron administration is a parenteral therapy (Schröder et al. 2005). A parenteral iron preparation must have a complex ability sufficient to avoid excessive blood concentration of iron and low toxicity to limit side effects (Perewusnyk et al. 2002). This route of iron administration is mainly used in treatment of anemia, in chronic haemodialysis patients and in serious iron deficiency such as in the last month of pregnancy. Great interest is turned on the development of new safe and low toxic iron carriers both for oral and intravenous administration.

Previously we have explored the ability of natural occurring molecules such as curcumin and its derivatives to chelate Fe^{3+} ion (Borsari et al. 2002). Curcumin [1,7-bis(3-hydroxy-4-methoxyphenyl)hepta-1,6-diene-3,5-dione], a yellow pigment obtained from the dried rhizomes of *Curcuma longa* L., is used in traditional medicine to treat a wide variety of disorders such as indigestion, urinary tract infections, liver disease, and rheumatoid arthritis (Goel et al. 2008). This phytochemical has gained interest due to its anti-inflammatory, anti-oxidant, anti-proliferative, anti-angiogenic, and anti-cancer properties (Sharma et al. 2005; Maheshwari et al. 2006; Ammon and Wahl 1991). The β -diketo moiety of curcumin undergoes a keto-enol tautomerism which enable Fe^{3+} coordination. Recently we have also tested the coordination properties of new glucosylated β -diketo compounds (Arezzini et al. 2008). The glucosyl moiety does not influence the coordination ability of β -diketo group therefore these ligands demonstrated a good Fe^{3+} chelating ability in acidic pH while in physiological condition suffer the competition of the insoluble $\text{Fe}(\text{OH})_3$.

As the main drawbacks in the clinical use of curcumin are: low solubility, high rate of metabolism, inactivity of metabolic products and/or rapid elimination from the body, we have synthesized curcumin galactosyl derivatives (Arezzini et al. 2004). These molecules were proposed for the treatment of iron

overload although their water solubility was not greatly improved ($\leq 5 \times 10^{-5}$). Now we have synthesized glucosyl curcuminoids (Scheme 1) with the aim to increase water solubility of curcumin. The compounds are here chemically characterized and the affinity towards Fe^{3+} is evaluated.

Lypophilicity is an important factor that influences drug absorption by gastrointestinal tract, the molecule needs to be quite lyposoluble to be absorbed by gastro intestinal tract, but on the other side with this feature it may penetrate critical barriers such as blood-brain barrier (BBB) and blood-placental barrier (BPB). Here the lypophilicity of the synthesized molecules together with that of their Fe^{3+} complexes is evaluated.

In order to estimate an important aspect of the behavior of the compounds in the tissues, we tested their antioxidant capability against the lipoperoxidation induced by cumene-hydroperoxide. This is to exclude, at least, one of the possible negative said effects on tissues after iron delivery.

Experimental

Synthesis

2,3,4,6-tetracetyl- α -D-glucopyranosyl bromide (**1**)

2,3,4,6-tetracetyl- α -D-glucopyranosyl bromide was synthesized quantitatively by reaction of 1,2,3,4,6-pentaacetyl- β -D-glucopyranose with HBr 30% in glacial acetic acid, at room temperature for 4 h (Fisher 1911).

4-(2,3,4,6-tetracetyl- β -D-glucopyranos-1-yloxy)benzaldehyde (**2**); 3-hydroxy-4-(2,3,4,6-tetracetyl- β -D-glucopyranos-1-yloxy)benzaldehyde (**3**); 3-methoxy-4-(2,3,4,6-tetracetyl- β -D-glucopyranos-1-yloxy)benzaldehyde (**4**)

The appropriate benzaldehyde were solubilized in NaOH, and then slowly dropped in an equimolar solution of **1** in acetone. The reaction was kept under stirring overnight at room temperature, and a precipitate splits up when water is added. The isolated product was recrystallized from EtOH.

Scheme 1 Synthesized compounds

Compound	R ₁	R ₂
Curcumin	OH	OCH ₃
Diacylcurcumin	OAc	OCH ₃
Glc-H (8)	 β-D-glucopyranos-1-yl	H
Glc-OH (9)		OH
Glc-OCH ₃ (10)		OCH ₃

1, 7-bis [4-(2,3,4,6-tetracetyl-β-D-glucopyranos-1-yloxy)phenyl]hepta-1,6-diene-3,5-dione (**5**); 1, 7-bis [3-hydroxy-4-(2,3,4,6-tetracetyl-β-D-glucopyranos-1-yloxy)phenyl]hepta-1,6-diene-3,5-dione (**6**); 1, 7-bis [3-methoxy-4-(2,3,4,6-tetracetyl-β-D-glucopyranos-1-yloxy)phenyl]hepta-1,6-diene-3,5-dione (**7**)

The condensation reaction between glucosilated benzaldehyde and acetylacetone was performed improving Pabon (1964) reaction. A suspension of 1.0 mmol of B₂O₃ and 1.0 mmol of acetylacetone in DMF (1.5 ml) was stirred for 30 min at 80°C, followed by the addition of 4.0 mmol of tributylborate and 1.8 mmol of the appropriate benzaldehyde after 30 min. About 0.4 mmol of *n*-butylamine in 0.5 ml of DMF were dropped during the following hour. The solution, kept under stirring at 80°C for 4 h, developed a yellow–orange color. A sticky orange solid separated after addition of 0.5 M HCl (8 ml) at room temperature. The crude product was filtered off, re-suspended in water, filtered again and dried in vacuum. Elemental analysis calc (exp.): (**5**) C₄₇H₆₀O₂₂: C 57.78 (58.57), H 6.19 (5.79), yield 51%; (**6**) C₄₇H₆₀O₂₄: C 55.95 (56.35), H 5.99 (6.07), yield 91%; (**7**) C₄₉H₆₄O₂₄: C 56.75 (57.09), H 6.22 (5.31), yield 77%. NMR chemical shifts (ppm in CDCl₃): (**5**) H-1 5.80, H-3 6.52, H-4 7.61, H-6 7.00, H-7 7.50, H-9 7.50, H-10 7.00, H-11 5.14, H-12 5.29, H-13 5.28, H-14 5.18, H-15 3.89, H-16 4.29, H-16' 4.18; C-1 99.5, C-2 180.7, C-3 120.7,

C-4 125.9, C-5 133.2, C-6 135.2, C-7 114.7, C-8 162.3, C-9 114.7, C-10 135.2, C-11 96.3, C-12 68.2, C-13 70.7, C-14 66.0, C-15 69.1, C-16 58.9 (**6**) H-1 5.80, H-3 6.51, H-4 7.55, H-6 7.18, H-9 6.96, H-10 7.02, H-11 5.00, H-12 5.28, H-13 5.32, H-14 5.17, H-15 3.88, H-16 4.31, H-16' 4.20; C-1 99.6, C-2 180.7, C-3 121.3, C-4 127.8, C-5 134.0, C-6 127.1, C-7 147.8, C-8 155.6, C-9 114.7, C-10 127.1, C-11 96.1, C-12 68.6, C-13 70.1, C-14 65.7, C-15 69.7, C-16 59.4; (**7**) H-1 5.82, H-3 6.51, H-4 7.58, H-6 7.10, H-9 7.04, H-10 7.05, H-11 5.02, H-12 5.27, H-13 5.28, H-14 5.16, H-15 3.80, H-16 4.26, H-16' 4.17; C-1 99.1, C-2 180.6, C-3 120.9, C-4 137.5, C-5 129.1, C-6 109.2, C-7 148.3, C-8 145.2, C-9 117.1, C-10 119.1, C-11 99.7, C-12 68.6, C-13 70.0, C-14 65.8, C-15 69.6, C-16 59.4.

1,7-bis(4-β-D-glucopyranos-1-yloxophenyl)hepta-1,6-diene-3,5-dione (Glc-H) (**8**); 1,7-bis(3-hydroxy-4-β-D-glucopyranos-1-yloxophenyl)hepta-1,6-diene-3,5-dione (Glc-OH) (**9**); 1,7-bis(3-methoxy-4-β-D-glucopyranos-1-yloxophenyl)hepta-1,6-diene-3,5-dione (Glc-OCH₃) (**10**)

Acetylated compounds were solubilized in 0.1 M CH₃ONa methanolic solution and kept under stirring for 2 h; pH was brought to neutrality by use of a DOWEX ion exchange resin, which was finally filtered off, and the solutions were evaporated in vacuum. Elemental analysis calc. (EXP.): (**8**) C₃₁H₃₆O₁₄: C 58.86

(58.10), H 5.74 (5.72). **(9)** $C_{31}H_{36}O_{16}$: C 56.02 (55.57), H 5.46 (6.02); **(10)** $C_{33}H_{40}O_{16}$: C 57.22 (56.75), H 5.82 (6.05). NMR chemical shifts (ppm in MeOD): **(8)** H-1 6.01, H-3 6.69, H-4 7.62, H-6 7.14, H-7 7.61, H-9 7.60, H-10 7.14, H-11 4.98, H-12 3.52, H-13 3.47, H-14 3.42, H-15 3.45, H-16 3.91, H-16' 3.71; C-1 102.8, C-2 185.6, C-3 124.5, C-4 142.1, C-5 131.6, C-6 131.6, C-7 118.9, C-8 161.6, C-9 118.9, C-10 131.6, C-11 96.5, C-12 68.4, C-13 71.2, C-14 66.7, C-15 68.7, C-16 58.3; **(9)** H-1 6.01, H-3 6.63, H-4 7.53, H-6 7.14, H-9 7.20, H-10 7.07, H-11 4.97, H-12 3.53, H-13 3.47, H-14 3.44, H-15 3.45, H-16 3.90, H-16' 3.71; C-1 102.8, C-2 185.4, C-3 124.5, C-4 142.1, C-5 132.6, C-6 116.2, C-7 147.7, C-8 149.4, C-9 118.8, C-10 122.8, C-11 96.3, C-12 68.1, C-13 71.4, C-14 65.0, C-15 70.1, C-16 59.2; **(10)** H-1 6.02, H-3 6.72, H-4 7.60, H-6 7.12, H-9 7.18, H-10 7.18, H-11 4.58, H-12 3.53, H-13 3.48, H-14 3.43, H-15 3.45, H-16 3.89, H-16' 3.71; C-1 102.8, C-2 185.6, C-3 124.5, C-4 142.1, C-5 132.1, C-6 112.1, C-7 149.6, C-8 150.1, C-9 117.3, C-10 122.5, C-11 99.4, C-12 68.1, C-13 70.5, C-14 66.2, C-15 69.7, C-16 59.2.

Spectroscopy

Spectrophotometric measurements and titrations were performed using Jasco V-570 spectrophotometer at $25.0 \pm 0.1^\circ\text{C}$ in the 200–600 nm spectral range employing a 1 cm quartz cell. 2.5×10^{-5} M aqueous solutions of **8**, **9**, and **10** were investigated. The pH-metric titrations of ligands and metal/ligand [(M/L); (1:1, 1:2, 1:3)] solutions were obtained varying the pH value by adding small amounts of concentrated NaOH or HCl in the pH range 1–11. The protonation constants of the ligands and the formation constants of metal complexes were evaluated from spectrophotometric titrations using the pHab2000 software (Gans et al. 1999).

Nuclear magnetic resonance spectra were recorded on a Bruker Avance AMX-400 spectrometer with a Broad Band 5 mm probe (inverse detection). Nominal frequencies were 100.13 MHz for ^{13}C and 400.13 MHz for ^1H . The typical acquisition parameters for ^1H were as follows: 20 ppm spectral bandwidth, 6.1 μs pulse width (90° pulse hard pulse on ^1H), 1 s pulse delay, 64 numbers of scan. About 0.5 ml of a CD_3OD 10^{-2} M solution of each ligand was prepared, then ten additions (10 μl each time) of $\text{Ga}(\text{NO}_3)_3$ or CaCl_2 solution (5×10^{-2} M in CD_3OD) were performed using a micropipette.

Partition coefficient

The shake-flask method was performed on the ligands in buffered condition (pH 7.0 $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$). About 25 ml of the ligand stock solution (10^{-4} M) or M/L 1:2 solution (10^{-4} M) were mixed in a vessel with a known volume of high purity analytical grade *n*-octanol saturated with water (15–30 ml) and shaken at 37°C for 1 h. After separation of two phases, the ligand concentration in the aqueous solution was determined by spectrophotometric method.

Antioxidant properties

Rat liver microsomes were prepared as previously described (Ferrali et al. 2002). The cellular fraction was incubated at 37°C for 10 min in 50 mM tris-maleate buffer (pH 7.4) to the concentration of 1.5 mg/ml of microsomal protein. Thiobarbituric reacting substances (TBARS) were measured at the end of the incubation as index of lipid peroxidation.

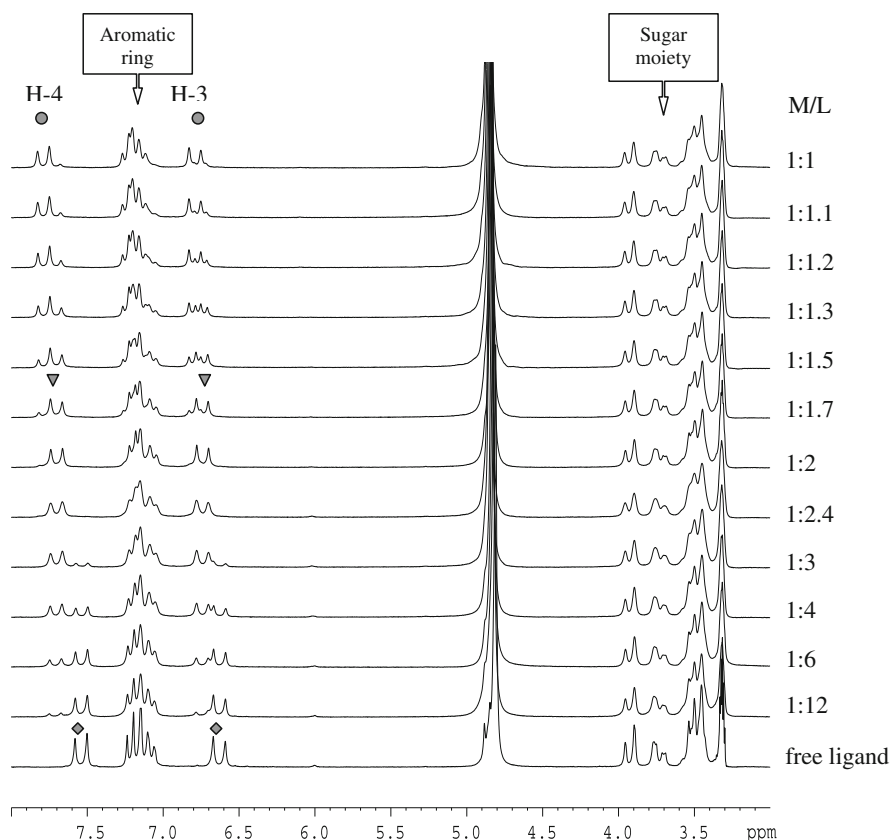
Results and discussion

NMR spectroscopy

All the molecules show a typical spectral pattern of a β -keto-enolic moiety (Borsari et al. 2002; Solčániová et al. 1982), the enolic proton is implied in a strong intra-molecular hydrogen bond, that makes it equally shared by the two oxygen atoms as confirmed by crystal structure of analogous compounds (Arrieta and Mostad 2001).

$\text{Ga}(\text{NO}_3)_3$ is used as NMR probe for Fe^{3+} (Atkinson et al. 1998; Hara et al. 2000), its addition to a solution of compounds **8**, **9**, **10** at acidic pH, immediately originates a complex species, in slow chemical exchange in NMR time scale, whose spectral pattern resembles the one of the free ligand but it is strongly downfield shifted (Fig. 1). By adding Ga^{3+} to the ligand solution, the integrated area of both H-3 and H-4 of the complexed form increases, while an equivalent diminishing of the same protons in the uncomplexed form is registered, until they disappear when 1:2 M/L molar ratio is reached. The greatest downfield shift is undergone by H-4 that probably “feels” the positive charge in the resonance structure of the bis-chelate complex, while a smaller shift is felt by H-3. As far as Gallium ion is added

Fig. 1 ^1H NMR titration of Glc-OH with a solution of $\text{Ga}(\text{NO}_3)_3$ in CD_3OD ; (◆) free ligand; (▼) M/L 1:2 complex; (●) M/L 1:1 complex



a new downfielded species appears, hinting the formation of another complex species, characterized by a M/L 1:1 independently on the ligand. In line with the behavior of curcumin (Borsari et al. 2002), the phenolic group of Glc-OH does not interact with the metal ion. Small changes are observed for the aromatic protons due to delocalization of the metal charge along the aliphatic chain and the aromatic rings. No evidence of an interaction of sugar moiety with metal ion is observed by NMR spectroscopy in acidic media, although we cannot exclude a possible glucoside interaction with the metal ion under physiological conditions, as reported in literature in the solid state (Hegetschweiler et al. 1995a, b, c). By adding Ca^{2+} to the ligand solution, no resonance shifts are observed, even in high excess of metal ion and increasing pH, suggesting a poor ability to bind Ca^{2+} ion.

UV-vis spectroscopy

All spectrophotometric measurements were performed in aqueous solution instead of alcoholic one

used for parent curcuminoids being the water solubility of the glucosilated ligands ($\sim 5 \times 10^{-3}$ M) greater than that of aglycone moiety ($< 5 \times 10^{-5}$ M). By plotting absorbance versus pH at 400 nm an equivalent point corresponding to enolic proton dissociation is observed for all the ligands, a further equivalent point corresponding to phenolic proton dissociation is found for Glc-OH; the pK_a values calculated from spectrophotometric data are reported in Table 1. The presence of sugar moiety does not affect the pK_a values which are similar to those found for curcumin and diacetylcurcumin (Borsari et al. 2002). The electron withdrawing feature of OH substituent on the aromatic ring stabilizes the conjugate base, lowering the pK_{a1} value, as a consequence Glc-OH is the most acidic one in aqueous solution.

By adding Ga^{3+} or Fe^{3+} solution to the free ligands in acidic condition ($\text{pH} \sim 4.5$) a general blue shift of the maximum is observed due to the interaction between the metal ion and the keto-enolic moiety with an anticipated enolic proton dissociation with respect to the free ligand ($\Delta \text{pK}_a \sim 4$). In the

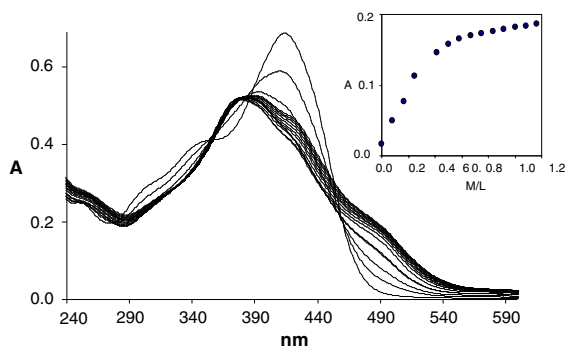
Table 1 Logarithms of protonation constants of the ligands and formation constants of complex species with Fe^{3+} , Ga^{3+} , and Ca^{2+} at 25°C, $I = 0.1 \text{ M}$ (NaNO_3)

		Glc-H			Glc-OCH ₃			Glc-OH			
L^-	Log K_{a1}	10.8			9.2			8.2			Log K_{a1}
								29.9			Log β_{013}
L^{2-}	Log K_{a2}							9.4			Log K_{a2}
								21.7			Log β_{012}
L^{3-}	Log K_{a3}							12.3			Log K_{a3}
								12.3			Log β_{011}
		Fe^{3+}	Ga^{3+}	Ca^{2+}	Fe^{3+}	Ca^{2+}	Ca^{2+}	Fe^{3+}	Ga^{3+}	Ca^{2+}	
$[\text{ML}]^{2+}$	Log β_{110}	12.2	12.1	2.03	10.7	11.8	2.12	32.5	32.2	24.23	Log β_{112}
								10.8	10.5	2.53	Log $\beta_{112}-\log \beta_{012}$
$[\text{ML}(\text{OH})]^+$	Log β_{11-1}	2.1	2.8		2.4	3.2		27.5	27.0		Log β_{111}
								5.8	5.3		Log $\beta_{111}-\log \beta_{012}$
$[\text{ML}_2]^+$	Log β_{120}	24.3	22.0	4.02	20.8	22.5	4.15	64.5	65.5	48.34	Log β_{124}
								21.1	22.1	4.94	Log $\beta_{124}-2\log \beta_{012}$
$[\text{ML}_2(\text{OH})]$	Log β_{12-1}	18.7	15.8		16.0	12.5		60.6	60.9		Log β_{123}
								17.2	17.5		Log $\beta_{123}-2\log \beta_{012}$

Estimated standard deviation of 0.1

L^- represents the ligand with the enolic group in the dissociated form

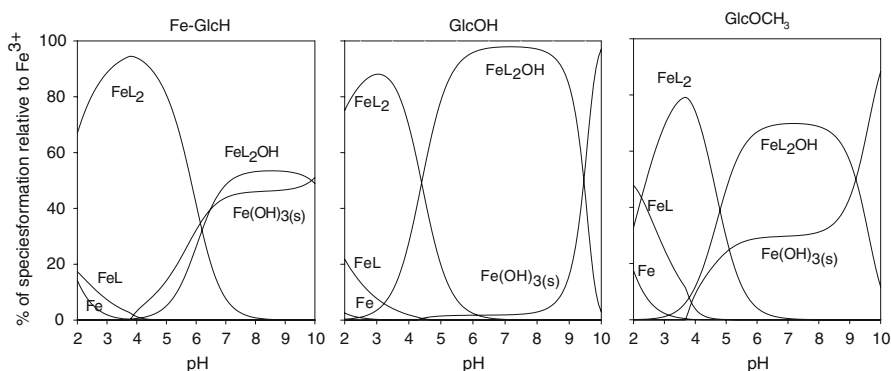
Fe^{3+} containing systems a new absorption at 500 nm appears (Fig. 2) which is due to a ligand to metal charge-transfer (LMCT) band (Lintvedt et al. 1970). The absorbance increases with the M/L molar ratio (inset Fig. 2) and reaches a maximum at a M/L value of 0.5 corresponding to 1:2 complex as detected by NMR spectra. By adding Ca^{2+} to the ligand solution, at acidic pH, no changes are observed in band position and absorbance, confirming no metal-ligand interaction as anticipated by NMR data.

**Fig. 2** Spectrophotometric titration of Glc-OH solution with $\text{Fe}(\text{NO}_3)_3$. The inset shows the plot of absorbance versus M/L molar ratio at 490 nm

The pH-metric titrations of Ga^{3+} or Fe^{3+} containing systems were performed in aqueous solution in the 1:1 and 1:2 M/L molar ratios and allowed to calculate the overall stability constants of the complexes reported in Table 1. The species distribution curves of Fe^{3+} complexes are reported in Fig. 3. Beginning from pH (~ 2) the prevailing species is FeL_2 , confirming UV and NMR data. In this complex the metal ion is coordinated by two ligand molecules acting as chelating agents through the dissociated keto-enolic moiety. In physiological conditions the prevailing species becomes FeL_2OH where an OH^- group is coordinated to FeL_2 species. On increasing pH the competition with hydroxyl groups leads to the precipitation of $\text{Fe}(\text{OH})_3$ as reported in the distribution curves obtained taking into account K_{PS} of $\text{Fe}(\text{OH})_3$ (Liu and Millero 1999); anyway over 50% of Fe^{3+} is still in the complexed form up to pH 9.

A comparison between Fe^{3+} -complexes with our ligands and those with gluconic acid (Escandar et al. 1994), which is the most widely used Fe^{3+} -supplier in the oral treatment of anemia, is forbidden on the basis of stability constant values due to the different stoichiometry of the formed species. Anyway the amount of free iron at acidic pH (~ 2) is around

Fig. 3 Species distribution curves of Fe^{3+}/L systems. $[\text{Fe}^{3+}]_{\text{tot}} = 1 \times 10^{-3} \text{ M}$, $[\text{L}]_{\text{tot}} = 2 \times 10^{-3} \text{ M}$. Complexes charges are omitted for clarity



40% for gluconic acid, 14% for Glc-H, 18% for Glc-OCH₃ and 2% for Glc-OH; in addition in the Fe/*gluconic acid* system at pH 5 the Fe^{3+} ion is almost completely precipitated as $\text{Fe}(\text{OH})_3$. These data suggest a higher stability of our complexes with respect to gluconic acid in a wide pH range; this is fundamental feature for a good gastrointestinal absorption in view of a potential oral administration. Glc-OH is the more effective in metal complexation probably due to the presence of phenolic functions that play an important role in the hydrogen bonds network involving water molecules and glucosidic moiety.

Ga^{3+} and Fe^{3+} give complexes with quite similar stability. Comparison of the behavior of glucosyl-curcuminoids with those of parent curcuminoids shows that the former prefer 1:2 M/L complex instead of 1:1 for parent curcuminoids (Borsari et al. 2002), despite the steric hindrance of the saccharide units. This is probably due to the polar/apolar character of the molecule, in fact, in aqueous solution the apolar core is tightly connected to the metal ion while the glucosidic polar units originate hydrophilic interactions (especially hydrogen bonds) with the solvent, giving a more stable structure and hindering the metal ion from additional ligands such as oxygen.

As Ca^{2+} is one of the most abundant metal ions in human body, its concentration in physiological fluids being about $1.2 \times 10^{-3} \text{ M}$ (Larsson and Magnusson 2004), we have evaluated its possible competition with iron in the interaction with our ligands. Figure 4 shows the distribution curves of $\text{Ca}^{2+}/\text{Glc-OH}$ system with respect to the ligand, calculated using the stability constants reported in Table 1. Up to physiological pH, the affinity of Glc-OH for proton is

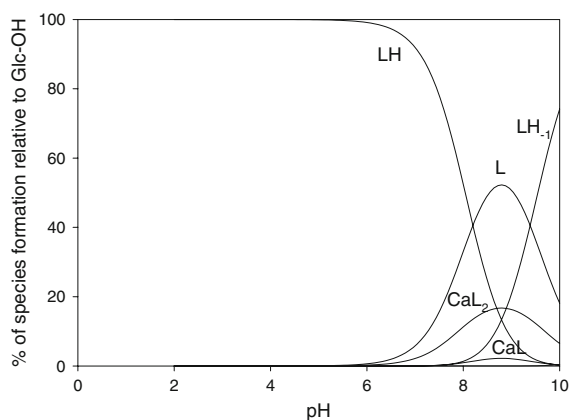


Fig. 4 Species distribution curves for $\text{Ca}^{2+}/\text{Glc-OCH}_3$ system. $[\text{Ca}^{2+}]_{\text{tot}} = 1.2 \times 10^{-3} \text{ M}$, $[\text{L}]_{\text{tot}} = 1.0 \times 10^{-3} \text{ M}$

higher than that for calcium, whereas in basic condition (pH ~ 9) only 20% of the ligand is coordinated to calcium ion. This percentage dramatically decreases in presence of iron (Fe/Ca 1:5) confirming the poor affinity for calcium ion as already observed by ^1H NMR data.

Lipophilicity

Lipophilicity is another important factor that influences drug absorption by gastro intestinal tract (GIT). Lipophilicity is commonly assessed by measuring partition coefficient (P) between two phases, typically *n*-octanol and water (Habgood et al. 1999). In order to achieve efficient oral absorption the compound should possess $\log P > -0.7$ (Liu and Hider 2002a). The measured $\log P$ for our ligands are reported in Table 2 while for curcumin and diacetyl-curcumin the residual concentration in the aqueous phase was too low to be determined. The presence of

Table 2 Logarithms of partition coefficient ($\log P$) defined as the concentration ratio (mol l^{-1}) in the two solvents (*n*-octanol and water)

	Ligand	Fe-ligand 1:2 system
Glc-H	0.14	−0.18
Glc-OH	0.28	0.13
Glc-OCH ₃	0.10	−0.20

sugar moiety strongly reduces the lipophilicity of the molecules anyway the $\log P$ value suggests a sufficient absorption by GIT.

The $\log P$ value of the ligands is mostly influenced by the nature of the substituent in *meta* position on the aromatic ring. The increased hydrophilic character of methoxyl group in Glc-OCH₃ with respect to hydrogen in Glc-H decreases the $\log P$ value, while the presence of hydroxyl group in Glc-OH favors the formation of intra-molecular hydrogen bond interactions with the glucosidic moiety diminishing the hydrophilicity of the compound. On the other hand lipophilicity favors the passive penetration through blood-brain barrier (BBB) and the existence of a good correlation between BBB permeability and P (*n*-octanol/water) for different *N*-alkylpyridinones is reported, in general $\log P < 1$ gives rise to a low BBB penetration (Habgood et al. 1999). The ability of our ligands to form intramolecular hydrogen bonds may decrease the barrier permeability.

The lipid solubility of Fe-complexes is lower than that of the corresponding ligand but it is sufficient to give an efficient absorption from the gastrointestinal tract. A linear relationship is often used to correlate the partition coefficients of the free ligand (L) and iron complex (FeL) (Edward 1998)

$$\log P_{\text{FeL}} = n \log P_{\text{L}} + k$$

where n is the stoichiometry of the complex and k is a constant representing the change in hydrophobicity in converting the ligating group of the ligand into the iron coordination sphere of the complex. Our data match this behavior with a calculated $n = 1.93$ and $k = -0.42$ predicting the stoichiometry of the prevailing complex species as suggested by spectroscopic data.

Antioxidant properties

An important aspect of the behavior of the drug vehicles is their fate after the drug delivery; it is

Table 3 Tiobarbituric acid reacting substances (TBARS) detection in rat liver microsomes (MC) exposed to the oxidant cumene hydroperoxide (ox)

	Time	
	0 min	10 min
MC (control)	0.26	0.69
MC + ox	0.36	8.77
MC + ox + Curcumin	0.33	0.79 (−90%)
MC + ox + Glc-H	0.36	7.69 (−22%)
MC + ox + Glc-OH	0.49	5.26 (−40%)
MC + ox + Glc-OCH ₃	0.36	4.38 (−50%)

Data expressed as nmol of TBARS/ml of incubation mixture

important they are rapidly excreted without any other activity. Unwanted effects such as enzyme inhibition, DNA damage and prooxidant activities are to be avoided. To assess the possible contributions from peroxidant activity, we assayed the behavior of the three compounds in the presence of the lipid peroxidation induced by cumene hydroperoxide. Should the compounds under investigation have oxidant activity they should stimulate hydroperoxide facilitated oxidations. As can be seen all three compounds show a partial antioxidant effect, in particular compounds Glc-H and Glc-OH despite much less than that exhibited by curcumin (Table 3). No oxidant activity was detected.

Conclusions

The synthesized glucosilated curcumin derivatives show a greater water solubility ($\sim 5 \times 10^{-3}$ M) compared with the parent curcumin, retaining the same Fe^{3+} coordination ability. The complex species are stable over a wide pH range, maintaining Fe^{3+} in a soluble form and preventing the precipitation of iron hydroxides. In addition, the ligands demonstrated to possess a poor affinity for the potentially competitive biological metal ion, Ca^{2+} . These metal coordination features, in comparison with those of other ligands commonly used in the treatment of iron deficiency, make these molecules worthy for consideration for clinical use. The factors influencing paracellular route of drug absorption are many such as: (1) molecular weight and steric hindrance, (2) hydrogen bond interactions, (3) lipophilicity. Although the MW of the 1:2 complexes is higher than the cut-off value of 500 Da for an efficient gastrointestinal absorption (Liu

and Hider 2002b), the good log P value of both ligand and iron complex, together with the possibility to form stable hydrogen bond interactions may favor drug absorption. Anyway further biological experiments will clarify these aspects. In addition the absence of pro-oxidant activity offers additional advantage.

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