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The investigation of the photophysical properties of α-chlorocurcumin and α-methylcurcumin

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ABSTRACT

The electronic properties of α-chlorocurcumin and α-methylcurcumin was theoretically investigated at the B3LYP/6-311++G(d,p) level of theory. The thermodynamics quantities were estimated by calculating the frequencies of the molecules. Three main isomers were predicted after full geometry optimization of various suggested isomers within the tautomeric mixture of each molecule; the *cis*-enol, *trans*-enol and the *trans*-diketo isomers. Their stability was in the sequence: *cis*-enol > *trans*-diketo > *trans*-enol. The stabilization energy for the *cis*-enol with respect to *trans*-diketo and *trans*-enol in chlorocurcumin is 8.44 and 12.59 kcal/mol, respectively, while in methylcurcumin, it is 4.80 and 10.79 kcal/mol, respectively. The fluorescence spectra were recorded for the investigated compounds in several protic and aprotic solvent with different dielectric constants and H-bonding abilities. The emission maxima are within the range 487 to 571 nm in ethylene glycol, while they are within the range 475 to 557 nm in *n*-hexane. The fluorescence quantum yields of both compounds are low and lower than those of curcumin. The quantum yield of chlorocurcumin ranges from Φ_{Fl} = 0.008 in MeOH to Φ_{Fl} = 0.058 in toluene, while for methylcurcumin it ranges from Φ_{Fl} = 0.007 in DMF to Φ_{Fl} = 0.0524 in ethylene glycol. The fluorescence of both compounds quenched by water and their fluorescence life times are estimated from the slopes of the linear curves that obtained from Stern-Volmer relationship to be 1.44 and 1.40 psec for chlorocurcumin and methylcurcumin, respectively.

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1. Introduction

Curcumin is a natural pigment with low toxicity and good stability obtained from the rhizomes of turmeric. It exhibits good optical and electrical properties owing to highly π-electron delocalized system and symmetric structure [1-3]. Combination with light induces additional biological activities in curcumin. Upon excitation to the S1-state it becomes phototoxic to bacteria [4-6] and mammalian cells both cancerous and healthy [7,8]. Curcumin as a β-diketone exhibits keto-enol tautomerism. The closed *cis*-enol tautomer which, is characterized by an intramolecular hydrogen bond between the keto and the enol moieties is the most stable tautomer [9]. Curcumin absorbs light in the visible region and fluoresces with low quantum yield [10] and its emission properties depend highly on the polarity of the environment. Curcumin has significant potential as an effective photodynamic therapy agent [10-18]. The presence of light has been shown to enhance the distraction of tumor cells [13-15]. It has been established that stable photoproducts are not the source of medical effect of curcumin but photolitically produced reactive oxygen species including singlet oxygen, hydroxyl radical, superoxide or hydrogen peroxide may be responsible for photoinduced activity [11,19,20]. Curcumin exhibits a marked solvent dependence of the electronic absorption and emission spectra as well as of the photolysis and photochemistry. Both solvent-dependent keto/enol tautomerism of the central intrahydrogen bonded ring and *cis*/*trans* isomerism might responsible for this behavior [21]. Despite several excellent papers that deal with the fluorescence properties of curcumin and some of its analogs in different solvents [22-25], and in different media [26-32], curcumin analogs [33-39], and curcumin complexes [40,41], to our knowledge, no study was concerned with fluorescence properties of curcumin substituted at the central carbon atom. The aim of this work is to investigate the photo properties of the curcuminoids α-chlorocurcumin and α-methyl curcumin in connection with their structural properties.

2. Experimental

α-Chlorocurcumin and α-methylcurcumin were prepared according to Al-Salim [42] and Siwak *et al.* [18], respectively. The UV-Vis absorption spectra were measured by CECIL 2700 spectrophotometer. Steady-state fluorescence measurements were done using Agilent supplied with the Agilent Cary Eclipse WinFLR Version 1.2 software. The entrance and exit slits were adjusted to 2 nm. The measurements were undertaken under thermostated environment at 25.0±0.1 °C.

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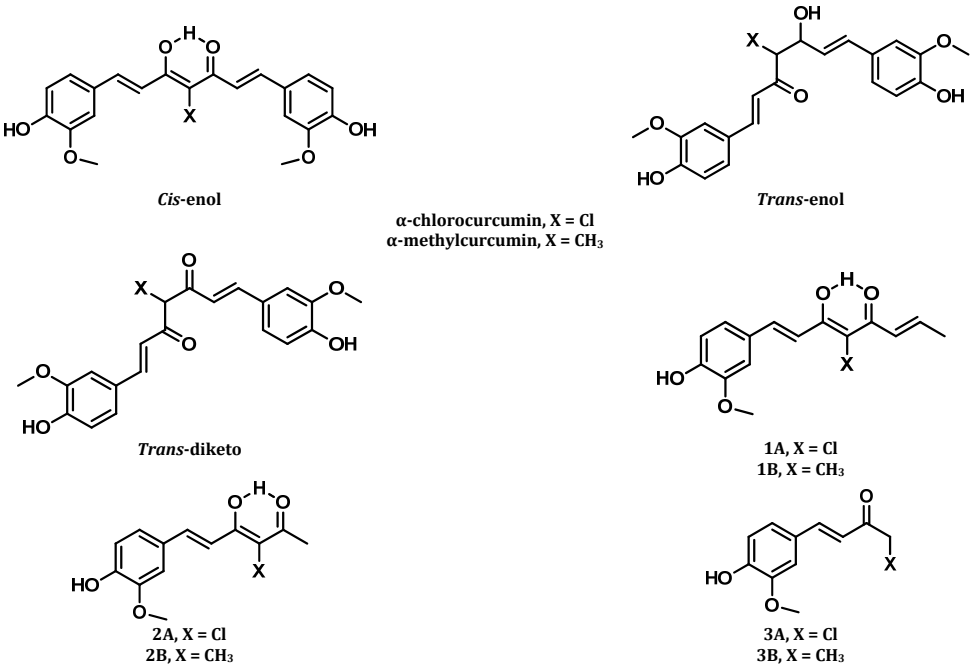

Figure 1. Structures of some important candidates of the studied curcuminoids.

The excitation wavelengths used were the respective absorption peaks of the solutions.

The fluorescence quantum yields of the studied compounds in various solvents were determined from their integrated fluorescence relative to quinine sulphate in 0.05 M H_2SO_4 as a standard with reference value Φ_{Ref} = 0.51 [43]. The reference was exited at its 344 nm absorption maximum. The quantum yields were systematically corrected for differences in the refractive index of the solvents and in absorption band absorbance. The software Spikwin32 Version 1.71.6.1 was used for the processing of some absorption and emission spectra.

All theoretical calculations were done using GAUSSIAN 09W [44] using the Density Functional Theory (DFT) [45]. The density functional theory approach utilizes the Becke's three parameter hybrid functional B3LYP [46]. The basis set 6-311++G(d,p) was used to fully optimize the ground state geometry of studied structures in the gaseous phase. The vibrational spectra of the *cis*-enol, *trans*-enol and *trans*-diketo have been determined to estimate their thermodynamics quantities and to check that all vibrational frequencies are real.

TD-DFT approach [47] was used to compute the electronic spectra at the theory level B3LYP/6-311++G(2d,p). The bulk solvent effects were evaluated via the Integral Equation Formalism Polarizable Continuum Model (IEFPCM) [48].

3. Results and discussion

3.1. Structure and energies

As is the case with curcumin both α-chlorocurcumin and α-methylcurcumin should exist as a balanced tautomeric equilibrium of the diketo and the enol forms [9,49] where the enol is the more stable form. In general, the enol form means the *cis*-enol form possessing the intrahydrogen bonded chelated ring (Figure 1), but on the basis of its photo properties the *trans*-enol with no H-bond was considered as an associated candidate within the overall tautomeric mixture of curcumin [20,22]. In addition, the *cis*- and *trans*-conformations are also suggested for the diketo tautomer.

Despite that the only resulting structures after the full geometry optimization of the studied molecules are the *trans*-diketo, the *cis*-enol and the *trans*-enol forms. The calculated thermodynamics energies at the B3LYP/6-311++G(d,p) level of theory are gathered in Table 1.

From Table 1, the most stable isomer is the *cis*-enol. This is in accordance with results reported for curcumin [49] and is attributed to both the intrahydrogen bonding and the through system π-conjugation. In chlorocurcumin, the *cis*-enol stabilization energies ($\Delta H°$) with respect to the diketo and the *trans*-enol are 6.91 and 12.92 kcal/mol, respectively, while in methylcurcumin the stabilizing energies are 2.79 and 9.38 kcal/mol, respectively. Stabilization energy of 6.7-7.5 kcal/mol was reported for curcumin depending on the level of theory used for the calculations [50]. This indicates that the equilibrium balance for the enol-diketo tautomerism is slightly corrupted towards the enol tautomer in chlorocurcumin, while the opposite is shown in the case of methylcurcumin. In this case, the Cl substituent tends to stabilize the *cis*-enol while the methyl substituent tends to stabilize the diketo form. It is known that in β-diketones alkyl groups attached to the α-position (central carbon atom) produces a decrease of the enol percent meanwhile stabilizing the enol form [51]. On the other hand, the electron withdrawing nature of the Cl group in β-diketones leads to the favor of the enol form due to the stabilization of the conjugate base enolate anion by the presence of the Cl, while in contrary the electron donating nature of the methyl group leads to favor the keto form [52]. Table 1 shows the *trans*-enol is less stable even than the diketo form. This is due to the lack to the intrahydrogen bonding that is responsible for the higher stability of the *cis*-enol and to the planar shape of the molecule which leads to strong electrostatic repulsions at several pairs of electronic centers which is nearly absent in the diketo form (Figure 1).

Table 1. B3LYP/6-311++G(d,p) calculated relative energies corrected for zero-point energy, enthalpy and Gibbs free energy for the *cis*-enol, *trans*-enol and *trans*-diketo forms.

Parameter	Chlorocurcumin			Methylcurcumin		
	***Cis*-enol**	***Trans*-enol**	**diketo**	***Cis*-enol**	***Trans*-enol**	**diketo**
Total energy (Hartree)	-1723.199303	-1723.179244	-1723.185852	-1302.865153	-1302.847667	-1302.857500
Enthalpy (Hartree)	-1723.260658	-1723.241851	-1723.249639	-1302.924664	-1302.909723	-1302.920218
Free energy (Hartree)	-1723.171450	-1723.150868	-1723.157428	-1302.837717	-1302.818895	-1302.828785
Δ*E* (kcal/mol)	0.00	12.587	8.44	0.00	10.79	4.80
Δ*H* (kcal/mol)	0.00	12.92	6.91	0.00	9.38	2.79
Δ*G* (kcal/mol)	0.00	11.80	8.80	0.00	11.81	5.60

Table 2. UV-Vis spectra of α- chlorocurcumin and α-methylcurcumin in different solvents.

Solvent	Chlorocurcumin		Methylcurcumin	
	λ_{max} (nm)	ε × 10^4 ($M^{-1}\cdot cm^{-1}$)	λ_{max} (nm)	ε × 10^4 ($M^{-1}\cdot cm^{-1}$)
n-Hexane	440	1.498	360	1.500
			428	1.120
Toluene	452	4.140	441	1.920
			367	1.920
Chloroform	450	4.100	364	2.500
			442	1.320
Ethyl acetate	448	3.820	436	1.380
			361	1.980
Acetone	451	3.255	440	1.500
			355	1.835
Acetonitrile	358 sh	-	439	1.260
	450	3.640	352	1.980
DMFA	370	2.085	429	2.125
	458	1.815	377	2.070
DMSO	359	2.020	369	2.220
	457	4.460	450	1.080
Isopropanol	454	1.910	442	1.410
			373	1.940
Ethanol	451	3.800	440	1.700
			367	2.100
Methanol	335	1.440	439	1.440
	449	2.940	366	2.560
Ethylene glycol	458	4.100	452	1.580
			363	1.820

3.2. The electronic spectra of the studied compounds

The absorption maxima (λ_{max}) and the molecular absorptivities (ε_{max}) of chlorocurcumin and methylcurcuminin the different solvents are gathered in Table 2 while some representative spectra are shown in Figure 2 and 3. The main character in the absorption spectra is a broad and composite band occurs within the ranges 440-458 and 428-445 nm in chlorocurcumin and methylcurcumin, respectively. The position of the band is in accordance with that reported for curcumin within the range 408-438 nm depending on the solvent [35].

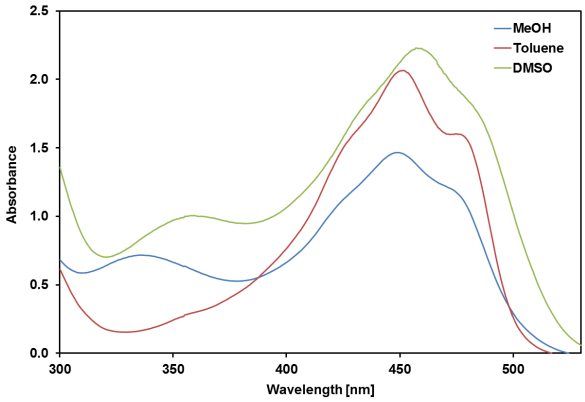

Figure 2. Absorption spectra of chlorocurcumin in DMSO, toluene and MeOH.

Figure 2 shows the spectra of α-chlorocurcumin in different solvents. It could be seen that in more polar solvents (DMSO and MeOH) a low intensity band appears at about 350 nm. In less polar solvents like toluene, it appears as a weak shoulder. This band is attributed to the diketo tautomer. Indeed, the compound half curcumin which is isoelectronic with any part of the diketo form has absorption band at 340 nm [53]. In addition, this is consistent with the fact that the diketo isomer is more polar than the enol isomer which illustrates its appearance in more polar solvents.

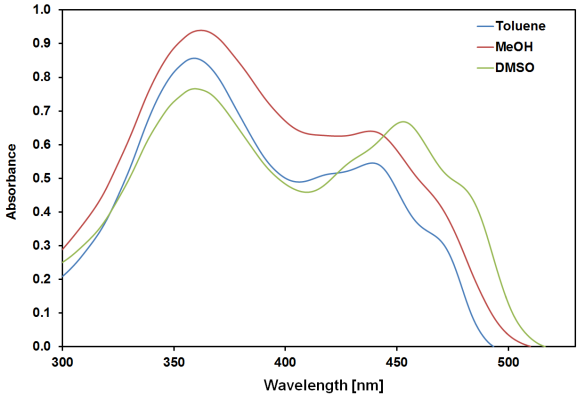

Figure 3. Absorption spectra of methylcurcumin in DMSO, toluene and MeOH.

The absorption spectra of methylcurcumin (Figure 3) have the same characteristics as with those of chlorocurcumin with some difference represented by the more intense band attributed to the diketo isomer appears at the range 352-372 nm. In all solvents this band is more intense (ε = 18200-25000 $M^{-1}\cdot cm^{-1}$) than the long wavelength band that attributed to the *cis*-enol (ε = 10800-19200 $M^{-1}\cdot cm^{-1}$).

Table 3. Comparison between theoretical and deconvoluted experimental main absorption bands in α-chlorocurcumin and α-methylcurcumin and theoretical λ_{max} (in nm) of some of their isomers and structural functionalities.

Molecule	α-Chlorocurcumin		α-Methylcurcumin	
	Theory	Deconvoluted	Theory	Deconvoluted
Cis-enol	474 (1.609)	481	462 (1.597)	473
Trans-enol	468 (1.152)	454	452 (1.046)	446
Diketo	421 (0.503)	-	388 (0.073)	-
1*	434 (1.139)	432	421 (1.183)	420
2*	404 (1.042)	427	391 (1.095)	417
3*	367 (0.631)	379	360 (0.652)	363

* 1, 2 and 3: structures related to both α-chlorocurcumin and α-methylcurcumin as shown in Figure 1.

This indicates a higher content of the diketo tautomer in the tautomeric equilibrium in this compound that will affects its fluorescence. This is consistent with that the alkyl substituents at the central carbon atom in β-diketones increase the diketo content [52].

From Figure 2 and 3, it appears that the long wavelength bands which are responsible for the yellow colors of the studied compounds are composite of several components with different intensities.

The deconvolution of these bands in toluene (Figure 4 and 5) shows that each one consists of five peaks at 481, 454, 432, 427 and 379 nm in chlorocurcumin and at 473, 447, 420, 417 and 363 nm in α-methylcurcumin. The most intense component in each case corresponds to the excitation wavelength for the fluorescence in the compounds. These bands can be appearing due to electronic transitions at various isomeric forms and various functionalities in each isomer.

In order to shed some light on the origin of the absorption bands a computational study of the absorption spectra of several possible candidates was done.

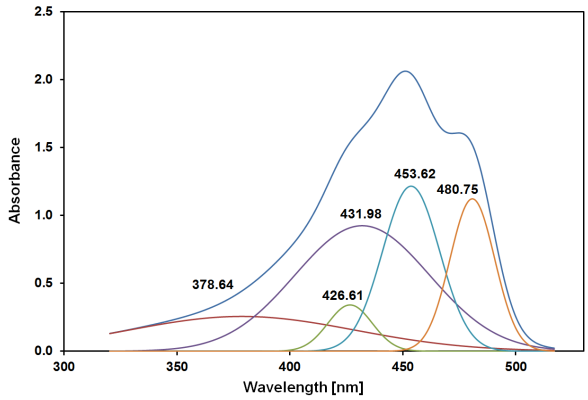

Figure 4. The absorption peak of chlorocurcumin after deconvolution.

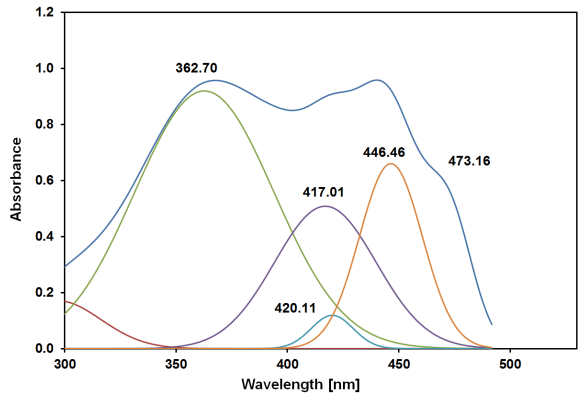

Figure 5. The absorption peak of methylcurcumin after deconvolution.

Since apart from the diketo, the *cis*-enol and the *trans*-enol isomers, all other rotamer isomers do not significantly different in their electronic structures and accordingly their spectra do not significantly different, the computations include only these isomers as well as some structural functionalities shown in Figure 1 and the results are gathered in Table 3. Table 3 includes the calculated spectra in solution at the B3LYP/6-311++G(2d,p) level of theory. Using higher level such as B3LYP/6-311++G(2df,p) did not improve the results over the previous level and the difference is only a fraction of nanometer. The calculations in the gas phase failed to reproduce the experimentally observed bands correctly. For example, the estimated bands in the gas phase for chlorocurcumin and methylcurcumin are 446 and 436 nm, respectively, while in the observed spectra there are bands (after deconvolution) at 481 and 473 nm, respectively.

The calculated spectra in the solution for the *cis*-enol forms are at 471 and 460 nm, respectively, which is more consistent with the observed bands. The estimated spectra showed that the enol isomers have absorptions at higher wavelengths and higher intensities than the diketo isomer in both molecules (Table 3). For chlorocurcumin the estimated peaks for *cis*-enol and the *trans*-enol at 471 and 468 nm while, in methylcurcumin, they are at 462 and 452 nm, respectively. For the diketo isomers the estimated peaks are 419 and 386 nm, respectively. This is interpreted as the previous isomers have longer conjugated π-systems and more planar structures. The presence of the intrahydrogen bonded ring in the *cis*-enol is responsible for its higher maxima than the *trans*-enol.

It is worth to note that the estimated spectra of some structural functionalities have λ_{max} fairly match the positions of some of the deconvoluted bands components. For example, the estimated peaks of the molecules **1a** (434 nm) and **1b** (419 nm) (Figure 1) are in good agreement with the components at 436 and 420 nm in α-chlorocurcumin and α-methylcurcumin, respectively. This is also the case with the peaks estimated for molecules **2a** (402 nm) and **2b** (389 nm) which may correlate with the components at 426 and 411 nm, respectively.

The calculation protocol overestimates the absorption peaks of the diketo isomer for both compounds in comparison with the observed peaks. This is may be due a problem with the computational algorithm. On the other hand the molecules **3a** and **3b** which are isoelectronic with the half molecule of chlorocurcumin and methylcurcumin have estimated peaks at 367 and 360 nm, respectively, which are closer to the observed peaks of the diketo isomers (Table 3).

From Table 3, it could be seen that the species which may be important for the fluorescence properties are the *trans*-enol isomer and the molecule **1a** and **1b** molecules since they have absorption maxima at the same regions of the excitation peaks of the fluorescence of the studied compounds. The excitation maxima of molecule **1a** and **1b** are within the ranges 440-458 and 418-458 nm, respectively. If this is true, it may rationalize for the low quantum yields of curcuminoids since the two species are of low probability.

Table 4. Spectral and photochemical properties of chlorocurcumin and methylcurcumin in different solvents.

Solvent	Chlorocurcumin			Methylcurcumin		
	λ_{FI} (nm)	Stokes Shift (nm)	Φ_{FI}	λ_{FI} (nm)	Stokes Shift (nm)	Φ_{FI}
n-Hexane	487, 514	47	-	475, 504	47	-
Toluene	497, 529	45	0.0583 (±0.001)	478, 515	41	0.0047 (±0.002)
Chloroform	511	55	0.0575 (±0.001)	497	59	0.0084 (±0.002)
Ethyl acetate	505	60	0.0286 (±0.002)	484, 515	66	0.0071 (±0.0008)
Acetone	537	92	0.0557 (±0.001)	503	63	0.0103 (±0.0001)
Acetonitrile	529	71	0.0241 (±0.001)	522	88	0.0084 (±0.0003)
DMFA	549	91	0.0161 (±0.002)	533	88	0.0076 (±0.001)
DMSO	547	90	-	537	89	-
Isopropanol	551	93	0.0380 (±0.0007)	541	95	0.0082 (±0.0003)
Ethanol	559	101	0.0365 (±0.003)	543	98	0.0121 (±0.0004)
Methanol	563	105	0.0083 (±0.0008)	545	105	0.0052 (±0.0001)
Ethylene glycol	571	113	0.0496 (±0.0008)	557	112	0.0524 (±0.005)

3.3. The fluorescence spectra

The fluorescence spectra of chlorocurcumin and methylcurcumin are shown in Figure 6 and 7 while the emission maxima, Stokes shifts and the quantum yields Φ_{FI} are listed in Table 4. The fluorescence bands of the two compounds are broad and structure less in all solvents, except in *n*-hexane and toluene where two emission maxima could be seen. This is similar to the emission spectra of curcumin [20,22]. The emission maxima λ_{FI} of chlorocurcumin in all solvents are higher than those of methylcurcumin. This is consistent with the higher absorption maxima of the former compound (Table 4).

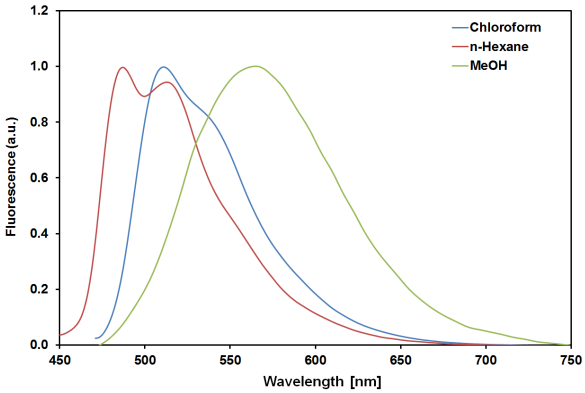

Figure 6. Normalized fluorescence spectra of chlorocurcumin in *n*-hexane, chloroform and MeOH.

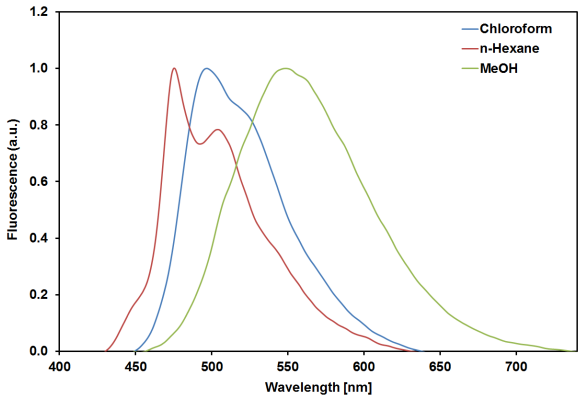

Figure 7. Normalized fluorescence spectra of methylcurcumin in *n*-hexane, chloroform and MeOH.

The emission maxima in both compounds generally undergo red shift when going from a solvent with low dielectric constant to a solvent with higher dielectric constant. The red shift is 60 and 33 nm when going from *n*-hexane to DMSO in chlorocurcumin and methylcurcumin, respectively, and becomes about 80 nm in alcohols (strong H-bonding ability) in both compounds.

It has been stated [20,22] that due to interhydrogen bonding with solvents molecules and perturbation by polar non-protic solvents, both *cis*-enol, *trans*-enol and anti-diketo isomers are present in the tautomeric mixture which may correlate with shoulders that appear in the absorption spectra. In this case the red shift observed in the emission maxima of the studied compounds is an indication that in less polar (Aprotic or protic) solvents the species who dominates the fluorescence activity is the species **1a** and **1b** while at higher polarity and higher H-bonding ability the *trans*-enol becomes of importance since it is more polar than the former species and has higher ability for interhydrogen bonding with solvent molecules. This is correlated with the change of Stokes shifts which, increase in the same direction (Table 4). Since the structures of the molecules become less rigid with the weakening of the intramolecular hydrogen bonding (at increasing polarity and H-bond ability of the solvent) and more liable to out-of-plane vibration higher Stokes shifts are expected [22].

We validate our measurement for the quantum yields and analyses by measuring quantum yields of curcumin in EtOH and acetonitrile which, have been reported in literature. Our results are 0.063±0.001 and 0.107±0.003 in EtOH and acetonitrile, respectively. The results are in fair agreement with the values reported by Chignell *et al*. [20] those are 0.063 and 0.104 in EtOH and acetonitrile, respectively.

Table 4 shows that the quantum yields of the both studied compounds are low and lower than that of curcumin in the same solvent [20,22]. The quantum yield of chlorocurcumin range from 0.0083 in MeOH to 0.0583 in toluene, while that of methylcurcumin is ranged from 0.0047 to 0.0520 in ethylene glycol. The low values are due to the presence of the Cl and the methyl substituents which are reduce the planarity of the molecules. On the other hand the higher content of the diketo isomer in the methylcurcumin is a crucial effect for reducing the quantum yield in this compound because of its expected very low fluorescence. This may illustrated on the basis of the fact that half curcumin isoelectronic compound with diketo isomer have very low fluorescence at 450 nm (Quantum yield < 0.0006) [20].

It is clear that the quantum yields of the two compounds are lower than that of curcumin. This could be as a result of the presence of the Cl and the methyl substituents at the α-position which lead to reduce the planarity of the molecules in addition the Cl substituent reduces the intrahydrogen bonding in the *cis*-enol isomer while the methyl substituent reduces the enol content which is responsible for the fluorescence activity of curcuminoids. Also, it had been concluded that electron withdrawing substituents like Cl at the α-position reduces the intrahydrogen bonding within the chelated ring [54] which may lead to reduce the rigidity of the molecule.

3.4. The temperature dependence of the fluorescence spectra

The emission spectra of the chlorocurcumin and methylcurcumin at different temperatures (10-50 °C) are presented in Figure 8 and 9. The emission curves indicate no changes with temperature within the range used. This implies that the compounds do not suffer important structural changes and that the relative concentrations of the diketo and the enol forms remain essentially unchanged.

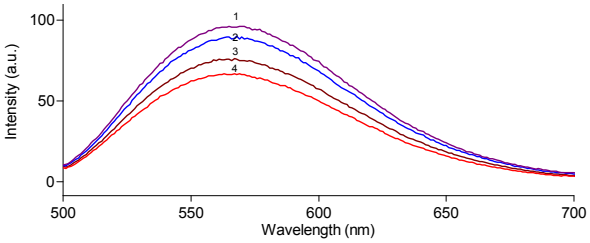

Figure 8. Fluorescence emission of chlorocurcumin in methanol at different temperatures. 1: 10, 2: 25, 3: 40 and 4: 50 °C.

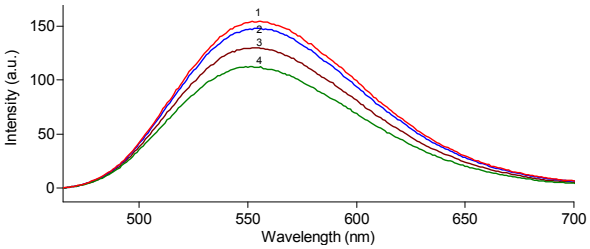

Figure 9. Fluorescence emission of methylcurcumin in methanol at different temperatures. 1: 10, 2: 25, 3: 40 and 4: 50 °C.

The same thing could be concluded from the relation between the emission spectra and concentration as shown in Figure 10 and 11 which display the fluorescence spectra of the molecules at different concentrations in methanol. No change detected in both the shape and the position of the emission bands as the concentration of the compounds varied. Meanwhile the intensity of the spectra changes at certain concentrations above which it decreases as the concentration increases. In Figure 10, the intensity of the emission of chlorocurcumin increases as the concentration increases from 1×10^{-6} to 1×10^{-4} M while it decreases at the concentration 2.5×10^{-4} M. This is also the case with Figure 11 in which the emission of methylcurcumin increases as the concentration increases from 1×10^{-5} to 5×10^{-5} M while it decreases as the concentration changes from 1×10^{-4} to 3×10^{-4} M. This change in intensity is due some kind of aggregation of the molecules leading to fluorescence non-reactive combinations.

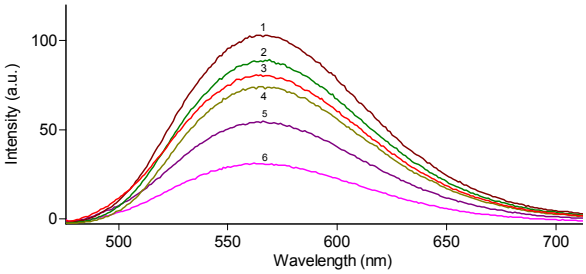

Figure 10. Fluorescence emission of chlorocurcumin in methanol at different concentrations: 1: 3×10^{-5}; 2: 4×10^{-5}; 3: 1×10^{-5}; 4: 5×10^{-5}; 5: 5×10^{-6}; 6: 2.5×10^{-6} M.

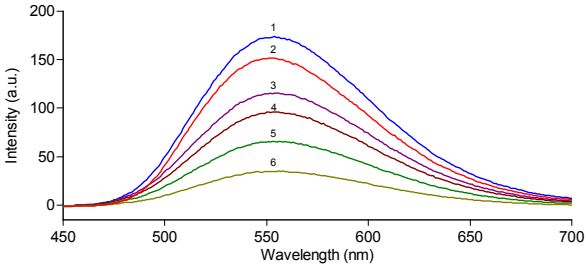

Figure 11. Fluorescence emission of methylcurcumin in methanol at different concentrations: 1: 5×10^{-5}; 2: 1×10^{-4}; 3: 3×10^{-4}, 4: 3×10^{-5}; 5:2×10^{-5}; 6:1×10^{-5} M.

Even though methylcurcumin clearly shows distinct bands due to its diketo and enol tautomers no wavelength dependence was recorded in its fluorescence excitation spectra. The excitation spectra of this compound recorded in toluene at several different emission wavelengths (Figure 12) and were identical and matched fairly the absorption peak at visible region.

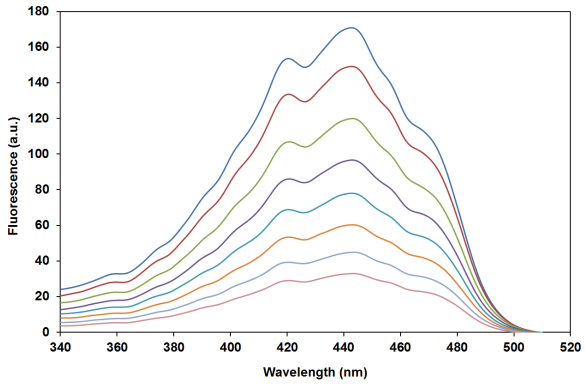

Figure 12. Fluorescence emission of methylcurcumin at different excitations. The excitation wavelengths from top to bottom are 520, 530, 540, 550, 560, 570, 580 and 590 nm.

The absence of the peak of the diketo at about 350 nm is an indication for the assignment of this peak and that the diketo isomer has no role in the fluorescence activity of this compound.

3.5. The fluorescence quenching by water of α-chloro- and α-methylcurcumin

It has been reported that water quenches curcumin [39] as a result of formation of a non-fluorescent complex between curcumin and water. Due to the solvent effect, linear plot was only obtained when using the modified Stern-Volmer relationship (1) to describe the quenching of curcumin by water [55],

$$I_{Fl}^{\circ}/I_{Fl} = (1 + k_q.\tau.[H_2O])/W \quad (1)$$

where I_{Fl}° and I_{Fl} are the fluorescence intensity in the absence and in the presence of water, respectively. k_q is the rate constant for quenching and τ is the life time of the excited singlet state. The fluorescence life time of curcumin was estimated to be 1.44 psec assuming that the quenching rate constant is equal to the rate constant of diffusion (k_{diff}) in MeOH.

In order to investigate the quenching effect of water on the fluorescence of α-chlorocurcumin and α-methylcurcumin, the fluorescence intensity of solutions of the compounds in MeOH containing different concentrations of water was measured. The results are gathered in Table 5.

Table 5. Effect of various concentrations for water (%*v*:*v*) on the fluorescence of α-chlorocurcumin and α-methylcurcumin.

[H_2O] (%*v*:*v*)	η°/η	α-chlorocurcumin			α-Methylcurcumin		
		I_{FI}	$I_{FI}°/I_{FI}$	$I_{FI}°/I_{FI} \times η°/η$	I_{FI}	$I_{FI}°/I_{FI}$	$I_{FI}°/I_{FI} \times η°/η$
0	1.00	121.325	1.000	1.000	140.602	1.000	1.000
3	0.99	96.223	1.261	1.248	111.819	1.257	1.244
5	0.96	80.493	1.487	1.427	94.569	1.487	1.428
7	0.95	72.514	1.673	1.589	85.903	1.637	1.555
10	0.92	62.237	1.933	1.778	71.347	1.971	1.813
20	0.85	41.468	2.926	2.487	45.806	3.069	2.609
30	0.79	28.368	4.277	3.379	34.547	4.070	3.215

Linear plots with slopes 1.39×10^{-2} and 1.34×10^{-2} M^{-1} for α-chlorocurcumin and α-methylcurcumin, respectively, were obtained using Equation 1 (Figure 13 and 14). Assuming that $k_q \approx k_{diff} = 9.6\times10^{9}$ $M^{-1}\cdot s^{-1}$ for MeOH [40], the fluorescence life times τ is 1.45 and 1.40 psec. The life times are comparable to the value reported previously for curcumin.

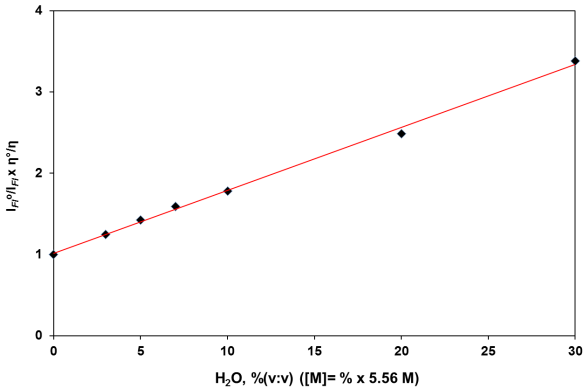

Figure 13. Stern-Volmer plot for the fluorescence quenching by water of chlorocurcumin in methanol.

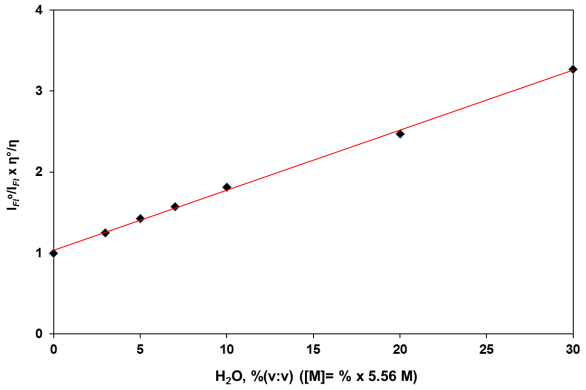

Figure 14. Stern-Volmer plot for the fluorescence quenching by water of methylcurcumin in methanol.

4. Conclusion

From the absorption spectra of the studied compounds and their calculated thermodynamics quantities it could be concluded that α-methylcurcumin has higher diketo content than α-chlorocurcumin. The maxima of the fluorescence bands are sensitive to both the polarity of the solvent and its H-bonding ability as an indication to the presence of several species responsible for emission activity. The quantum yields of the fluorescence are lower than those of curcumin in all solvents which, implies that the chloro and the methyl substituents reduce the quantum yield. The methyl substituent has greater effect in reducing the quantum yield than the chloro due its role in increasing the diketo content.

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