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New insights on the Chemical Stability of Curcumin in Aqueous Media at Different pH: Influence of the Experimental Conditions

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From spectrophotometric and electrochemical measurements recorded in curcumin aqueous solutions at different pH values, both the stability and the acidity constants of curcumin were evaluated. It was found that contrary to hitherto acceptances, curcumin becomes stable at basic pH values while at acid ones curcumin rate of degradation is around 20 times higher than in neutral or basic conditions. Notwithstanding, by controlling the experimental conditions the degradation rate of curcumin can be diminished even at acid and neutral conditions. Our results show that curcumin has 3 pKa values, namely: 7.428 ± 0.015, 9.552 ± 0.024 and 10.946 ± 0.034, and for the first time, the molar absorptivity coefficients of the curcumin species in aqueous media: H_3Cur, H_2Cur^-, $HCur^{2-}$ and Cur^{3-}, were reported as a function of the wavelength.

Keywords: Curcumin; stability; aqueous solution; pH; pKa

1. INTRODUCTION

Curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione] the main component of turmeric curcumin, also known as curcuma, is constituted by demethoxycurcumin, the bisdemethoxycurcumin and the cyclocurcumin. It is found in the roots of the *Curcuma Longa L*. plant [1]. The curcumin is a phenol compound with antiinflamatory [1, 2], anticarcinogenic [1, 3-5], antioxidant [1, 3, 6-8] and antiviral [9-13], properties that have been amply reported. Likewise, is used as coloring and spice in the textile and food industries [14], respectively. However, its low solubility in water gives rise to a low biodisponibility, which has hindered its potential application in the

pharmaceutical industry in spite that the Food and Drug Administration Agency, (FDA), of the U.S. federal government states that curcumin is not toxic. It has been reported that aqueous curcumin dissolutions undergo degradation processes. Tønnesen and Karlsen [15] evidenced curcumin degradation in the 7 to 10 pH interval that led them to maintain the working pH below 7 to ensure stability, although they also found that vanillin, ferulic acid and diferuloylmethane are the main degradation products [16]. Further, Wang et al. [17], evidenced the curcumin degradation in different buffer systems (citrates, phosphates and carbonates) at 0.1 M and determined a first order kinetics at 37 °C, reporting also increasing curcumin stability at acid pH in an aqueous medium, with an apparent half-life time, $t_{1/2}$, that increased to 118.63 minutes as the pH value turned more acid. Similarly, the same three degradation products were reported as those by Tønnesen and Karlsen [16] and concluded that the vanillin was the main degradation product. Tønnesen and Karlsen [16] and Wang et al. [17], imposed the curcumin solutions under study, at very acid pH, where the latter authors [17] added HCl 6N until attaining pH = 3. Recently, Gordon et al. [18], proposed an autooxidation process as the curcumin degradation mechanism (conducted in 500 μL of 10 mM NH_4OAc buffer, pH 7.4, at room temperature) to enable formation of bicyclopentadione as the main degradation product, suggesting thereby that the products reported by Tønnesen and Karlsen [15, 16] and by Wang et al. [17], are not the main ones for this degradation mechanism. On consideration of the initial pH difference of the curcumin solutions between these research works, there exists the possibility that in case of Tønnesen and Karlsen [15, 16] and Wang et al. [17], the curcumin used had already been degraded by the highly acidic media, to which it had been subjected prior to extraction and chromatographic injection, thus giving rise to a positive false with respect to its stability in acid media. Many authors have assumed valid these curcumin stability results in acid pH [19-21] and then proceeded to study diverse relevant curcumin aspects, namely: acidity constants and β-cyclodextrin-Curcumin inclusion complex constant determination [19], curcumin antioxidant capacity [20], curcumin quantification [19, 21] and its anticarcinogenic properties [5]. Therefore, this work considers very important to study, once more, the curcumin stability in aqueous media at different pH values.

2. EXPERIMENTAL

2.1 Reactants.

Curcumin R. A. (≥ 99.5 %) was from Sigma Aldrich, the NaOH from Macron, concentrated HCl from Merck and deionized water (18.2 MΩcm), free from organic matter from a PURE-LAB Plus UV unit.

2.2 Spectrophotometry study.

Solutions preparation. An aqueous solution containing 0.002 M curcumin and 5 mM NaOH (pH = 13.646 ± 0.001) was prepared and stored in a refrigerator. From this stock solution, a 120 μL

aliquot was taken to prepare the working solution, having a 33 μM curcumin concentration, along with those of NaOH and HCl at various concentrations to set the pH adequately.

2.2.1 Acquisition of the absorption spectra.

All absorption spectra were recorded aided by a spectrometer Lambda 20 UV Vis fitted with 1 cm optical path quartz cells. The spectra acquisition interval was 200 to 700 nm at 1960 nm min^{-1} rate.

2.3 pH measurement.

A sensION potentiometer (HACH Sension+ PH31) fitted with a Ag / AgCl (KCl, 3 M) refillable, double union with silver ion barrier glass electrode (Sension+ + CAT, 5014T, (high performance); the pH range was from 0 to 14, from -10 to 100 ºC, which allowed measurements of the pH up to three significant figures.

2.4 Stability study.

The curcumin working solution absorbance was monitored as a function of time at three different pH values: 3.576 ± 0.001, 7.025 ± 0.001 and 10.526 ± 0.001 under experimental conditions termed "without control" and "controlled", the latter to prevent ambient light impinging on the solutions, after $N_{2(g)}$ bubbling for 5 min, also keeping a gas shroud over the dissolution surface throughout the duration of the experiment while imposing a constant temperature, T = (21.0 ± 0.1) °C.

2.4.1 Electrochemical measurements

Cyclic voltammetry experiments were conducted, at different times, in each of the curcumin aqueous solutions having different pH values using a typical three-electrode electrochemical cell where the working electrode was a carbon paste electrode, CPE, (see Ramírez-Silva et al. [22, 23]), the reference and the counter electrodes were: Ag /AgCl [KCl, 3 M], BAS MF-2052, and a platinum wire, BAS MW-1032, respectively, both from BASi. The working electrode potential was controlled with a potentiostat/galvanostat Epsilon-Basi coupled to a computer running the software Basic Plus for the experiment control and data acquisition.

2.5 Determination of the acidity constants.

The software known as SQUAD [24] was used for the estimation of the curcumin acidity constants through analysis of the curcumin UV Vis absorption spectra as a function of pH in aqueous media.

3. RESULTS AND DISCUSSION

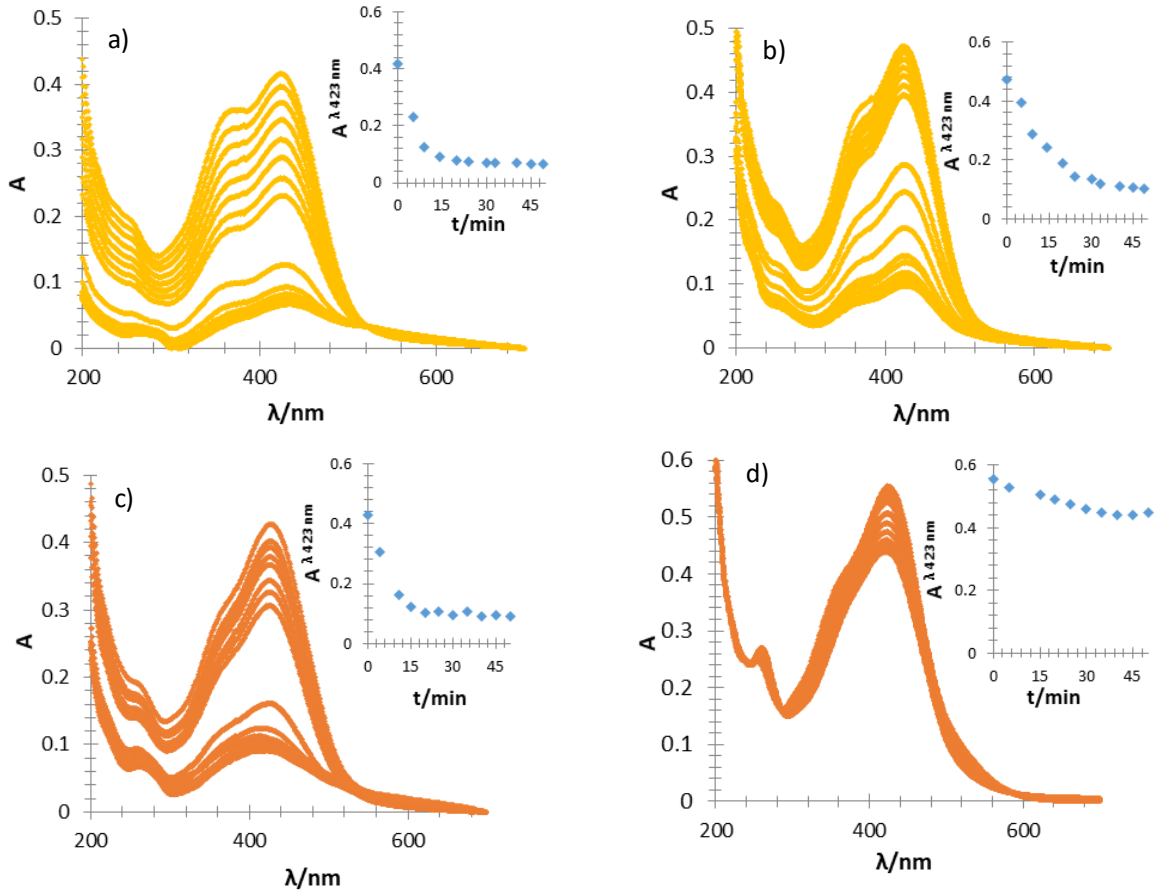
3.1 Curcumin stability in aqueous solution at different pH values

3.1.1 Spectrophotometric measurements

Figure 1 shows families of absorption spectra recorded from curcumin solutions at various pH and experimental conditions set as well. Note that at acid pH, see Figures 1a and 1b, the absorbance diminished drastically as a function of time regardless of the control on the experimental conditions, see insets in Figures 1a and 1b. At a pH close to neutral, Figures 1c and 1d, the absorbance variation also dependent on time, see inset in Figure 1c, is indeed similar to what was observed at acid pH, under uncontrolled experimental conditions. However, when controlling these, see Figure 1d inset, the absorbance variation as a function of time does not change drastically at all. This same can be observed for basic pH independently of the experimental control imposed, see insets in Figures 1e and 1f. Based on the absorbance temporal variation, a curcumin degradation percent (%CD) can be defined as follows:

$$\%CD = 100\ (A_0 - A_t / A_0) \quad (1)$$

where A_0 is the initial absorbance and A_t is the absorbance at time t.



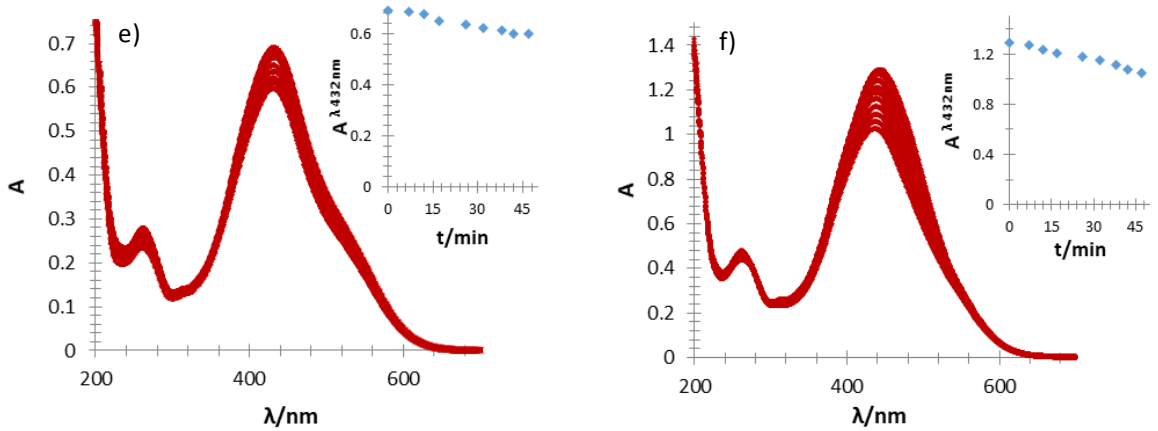


Figure 1. Absorbance spectra recorded in an aqueous solution containing 33 μM curcumin at different pH values and experimental conditions: a) pH = 3.576 ± 0.001 without control of the experimental conditions, b) pH = 3.576 ± 0.001 controlling the experimental conditions, c) pH = 7.025 ± 0.001 without control of the experimental conditions, d) pH = 7.025 ± 0.001 controlling the experimental conditions, e) pH = 10.526 ± 0.001 without control of the experimental conditions and f) pH = 10.526 ± 0.001 controlling the experimental conditions. The insets depict the variation of the absorbance recorded at 423 nm (a-d) or at 432 nm (e and f) as a function of time.

Figure 2 shows the %CD variation as a function of time for the different pH and experimental conditions considered.

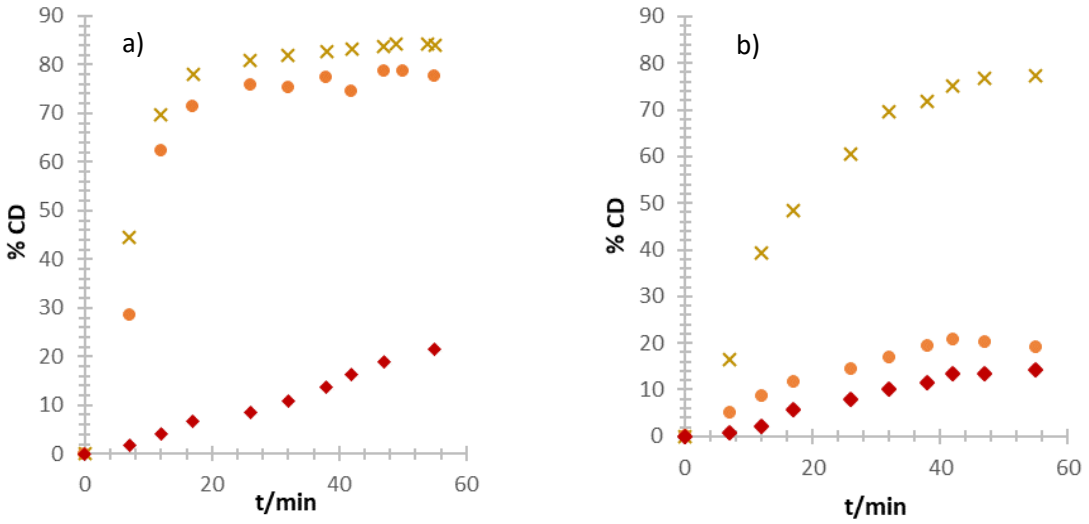


Figure 2. Percent curcumin degradation (%CD) as a function of time in an aqueous solution containing 33 μM curcumin at different pH values: (**X**) 3.576 ± 0.001, (●) 7.025 ± 0.001 and (♦) 10.526 ± 0.001, and different experimental conditions: a) without control of the experimental conditions and b) controlling the experimental conditions.

From Figure 2a, recorded without controlling the experimental conditions and for pH acid or neutral, the degree of curcumin degradation increased abruptly with time. Note that at 7 minutes the %CD is greater by about 45 % in acid medium and by 30 % in neutral, however at basic pH it is only 2 % but controlling the experimental conditions, see Figure 2b, the degradation at 7 minutes is 16 % at

acid pH, and 5% and 1 % for neutral and basic pHs, respectively. In all cases, the curcumin is more unstable at acid pHs, which is quite the contrary to that ascertained by Tønnesen and Karlsen [15, 16] and Wang et al. [17]

From the plots in Figure 2a and 2b the rate of degradation, V_{deg}, can be defined as the time derivative of the curcumin degradation percent:

$$V_{deg} = d/dt\ (\%CD) \quad (2)$$

For the first 20 minutes, the %CD variation as a function of time was practically linear, then the degradation rate can be estimated from the lines slope, see Table 1, from which it can be observed that the greatest degradation rate takes place at acid pH.

From the V_{deg} data, see Table 1, and that shown in Figure 1 and Figure 2 it may well be concluded that the curcumin in aqueous solution turns more stable as the pH becomes basic. Notwithstanding, control over the experimental conditions inevitably diminishes the degradation rate. It is quite important to mention that during all these experiments the presence of precipitates was not observed, therefore, the absorbance changes described of the curcumin solutions used are due solely to curcumin degradation and not to variations in the curcumin solubility.

Table 1. Values of the curcumin degradation rate, $V_{deg} = d/dt(\%CD)$, in an aqueous solution at different pH values and control of the experimental conditions.

	V_{deg}/ %CD min^{-1}	
pH	Without control of the experimental conditions	Controlling the experimental conditions
3.576 ± 0.001	7.80 ± 0.75	2.86 ± 0.29
7.025 ± 0.001	4.78 ± 0.59	0.47 ± 0.03
10.526 ± 0.001	0.39 ± 0.02	0.34 ± 0.02

3.1.2 Electrochemical measurements

In order to further verify the stability of curcumin, cyclic voltammetries were conducted, at different times, in each of the curcumin aqueous solutions having different pH values. Figure 3 depicts CVs where it is possible to note that for acid and neutral pH values, see Figure 3a and 3b, the CVs taken at the beginning of the experiment, named as 0 min, are quite different from those taken after 60 min, indicating that different curcumin chemical species were formed with time, however, for basic pH, see Figure 3c, the CVs are practically the same which indicates that curcumin was stable at this pH condition as the spectrophotometric studies previously revealed.

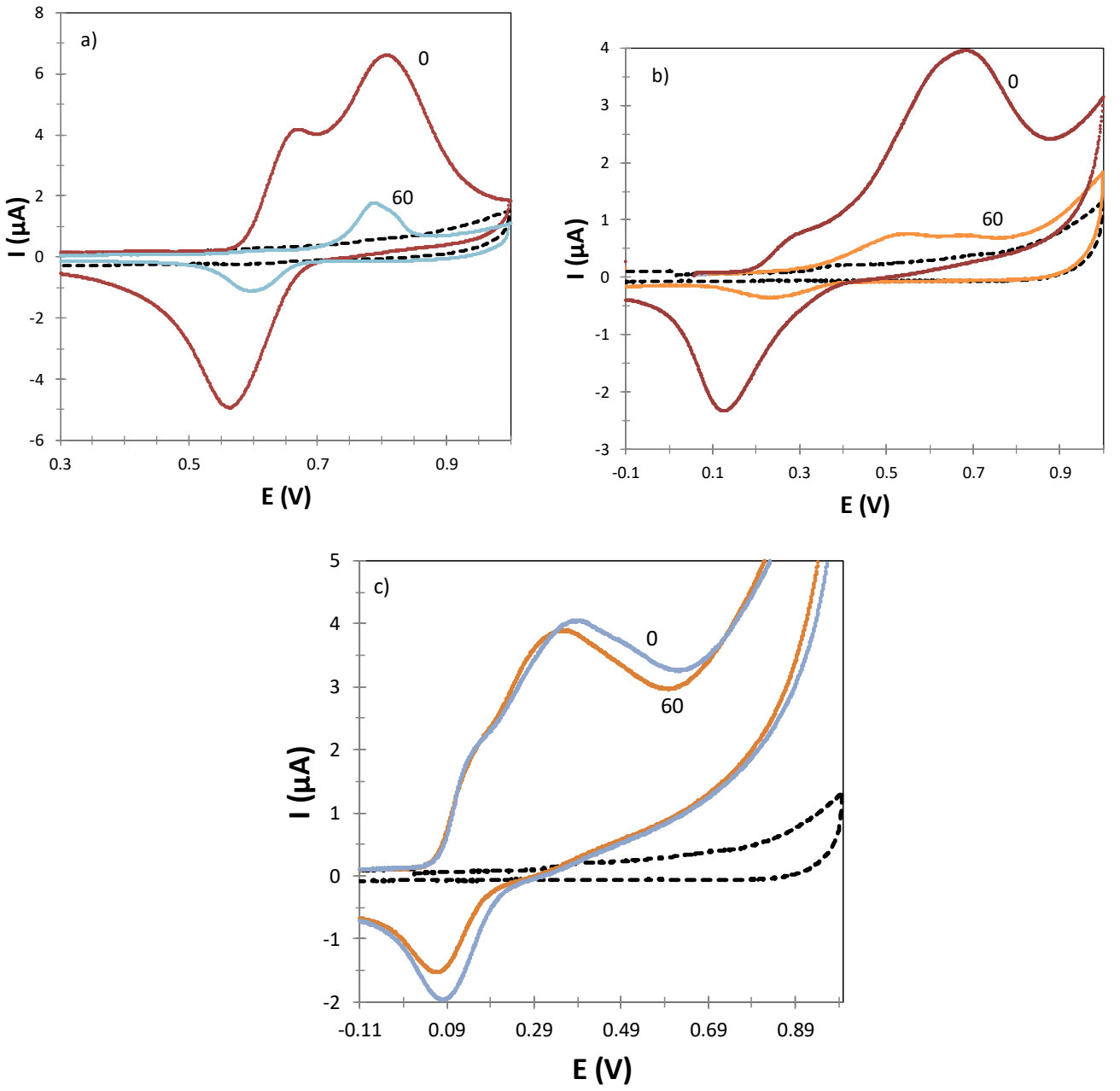
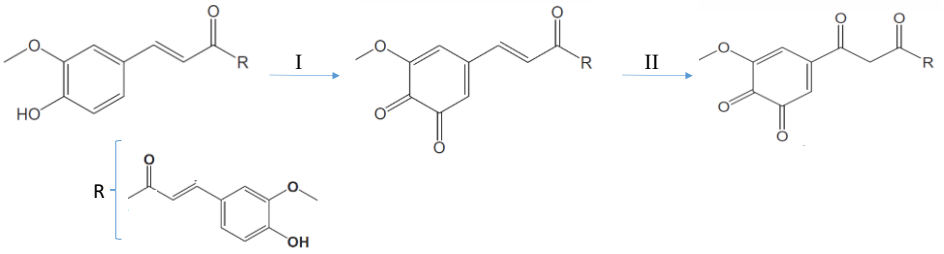


Figure 3. Experimental CVs recorded in the system CPE / Curcumin 33 µM at different pH values: a) 3.576 ± 0.001, b) 7.025 ± 0.001 and c) 10.526 ± 0.001 and two different times: 0 (experiment beginning) and 60 min. In all cases the potential scan started at −0.1 V in the positive direction at 100 mVs^{-1}. The dashed lines correspond to the CVs recorded at each pH in the absence of curcumin (blanks).

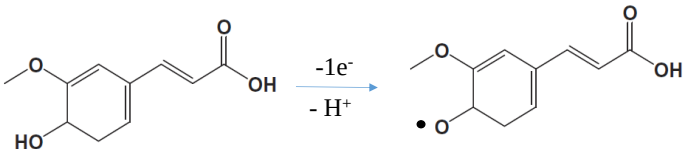
Manaia et al. [25], have proposed the electrochemical reactions, see Reac. (1), involved during curcumin oxidation, which are associated with the two anodic peaks observed in Figure 3 (a, b and c) for the CVs recorded at time 0 (experiment beginning). During curcumin electrochemical oxidation, at the first anodic peak, there occurs formation of the phenoxy radical, which undergoes hydrolysis at the ortho-position, and the two hydroxyl groups in the benzene ring of an orthoquinone moiety

electrochemically generated are immediately oxidized (stage I in Reac.(1)). At higher potentials (stage II in Reac. (1)) also occurs due to an oxidation, after hydroxylation at position 1 and/or 7.



REAC. (1)

The CVs depicted in Figure 3 (a and b) recorded after 60 min can be associated with the electrochemical oxidation of ferulic acid (3-methoxy-4-hydroxycinnamic acid), one of the curcumin degradation products that have been observed [16] and the electrochemical reaction associated (see React. (2)) have been proposed by Kallel Trabelsi et al. [26]



REAC. (2)

3.2 Spectrophotometric estimation of the curcumin acidity constants under controlled experimental conditions

Figure 4a shows a family of absorption spectra recorded from aqueous curcumin solutions at different pH values. A hypsochromic shift occurs in the absorption maximum located at 471 nm as the pH turns more acid. Further, insofar as the pH becomes more acid, the absorption spectra develop a new, well defined band at 357 nm. Likewise, note several isosbestic points at 306, 327, 382, 421, 428 and 530 nm, which suggest that more than one curcumin acid-base equilibrium occur in solution. Their acid-base nature corresponds to successive dissociations of the acid protons of the curcumin molecule, which in agreement with its enol form structure they could be three. In order to consider the curcumin three deprotonations in aqueous media, these are input to SQUAD [24] along with information of the 25 absorption spectra as a function of pH every 4 nm (from Figure 4): for further details of such procedure see Palomar-Pardavé et al. [27] Table 2 shows the refined values of the acidity constant from the SQUAD software for curcumin, that are compared with those reported in the literature.

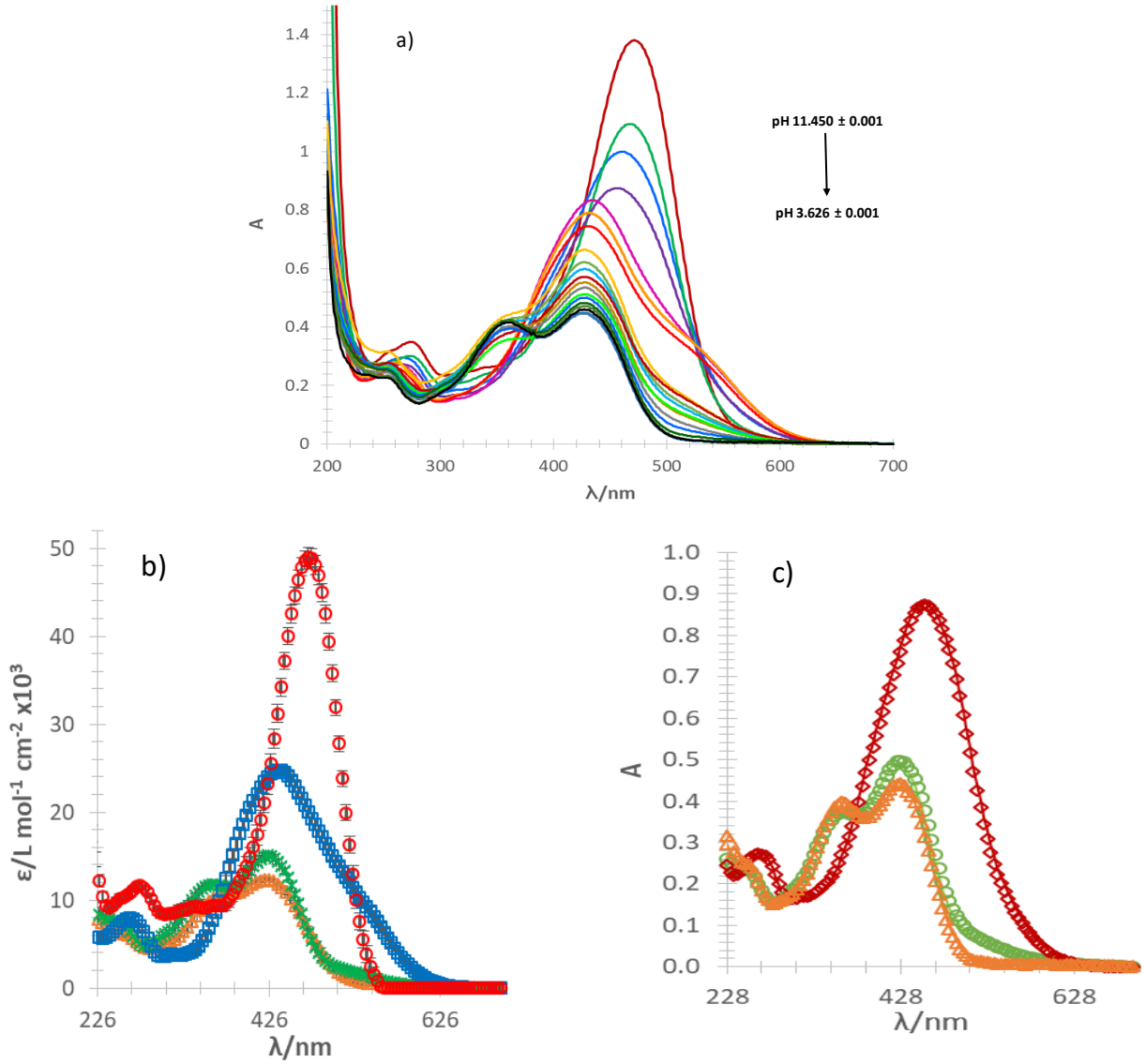


Figure 4. a) UV-Vis spectra recorded in aqueous solution containing 33 μM curcumin at different pH values shown in the figure. Experimental conditions were controlled (protection from light, N_2(g) bubbling and temperature control (21.0 ± 0.1) °C). b) Molar absorptivity coefficients for the curcumin species in aqueous media: (Δ) H_3Cur (X) H_2Cur^- (□) $HCur^{2-}$ (O) Cur^{3-}, estimated from analysis of the spectra in Figure 3a using the SQUAD software. c) Comparison of the theoretical spectra (lines) with the corresponding experimental spectra (points) for the different pH: (♦) 10.526 ± 0.001, (●) 7.025 ± 0.001 and (Δ) 3.576 ± 0.001.

From the comparison of the values obtained for the curcumin acidity constant in this work, it becomes apparent that there is a difference of two units with respect to the other two values. The difference between the values of pKa_2 and pKa_3 is only 1.4 units approximately. Such closeness of the last two pKa values indicates that pKa_2 and pKa_3 correspond to deprotonation of the phenols located at each end of the molecule (due to the symmetry of the curcumin molecule), whereas that of pKa_1 is associated with a keto-enol equilibrium.

From the comparison of this acidity constant value with those shown in Table 2 the closeness between them becomes clearly apparent with only a difference not larger than a unit for pKa_2 and

pKa_3. Therefore, the most significant difference is over the first acidity constant, because the curcumin is largely degraded in acid solutions, as clearly shown before. In this sense, it is relevant to add that in this work instead of working with only one curcumin solution to modify its pH, there were prepared 32 curcumin solutions setting them under controlled conditions with different pHs, and then recording their respective absorption spectra, in order to minimize the period during which a particular curcumin sample was exposed to its respective acidity condition. This form of point analysis together with the use of the software SQUAD allows working with large quantities of absorbance and wavelength data (4975 data from 25 absorption spectra sampled every 4 nm), thus granting a higher degree of reliability to the pKa values reported.

Table 2. Acidity constant values reported for curcumin in aqueous solution

Method	***pKa₁*** $H_3Cur = H_2Cur^{-} + H^{+}$	***pKa₂*** $H_2Cur^{-} = HCur^{2-} + H^{+}$	***pKa₃*** $HCur^{2-} = Cur^{3-} + H^{+}$	***Ref.***
HPLC with florescence detector	7.75 - 7.80	8.55 ± 0.05	9.05 ± 0.05	15
UV Vis	8.10 ± N.R.	10.45 ± N.R.	N. R.	19
UV-Vis	8.55 ± 0.05	10.41 ± 0.05	N. R.	28
Spectrophotometric titration	8.38 ± 0.04	9.88 ± 0.02	10.51 ± 0.01	29
Spectrophotometric	7.428 ± 0.015	9.552 ± 0.024	10.946 ± 0.034	**This work**

N.R. Not reported.

Figure 4b shows the curcumin absorptivity coefficient values for the 4 curcumin species present in this system and the comparison of the theoretical and experimental spectra, see Figure 4c, obtained through figure 3a experimental data analysis with SQUAD. Note from Figure 4c that the fitting of the theoretical data on the experimental ones is quite good, showing thus that the acidity constant values estimated through this methodology are plainly reliable.

With the curcumin acidity constants data obtained in this work, a species distribution diagram was processed, see Figure 5a, and from the predominant zone diagram depicted in Figure 5b (see Rojas-Hernández et al. [30, 31]) it is possible to note that below pH 7.428 ± 0.015, the predominant

species is the curcumin fully protonated (H_3Cur), whereas above a pH value of 10.946 ± 0.034 the fully deprotonated form predominates (Cur^{3-}). Between these values, there exists a mix of the mono ($HCur^{2-}$) and diprotonated (H_2Cur^{-}) species.

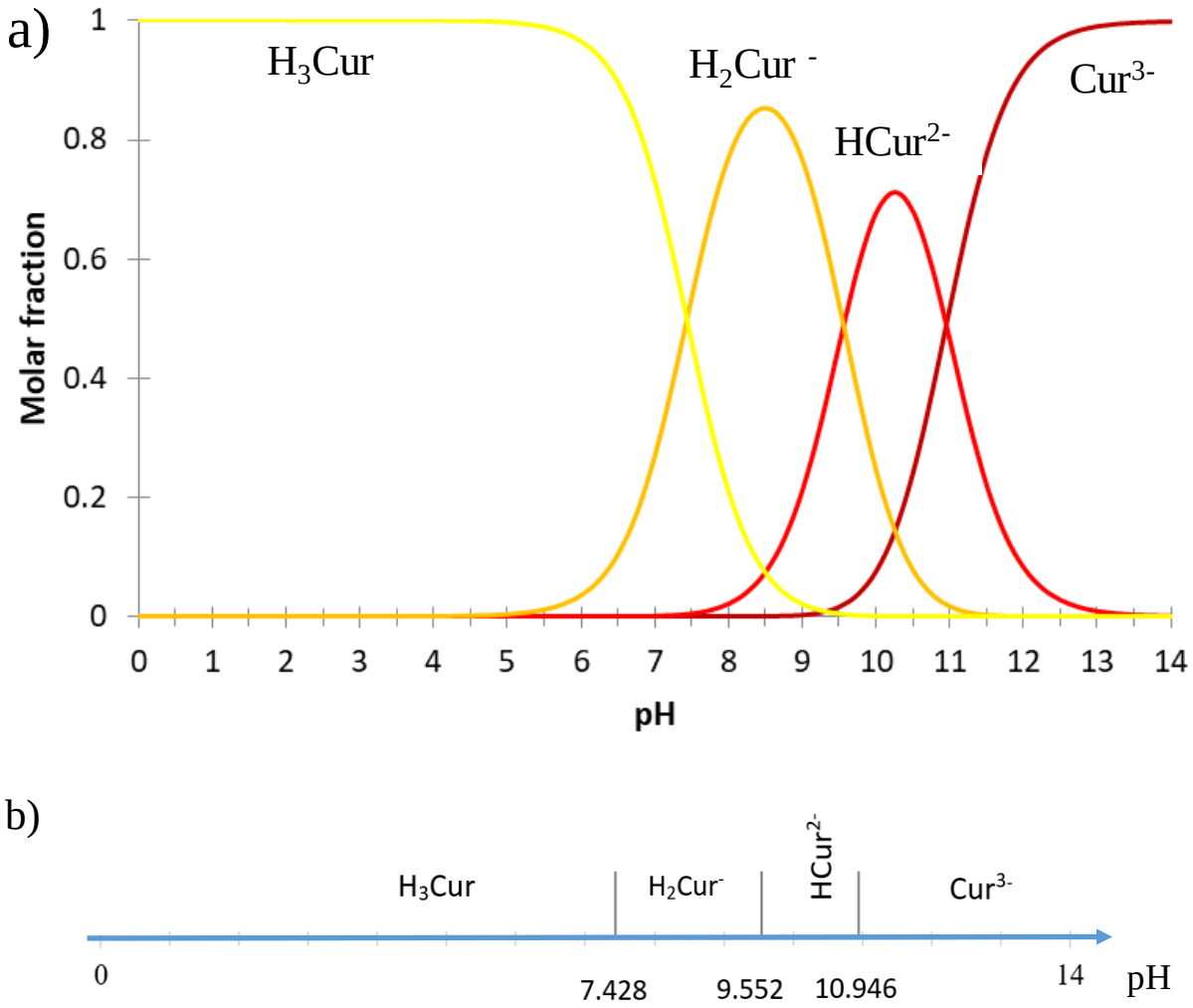


Figure 5. a) Curcumin molar fraction diagram in an aqueous medium using the pKa values reported in this work, see Table 2; b) the corresponding predominance zone diagram.

4. CONCLUSIONS

It was determined that the stability of curcumin is greater as the pH value increases and not under conditions of acid pH as several authors have claimed **[15-17]**. The stability of curcumin in aqueous medium is improved in all cases when working solutions are protected from incident light, work carried out under a N_2 (g) atmosphere (after bubbling with this gas for 5 minutes) and controlled temperature. A good fit was found between the experimental and theoretical spectrophotometric data

(obtained with the SQUAD software) giving reliability to the pKa values calculated in this work, which are duly contrasted with those already reported.

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