CURCUMIN AS ACID/BASE INDICATOR: A STATISTICAL ANALYSIS

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ABSTRACT: Since the world became aware of environmental issues, natural pH indicators have been widely study to replace those synthetics to identify the end point of acid-base titrations. However, most studies only present qualitative evidence for the use of these indicators. In this paper, the Curcumin (CUR) application viability in volumetric titrations as pH natural indicator was performed not only qualitative but also quantitative analysis in comparison to phenolphthalein. The methodology employed was visual, spectroscopic and statistical hypothesis tests ($p< 0.05$). The qualitative results showed that the CUR changed color as a function of pH and its application was clear, fast and satisfactory as an acid-base indicator. Such color changes were assigned to the CUR keto-enol tautomerism and characterized by UV-Vis. By quantitative analysis, it was observed that there were no significant differences between the both confidence interval of the means for the calculated hydrochloric acid (HCl) and acetylsalicylic acid (ASA) concentration titrated by CUR and phenolphthalein as an indicator. The same observations was done by the paired t-test with comparable standard deviation. It suggests that CUR can easily replace phenolphthalein on the acid-base volumetric in a low-cost, affordable and eco-friendly way, especially on the chemical education.

KEYWORDS: Statistic. Titrations. Analytical Chemistry.

CURCUMIN COMO INDICADOR DE ÁCIDO/BASE: UMA ANÁLISE ESTATÍSTICA

RESUMO: Desde que o mundo se conscientizou das questões ambientais, os indicadores naturais de pH têm sido amplamente estudados para substituir os sintéticos para identificação do ponto final nas titulações ácido-base. No entanto, a maioria dos estudos apresenta apenas evidências qualitativas para o uso desses indicadores. Neste trabalho, a viabilidade de aplicação da Curcumina (CUR) em titulações volumétricas como indicador natural de pH foi realizada não apenas qualitativa, mas também quantitativamente em comparação com a fenolftaleína. A metodologia empregada foi visual, espectroscópica e testes estatísticos de hipóteses ($p< 0,05$). Os resultados qualitativos mostraram que a CUR mudou de cor em função do pH e sua aplicação foi clara, rápida e satisfatória como indicador ácido-base. Tais mudanças de cor foram atribuídas ao tautomerismo ceto-enólico da CUR e caracterizadas por UV-Vis. Pela análise quantitativa, observou-se que não houve diferenças significativas entre os dois intervalos de confiança das médias para a concentração calculada de ácido clorídrico (HCl) e ácido acetilsalicílico (ASA) titulada pela CUR e fenolftaleína como indicador. As mesmas observações foram feitas pelo teste t pareado com desvio padrão comparável. Sugere-se que a

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CUR pode substituir facilmente a fenolftaleína na volumetria ácido-base de forma econômica e ecologicamente correta, principalmente no ensino de química.

PALAVRAS-CHAVE: Estatística. Titulação. Química Analítica.

1. INTRODUCTION

Acid-base indicators are substances that due to their physicochemical and structural properties, shows chromophores groups that can change their color in the presence of H^+ (acid form) or OH^+ (basic form) ions concentration (Skoog et al, 2016). These indicators can be synthetic or natural and are widely used in laboratories when it is needed to determine the acidic-basic character of a solution or to indicate the end point of a volumetric titration (Khan and Farooqui, 2011).

The natural indicators are organic substances, with weakness acid or basic character. They are found in leaves, flowers or fruit plants and change of coloration according to the pH (Terci and Rossi, 2002). Natural dyes have been used as “green” acid-base indicators, as an alternative to commonly used indicators, such as phenolphthalein, bromothymol blue, methyl red and others (Mendham et al, 1999).

Curcumin (CUR, 1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-hepta-diene-3,5-dione) is one of the three curcuminoid extracted from turmeric (Leung and Kee, 2009). It is well known that its molecular structure (Figure 1) shows high conjugation, two ionizable hydroxyls as well as an unsaturated diketone that can undergo keto-enolic tautomerism as a function of pH (Chignell et al, 1994; Priyadarsini, 2009; 2014). All these characteristics make this molecule a promising natural acid-base indicator, since CUR exhibits a color in acidic or neutral solution (diketone form) and a different color in alkaline conditions (keto-enol form). (Jovanovic et al, 1999; Supharoek et, 2018).

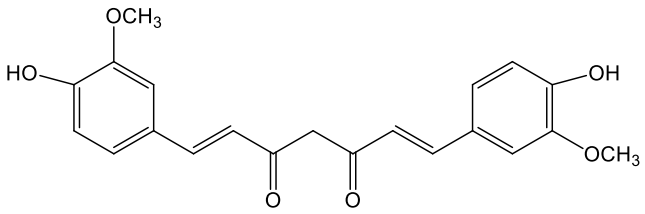

Figure 1- Molecular structure of Curcumin.

In general, the CUR and others natural pH indicators studies in titrations had the focus in qualitative analysis (Terci and Rossi, 2002; Abugri, Apea and Pritchett, 2012; Bahadori and Maroufi, 2016; Kapilraj, Keerthanan and Sithambaresan, 2019). Until now, have almost no papers to prove the quantitative efficiency of CUR as an indicator in acid-base titrations. To fill this gap, this study reports the statistical evidence of the applicability of CUR as a pH indicator in neutralization volume, comparing the CUR against to phenolphthalein (the synthetic indicator commonly used) by the confidence interval for the mean comparison and the paired and unpaired t-test applied to the titrations data.

2. EXPERIMENTAL

CUR was kindly provided for our study from local pharmacy (Barra do Garças-MT, Brazil) and used as received. A stock solution of CUR (5.49×10^{-2} mol L^{-1}) was prepared in ethanol and stored in the dark at 4ºC. This solution was diluted in aqueous solutions as function of the pH from 1 to 14 using H_3COOH (Dinâmica), HCl (Merck), NH_4OH (Neon) or NaOH (Dinâmica) 6 mol L^{-1} solutions. The pH was tested using a universal indicator.

The qualitative analysis of CUR application viability in volumetric titrations as acid-base indicator was done as function of pH by spectroscopy and comparing visually the colors of CUR aqueous solutions before/after the volumetric titration end point (Silva Brito and Gonçalves, 2018). Ultraviolet-visible absorption spectra were recorded on a Perkin Elmer-Lambda 25 Spectrophotometer in the range of 300–700 nm. For these measurements, CUR solution concentrations were of the order of 1 μ mol L^{-1}.

The quantitative analysis were done by classic acid-base titrations (Harris, 2010; Skoog et al, 2013) using five drops of CUR or three drops of phenolphthalein (Neon) 1% as an end point indicator. The titrant was a [NaOH] = 0.09724 mol L^{-1} solution previously standardized with potassium biphthalate as the primary standard. The titles were (i): [HCl]= 0.1000 mol L^{-1} solution previously standardized with sodium tetraborate as the primary standard and (ii) commercial tablets of ASA, which the indicated amount by the producer was 500mg. All solutions were standardized by conventional methodology (Mendham et al,1999; Harris, 2010; Skoog, 2013). All the titrations were leaded at 25ºC.

For statistical evidence, initially, these data were submitted to the Q-Dixon test for the identification and rejection of anomalous values by the follow equation:

$$Q = \frac{|V_s - V_n|}{V_h - V_l} \quad (1)$$

which V_s is the suspicion value, V_n is the nearest value from the suspicion value, V_h is the highest value, V_l is the lowest value and Q is the Q calculated value, Q_{cal}. The Q_{cal} and the Q tabulated value, Q_{tab}, are then compared. All values were considered, which the Q_{cal} value was less than the Q_{tab} value (Mendham et al,1999; Harris, 2010), with 95% of confidence level.

All results were expressed as the mean ± S.D. (n= 3). CUR has been compared to phenolphthalein as indicator based on the hypothesis tests. In order to evaluate the significant differences between the means of the acid concentration obtained by titration using CUR or phenolphthalein as an indicator, the experimental data was performed using confidence interval for the mean ($p<0.05$) calculated using Equation 2:

$$\mu = x \pm t_{N-1} \times \frac{s}{\sqrt{N}} \quad (2)$$

which x is the mean value of the acid concentration obtained by titration using CUR or phenolphthalein as an indicator, t is the t tabulated value, t_{tab} ($p<0.05$, df=2), s is the standard deviation, N is the replicates number and μ is mean value of the calculated confidence interval ($p<0.05$). It is well known that the null hypothesis cannot be rejected if there is no significant statistic difference between both confidence interval for the mean (Mendham et al,1999; Harris, 2010; Pfister and Janczyk, 2013; Miller, Miller, and Miller, 2018).

In the same hand, it was applied the paired t-test for samples with comparable standard deviation ($p<0.05$) calculated using Equation 3:

$$t = \frac{(x_1 - x_2)}{s\sqrt{(\frac{1}{N_1} + \frac{1}{N_2})}} \quad (3)$$

which x_1 and x_2 are the mean value of the acid concentration obtained by titration using CUR or phenolphthalein as an indicator, s is the global standard deviation, N is the replicates number and t is the calculated value, t_{cal}. Likewise for test Q, in the t-test the t_{cal} and the t tabulated value, t_{tab}, are then compared. If the t_{cal} was less than the t_{tab}, the null hypothesis is accepted and there were no significant differences between the samples (Harris, 2010; Miller, Miller, and Miller, 2018).

In order to confirm that the standard deviations for the data were comparable, it was used an F-test by the following equation:

$$F = \frac{s_1^2}{s_2^2} \quad (4)$$

which s_1^2 and s_2^2 are the square of standard deviation for the acid concentration obtained by titration using CUR or phenolphthalein as an indicator, respectively, and F is the F calculated

value, F_{cal}, that should be always more than 1. Once more, in the F-test the F_{cal} and the F tabulated value, F_{tab}, are then compared. If the F_{cal} was less than the F_{tab}, the null hypothesis is accepted and there were no significant differences between both standard deviation (Mendham et al,1999; Pfister and Janczyk, 2013) Therefore they could be put together into a global variance (Harris, 2010) described by the Equation 5:

$$s^2 = \frac{[(N_1-1)s_1^2+(N_2-1)s_2^2]}{(N_1+N_2)-2} \quad (5)$$

which $N_1$is the replies number of titration using CUR as an indicator, N_2 is the replies number of the titration using phenolphthalein as an indicator, s_1^2 and s_2^2 are the square of standard deviation for the acid concentration obtain by titration using CUR or phenolphthalein as an indicator, respectively and s^2 is the global variance with $[(N_1 + N_2) - 2]$ degrees of freedom (df).

In addition, it was applied an unpaired t-test that is used to compare data obtained in a laboratory and/or method with a certified value used as a reference (Equation 6). Just to the weak acid samples were submitted to this test because the reference value about the amount of ASA contained in each tablet was given by the producer.

$$t = \frac{\frac{x-\mu}{s}}{\sqrt{N}} \quad (6)$$

which x is the mean value of the ASA concentration obtain by titration using CUR or phenolphthalein as an indicator, μ is the reference value, s is the standard deviation, N is the replicates number and t is the calculated value, t_{cal}. One more time, in the unpaired t-test the tcal and the t tabulated value, t_{tab}, are then compared. The results are interpreted as the same to the paired t-test described above (Mendham et al,1999; Bruns, Scarminio and Barros Neto, 2006; Harris, 2010; Pfister and Janczyk, 2013)

3. RESULTS AND DISCUSSION

The absorption spectra of CUR were analyzed at acid, neutral and alkaline pH conditions (Figure 2). At pH=1.0 CUR exhibits a main band centered at 434 nm attributed to and π - π * transitions and a weaker band centered at 354 nm due to the n-π* transitions (Jagannathan, Abraham and Poddar, 2012; Gonçalves et al, 2017). It was observed a hypochromic shift on the main band to 423 nm as pH increasing until neutral conditions. Therefore, there was a band broadening and a bathochromic shift up to 462 nm at pH=14. All

these considerable spectral changes as pH function have been widely assigned due to the CUR keto-enol tautomerism (Chignell et al, 1994; Priyadarsini, 2009; 2014).

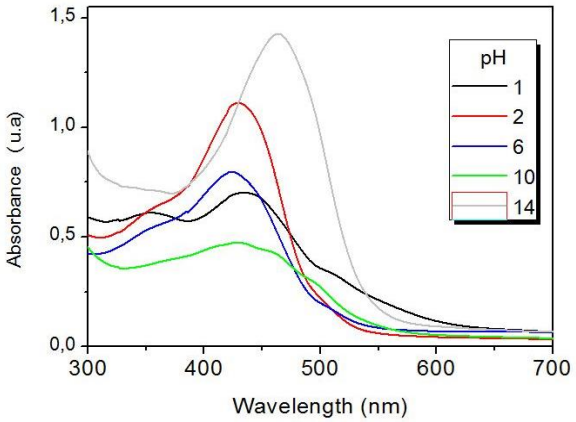

Figure 2- Absorption spectra of CUR as function of the pH.

In a similar study, Suphoroek *et. al.* (2018) had already observed the same spectral shifts as function of pH for a turmeric extract- which active substance as an indicator is curcumin. The authors proposed the equilibrium for such an extract based on these shifts and the changing colors from orange/ reddish-brown (base) to yellow by the acid acetic addition. In acid/neutral and alkaline conditions, the CUR are present in diketone and enolate form, respectively (Suphoroek et al, 2018). As the aim of this paper was a qualitative and quantitative evidence for the use of CUR as an indicator, most of the following discussion will be focused on the acid-basic titration and the statistics of those data.

The qualitative analysis of CUR application viability as acid/base indicator showed that CUR had different colors as function of pH that were easily visually identified suggesting its use as a natural pH indicator as presented in Figure 3. It was observed a yellow solution about to pH 1 to 6 and an orange/red solution above pH.

Figure 3- Color variation of CUR as a function of pH. Observations from left to right pH=1; 2; 6; 10 and 14.

In a similar analysis, Bahadori and Maroufi (2016) had already observed the same color changes to CUR in acidic/base medium for toluene, tetrachloride, chloroform, ethanol and methanol (Bahadori and Maroufi, 2016). As expected, the end point of titration employing both strong and weak acid versus strong base was readily identified by these color changes (Figure 4). Such observations were in agreement with the spectral shifts previously described.

Figure 4- HCl solution titrated with [NaOH] = 0,09724 mol L^{-1} employing CUR as an indicator before (left) and after (right) the titration end point.

The pH range which the indicator can be used is defined as the pH range that the indicator acidic color changes to the basic color and is given by pKa ± 1 (Skoog et al, 2013; Kapilraj, Keerthanan and Sithambaresan, 2019). Jovanovic *et. al.* (1999) had described that pKa_1 of CUR in aqueous solution is 8.55±0.05 and the pKa_2= 10.41±0.05 (Jovanovic et al, 1999). This lead one to believe that CUR can be used as indicator only in titrations whose end point is around pH 7.50 to 9.50 due to this, it was impossible to note the change color from pKa_2. In addition, it had been described that CUR is instable in alkaline conditions (Kumavat et al, 2013; Hazra, Roy and Bagchi, 2014).

The titrations data were shown in the Table 1. In the first step, it had been applied the Q-Dixon test by Equation 1 on the spent titrant volume on titrations using CUR as an indicator. Then, the same test was applied for phenolphthalein results. It was observed that both Q_{cal} were less than the Q_{tab} (0,970 to $p<0.05$, n=3, data not shown). Thus, no value can be rejected –i.e. there were no anomalous volume values (Miller and Miller, 1988). This suggests that

discrepancies in titrant volumes spent are entirely due to indeterminate errors, so all the spent titrant volumes were used to calculate the mean volume and hence the HCl concentrations.

Table 1- Titration date and statistical analysis
(n=3, p<0.05)

	Data analysis	*CUR*	*Phenolphthalein*
Strong acid	Mean volume spent (mL)	9.81±0.06	9.75±0.05
	[HCl] (x10-2 mol L^{-1})	9.49±0.06	9.44±0.05
	Confidence interval for the mean (x10^{-2} mol L^{-1})	9.32<μ<9.56	9.31<μ<9.56
Weak acid	Mean volume spent (mL)	28.85±0.05	28.72±0.10
	[ASA] (mg)	503±2	505±1
	Confidence interval for the mean (mg)	499<μ<508	503<μ<508

In the second step, it had been focused on a simple and often applied statistical analysis: the comparison of two means. Such comparison was done by the confidence interval for the mean at appropriate confidence level ($p< 0.05$) that only includes the mean itself, a standard deviation, the replies number and a coefficient that is derived from the bicaudal t-distribution (Pfister and Janczyk, 2013). This confidence interval can be used for inferring if

the null hypothesis is accept, i.e. both means are equal or if it should be rejected charactering that there are significant difference between then.

It was applied the Equation 2 for the two means obtained through the calculated values for the titrated HCl concentration using CUR and phenolphthalein as an indicator (Table 1). The result showed that the confidence interval for the HCl concentrations means obtained by phenolphthalein completely overlapped to those obtained by CUR as indicator, which made that null hypothesis be accepted. In other words, there were no significant differences between the two means and HCl concentration calculated using both indicators were the same, so CUR and phenolphthalein can be used as an indicator in this kind of neutralization volume as equal statistics.

In the final step, a paired t- test ($p<0.05$) was used as statistical tool to confirm the equality of indicators used in this paper. It was applied an F-test (Equation 4) to confirm that the standard deviations for HCl concentration calculated by titration employing CUR or phenolphthalein as indicator were comparable ($p <0.05$). As expected, the F_{cal} was lower than the F_{tab} (1.314 and 19.000, respectively), reveling that the standard deviations between both indicators did not present significant differences and could be put together into a global variance calculated by Equation 5.

Afterwards, it was applied the t-test by Equation 3 which resulted in a calculated value of t_{cal} = 1,314 that was lower than the t_{tab}=2,776 ($p< 0,05$; df=4), consequently accepting the null hypothesis (Miller and Miller, 1988; Feng et al, 2006). Once more, no significant differences were identified between acid concentrations obtained by titration using CUR or phenolphthalein as indicators. This t-test analysis corroborated not only the qualitative analysis but also the qualitative analysis by confidence interval for the mean.

Abugri *et. al.* (2012) had already used the t-test to statistically prove the equivalence of a natural extract as an indicator in volumetric analysis. Their results showed that the waakye leaves extract (natural indicator) successfully substituted the methyl orange, methyl red and phenolphthalein indicators for titrations with (i) strong acid versus strong base, (ii) strong acid versus weak base, (iii) weak acid versus strong base and (iv) weak acid versus weak base (Abugri et al, 2012).

In order to prove the usefulness of the proposed method, all experiments realized until now were also done for a weak acid - ASA. Just like that occurred to HCl, the titration end point was easily identified by the color change from green to orange. For the quantitative analysis, it was not found any outliers value applying the Q- test (Equation 1, data not shown), thus the mean of the spent volume in the titration was done by the three replies for both indicators. Also

for HCl, the confidence interval for the mean (Equation 2) obtained from the calculated values for the ASA titrated using phenolphthalein as an indicator completely overlapped to those obtained by CUR as an indicator (Table 1). Therefore, the null hypothesis was not rejected and it had been suggested that there were no significant differences on these indicators. However, a systematic error can be present just on the phenolphthalein data because all the confidence interval of the mean was above the reference value (Miller, Miller and miller, 2018). The paired t- test was applied (Equation 3) considering that the standard deviation was comparable since in the result for the F-test (Equation 4) the F_{cal} was lower than the F_{tab} (2.003 and 19.000, respectively). The t calculated value t_{cal}= 0.973 was lower than the t_{tab}=2.776 ($p< 0.05$; df=4). Another time, the results make the null hypothesis be accepted characterizing that there were no statistic differences between CUR and phenolphthalein as an indicator on acid/base titration.

Moreover, because commercial tablets have been used to obtain the ASA, the results can be compared with the reference value (500mg) described on the packaging by an unpaired t-test (Equation 6). The t_{cal} for the titration data was 0.972 and 3.564 using CUR or phenolphthalein as an indicator, respectively. For both cases, it was observed that the tcal is lower than the t_{tab} (4.303, $p< 0.05$, df=2), leading to the acceptance of the null hypothesis (Pfister and Janczyk, 2013). This denotes that no significant differences were identified between the amounts of ASA determined by titration and the reference value.

4. CONCLUSIONS

The equity of CUR as acid-base indicator in acid-base titrations was attested not only qualitative but also quantitative by comparing to phenolphthalein. The qualitative analysis showed that CUR had different colors as function of pH that were spectroscopically characterized. The main absorption band of CUR underwent a bathochromic shift from 434 nm to 462 nm in acidic and basic media, respectively. This resulted in a visual color change from yellow to orange/red around pH 8.5. These color changes were extremely useful to identify the end point of titration of strong or weak acid with strong base suggesting the use of CUR as an alternative indicator for acid-base titrations whose end point is between 7.5 and 9.5.

The quantitative analysis showed that the volumes of titrant spent in the HCl versus NaOH titrations were 9.81±0.06 mL to CUR or 9.75±0.05 to phenolphthalein as an indicator, respectively. According to the confidence interval of the mean there were no significant differences between the HCl concentrations obtained by titration using CUR ($9.32<\mu<9.56 \times 10^{-2}$ mol L^{-1}) or phenolphthalein ($9.31<\mu<9.56 \times 10^{-2}$ mol L^{-1}) as indicators ($p<0.05$). The same

conclusion was taken using a paired t-test with comparable standard deviation with 95% of confidence level.

For the ASA measurements, the volumes of titrant spent were 28.85±0.05 mL to CUR and 28.72±0.10 mL to phenolphthalein. The confidence interval of the mean quantities was 499<μ<508mg to CUR and 503<μ<508mg to phenolphthalein as indicators. Once more, the null hypothesis was accepted and no statistic differences were identified between these two indicators ($p<0.05$). It was also described that there might be evidence of a systematic error for the data using phenolphthalein as an indicator due to the all confidence interval for the mean was above the reference value.

Furthermore, the unpaired t-test ($p<0.05$) comparing with the reference packaging value (500 mg) clearly demonstrated that the use of CUR as an indicator in titration is perfectly acceptable. The t_{cal}= 0.972 was lower than those tabulated (t_{tab}=4.303), evidencing that there were no significant differences about the quantities obtained by titrations using CUR as an indicator and the reference value.

The use of natural reagents to replace those synthetics that may, in some way, harm the environment is strongly encouraged by green Chemistry. Thus, the proof that a natural product such as CUR presented qualitative and quantitative equity suggests its use as an alternative to phenolphthalein and should be widely used in Analytical Chemistry.

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