

# RAPID ASSAY TO EVALUATE THE POTENTIAL OF *Caesalpinia pulcherrima* FLOWER EXTRACT IN *HCl* SOLUTION STANDARDIZATION

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## ABSTRACT

Artificial indicators are toxic to the ambient. An alternative to minimize waste generation is the use of natural indicators, meeting the Green Chemistry. The aim of this work was to evaluate the potential of *Caesalpinia pulcherrima* flower extract in the standardization of hydrochloric acid (*HCl*) solution, in comparison with the artificial Methyl Red indicator, using a titrimetric technique. This acidic solution standardization procedure using natural indicator is pioneer and should be studied. Flower petals were macerated with PA 1 : 1(w/v) ethyl alcohol, and the extract was filtered through a fiberglass filter and stored in a test tube wrapped in aluminum foil. Titration procedures were performed in triplicate, with artificial and natural indicator. The natural indicator was able to detect the end point of the titration of the *HCl* with Borax, showing the same pH (around 5.0) indicated by the artificial indicator. The colors observed at the beginning and end of the titration were, respectively, yellow and salmon pink with the use of methyl red and, yellow and light lilac with the extract of *C. pulcherrima*. Concentration results for *HCl* in the standard solution were similar to the natural indicator. Thus, using the *C. pulcherrima* flower extract evaluated in the standardization of strong acid solution (*HCl*) with the weak base (borax) is advantageous.

**Keywords:** Acid-base titration. pH indicator. Green Chemistry.

## RESUMO

Indicadores artificiais são tóxicos ao meio ambiente. Uma alternativa para minimizar a geração de resíduos é a utilização de indicadores naturais, atendendo à Química Verde. O objetivo deste trabalho foi avaliar o potencial do extrato da flor de *Caesalpinia pulcherrima* na padronização da solução de ácido clorídrico (*HCl*), em comparação com o indicador artificial de Vermelho de Metila, por meio da técnica titulométrica. Esse procedimento de padronização de solução ácida usando indicador natural

é pioneiro e deve ser estudado. Pétalas de flores foram maceradas com álcool etílico PA 1:1 (m/v), e o extrato foi filtrado em filtro de fibra de vidro e armazenado em tubo de ensaio envolvido em papel alumínio. Os procedimentos de titulação foram realizados em triplicata, com o indicador artificial e o natural. O indicador natural foi capaz de detectar o ponto final da titulação do *HCl* com Bórax, apresentando o mesmo pH (em torno de 5,0) indicado pelo indicador artificial. As cores observadas no início e no final da titulação foram respectivamente, amarelo e rosa salmão com o uso do vermelho de metila e amarelo e lilás claro com o extrato de *C. pulcherrima*. Os resultados da concentração de *HCl* na solução padronizada foram semelhantes para o uso dos dois indicadores (natural e artificial). Assim, a utilização do extrato de *C. pulcherrima* avaliado na padronização da solução de ácido forte (*HCl*) com a base fraca (bórax) é vantajosa.

**Palavras-chave:** Titração ácido-base. Indicador de pH. Química Verde.

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## INTRODUCTION

In chemical laboratory procedures, such as reactions involving the use of acids and bases, using artificial pH indicators, such as phenolphthalein, Bromothymol Blue, Methyl Orange, among others is very common. Artificial indicator is a substance that changes color with pH variation (SKOOG et al., 2013, p.2). Artificial indicators also can cause toxicity to human health. Phenolphthalein, for example, widely used in practical classes and scientific research, also present in laxative drugs can cause toxic epidermal necrosis (ARTYMOWICZ; CHILDS; PAOLINI, 1997, p. 2). Methyl Red, while not a hazardous chemical indicator, can be toxic to the flora and fauna of the aquatic environment if discarded in natura (??, p. 2).

Due to concern for the environment, researchers have been testing and already using natural pH indicators. In nature, there are substances, as such as anthocyanin, that can be extracted from plant species, acting as pH indicators in acid-base reactions. Anthocyanin is also present in the sap of many vegetables, such as grapes, jaboticabas, blackberries, as well as in red leaves and colorful petal flowers. The use of flower extracts as natural pH indicators has been highly recommended (GOUVEIA-MATOS, 1999; RAMOS; CAVALHEIRO; CAVALHEIRO, 2006; LOPES et al., 2007; ANTUNES et al., 2009; GUIMARÃES; ALVES; FILHO, 2012; DOMINGUINI et al., 2014, p. 2), as it is a relatively inexpensive method and can be used by people overall.

The use of natural indicators meets the principles 1 (prevention), 4 (design of safe products) and 7 (use of renewable sources) of Green Chemistry. The first principle says preventing generation is cheaper than treating chemical waste. The fourth principle is about the design of products that perform the desired function and are non-toxic. Principle 7 encourages the use of renewable sources (LENARDÃO et al., 2003, p. 3). In the case of natural indicators, they come from plants, which can be constantly renewed.

This research aimed to evaluate the potential of the extract of the *C. pulcherrima* plant (Fig.

1) flower in the standardization of  $0.01 \text{ mol L}^{-1}$  hydrochloric acid solution. But, the use of this species extract in the standardization of *HCl* solution is pioneer. The idea for the use of *C. pulcherrima* flower extract in the standardization of *HCl* with borax came from a work developed with undergraduate students, in the General Chemistry course, resulting later in a research (unpublished). PH scales were arranged in test tubes with solution in the pH range of 0 to 12. Extracts from flowers of different species were tested. *C. pulcherrima* extract showed a clear color change in pH around 5, which is the same turning pH of a titration between *HCl* and borax using Methyl Red.

The standardization of *HCl* solution can be done using the traditional titrimetric method, in which it uses a weak base solution (example, borax, which is a primary standard) (VOGEL, 1989, p. 3) and usually an artificial indicator (Methyl Red or Orange) to detect visual change of turning color at end point of titration. These indicators detect an end point with color change in the pH range between 4.4 and 6.2.

Figure 1 – *Caesalpinia pulcherrima* specie



Fonte – Prepared by the author

## MATERIAL AND METHODS

All material (except metal) used in the chemical analysis was previously washed with  $50\%(v/v)$  *HCl* solution, rinsed with deionized water and dried naturally. The solutions were prepared with good quality deionized water (conductivity =  $1.16 \mu\text{Scm}^{-1}$ ).

The  $0.0100 \text{ mol L}^{-1}$  *HCl* solution to be standardized was prepared by dilution from a  $0.1 \text{ mol L}^{-1}$  *HCl* solution. For standardization, a so-

lution of sodium tetraborate,  $Na_2[B_4O_5(OH)_4] \cdot 8H_2O$  (known as borax)  $0.005 \text{ mol L}^{-1}$  was used, for which the reagent was previously recrystallized to remove impurities. The salt (15.0g) was recrystallised from deionized water (50.0 mL) in temperature minor that  $55^\circ C$ . The crystals were filtered, washed twice with water, then twice with portions of 95% ethanol, followed by two portions of diethyl ether. The solid was spread in a clock-glass and allowed to stand at room temperature for 12–18 hours. The pure sodium tetraborate was then dry and stoppered in plastic flask (VOGEL, 1989, p. 4).

After preparation of the solutions, a system for titration of the  $HCl$  solution was set up using a 25.0 mL glass burette, claw holder, 50.0 mL beaker, stirrer and magnetic bar. The magnetic stirrer was placed at the bottom of the burette holder. The burette was filled with  $HCl$  solution. Then, using a micropipette, the volume of 5.00 mL of the borax solution was pipetted and poured into the beaker containing the magnetic bar. An amount equivalent to 3 drops ( $\sim 0.05 \text{ mL}$ ) of artificial Methyl Red indicator was added to the beaker containing the solution. Stirring of the solution was controlled so as not to form bubbles and to prevent unnecessary oxygenation. Determinations were made in triplicate to verify analytical accuracy.

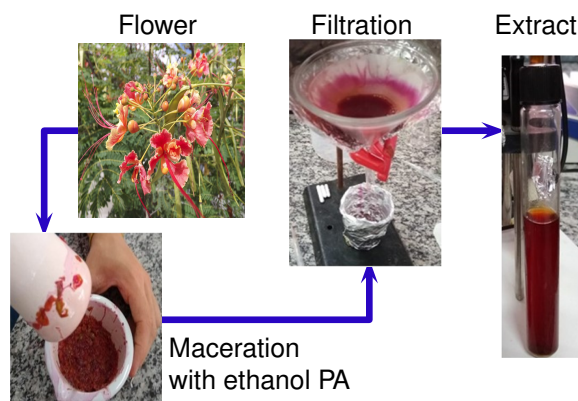
A flower alcoholic extract (pH 6) was prepared one hour before the titration procedure, being the pH was measured with universal indicator tape (scale 0 to 14).

The collection of petals (pink red in color) of the *C. pulcherrima* plant, commonly known as Flamboyanzinho, occurred at the UFRB campus in Cruz das Almas, Bahia, in November 2019, and stored in a plastic bag. In the laboratory, the red parts of the flower were separated and a fresh mass of 7.00g was weighed, which was ground with a glass stick in 7.00 mL of absolute ethyl alcohol (99.5%) in a 50.0 mL centrifuge tube (Figura 2). According to Dominguni et al. (2014), ethyl alcohol is the best way to preserve the chemical properties of natural indicators.

The mentioned sample to alcohol ratio was defined according to preliminary laboratory tests.

This proportion was found to leave the indicator solution stained strong enough to use only 3–5 drops, mimicking the artificial indicator. The mixture was allowed to stand for a few minutes and then squeezed with hands dressed in a nitrile glove, the extract being filtered through a glass funnel, 47cm diameter glass fiber filter and glass vial. The extract was reserved for further titration analysis.

Figure 2 – Maceration and filtration procedure of *C. pulcherrima* flower extract



Fonte – Prepared by the author

The first three measurements were made using three (3) drops of the Methyl Red pH artificial indicator solution to ascertain the indicator color at the titration end point, and the amount of acidic solution spent and the pH of the sample were noted. Between each replica, the burette was properly filled with acidic solution, the beaker and magnetic bar were washed with tap water, 50%(v/v) $HCl$  solution and rinsed 4 times with deionized water. The other three measurements were made using five (5) drops of the flower extract and the same procedure as that used with the artificial indicator.

In the titrations, volumes of 5.00 mL were used for replicates and low concentration solutions (borax  $0.00500 \text{ mol L}^{-1}$  and hydrochloric acid  $0.0100 \text{ mol L}^{-1}$ ). The total volume of tailings for the analysis in triplicate, considering the test samples, was less than 150 mL. After the end of the analysis, the residues containing the artificial indicator were discarded in an appropriate gallon for inorganic residue and the residues with natural indicator were disposed of in the sink. The acid solution of the burette was neutralized with  $NaOH$  solution and the saline solution also discarded in the sink.

The data treatment and the basic statistics were performed in Excel 2007 and a Student *t* test was performed in Origin 6.0. Student *t* test was used to assess a possible difference between volumes of acids from two samples (artificial and natural indicator).

## RESULTS AND DISCUSSION

Green Chemistry encourages the reduction of the amount of reagents and volumes of solution in laboratory experiments, aiming to generate less waste. The small volume per replica and the low concentrations of the acidic and basic solutions used in this work were much smaller than that used in other studies (SINGH et al., 2011; MUHAMMAD et al., 2016; BAYE; LESHE, 2019, p. 6).

Table 1 – Values found in the standardization of  $0.01 \text{ mol L}^{-1} \text{HCl}$  solution using borax and artificial (Methyl Red) and natural (*C. pulcherrima* flower extract) indicators.

Replica	borax	Indicator	$\text{HCl} (0.01 \text{ mol L}^{-1})$	Turning pH	Color before turning	Turning color
A1	5 mL	Artificial	4.80 mL	~ 5	Y	Sr
A2	5 mL	Artificial	4.80 mL	~ 5	Y	Sr
A3	5 mL	Artificial	4.80 mL	~ 5	Y	Sr
Mean vol.			4.80 mL			
B1	5 mL	Natural	4.90 mL	~ 5	O	LI
B2	5 mL	Natural	4.80 mL	~ 5	O	LI
B3	5 mL	Natural	4.80 mL	~ 5	O	LI
Mean vol.			4.83 mL			

Legend - Y: Yellow O: Orange Sr: Salmon rose LI: Light lilac

Fonte – Elaborated by the author

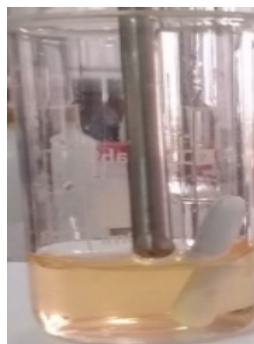
The results obtained from the titration procedure are described in Tabela 1. At the end point of titration with the artificial indicator, the average spent volume of  $\text{HCl}$  solution was  $4.80 \text{ mL}$  to neutralize the  $5.00 \text{ mL}$  volume of borax solution. The

color of this indicator changed from yellow (before turning, Figura 3) to salmon rose at the turning point (Figura 4) at pH around 5. The pH was measured with benchtop pHmeter and pH tape. With benchtop pHmeter, the pH of the borax solution was 8.5. At the turning point the pH is around 5.5. The tape showed pH in the range of 5 to 6.

Figure 3 – color yellow of the artificial indicator Methyl Red in the borax solution before titration (pH = 8.5)



Figure 4 – Methyl Red color (salmon rose) in the borax solution at the titration end point (pH ~ 5) after the addition of  $4.8 \text{ mL}$  of  $\text{HCl}$



Fonte – Prepared by the author

Using the natural indicator, a similar expense of  $\text{HCl}$  ( $4.83 \text{ mL}$ ) was observed in order to reach the titration end point, at a pH in the range of 5 to 6. The color of the solution before turning was orange and light lilac in the end. However, some important observations were made throughout the titration. With the addition of the  $\text{HCl}$  solution to the borax solution, the color of the mixture changed in the following order: orange (pH = 8.5 without  $\text{HCl}$ , Figura 5a) → yellow (after addition of  $3.5 \text{ mL}$ , Figura 5b) → yellowish beige (after the addition of  $\sim 4.0 \text{ mL}$ , Figura 5c) → light beige (after the addi-

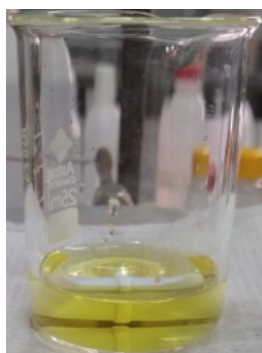
tion of  $\sim 4.4 \text{ mL}$ , [Figura 5d](#))  $\rightarrow$  light lilac (pH  $\sim 5$ , after addition of  $\sim 4.8 \text{ mL}$ , [Figura 5e](#)). After the equivalence point, the solution with the natural indicator became pink, tending to darker pink with increasing acidity of the solution.

Figure 5 – Color of natural flower indicator of *C. pulcherrima* species borax solution

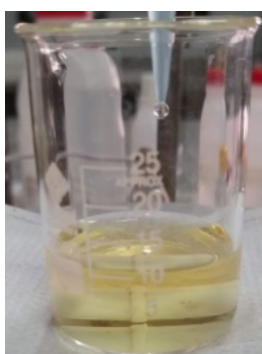
a – orange, at pH = 8.5 before titration



b – yellow after the addition of  $3.5 \text{ mL}$  of *HCl*



c – yellowish beige after addition of  $\sim 4.0 \text{ mL}$  of *HCl*



d – light beige after the addition of  $\sim 4.4 \text{ mL}$  of *HCl*



e – light lilac, at pH 5, after addition of  $\sim 4.8 \text{ mL}$  of *HCl* = titration end point



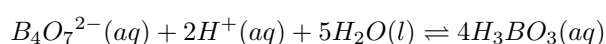
The color change is due to the form of anthocyanin present at each pH. Anthocyanin has different chemical structures (flavylic cation, carbinol, anhydrobases and chalcones), which vary according to the pH of the solution in which it is present. When the indicator molecule loses or gains  $H^+$  ion, its structure is changed and the color of the solution changes as a function of the pH. At pH 1 to 2, for example, the solution may be red due to the presence of flavyl cation ( $AH^+$ ). At pH 6 there is carbinol and the solution is colorless. Between pH 7 and 8 there is a violet color and there is the presence of anhydrobases ([ROSSI; SHIMAMOTO, 2010](#), p. 8). In this research, the color of the indicator at the end point was very light lilac, almost colorless, suggesting the predominance of the carbinol form of anthocyanin.

The light lilac color noted at the end point of the titration with the natural indicator of the flower under study was almost colorless. The indicator in the borax sample showed four different colors throughout the titration process, according to the pH change, as previously reported, but this did not prevent the color detection at the end point of the

titration. Thus, it was possible to identify the volume of the titrant at the end point, at a pH of around 5. This showed that the indicator was efficient in standardizing strong acid (*HCl*) with the weak base (borax). Next, it will be shown how the reaction occurs throughout the studied titrimetric procedure.

In the titrimetric process, the borax ( $B_4O_7^{2-}$ ) reacts with *HCl* (VOGEL, 1989; SKOOG et al., 2013, p. 8), which in aqueous medium releases the  $H^+$  ions, which tends to turn the solution pinkish. The equilibrium reaction occurs at a ratio of 1 : 2, i.e. 1 mol of borax for every 2 mol of *HCl*.

Chemical reaction:



Ratio: 1 mol : 2 mol

As titration was performed using  $0.00500 \text{ mol L}^{-1}$  borax solution and  $0.0100 \text{ mol L}^{-1} \text{ HCl}$ , at the titration end point the amount of matter (mol number =  $n$ ) present in the used borax solution volume was similar to the amount of matter contained in the spent *HCl* volume (Equação 1) at the titration end point. The amount of matter is equal to the concentration times the volume ( $C \cdot V$ ). Thus, at the titration equivalence point, for  $n_{HCl}$  to be similar to  $n_{borax}$ , it is necessary to multiply  $n_{borax}$  by 2 (Equação 2), since the reaction occurs in a 1 : 2 ratio.

$$n_{HCl} = n_{borax} \quad (1)$$

$$C_{HCl} \cdot V_{HCl} = 2(C_{borax} \cdot V_{borax}) \quad (2)$$

With the rearrangement of the Equação 2, the concentration of the titrated acid solution Equação 3 was obtained:

$$C_{HCl} = 2 \left( \frac{C_{borax} \cdot V_{borax}}{V_{HCl}} \right) \quad (3)$$

From Equação 3, the *HCl* concentration of the standardized solution was calculated. The triplicate mean volume was used, being  $4.80 \text{ mL}$  ( $0.0048 \text{ L}$ ) for the acid solution and  $5.00 \text{ mL}$  ( $0.00500 \text{ L}$ ) for the borax solution. The calculations showed an actual (standard) *HCl* concentration equal to:

$$a) C_{HCl} = 2(0.00500 \text{ mol L}^{-1} \cdot 0.00500 \text{ L}) / 0.00480 \text{ L} = 0.00960 \text{ mol L}^{-1}, \text{ with use of artificial indicator};$$

$$b) C_{HCl} = 2(0.00500 \text{ mol L}^{-1} \cdot 0.00500 \text{ L}) / 0.00483 \text{ L} = 0.00966 \text{ mol L}^{-1}, \text{ with use of natural indicator.}$$

The difference in *HCl* concentration between titrations (with artificial and natural indicator) was very small,  $0.0000600 \text{ mol L}^{-1}$ , showing that the indicator is efficient in the process of standardizing *HCl* (strong acid) with borax (weak base).

The titration error ( $E_t$ ) was also calculated and involved the volume of equivalence (theoretical =  $V_{eq}$ ) and the final volume at the end point ( $V_{fi}$ ) of the (real) titration obtained through Equação 4:

$$E_t = V_{eq} - V_{fi} \quad (4)$$

a) with use of artificial indicator:

$$E_t = 5.00 \text{ mL} - 4.80 \text{ mL} = 0.200 \text{ mL};$$

b) with use of natural indicator:

$$E_t = 5.00 \text{ mL} - 4.83 \text{ mL} = 0.170 \text{ mL}.$$

The results showed very close value between the theoretical and final equivalence volume, indicating an error of  $0.200 \text{ mL}$  or 4% for titration with Methyl Red and  $0.170 \text{ mL}$  or 3.4% for titration performed with the flower extract. Student's t test (Tabela 2) showed also that there was no significant difference ( $p = 0.374$ ; 0.05 level) between the volumes of acid used for the artificial and natural indicator.

Table 2 – Student's t test between the two samples of indicators (artificial and natural).

Indicator	Mean	Variance	N
Artificial	4.80	0	3
Natural	4.83	0.00333	3

0.05 level and  $p = 0.3739$

Fonte – Prepared by the author

The titrimetric procedure performed was simple and is still widely used in chemistry, using artificial indicators. In literature, several studies report

the use of natural indicators in acid-base titrations to replace synthetic indicators (MUHAMMAD et al., 2016; PATRAKAR; GOND; DHANRAJ, 2010; ABUGRI; APEA; PRITCHETT, 2012; EZE S. O.; OGBUEFI, 2014; PIMPODKAR; SURVE; BHISE, 2014, p.10). However, there is still a lack of knowledge in chemistry about the use of a natural indicator in the standardization of solutions. In this sense, this research comes to complement the scientific knowledge showing the potential of the extract of *C. pulcherrima* in the standardization of *HCl* solution, with the use of relatively small volumes and low concentrations of the solutions, corroborating with the first principle of Green Chemistry (prevention). It is suggested that future research can test the natural indicators in the standardization of solutions.

## CONCLUSIONS

The natural pH indicator from *C. pulcherrima* flower in the titrimetric was efficient in the standard of hydrochloric acid solution. Thus, in regions where there are flamboyanzinho shrubs, using its flower extract as a pH indicator in strong acid and weak base reactions is advantageous. It is also interesting to further test other types of natural indicators in strong acid and strong base, weak acid and strong base titrimetric procedures.

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