Artículos científicos

Microtitulación para la determinación de la acidez titulable de tés (Camellia sinensis)

Microtiter for the Determination of Titratable Acidity of Teas (Camellia sinensis)

Microtitulação para determinação da acidez titulável de chás (Camellia sinensis)

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Resumen

Se desarrolló un método a microescala para la determinación de la acidez titulable de té (*Camellia sinensis*) y se comparó con el método recomendado. Los resultados obtenidos muestran que el método desarrollado es satisfactorio: los valores obtenidos son muy similares a los del método de referencia y mostraron una correlación lineal con un valor de $R^2 = 0.8901$. El método desarrollado se puede emplear como método de enseñanza en los laboratorios de química para la caracterización fisicoquímica de bebidas elaboradas a base de *Camellia sinensis*.

Palabras clave: alcalimetría, microescala, potenciometría.

Abstract

A microscale method was developed for the determination of titratable acidity of tea (*Camellia sinensis*) and compared with the recommended method. The results obtained show that the method developed is satisfactory: the values obtained are very similar to those of the reference method and showed a linear correlation with a value of $R^2 = 0.8901$. The method developed can be used as a teaching method in chemistry laboratories for the physicochemical characterization of drinks made from *Camellia sinensis*.

Keywords: alkalimetry, microscale, potentiometry.

Resumo

Um método em microescala para a determinação da acidez titulável do chá (Camellia sinensis) foi desenvolvido e comparado com o método recomendado. Os resultados obtidos mostram que o método desenvolvido é satisfatório: os valores obtidos são muito semelhantes aos do método de referência e apresentaram uma correlação linear com um valor de R2 = 0,8901. O método desenvolvido pode ser utilizado como método de ensino em laboratórios de química para a caracterização físico-química de bebidas à base de Camellia sinensis.

Palavras-chave: alcalimetria, microescala, potenciometria.

Introduction

Tea (Camellia sinensis) is a very popular drink with high consumption by the world population. As a food, its quality must be guaranteed, and two of the main physicochemical characteristics in a beverage are pH and titratable acidity. These parameters help detect alterations or adulterations in beverages, such as dilution with water or the addition of minerals (Dornelles and Porto, 2014; Lunkes and Hasizume, 2014). The titratable acidity of a beverage is frequently determined by alkalimetry using phenolphthalein as an indicator (pH = 8.3) and is expressed as the amount of citric acid contained in a given volume of sample (Mettler and Weibel, 2018; Parra, 2013). One difficulty in this analytical process is that the tea has a very strong color and, even if it is diluted, it can affect the determination of the end point of the titration, that is, the change in phenolphthalein (dos Santos, dos Santos, da Silva, Constant and Belmino, 2014; Mettler and Weibel, 2018). In the methods of quantification of analytes in solution by titrimetry, the most important aspect is the determination of the equivalence point that represents the amount of titrant that is equivalent to the substance that is being determined, and because in practice the determination of this point is affected by different factors is called the end point to the experimental equivalence point (Morales, González, Abella and Ahumada, 2019; Morita, Yanagisawa, Maeda, Takatsu and Ikka, 2011; Torres, Durán and Rodríguez, 2009).

Experimental techniques allow variation and error in the determination of the equivalence point to be reduced because they are not based on the visual appreciation of the analyst, but instead measure a physicochemical property associated with the analyte, such as pH (Adiba, Rabia and Anwar, 2018; Morales et al., 2019; Nunes et al., 2016). An alternative to perform the titration are potentiometric techniques that monitor the pH during the titration and determine the equivalence point by the abrupt increase in pH in response to small volumes of added titrant. The advantage of this method is that the color of the beverage does not interfere with the measurements (Adiba et al., 2018; Cáñez, García, Bernal, Federico, & Wicochea, 2011; Friedman, Levin, Lee, & Kozukue, 2009).

In this research, a microscale potentiometric technique was implemented to determine the titratable acidity of tea and was compared with the macroscale technique to have a simpler and cheaper method that allows quantification with fewer reagents and generates less chemical waste.

Methodology

A box of tea bags (Camellia sinensis) was purchased and approximately 250 mL of aqueous infusions were obtained from the bags. The infusions were acidified with a few drops of lemon and added with sugar. This procedure was performed 10 times to obtain 10 tea samples. In each tea sample, the pH and titratable acidity were determined by two methods. The macroscale method was performed as described in the literature: 10 mL of tea at room temperature was transferred to a flask and 100 mL of distilled water and four drops of 0.1% phenolphthalein in ethanol were added. The sample was titrated with a standardized NaOH solution with an approximate concentration of 0.01N until the indicator turned. The microscale method was carried out in a 50 mL beaker to which 10 mL of the tea infusion and 25 mL of water were added. A magnetic stirrer was placed inside the beaker and placed on a stir plate. The potentiometer was introduced and the pH was monitored after increments of 0.1N NaOH of 50 $\Box$L until a pH greater than 11.0 was obtained. With the pH values obtained, the titration curve was plotted and, from these data, it was processed to obtain the graph of the first and second derivatives, which are used to determine the equivalence point. Also, a 0.1M citric acid solution was used as a control; 1.0 mL of the control solution was titrated with 0.1M NaOH using the potentiometer.

Results

The results obtained in the determination of the pH and the titratable acidity of the analyzed infusions are shown in table 1. These values correspond to those referred to in the literature for samples of Camellia sinensis. It can be seen that the determination of acidity by the microscale potentiometric method yields a lower value and with a lower standard deviation, which is due to the fact that the visual method requires a small excess of sodium hydroxide to cause the phenolphthalein to turn. , which is overshadowed by the color presented by the infusions, which sometimes causes doubts as to whether the end point has already been reached and to make sure, more reagent is added. In contrast, with the potentiometric method the end point is determined graphically (Mansilla, 2014; Sotomayor *et al.,* 2019).

Tabla 1. Resultados de la determinación del pH y la acidez titulable de las muestras de té

Muestra	pH	ATP Micro	ATD Micro	ATP Macro	ATD Macro
1	5.75	9.84	0.01	10.28	0.15
2	5.67	10.36	0.02	11.66	0.02
3	5.62	10.67	0.00	11.76	0.04
4	5.55	10.83	0.02	11.98	0.04
5	5.54	11.25	0.02	12.11	0.07
6	5.53	11.34	0.02	12.35	0.11
7	5.47	11.42	0.01	12.50	0.06
8	5.33	11.64	0.01	12.48	0.04
9	5.23	11.83	0.01	12.61	0.09
10	5.19	11.95	0.01	12.81	0.06
Nota: ATP = promedio de la acidez titulable, ATD = desviación de la acidez titulable.					

Fuente: Elaboración propia

The graphs of the citric acid titration curve are shown in figure 1. There it can be seen that the distinguishable equivalence point corresponds to the total neutralization of sodium hydroxide. This is due to the fact that the pKa values of citric acid are very similar and therefore the two previous points cannot be established, but only when the pH change is more notable due to the complete neutralization of the acid (Marín, García, Santiago and Baez, 2014). The NaOH concentration calculated with the neutralization values with citric acid were the same as those obtained by the potassium biphthalate standard and this corroborates that the distinguishable point in the titration corresponds to the third neutralization.

Figura 1. Gráficos de primera derivada (izquierda) y segunda derivada (derecha) para la titulación de ácido cítrico con NaOH
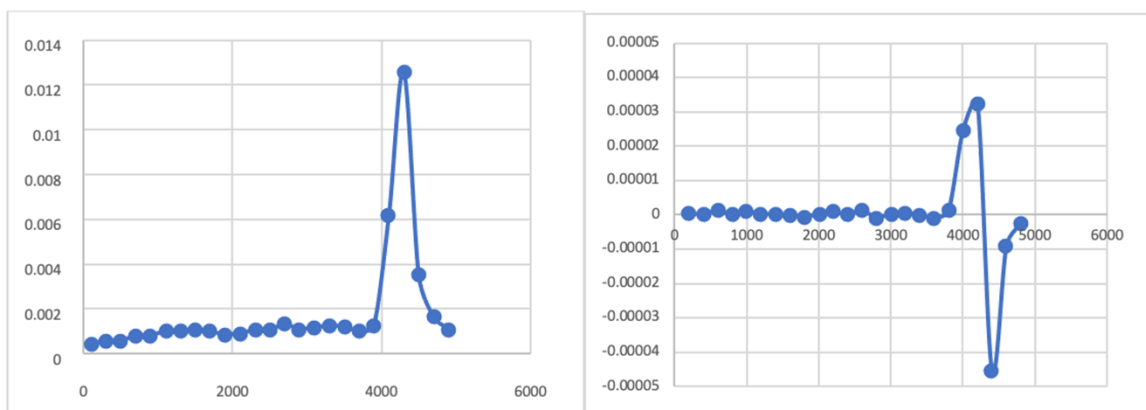
Fuente: Elaboración propia

Figure 2, on the other hand, shows the graphs of the titration curve of tea with NaOH. Although the graphs present a greater irregularity (compared to the pure citric acid solution), the final point can be easily appreciated (largest peak in the case of the first derivative and intersection in the case of the second derivative), since, if Although the results of titratable acidity are usually expressed as grams of citric acid per volume of sample, in reality the tea contains other organic acids that contribute to the total acidity of the drink, so the final record would correspond to the total neutralization of all acids (when the pH becomes alkaline from excess NaOH) (Alarcón, Barreiro, Boicet, Ramos y Morales, 2018; Beretta, Bassahun, Torres, Musselli y García, 2017; Torres *et al.,* 2009).

Figura 2. Gráficos de primera derivada (izquierda) y segunda derivada (derecha) para la acidez titulable de las infusiones de té
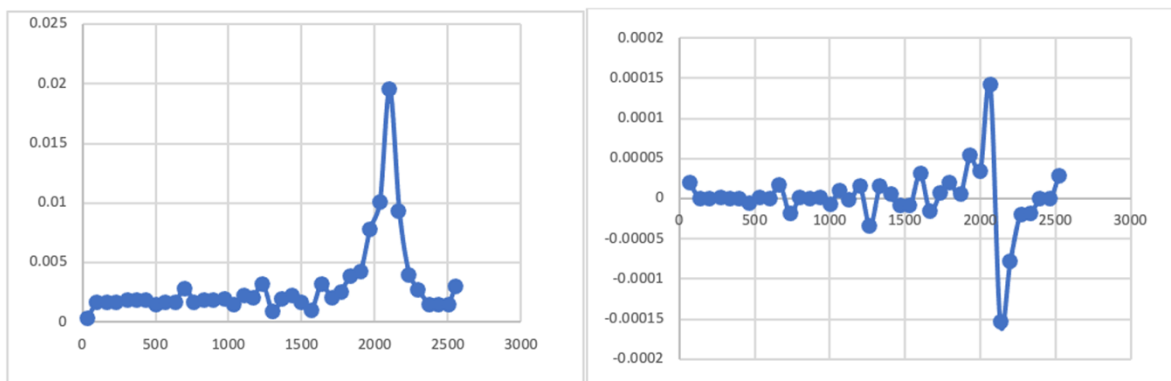
Fuente: Elaboración propia

pH and acidity are related because pH is a measure of the concentration of hydrogen ions in the solution and, although not linear, there is a proportional relationship between the concentration of acids and pH that is influenced by the type of acid, that is, due to the chemical nature of the acids present in the solution, especially due to their concentration and pKa (Alarcón et al., 2018; Mansilla, 2014). Figure 3 shows that there is a negative correlation between the concentration of acids and the pH, since as the acidity increases, the pH will drop because the concentration of hydrogen ions increases [pH = -log (hydrogen ion concentration)]. In the visual titration method, the Ka of the titrated acid cannot be appreciated. (Mansilla, 2014; Sotomayor *et al.*, 2019).

Figura 3. Correlación entre el pH (abscisas) y la acidez titulable de las infusiones de té (ordenadas) obtenida por ambos métodos
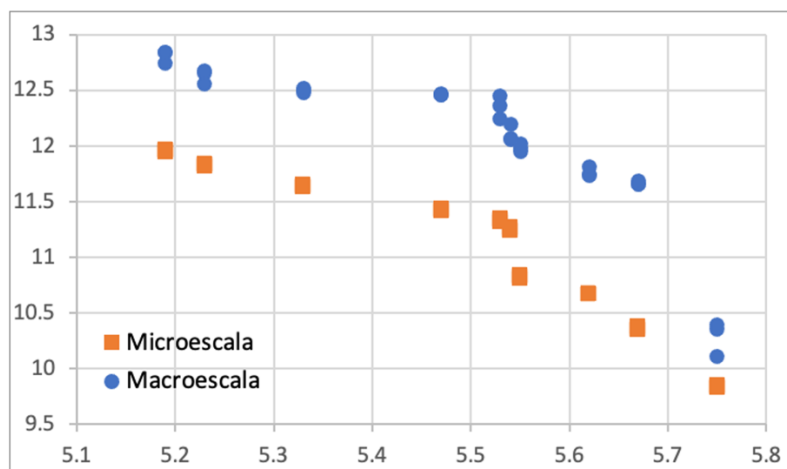
Fuente: Elaboración propia

Finally, the titratable acidity values obtained by both methods correlate well, as can be seen in figure 4; however, the greater variation in the macroscale method decreases the value of the correlation with the microscale method; It would be necessary to evaluate the behavior of the method by adding an internal standard to estimate the recovery percentage to corroborate this statement.

Figura 4. Correlación entre los valores de acidez titulable obtenido por los dos métodos (abscisas método microescala potenciométrico y ordenadas método macroescala)
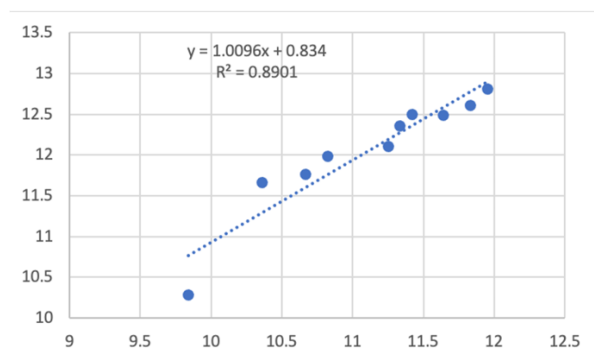
Fuente: Elaboración propia

Discussion

The proposed microscale method represents a viable alternative for the quantification of the quality of commercial tea samples because the results obtained are analytically equivalent to those obtained by the traditional method. In this particular case, due to the collation of the analysis matrix, potentiometric methods are preferred; traditional titrating involves the use of phenolphthalein as an indicator, the color change of which is often masked by the pigmentation of tea metabolites (Dornelles and Porto, 2014; Friedman et al., 2009; Lunkes and Hashizume, 2014).

The use of a potentiometer, however, limits the application of the microscale technique: it will depend on the physical characteristics of the equipment to be able to use it in an adequate sample volume, to correctly carry out a measurement with the smallest possible volume that guarantees an exact measurement. , in addition to being equipped with a magnetic stirrer with its magnet to homogenize the sample after adding the alkali to avoid losses or false results due to manual stirring (Cáñez et al., 2011).

This method was developed in the drug analysis laboratory, first by analytical chemists and later with the students participating in the research, on both occasions with similar results. This is a test of ease of implementation. And for the same reason, its use is proposed in experimental laboratories of subjects such as Pharmacognosy or Analytical Chemistry, where samples of medicinal plants or foods are analyzed to determine their quality and which, by their nature, contain interfering pigments with acid-base indicators. . In addition, the use of microscale methods gives the experimental sessions an approach of environmental care and social responsibility by generating less waste and less expense due to the use of chemical reagents. (dos Santos *et al.,* 2014; Lunkes y Hashizume, 2014; Mettler y Weibel, 2018).

It is required to continue with the study of the method to test its relevance and efficacy as an alternative method to determine the total and titratable acidity of commercial samples for which it is desired to know these parameters as a quality criterion. Likewise, comparing the results obtained with those obtained through the official methods allows knowing its analytical usefulness and validating it scientifically, for this reason its characterization as an analytical method must be carried out, especially in the determination of its accuracy, precision, repeatability and reproducibility. (Adiba *et al.,* 2018; Marín *et al.,* 2014).

Conclusion

The developed microscale potentiometric technique was satisfactory because with it the titratable acidity of tea samples was determined with an adequate correlation with the values obtained by the recommended method and with greater precision. This microscale method can be used appropriately in the practical laboratories of subjects such as food analysis, instrumental analysis or chemistry of natural products.

Future lines of research

According to the results obtained in this investigation, it would be advisable to evaluate the method applied as a laboratory practice and in the analysis of commercial samples, comparing the proposed method with that of references in officially recognized techniques such as those reported in the Mexican official standards or pharmacopoeias.

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