Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Convenient UV-spectrophotometric determination of citrates in aqueous solutions with applications in the pharmaceutical analysis of oral electrolyte formulations

Sylwester Krukowski[*], *Mateusz Karasiewicz*, *Waclaw Kolodziejski*

Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Warsaw, Poland

ARTICLE INFO

Article history:
Received 19 October 2016
Received in revised form
22 January 2017
Accepted 24 January 2017
Available online 17 March 2017

Keywords:
Citrates
Oral rehydration
Ultraviolet spectrophotometry
Validation studies

ABSTRACT

Herein, we present a convenient method for quantitative spectrophotometric determination of citrate ions in aqueous solutions in the middle-UV range. It involves measuring the absorbance of citric acid at 209 nm under suppressed dissociation at pH < 1.0 in the presence of hydrochloric acid. Validation of the method was performed according to the guidelines of the International Conference on Harmonization. A very good linear dependence of the absorbance on concentration (r^2 = 0.9999) was obtained in a citrate concentration range of 0.5–5.0 mmol/L. This method is characterized by excellent precision and accuracy; the coefficient of variation in each case is below the maximal permissible value (%RSD < 2). The proposed analytical procedure has been successfully applied to the determination of citrates in oral electrolyte formulations.

1. Introduction

Citric acid ($C_6H_8O_7$) and its salts are widely used in food and pharmaceutical industries. One of the common pharmaceutical applications of citrates is their use in oral rehydration treatment [1]. For this purpose, electrolytes are normally supplied in the form of effervescent tablets or powders for the preparation of oral solutions. In addition to the citrate salts (most often trisodium citrate) [1], they usually contain glucose, inorganic sodium, and potassium salts (chlorides or bicarbonates). In these formulations, citric acid is typically an acidity regulator. As a rule, citric acid is regarded as a triprotic acid, having three dissociation constants (pK_a = 3.13, 4.76 and 6.40) [2] and forming mono-, di- or tribasic salts. However, there are some reports of a fourth dissociation constant related to the deprotonation of the hydroxyl group at the central carbon atom in strongly basic solutions (pK_a = 13.0) [2,3]. The unionized acid and related citrates exist in aqueous solutions in specific pH ranges (Figure 1) [2–7].

* *Corresponding author*. Department of Inorganic and Analytical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, ul. Banacha 1, 02-097 Warsaw, Poland.
E-mail address: sylwester.krukowski@wum.edu.pl (S. Krukowski).

http://dx.doi.org/10.1016/j.jfda.2017.01.009

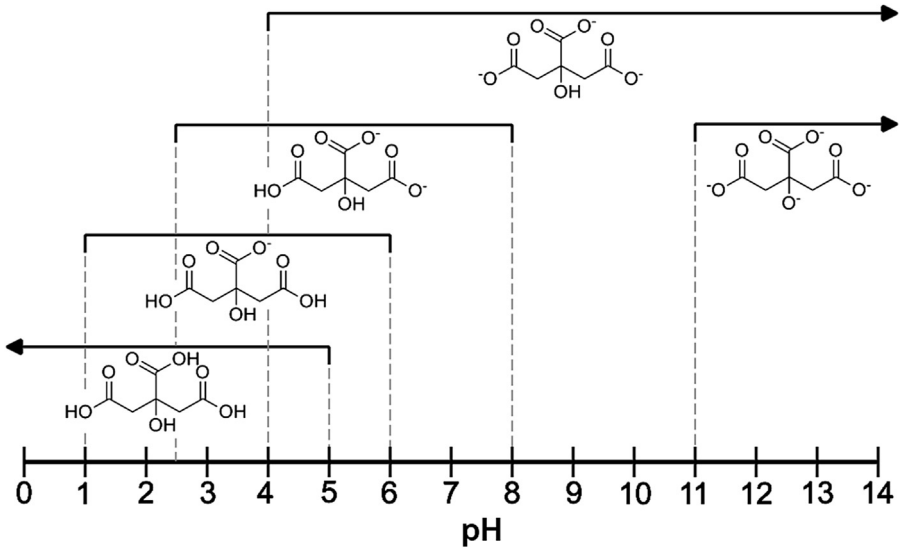

Figure 1 – Specific pH ranges of the occurrence of citric acid and various citrates in aqueous solutions (25°C, $I = 0.1$ M) [2–7].

Aqueous solutions of citric acid and its salts are colorless, since they absorb in the middle-UV range. This absorption is attributed to the carbonyl groups (C=O) of unionized or ionized carboxyl groups (electronic $n \rightarrow \pi^*$ transitions). The absorption maximum of citrates in aqueous solutions is observed at about 200 nm [8,9]. However, because citrates produce broad UV absorption bands close to the lower detectable wavelength limit accessible for standard spectrometers, the direct spectrophotometric method is rarely used. Usually, citrates are analyzed using an indirect procedure necessitating their complexation with transition metal ions [10–12] or enzymatic derivation to achieve clear absorption bands, preferably in the visible range [13,14]. Rather than the spectrophotometric method, chromatographic analysis [6,15–18], capillary electrophoresis [19,20], or classical titration [18] are typically used.

In aqueous solutions of citric acid and citrate salts, the processes of dissociation and hydrolysis lead to complex equilibria which are dependent on nominal concentrations of solutes, pH, and temperature. Therefore, at a constant temperature, an analyst is concerned with a mixture of various forms of citrates (H_3Cit, H_2Cit^-, $HCit^{2-}$, Cit^{3-}) present in the solution at a given pH. The spectrophotometric determination of citrates proposed in this paper requires neither complicated reactions nor the use of specialized reagents. According to the Le Chatelier–Braun principle, the addition of strong acid to a solution of citrates shifts the dissociation backwards towards a less dissociated species. In view of the pK_a values of citric acid, only undissociated H_3Cit molecules are present in aqueous solutions at pH < 1.0 in practice. Thus, the spectrophotometric analytical conditions are significantly improved, as shown in this paper: the absorption maximum moves to a higher wavelength and becomes more discernible, while the absorption bands become narrower.

This study is aimed at the quantitative analysis of citrates in highly acidic aqueous solutions using UV spectrophotometry. Citric acid and trisodium citrate solutions have been studied with the admixture of hydrochloric acid to lower the pH below 1.0. The spectrophotometric analytical procedure is carefully examined and validated on standards. Following this, it is verified against five different commercial medications, which are distributed in sachets as powders containing trisodium citrate (oral electrolyte medications for rehydration therapy). Our method can be applied in pharmaceutical, clinical, and chemical analyses provided that there is no spectral or chemical interference from accompanying compounds.

2. Materials and methods

The commercial oral electrolyte formulations used in this study were procured from the local drugstore; they are listed as follows: Acidolit powder – code E1-AC (Z.F. Polpharma S.A., Starogard Gdanski, Poland), composition: trisodium citrate, glucose, sodium chloride, potassium chloride; Dicodral 60 powder – code E2-DI (Dicofarm S.p.A., Rome, Italy), composition: trisodium citrate, glucose, sodium chloride, potassium chloride, banana flavor; Floractin electrolytes – code E3-FL (Novascon Pharmaceuticals Sp. z o.o., Warsaw, Poland), composition: trisodium citrate, tripotassium citrate, glucose, sodium chloride, orange flavor; Hydronea Baby – code E4-HY (Aflofarm Farmacja Polska Sp. z o.o., Pabianice, Poland), composition: trisodium citrate, glucose, sodium chloride, potassium chloride, lemon flavor; and Orsalit – code E5-OR (Biomed S.A., Cracow, Poland), composition: trisodium citrate, glucose, sodium chloride, potassium chloride.

Validation of the analytical method was performed according to the guidelines of the International Conference on Harmonization [21]. The statistical analysis of the results was done using STATISTICA software (StatSoft, Inc.; version 12; 2014).

2.1. *Preparation of standard solutions for the method validation*

Two series of standard solutions (A and B), each containing 11 solutions, were prepared using anhydrous citric acid (ACS reagent, ≥99.5%, Sigma-Aldrich) and trisodium citrate