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ARTICLES

Simultaneous Determination of Ascorbic, Citric, and Tartaric Acids by Potentiometric Titration with PLS Calibration[1]

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Abstract—The partial least squares (PLS) modeling method was used in the analysis of mixtures of ascorbic, citric, and tartaric acids by potentiometric titration. Binary mixtures of tartaric and citric acids, as well as ternary mixtures of tartaric, citric, and ascorbic acids, were titrated by sodium hydroxide pH-metrically. The linear relationship between the volumes of titrant and the concentrations of analytes was obtained by PLS regression. The designed model was then used to predict the concentrations of components in unknown samples. The practical utility of this method was demonstrated for the simultaneous determination of acids in binary and ternary mixture systems within concentration range from 4×10^{-4} to 2×10^{-3} M, and acceptable results were obtained.

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The simultaneous determination of several analytes in a given sample is now an interesting area in chemometrics [1–3]. Multivariate calibration methods are the basis of such determinations, and over the past several decades advances in chemometrics have led to the development of a multitude of multivariate calibration methods for the analysis of chemical mixtures [4–8]. Classical least squares (CLS), principal component regression (PCR), and partial least squares (PLS) are three multivariate calibration methods that have received considerable attention in the chemometric literature [9–11], and, in recent years, many applications of these chemometric methods have been reported in chemical [12, 13] and pharmaceutical [14–20] analysis.

In the titration of acid–base systems, the detection of endpoints usually depends on using visual indicators or potentiometric methods. These methods are essentially based on the inflection point at which there is a maximum change in pH or potential. In binary or ternary mixed acid systems, if ΔpK_a (K_a is the acid dissociation constant) between any two acids is less than 4, the titration steps of the acids overlap, and it is very difficult to determine the concentration of each acid in these cases.

The simultaneous determination of analytes by potentiometric titration was initiated by Gran [21, 22] and Burns et al. [23]. They deduced a linear plot method for the simultaneous determination of halides and thiocyanate mixtures. The application of multivariate calibration to potentiometric titration data was introduced by Lindberg and Kowalski [24] in 1988 for the simultaneous determination of acid mixtures using PLS regression. After that, this PLS calibration method was applied to acid–base titration [25], complexometric titration [26], and potentiometric precipitation titration [27] by different researchers. In this method, no explicit model is assumed, and the model error is thereby significantly reduced or even completely eliminated. However, it is necessary to have similar samples with known analyte concentrations and the same interference as in the actual samples. Very recently, artificial neural network (ANN) calibration has been applied for the processing of potentiometric titration data of acid mixtures [28–30].

Citric acid ($K_1 = 7.4 \times 10^{-4}$, $K_2 = 1.8 \times 10^{-5}$, $K_3 = 4.0 \times 10^{-7}$) [31] is a natural fruit acid that has been produced commercially by microbial fermentation of a carbohydrate substrate. Citric acid is the most widely used organic acidulant and pH-control agent in foods, beverages, pharmaceuticals, and technical applications. Tartaric acid ($K_1 = 9.1 \times 10^{-4}$, $K_2 = 4.3 \times 10^{-5}$) [31] can be found in many plants. The acid potassium salt is derived as a deposit from fermented grape juice. Ascorbic acid, or vitamin C ($K_1 = 9.1 \times 10^{-5}$, $K_2 = 4.6 \times 10^{-12}$) [31], which is found in many fruit and vegetables, is important in the formation and maintenance of collagen, a protein that supports many-body structures, and plays a major role in the formation of bones and teeth.

[1] The text was submitted by the authors in English.

In our study, the chemical equilibrium of mixtures of ascorbic, citric, and tartaric acids in the titration procedure was investigated. The linear titration equation [25] was used, and the PLS method was applied to the evaluation of potentiometric titration data of the acid mixtures. Mathematical models for multivariate calibration (procedure of calibration) were used to predict the concentration in unknown samples.

PRINCIPLES

Ni [25] showed that, if there are P different acids in the mixture, there is an approximate additive relationship between the volume of titrant added to reach a predetermined pH value and the molar concentration of acids (C), and the following equation was obtained:

$$V_j = k_{j0+}k_{j1}C_1 + \ldots + k_{ji}C_p = k_{j0} + \sum_{i=1}^{P} k_{ji}C_i \quad (1)$$

$$(j = 1, 2, \ldots, J),$$

where C_i refers to acid concentration and k_{ji} is a proportional constant.

It can be found that the potentiometric titration equation of mixed acids is linear. Brown's method [32] was used, and Eq. (1) was written, in simple form, as

$$V_j = \sum_{i=1}^{p} k_{ji}C_i \quad (j = 1, 2, \ldots, J) \quad (2)$$

and in matrix form as

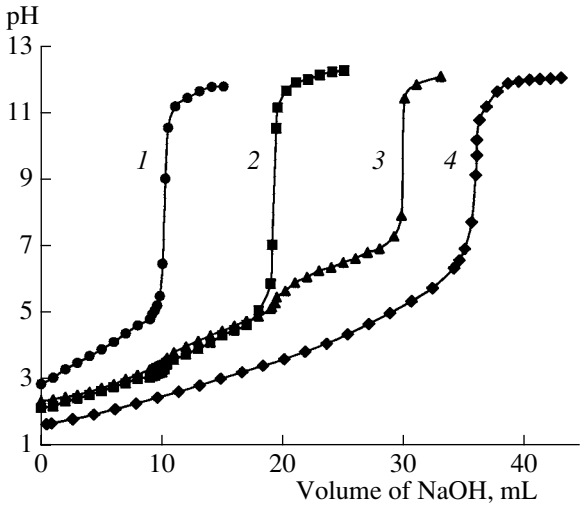
$$\mathbf{V} = \mathbf{KC}, \quad (3)$$

where $\mathbf{V}$ is a $(m \times n)$ matrix of volume of titrant and m and n are the number of samples and number of volumes reading per sample, respectively. If there are p analytes in the system, $\mathbf{C}$ will be a $(m \times p)$ matrix of analyte concentrations, and $\mathbf{K}$ is a $(p \times n)$ matrix of proportionality constants. From this description, it is clear that, although the chemical equilibrium in the titration procedure of acid mixtures is very complex, it can be simplified and treated by suitable chemometric methods.

EXPERIMENTAL

Reagents. All reagents were of analytical reagent grade. Sodium hydroxide, 0.01 M, was prepared by diluting a solution of 0.1 M NaOH. Since no standardization procedure for titrant was necessary, the prepared solution of sodium hydroxide was used both in the calibration and prediction steps. Potassium chloride, 2.0 M, and solutions (0.1 M) of ascorbic, citric, and tartaric acids were prepared according to usual methods.

Apparatus. Titrations were performed by stirrer, burette, and the glass vessels, which were standard equipment. An Eppendorf micropipette (0.5–10 μL) was used to add small volumes of titrant in each titration. pH measurements (±0.001) were carried out with a Corning 125 pH meter (Metrohm) using a combined glass electrode. All experiments were performed at 25°C. Calculations were performed on a PC (Pentium) with the Windows operating system, which was equipped with the Matlab and Excel programs.



Titration curves for (*1*) 10.0 mL of 0.1 M of ascorbic acid, (*2*) 10.0 mL of 0.1 M tartaric acid, (*3*) 10.0 mL of 0.1 M citric acid, and (*4*) their ternary mixtures containing 10.0 mL of 0.1 M ascorbic acid, 5.0 mL of 0.1 M citric acid, and 5.0 mL of 0.1 M tartaric acid with sodium hydroxide.

Procedure. The proper amount of a mixture of acids was placed in a 50-mL vessel, and 5.0 mL of 2.0 M potassium chloride was added to adjust the ionic strength to 0.2 M. The solution was diluted up to 50.0 mL with distilled water. The mixture was then stirred and titrated with the standard solution of sodium hydroxide. The pH meter was used to monitor the pH values during the titration, and the titrant volumes added to reach the predetermined pH values were recorded. Two matrices of data were built; the volume of base at each selected pH point (0.1 pH interval) formed the first matrix, while the concentration of acids formed the second matrix. In addition, the data were mean-centered before the calibration and prediction steps. All the necessary programs for the PLS modeling computing process were written in Matlab.

RESULTS AND DISCUSSION

Experimental design of the calibration sets. In primary experiments, 10 mL of solution of each acid with 0.1 M concentration and a ternary mixture of acids were titrated with 0.1 M sodium hydroxide. The figure shows the titration curves of individual acids and also the titration curve of a mixture of three acids. The curves (figure) show that the first equivalence point for ascorbic acid, second equivalence point for tartaric acid, and third equivalence point for citric acid were

Table 1. The composition of calibration sets for binary and ternary mixtures

System 1 of mixture of acids, mM			System 2 of mixture of acids, mM			
no.	tartaric acid	citric acid	no.	tartaric acid	citric acid	ascorbic acid
1	2.0	1.8	1	0.0	0.4	0.0
2	1.0	2.0	2	0.0	0.0	1.4
3	1.4	0.8	3	0.0	1.2	2.0
4	0.6	0.6	4	0.0	1.8	6.0
5	1.6	0.6	5	0.4	4.0	1.4
6	1.0	1.0	6	0.4	1.8	2.0
7	0.0	1.2	7	1.0	0.0	1.0
8	8.0	1.8	8	1.0	1.8	0.0
9	4.0	1.4	9	1.6	0.4	0.6
10	1.4	2.0	10	1.6	1.2	1.4
11	1.2	0.0	11	1.6	1.8	0.0
12	0.4	1.0	12	1.4	1.4	1.8
13	0.8	1.0	13	2.0	2.0	0.4
14	0.6	2.0	14	2.0	0.4	1.8

monitored. For their mixture, the titration curve shows one inflection point, and, as is obvious, the determination of each acid by usual methods is not possible.

Two systems of acids were prepared. The first system was a binary mixture of different concentrations of tartaric and citric acids, and the second system contained a ternary mixture of various concentrations of tartaric, citric, and ascorbic acids. Table 1 shows the composition of these two systems. The calibration sets for multivariate calibration were prepared according to a random design. The composition of the acid mixtures was chosen to be orthogonal, so that there was no relationship between the concentrations of acids in each system, and the concentrations were not collinear. Each of the acid mixtures (binary and ternary) was titrated with 0.01 M NaOH. In the titration curve of the acid mixtures, most titration points correspond to a range of pH 2.0–9.0, which most clearly reflects the properties and features of acids, so the potentiometric titration data in this pH region were used for calculation.

The factor-analysis-based method of PLS was used to investigate the potentiometric titration data and to determine the acid concentrations in the different mixtures. Therefore, m samples containing different concentrations of acids are titrated and n volumes of base are measured at preselected pH values. Since in the PLS regression (PLS1) the method runs and optimizes for each component separately, the corresponding known concentrations should be arranged in an $m \times l$ vector of **c**. In our study, the volume of base needed to change the pH by an interval of 0.1 was arranged in a **V** matrix, and a matrix with 14 rows and 70 columns was obtained and used to model the systems where the vector of **c** was 14×1.

In order to obtain more accurate results, the pH regions for volume measurements were optimized; therefore, selected pH regions rather than the full pH range were used. For this purpose, different starting and ending pH were selected, and titrant volumes in these regions were entered into the PLS model. Thus, different volume data submatrices (**V**) were built, PLS regression was run for each matrix, and the pH region in which a lower standard error of prediction was obtained was chosen as the working pH region. It was found that a tartaric acid pH range of 4.0–8.0, a citric acid pH range of 4.0–6.0, and an ascorbic acid pH range of 6.3–8.0 give the best results; therefore, these regions were chosen for further study.

Selection of the optimal number of factors. PLS is a factor-analysis-based method. A very important step is the selection of the number of latent variables (factors) in order to model the systems. If we decided to retain more factors than we should, we would be retaining some factors that can only add more noise to our data. On the other hand, if we did not keep enough factors, we would be discarding potentially meaningful information that could be necessary for a successful calibration. Fortunately, there are a number of tools to help us make the decision, such as indicator functions, PRESS for validation data, and cross-validation [34]. In our study, the "leave one out" cross-validation procedure was used to find the optimum number of factors. For each system of mixture of acids that gives a set of 14 calibration titration curves corresponding to the samples listed in Table 1, PLS calibrations on 13 calibration titration curves were performed; using this calibration, the concentrations of compounds in the sample left out during calibration were predicted. The predicted and actual compositions of the samples were compared, and the prediction residual error sum of squares (PRESS) was calculated. The PRESS value was calculated in the same manner. Each time, a new

Table 2. Statistical parameters obtained at selected pH values and optimum number of factors for each acid

Acid in both systems	Number of factors	PRESS	Recovery, %	R^2	RPE_S, %	RPE_T, %
Tartaric acid (system 1)	4	0.30	98.4	0.994	4.93	4.40 (sys1)
Citric acid (system 1)	3	0.06	101.6	0.990	3.60	
Tartaric acid (system 2)	4	0.86	101.2	0.973	6.25	
Citric acid (system 2)	4	0.25	99.8	0.981	5.00	6.01 (sys2)
Ascorbic acid (system 2)	4	0.16	99.2	0.973	6.10	

Table 3. Prediction set composition, predicted values, and respective relative errors

Solution number	Tartaric acid			Citric acid			Ascorbic acid		
	actual value, mM	predicted value, mM	RE, %	actual value, mM	predicted value, mM	RE, %	actual value, mM	predicted value, mM	RE, %
Binary									
1	1.8	1.70	–5.6	1.6	1.65	3.1	–	–	–
2	1.8	1.81	0.6	0.6	0.66	10.0	–	–	–
3	0.4	0.44	10.0	1.2	1.2	0.0	–	–	–
4	2.0	1.89	–5.5	1.4	1.41	0.7	–	–	–
5	0.8	0.74	–7.5	1.8	1.72	–4.4	–	–	–
Ternary									
1	1.6	1.58	–1.3	1.2	1.23	2.5	2.0	1.84	–8.0
2	1.0	1.02	2.0	0.4	0.36	–10.0	1.4	1.34	–4.3
3	0.4	0.44	10.0	1.4	1.52	8.6	0.6	0.65	8.3
4	1.0	0.90	–10.0	1.2	1.15	–4.2	1.0	1.07	7.0
5	1.0	1.1	10.0	1.8	1.76	–2.2	1.8	1.84	2.2
6	0.6	0.58	–3.3	1.4	1.46	4.3	1.0	0.9	10.0

factor was added to the models, and, finally, the number of factors that gives the best results was chosen. One reasonable choice for the optimum number of factors would be the number that yielded the minimum PRESS.

In our case, we found that, for the binary system, four factors for tartaric acid and three factors for citric acid and, for the ternary system, four latent variables for all acids were optimum for the PLS method.

Prediction of synthetic mixtures of acids. Sets of synthetic two- and three-component mixtures of acids within a relatively low concentration range (0–2.0 mM) were analyzed by the proposed method. According to the results, the recovery and prediction errors were calculated. The individual and total prediction errors for mixtures in both systems were calculated in terms of the relative predictive error of the predicted concentration, RPE_S and RPE_T, respectively, and the fitting of data in a straight line was determined by R^2:

$$RPE_S(\%) = 100\left[\sum (C_{ij}^{found} - C_{ij}^{actual})^2 / \sum (C_{ij}^{actual})^2\right]^{\frac{1}{2}}, \quad (4)$$

$$RPE_T(\%) = 100\left[\sum\sum (C_{ij}^{found} - C_{ij}^{actual})^2 / \sum\sum (C_{ij}^{actual})^2\right]^{\frac{1}{2}}, \quad (5)$$

$$R^2 = 1 - \sum_{i=1}^{n} (C_i^{found} - C_i^{actual})^2 / \sum_{i=1}^{n} (\bar{C}_i - C_i^{actual})^2, \quad (6)$$

where C_{ij} is the concentration of the jth component in the ith sample and n is the number of samples.

The values, number of factors, PRESS in the optimum number of factors, percent of recovery, and RPE_S and RPE_T that was calculated for tartaric, citric, and ascorbic acids in the prediction set are summarized in Table 2. The prediction set's composition, predicted values, and respective relative errors are shown in Table 3. As is obvious, relative prediction errors less than 10% are obtained. From repeated titration of tartaric acids with 1.0 M in the ternary mixture, the following average predicted values, relative standard deviation, and confidence interval were obtained: 1.01, 0.1, and 0.12 mM, respectively. The results demonstrate that the factor-analysis-based method of PLS gives good results for resolving the overlapping titration curves of ascorbic, citric, and tartaric acids in their binary and ternary mixtures.

CONCLUSIONS

Simultaneous determination of ascorbic, tartaric, and citric acids by using PLS modeling was established with good prediction ability in artificial samples. This work indicates that, although the solution equilibrium in the acid–base titration procedure is complex, especially for a mixture of polyprotic acids, the titration equation is linear, and the factor-analysis-based method of PLS can resolve the overlapping titration curves of these acids in their mixtures.

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