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A STUDY OF A REACTION SYSTEM FOR ORGANIC ACID ANALYSIS USING A pH INDICATOR AS POST-COLUMN REAGENT

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SUMMARY

A post-column reaction system using a pH indicator for organic acid analysis has been studied theoretically with the aid of computer calculation. This examination has revealed that bromothymol blue (BTB) is a most suitable pH indicator for this purpose. The analysis system using BTB gives linear calibration curves up to 50 μg of organic acids. The detection limit is about 20 ng (signal-to-noise ratio = 2) for acetic acid, and the between-analysis coefficients of variation were 0.6% for injection of 10 μg pyroglutamic acid, and $\leq 0.6\%$ for citric, malic, succinic, lactic and acetic acids.

INTRODUCTION

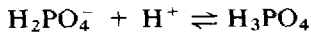
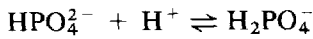
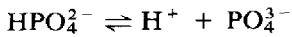
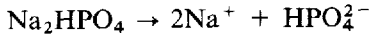
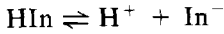
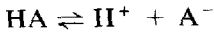
In high-performance liquid chromatographic (HPLC) analysis, organic acids are commonly monitored by refractive index (RI) detection or UV detection at around 210 nm. However, these methods of detection are not specific for organic acids. Therefore, selective detection methods are desired.

For pre-column derivatization of carboxyl compounds, *p*-bromophenacyl bromide¹, bromomethoxycoumarin²⁻⁴, 9-anthryldiazomethane (ADAM)⁵, etc. have been employed as the reagent. For post-column reaction, dicyclohexylcarbodiimide⁶, bromocresol purple (BCP)^{7,8} and bromophenol blue⁹ have been used. Of these reagents, BCP gives higher sensitivity, even with a simpler reactor, due to its fast reaction rate, although it gives a less linear calibration curve. It could be a superior method if the problem of linearity of response were overcome.

The principle of detection using a pH indicator such as BCP is as follows. When an organic acid is added to a buffer solution, which contains the pH indicator, the pH of the buffer solution decreases causing the indicator to change colour. Therefore, the organic acid can be detected photometrically by monitoring at the wavelength of maximum absorbance of the pH indicator in its acidic form. We have analyzed various combinations of pH indicators and buffer solutions by utilizing a small computer for numerical calculation of equilibrium, and studied the reaction system.

THE REACTION SYSTEM

One example of the theoretical analysis will be demonstrated. In the reaction system chosen, Na_2HPO_4 and HClO_4 act as the buffer solution and eluent, respectively. All the reactions in this system are those of acid-base titrations



where HA represents an organic acid and HIn the pH indicator. The concentrations of free and dissociated organic acid, [HA] and [A], are related to its dissociation constant, K_a , by:

$$\frac{[\text{H}^+][\text{A}^-]}{[\text{HA}]} = K_a$$

The concentrations of other ions are related by the following equations:

$$\frac{[\text{H}^+][\text{In}^-]}{[\text{HIn}]} = K_{\text{in}} \quad \frac{[\text{H}^+][\text{PO}_4^{3-}]}{[\text{HPO}_4^{2-}]} = K_3$$

$$\frac{[\text{H}^+][\text{H}_2\text{PO}_4^-]}{[\text{H}_3\text{PO}_4]} = K_1 \quad [\text{H}^+][\text{OH}^-] = K_w$$

$$\frac{[\text{H}^+][\text{HPO}_4^{2-}]}{[\text{H}_2\text{PO}_4^-]} = K_2$$

Then, the total concentrations of organic acid, C_a , pH indicator, C_{in} , Na_2HPO_4 , C_B , and H^+ ion are

$$[\text{HA}] + [\text{A}^-] = C_a$$

$$[\text{HIn}] + [\text{In}^-] = C_{\text{in}}$$

$$[\text{H}_3\text{PO}_4] + [\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{PO}_4^{3-}] = C_B$$

$$3[\text{H}_3\text{PO}_4] + 2[\text{H}_2\text{PO}_4^-] + [\text{HPO}_4^{2-}] + [\text{HA}] +$$

$$+ [\text{HIn}] + [\text{H}^+] - [\text{OH}^-] = C_A + C_B + C_a + C_{\text{in}}$$

where C_A represents the concentration of HClO_4 .

Now, as ten equations are given for the ten variables, these equations can be solved and it is theoretically possible to obtain the relationship between the concentration of the pH indicator in its acidic form, $[\text{HIn}]$, and the amount of organic acid, C_a . However, this requires complicated calculations. Therefore two approximations are made: (i) C_a can be considered as an increment of C_A ; (ii) the amount of protons originating from the pH indicator is negligible. The former is based on the fact that almost all the organic acids are dissociated completely at around pH 7, and the later on the fact that C_{in} is negligibly small compared with C_B . With these approximations the equations led to the following result

$$C_A = C_B \frac{2\beta_3[\text{H}^+]^3 + \beta_2[\text{H}^+]^2 - 1}{\beta_3[\text{H}^+]^3 + \beta_2[\text{H}^+]^2 + \beta_1[\text{H}^+] + 1} + [\text{H}^+] - \frac{K_w}{[\text{H}^+]}$$

where:

$$\beta_1 = \frac{1}{K_3}, \quad \beta_2 = \frac{1}{K_2K_3}, \quad \beta_3 = \frac{1}{K_1K_2K_3}$$

This equation describes the titration curves of Na_2HPO_4 with HClO_4 . Fig. 1 shows the relationship between C_A and $[\text{H}^+]$ calculated for a C_B value of 0.0075 M using this equation and a small computer. The change in pH on adding organic acid can be evaluated from the pH values at C_A and $C_A + C_a$.

The colour change of the pH indicator can also be represented as an acid dissociation equilibrium, and the indicator transition, φ , can be related to $[\text{H}^+]$ by:

$$\varphi = \frac{[\text{HIn}]}{C_{in}} = \frac{[\text{H}^+]}{[\text{H}^+] + K_{in}}$$

This relationship between φ and pH is plotted in Fig. 2 for three pH indicators with

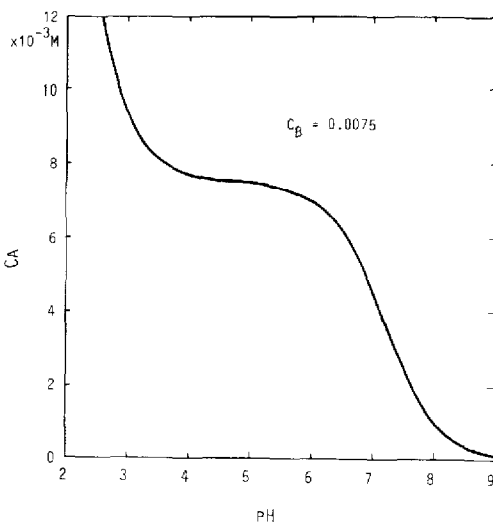


Fig. 1. Relationship between the concentration of acid, C_A , and pH. $C_B = 7.5 \text{ mM}$.

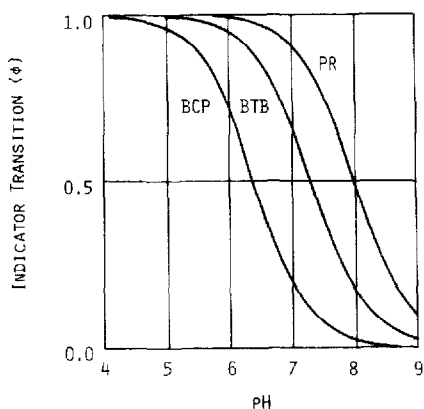


Fig. 2. Relationship between the indicator transition, ϕ , and pH. BCP = Bromocresol purple ($pK_{in} = 6.4$); BTB = bromothymol blue ($pK_{in} = 7.3$); PR = phenol red ($pK_{in} = 8.0$).

their pK_{in} values of around 7: BCP ($pK_{in} = 6.4$), bromothymol blue (BTB) ($pK_{in} = 7.3$) and phenol red (PR) ($pK_{in} = 8.0$). C_A is also related to ϕ via the pH, as illustrated in Fig. 3, where the derivative $\partial\phi/\partial C_A$ is plotted against C_A . Now, $\partial\phi/\partial C_A$ is directly related to the gradient of the calibration curve, and a large and constant value of $\partial\phi/\partial C_A$ is preferable for a high sensitivity and linear response. It is apparent from Fig. 3 that BTB is the most suitable pH indicator.

Studies were also made at several other values of C_B , and it is found that the larger the value of C_B the wider is the dynamic range and the lower the sensitivity. It is concluded that $0.0075 M$ is the most suitable concentration for the Na_2HPO_4 buffer in order to obtain both a wide dynamic range and high sensitivity.

Similar studies of other buffer solutions, such as Na_2CO_3 , Na_3BO_3 , CH_3COONa , etc., gave no better results.

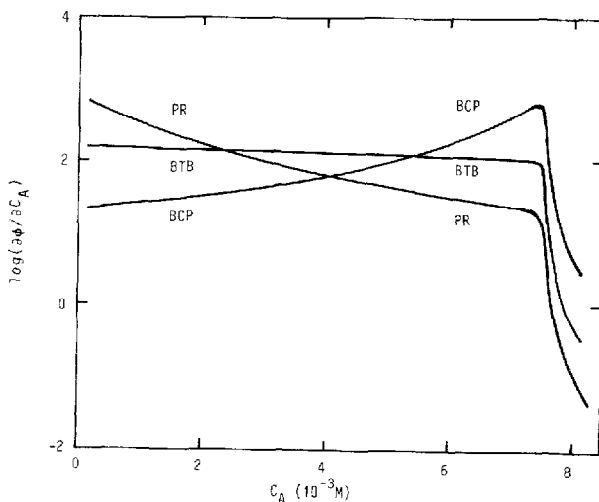


Fig. 3. Relationship between the indicator transition, ϕ , and the concentration of acid, C_A . The value of $\partial\phi/\partial C_A$ is plotted on a logarithmic scale.

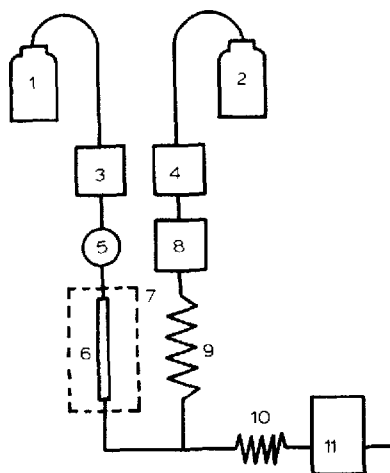


Fig. 4. Block diagram of the system. 1 = Eluent; 2 = reagent; 3 = Trirotar-V eluent delivery pump; 4 = SP-078 reagent delivery pump; 5 = VL-614 sample injector; 6 = Shodex Ionpak C-811 column; 7 = TU-100 column oven; 8 = PG-350 D pressure gauge; 9 = back-pressure coil; 10 = reaction coil; 11 = UVIDEC-100-IV detector.

EXPERIMENTAL

Based on the results of the calculations, a reaction system was designed and tested. A block diagram of this system is shown in Fig. 4. The system includes a JASCO Model Trirotar-V eluent delivery pump, a Model SP-078 reagent delivery pump, a Model VL-614 sample injector, a Model TU-100 column oven and a Model UVIDEC-100 IV UV-VIS detector. The separation column was a Shodex Ionpak C-811 (500 × 8.0 mm I.D.) packed with strong cation-exchange resin. Analytical conditions are listed in Table I. A 0.002 *M* solution of NaOH was added to the reagent solution in order to obtain a wider dynamic range by partially neutralizing HClO₄.

All chemicals used were of reagent grade and obtained from commercial sources.

RESULTS AND DISCUSSION

Fig. 5 shows a typical chromatogram obtained for 10 μl of a standard mixture

TABLE I
OPERATING CONDITIONS

Eluent	3 <i>mM</i> HClO ₄
Flow-rate	1.0 ml/min
Reagent	0.2 <i>mM</i> BTB, 15 <i>mM</i> Na ₂ HPO ₄ , 2 <i>mM</i> NaOH
Flow-rate	1.0 ml/min
Column	Shodex Ionpak C-811
Temperature	60°C
Wavelength	445 nm

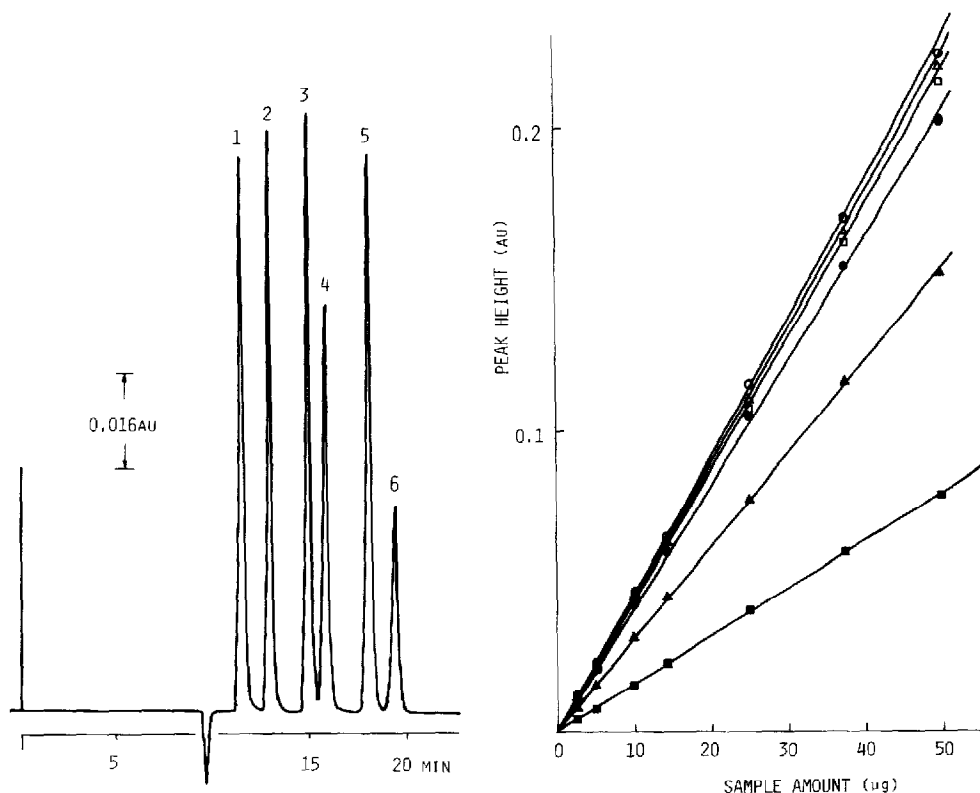


Fig. 5. Typical chromatogram of 10 μ l of standard mixture. Peaks: 1 = citric acid; 2 = malic acid; 3 = succinic acid; 4 = lactic acid; 5 = acetic acid; 6 = pyroglutamic acid (each 10 μ g).

Fig. 6. Calibration curves for citric (\square), malic acid (\triangle), succinic acid (\circ), lactic acid (\blacktriangle), acetic acid (\bullet) and pyroglutamic acid (\blacksquare).

containing 10 μ g each of six organic acids, citric, malic, succinic, lactic, acetic and pyroglutamic acid.

A crucial aspect of this study is the linearity of the calibration curves. Fig. 6 shows the calibration curves obtained for the six organic acids, and the equations describing these curves and the correlation coefficients are listed in Table II. All the

TABLE II

EQUATIONS OF CALIBRATION CURVES AND CORRELATION COEFFICIENTS

The equation is defined as $h = ax + b$, where h is the peak height ($\times 10^{-3}$ absorbance) and x is the sample amount (μ g).

Organic acid	Equation	γ
Citric acid	$h = 4.250x + 1.130$	0.9998
Malic acid	$h = 4.329x + 2.408$	0.9997
Succinic acid	$h = 4.413x + 2.598$	0.9997
Lactic acid	$h = 3.030x + 1.339$	0.9999
Acetic acid	$h = 3.987x + 2.836$	0.9997
Pyroglutamic acid	$h = 1.578x + 0.346$	0.9999

TABLE III
REPRODUCIBILITY OF PEAK HEIGHTS FOR ORGANIC ACIDS

$n = 10$.

Organic acid	Peak height (absorbance)	σ ($\times 10^{-4}$ absorbance)	C.V. (%)
Citric acid	0.2111	6.9	0.33
Malic acid	0.2167	5.4	0.25
Succinic acid	0.2203	9.0	0.21
Lactic acid	0.1517	6.0	0.39
Acetic acid	0.1990	8.6	0.43
Pyroglutamic acid	0.0790	4.7	0.60

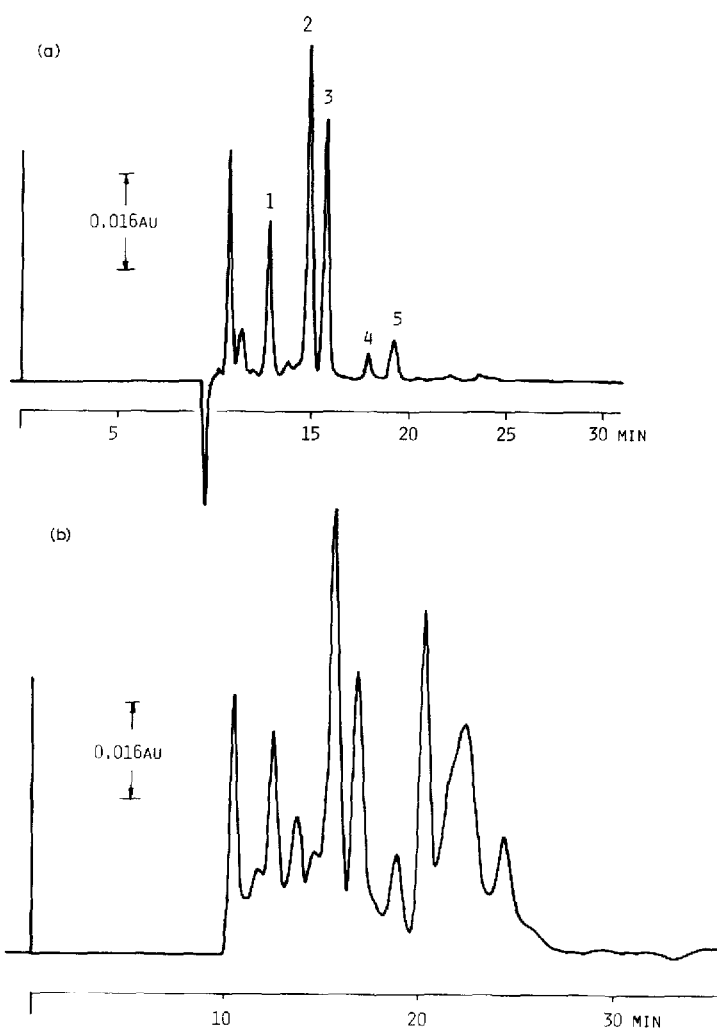


Fig. 7. Chromatograms of Japanese "Sake" obtained using the described system. (a) and by UV detection at 210 nm (b). Peaks: 1 = malic acid; 2 = succinic acid; 3 = lactic acid; 4 = acetic acid; 5 = pyroglutamic acid.

correlation coefficients are almost equal to 1.0, thus verifying the excellent linear relationships between the amounts (up to 50 μg) and peak heights. The detection limit defined as $S/N = 2$ was found to be 20 ng for acetic acid.

The reproducibility of this method was evaluated by repeated analysis of the standard mixture, and excellent results were obtained as listed in Table III. The coefficient of variation (C.V.) for pyroglutamic acid was 0.6% ($n = 10$) and the C.V.s for the other five organic acids are less than this value.

This system gives sufficient selectivity for organic acid analysis in food. Fig. 7a shows a chromatogram obtained by injection of 30 μl of a Japanese "Sake" without any pretreatment. The peaks of malic, succinic, lactic, acetic and pyroglutamic acids are clearly separated from each other. On the other hand, a chromatogram of the same sample, shown in Fig. 7b, obtained by using an UV detector at 210 nm without the reaction system, contains so many interfering peaks that the peaks of the organic acids cannot be distinguished.

In conclusion, we have shown that BTB is a suitable pH indicator for organic acid analysis by analyzing theoretically the reaction system with the aid of computer calculation. Due to its superior linearity, the resulting system can serve as a useful tool for quantitative analysis of organic acids in foods, biological samples, etc.

REFERENCES

- 1 H. D. Durst, M. Milano, E. J. Kitka, Jr., S. A. Connelly, and E. Grushka, *Anal. Chem.*, 47 (1975) 1797.
- 2 W. Dunges, *Anal. Chem.*, 49 (1977) 442.
- 3 S. Lam and E. Grushka, *J. Chromatogr.*, 158 (1978) 207.
- 4 E. Grushka, S. Lam and J. Chassin, *Anal. Chem.*, 50 (1978) 1398.
- 5 N. Nimura and T. Kinoshita, *Anal. Lett.*, 13-3A (1980) 191.
- 6 Y. Kasai, T. Tanimura and Z. Tamura, *Chem. Pharm. Bull.*, 20 (1972) 1845.
- 7 *Jap. Pat.*, S57-34502, 1982.
- 8 R. Faromptto, M. Cande, G. Mahuzier and R. Rosset, *Analisis*, 7 (1979) 449.
- 9 A. Egashira, *Bunseki Kagaku (Jap. Anal.)*, 15 (1966) 1356.