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Identification and determination of active components in *Gastrodia elata Bl.* by capillary electrophoresis

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Abstract

Capillary zone electrophoresis (CZE), using a 25 m*M* borate buffer (pH 10.0) with 10% (v/v) acetonitrile, was established for the identification and determination of five constituents – gastrodin (GA), 4-hydroxybenzyl alcohol (HA), vanillyl alcohol (VA), 4-hydroxybenzaldehyde (HD) and vanillin (VL) – in the extracts of *Gastrodia elata Bl.* roots. Regression equations revealed linear relationships (correlation coefficients: 0.9992–0.9999) between the peak area of each constituent (GA, HA, VA, HD and VL) and its concentration. The relative standard deviations of the migration times of five constituents ranged between 0.99 and 1.43%. The recoveries of five constituents ranged between 94.3 and 109.2%. The GA, VA and HA contents measured 6.18 mg/g (1.11% RSD), 0.71 mg/g (0.86% RSD) and 0.25 mg/g (8.56% RSD), respectively, in the sample of *Gastrodia elata Bl.* collected in Sichuan province. Also, the dissociation constant of GA was determined by a CE method.

Keywords: *Gastrodia elata*; Plant materials; Pharmaceutical analysis; Gastrodin; Alcohols; Vanillin; Aldehydes

1. Introduction

Traditional Chinese medicines have been used to treat human diseases in China for centuries. In the last few decades, the research and development of bioactive ingredients of these traditional medicines has attracted serious international scientific attention [1]. Because a great number of these medicinal plants show complicated profiles of constituents, work on the qualification control and quantitative analysis of active components in traditional and herbal medicines has become necessary.

Gastrodia elata Bl. is a notable Chinese medicine. The steamed and dried roots of this plant are commonly used as a folk medicine under the name of ''Tianma''. The medicine is considered to have very beneficial properties, it is said to improve the circulation, and it is prescribed for rheumatism, sedative, paralysis, hemiplegia, lumbago, headaches, vertigo and anticonvulsant actions [2,3]. The activity has been demonstrated in animal experiments in which rats, monkeys pigeon and domestic rabbits were used as experimental objects. Usually, it takes a long time to decoct these medicines with water in the traditional preparation process. This method limits the wide use of Chinese medicine. In order to overcome the shortcoming, now, the extracts of *Gastrodia Bl.* have been manufactured by many Chinese pharmaceutical companies on a large scale. This extract product has been widely employed in

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the treatment of some diseases, and it has also been used as an important raw material in the pharmaceutical industry. It has been demonstrated that the major active components in *Gastrodia Bl.* are gastrodin (GA), 4-hydroxybenzyl alcohol (HA) and vanillyl alcohol (VA) [2,3], it was reported that 4-hydroxybenzaldehyde (HD), vanillin (VL), succinic acid, β-sitosterol and sucrose were isolated from the plant [4,5]. Thus, the three important bioactive components can be considered as a quality index for evaluation of *Gastrodia Bl*. It is therefore necessary to develop a simple and quality control method for identifying and determining the active components.

Although a wide variety of analytical methods including spectrophotometry [6] and high-performance liquid chromatography (HPLC) [7–9] were used for determination of GA and/or HA, these methods suffer from limitations such as material- and time-consuming, since a number of prior steps are often required to obtain species of interest from the sample matrix and isolate them from potentially interfering compounds. Recently, owing to its higher resolving power, short analysis time and simple pretreatment, capillary electrophoresis (CE) has been used as an attractive method for the separation and monitoring of Chinese traditional medicine [10–16]. CE methods have developed for the determination GA in Tianma injection [17], and some other constituents parishin, parishin B and parishin C [18,19] in traditional Chinese medicinal formulas including *Gastrodiae rhizoima*. There are no reports on the determination of the three major active components in *Gastrodia Bl.* by CE. This paper firstly developed a CE method for identification and determination of three active components with VL and HD from *Gastrodia Bl.*

2. Experimental

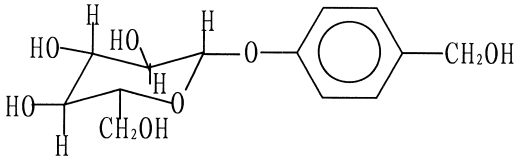
2.1. Instrumental

All experiments were carried out on a Waters Quanta 4000 system (Waters, Milford, MA, USA). The temperature was kept at 23°C and the wavelength of the UV detector was set at 214 nm. Hydrodynamic injection (10.0 cm) was for 4 s. A 50.0 cm (distance between injector and detector 42.4 cm)×75 μm I.D. fused-silica capillary from Waters Accasep, was used. A 5 min wash cycle with 0.1 *M* NaOH followed by 3 min distilled water, and 5 min run buffer was necessary to condition the capillary. Between the runs, the capillary was washed with run buffer.

2.2. Materials and reagents

The samples of the roots of *Gastrodia Bl.* produced in the Gansu and Sichuan provinces (marked as sample 1 and sample 2, respectively) were purchased from local drugstores. Gastrodin (GA) was provided by Kunming Pharmacy Industry, China. HA, VA, HD and VL (structures shown in Fig. 1) were purchased from Beijing Chemicals Reagents Plant, acetonitrile was from Tianjing Chemistry Reagents Plant. Unless otherwise specified, all reagents were analytical grade. All solutions and samples were prepared by filtered, degassed.

The run buffer solutions were prepared as to contain 25 m*M* sodium tetraborate, and their desired pH was adjusted by addition of an appropriate volume of 0.1 *M* NaOH. Ethanol was used as the solvent and the electroosmotic marker.



Gastrodin (GA)

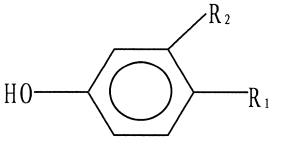


Fig. 1. Molecular structure of the analytes 4-hydroxybenzyl alcohol (HA): $R_1=CH_2OH$, $R_2=H$, vanillyl alcohol (VA): $R_1=CH_2OH$, $R_2=OCH_3$, 4-hydroxybenzaldehyde (HD): $R_1=CHO$, $R_2=H$, vanillin (VL): $R_1=CHO$, $R_2=OCH_3$.

2.3. Sample preparation

Three methods were used to prepare the extract from *Gastrodia Bl.* (1) Crashed roots (1.0 g) of *Gastrodia Bl.* were extracted successively three times (2 h each time) with alcohol at 80°C. The total extract was concentrated to 10 ml as injection sample after filtering. (2) Methanol was used as the extractant. (3) A 1.0-g sample was extracted with distilled water (2 h each time) at 95°C, the extract solution was evaporated to dryness. The residue was dissolved in 10 ml alcohol.

2.4. Calculation

In conventional CE, μ_{eff}, the effective mobility of the analyte is easily determined by the classical relationship:

$$\mu_{eff} = \frac{l \cdot L}{V}\left(\frac{1}{t_{eof}} - \frac{1}{t_M}\right) \quad (1)$$

where, t_M and t_{eof} are, respectively, the migration time(s) of the analyte and a neutral marker. L, l and V are, the total length of capillary (cm), the capillary length to the detector (cm) and the applied voltage (V), respectively.

3. Results and discussion

3.1. Optimization of separation

The structures of GA, HA, VA, HD and VL suggested that they could be analyzed as anions, borate was selected as the background electrolyte. The separation was achieved by optimizing the pH of the buffer, borate concentration, organic modifier and the voltage.

To verify the effect of buffer pH on migration behavior, preliminary experiments were performed with 25 m*M* borate without acetonitrile in the electrophoretic medium, results are shown in Fig. 2. It can be observed that the almost co-migration of GA, VA and HA with electroosmotic flow (EOF) occurred at pH 8.6, which suggested that ionization of the three compounds had not taken place. With the increase in pH of the electrolyte, the separation of GA, VA and HA was improved. In addition, Fig. 2 shows that the migration time of EOF, GA, VL and

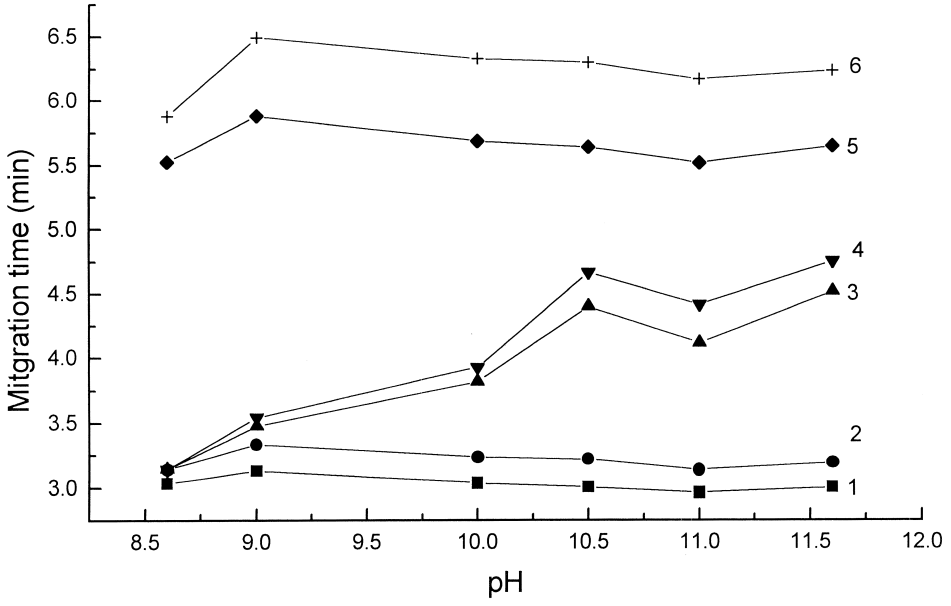


Fig. 2. Effect of pH on the migration time: 1=marker; 2=GA; 3=VA; 4=HA; 5=VL; 6=HD. Analytical conditions, 25 m*M* borate, voltage 18 kV, sample time 4 s.

HD almost remained unchanged when the pH was above 9.0. However, the migration time of VA and HA increased rapidly. Therefore, pH 10 was selected. When the borate concentration varied from 10 to 20 m*M*, the migration times of the different solutes increased rapidly, showing there should be a strong interaction between borate and the hydroxyl group of these compounds [12]. After that, the migration times were found to increase gradually for GA, VA and HA, and even slightly decreased. 25 m*M* borate was chosen for the separation. A higher voltage was necessary for rapid CE analysis. It was found that with the applied voltage ranging from 10 to 26 kV, the separation efficiency of these compounds was not improved. But, when there was a lower voltage, the migration time increased. 18 kV was used as the run voltage.

Although these compounds were completely separated under the above optimal conditions, when the samples were determined, VA and HA were still not free of interference from other coexisting constituents (see Fig. 4A). Therefore, further attempts were made to improve the resolution by adding the organic modifier acetonitrile to the electrolyte. Different acetonitrile concentrations ranging from 5 to 20% were used to study the effect of acetonitrile concentration on the resolution. The experimental results indicated that the migration times of GA, VL and HD increased as the acetonitrile concentration increased, but the migration times of VA and HA decreased when the acetonitrile was absent. Thus, VA and HA could be separated from coexisting constituents (see Fig. 4B). Meanwhile, the addition of acetonitrile to the buffer also resulted in the measured peak area of the solute studied increasing with increasing acetonitrile concentration. The increase of the peak area suggests the addition of acetonitrile could improve the detection sensitivity of the five compounds.

According to the factors mentioned above, the best resolution was obtained with a 25 m*M* borate, 10% acetonitrile, at pH 10, and voltage 18 kV.

3.2. The determination of the dissociation constant (pK_a) of gastrodin by CE

The pK_a value is an important physiochemical property of drug. Potentiometric, spectrophotometric, solubility and paper electrophoretic methods have been employed for the determination of pK_a values. It was reported that CE was also one of the better methods to determine pK_a values [20–22]. This was based on the fact that pH plays a fundamental role in the ionization of the analyte, and this can be used for the determination of pK_a by measuring the electrophoretic mobility. The influence of buffer pH on the effective mobility of GA is shown in Fig. 3. Using the method [22], the apparent pK_a of gastrodin was calculated to be 9.10. Similarly, the pK_a values of VA, HA, VL and HD were obtained. Table 1 gives the list of the pK_a values of GA, VA, HA, VL and HD in aqueous solution using CE, compared to the literature data [23]. From Table 1, it was seen that the pK_a values of HA, VL and HD determined by CE were in a agreement with those in the literature.

3.3. Regression equations and linear ranges of GA, VA, HA, VL and HD

The linear relationships between the concentrations of five compounds and the corresponding peak areas were found in the concentration range of 25.0~1000 μg/ml for GA, 12.5~500 μg/ml for VA and HA, 5.0~200 μg/ml for VL, 10.0~250 μg/ml for HD. The regression equations of these curves and their correlation coefficients (r) were calculated as follows: GA, $y=11.77x+229.83$ ($r=0.9995$); VA, $y=28.65x+36.22$ ($r=0.9999$); HA, $y=13.13x+5.21$ ($r=0.9999$); VL, $y=55.21x+114.22$ ($r=0.9992$); HD, $y=31.92x-211.39$ ($r=0.9996$); where y and x are the peak area and the concentration (μg/ml) of the analytes, respectively.

3.4. System suitability test

The method was validated for reproducibility of the migration time and the peak area of the analytes. The relative standard deviation (RSD) values of the migration time and the peak area of each peak for three replicate injections were 0.99–1.43% and 1.52–4.86%, respectively. The accuracy and recovery of the method were determined with the standard addition method for GA, VA, HA, VL and HD in *Gastrodia Bl.* samples 1 and 2, with results ranging from 94.3–109.2% for sample 1, and 100.9–107.5% for sample 2.

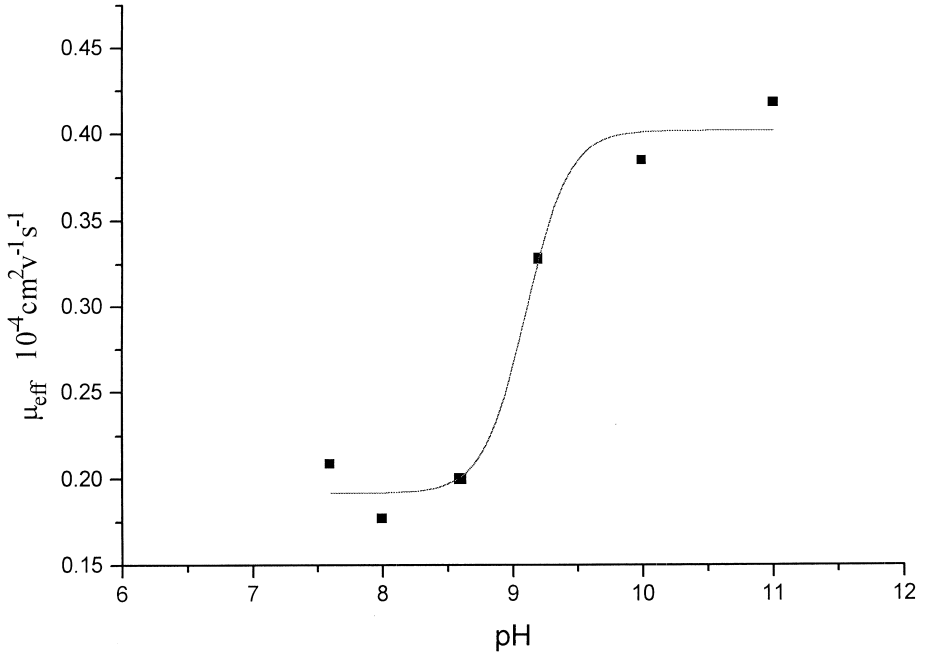


Fig. 3. Effect of pH on the effect mobility of gastrodin. Conditions as in Fig. 2.

3.5. Extraction and determination of the analytes in Gastrodia Bl.

Under the above conditions, GA, VA, HA, VL and HD in *Gastrodia Bl*. were all successfully separated. A typical electropherogram is shown in Fig. 4B (sample 2). Peaks were identified by addition standard substances of GA, VA, HA, VL and HD. It was observed that GA, VA and HA were baseline resolved, but VL and HD were not detected. Another sample 1 gave a similar electropherogram without VL and HD peaks. It shows that the contents of VL and HD in both *Gastrodia Bl*. samples are lower.

Table 1
Dissociation constants of GA, VA, HA, VL and HD

Analytes	CE	Literature [23]
Gastrodin	9.10	No
Vanillyl alcohol	11.06	No
4-Hydroxybenzyl alcohol	9.93	9.82
Vanillin	7.74	7.40
4-Hydroxybenzaldehyde	7.76	7.62

Furthermore, the three extraction methods described in the above experiments were compared, their analytical results are listed in Table 2. It is apparent that methods I and II gave higher contents than method III, that is, use of ethanol or methanol extracting bioactive components from *Gastrodia Bl.* is better than the use of water. This result suggests that it is important to select a suitable solvent for extracting bioactive components from traditional Chinese medicines. Also, Table 2 shows that the contents of the constituents of *Gastrodia Bl.* grown in different places vary.

4. Conclusion

The results demonstrate that CE is a useful, simple and rapid technique for identification and determination of GA, VA, HA, VL and HD in *Gastrodia Bl.* The method also promises to be applicable to the quality control of traditional Chinese medicines. CE is suitable for the determination of the pK_a of compounds.

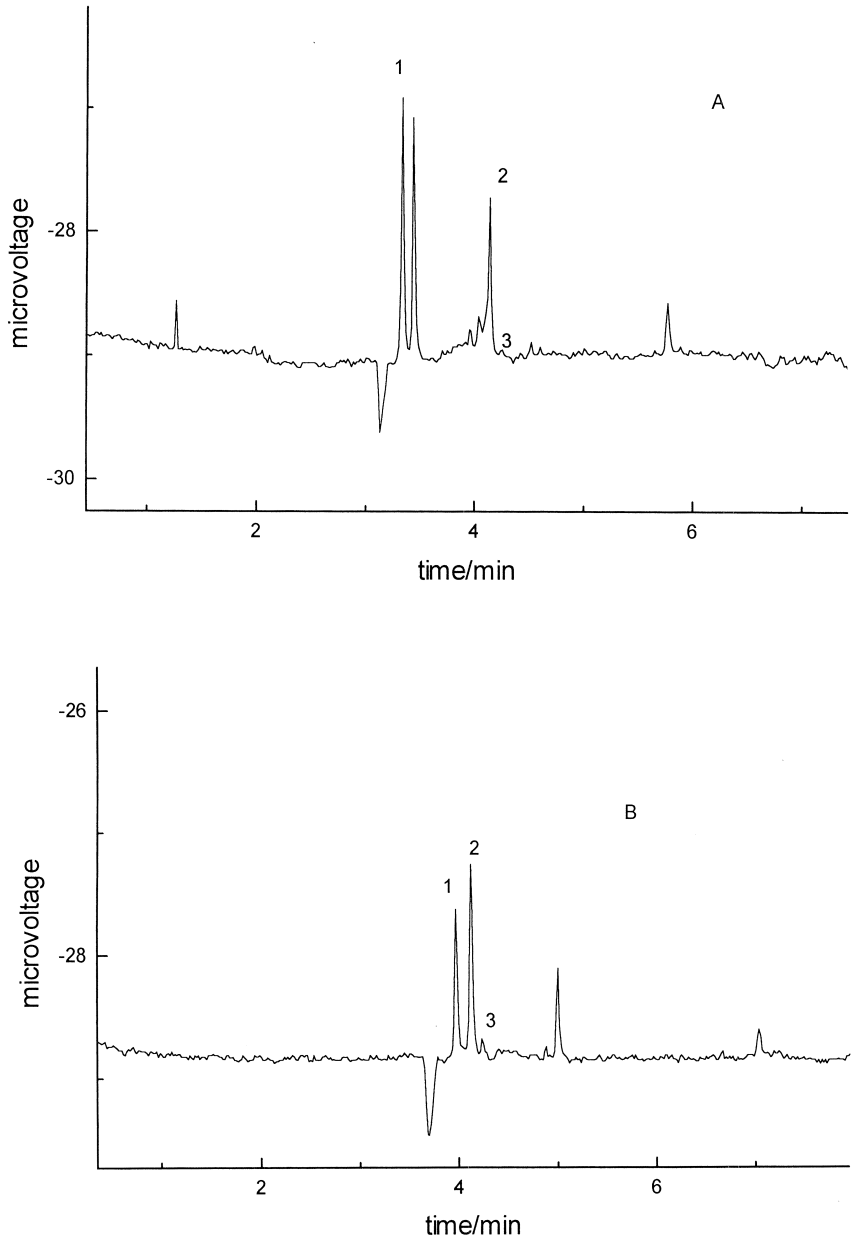


Fig. 4. Electropherogram of alcohol extract of *Gastrodia Bl.* sample 2. (A) Without acetonitrile; (B) acetonitrile concentration (10%, v/v); other conditions as in Fig. 2. Peaks: 1=GA, 2=VA, 3=HA.

Acknowledgements

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Table 2
Content of the analytes in *Gastrodia Bl.* ($n=3$)

Composition	Sample 1						Sample 2,	
	Method I		Method II		Method III		Method I	
	mg/g	RSD (%)	mg/g	RSD (%)	mg/g	RSD (%)	mg/g	RSD (%)
Gastrodin	2.59	7.08	2.62	8.32	1.45	6.25	6.18	1.11
Vanilly alcohol	1.66	6.24	1.32	4.59	0.59	2.67	0.71	0.86
4-Hydroxybenzyl alcohol	0.21	3.92	0.15	4.18	0.17	3.41	0.25	8.56

References

[1] L.N. Li, Pure Appl. Chem. 70 (1998) 547.
[2] Jiangsu New Medical College, in: Dictionary of Traditional Chinese Medicines, Shanghai Scientific and Technological Publ, Shanghai, 1996, p. 315.
[3] G.J. Xun (Ed.), Pharmacognosy, Peoples' Hygiene Publ, Beijing, 1997, p. 121.
[4] J. Zhou, Y.B. Yang, T.R. Yang, Acta Chem. Sinica 37 (1979) 185.
[5] X.Z. Feng, Y.W. Chen, J.S. Yang, Acta Chem. Sinica 37 (1979) 175.
[6] Z.Y. Zhu, Fenxi Ceshi Tongbao 8 (1989) 43.
[7] C.Y. Tong, Yaowu Fenxi Zazhi 9 (1989) 182.
[8] Y.Y. Luo, M. Liu, Q. Zhu, G.H. Li, Zhongguo Yaoxue Zazhi 29 (1994) 684.
[9] S.Z. Chen, C.Z. Liang, M.X. Ren, Yaowu Fenxi Zazhi 4 (1984) 144.
[10] C.T. Chen, S.T. Shen, J. Chromatogr. A 710 (1995) 323.
[11] C.Y.C. Chou, T.H. Tsai, M.F. Lin, C.F. Chen, J. Liq. Chromatogr. Rel. Technol. 19 (1996) 1909.
[12] Z.P. Zhang, Z.D. Hu, G.L. Yang, Chromatographia 44 (1997) 162.
[13] G.B. Li, X.G. Chen, M.C. Liu, Z.D. Hu, Analyst 123 (1998) 1501.
[14] H.M. Liebich, R. Lehman, C. Di Stefano, H.U. Haring, J.H. Kim, K.R. Kim, J.R. Kim, J. Chromatogr. A 795 (1998) 388.
[15] J.J. Yang, H. Long, H.W. Liu, A.J. Huang, Y.L. Sun, J. Chromatogr. A 811 (1998) 274.
[16] M.C. Lee, S.J. Shen, J. Liq. Chromatogr. Rel. Technol. 20 (1997) 63.
[17] F.M. Han, Y. Chen, Fenxi Kexue Xuebao 14 (1998) 135.
[18] Y.R. Ku, Y.T. Lin, J.H. Lin, K.C. Wen, C.H. Liao, J. Chromatogr. A 805 (1998) 301.
[19] Y.R. Ku, Y.T. Lin, J.H. Lin, K.C. Wen, C.H. Liao, J. Chromatogr. A 805 (1998) 330.
[20] J.L. Beckers, F.M. Everaerts, M.T. Ackermans, J. Chromatogr. 537 (1991) 407.
[21] J.A. Cleveland, M.H. BenKo, S.J. Gluck, Y.M. Walbroehl, J. Chromatogr. A 652 (1993) 301.
[22] Y. Mrestani, R. Neubert, A. Munk, M. Wiese, J. Chromatogr. A 803 (1998) 273.
[23] J.A. Dean (Ed.), Lange's Handbook of Chemistry, 13th ed, McGraw-Hill, New York, 1985.