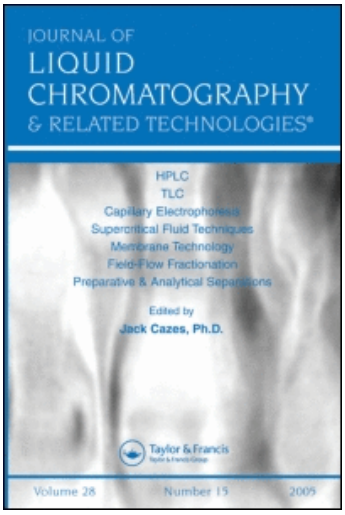

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Chen, Lu , Johnson, Bruce D. , Grinberg, Nelu , Bickery, Gary R. and Ellison, Dean K.(1998) 'Kinetic Characterization of the Esterification of Sulfuric Acid by Ethanol Using Capillary Electrophoretic Ion Analysis', Journal of Liquid Chromatography & Related Technologies, 21: 9, 1259 — 1272
To link to this Article: DOI: 10.1080/10826079808005876
URL: http://dx.doi.org/10.1080/10826079808005876

J. LIQ. CHROM. & REL. TECHNOL., 21(9), 1259–1272 (1998)

KINETIC CHARACTERIZATION OF THE ESTERIFICATION OF SULFURIC ACID BY ETHANOL USING CAPILLARY ELECTROPHORETIC ION ANALYSIS

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ABSTRACT

A capillary electrophoretic ion analysis method was developed for monitoring esterification of sulfuric acid by ethanol to form monoethylsulfate. Since the analytes of interest have no chromophores, detection was performed utilizing indirect photometric detection. Several background electrolyte systems were evaluated to optimize the efficiency of the separation. The method was more sensitive and specific in comparison to alternative techniques. Pseudo-first-order rate constants for the esterification reaction were determined as a function of temperature and the activation energy was calculated.

INTRODUCTION

Quantitation of small ionic species utilizing capillary zone electrophoresis has been of great interest recently.[1,2] In contrast to the conventional ion chromatography, which is based on analytes interacting

with an ion exchange packed column, capillary ion analysis (CAI) achieves separation through differentiation of solutes electrophoretic mobility within an open tubular capillary. Characterized for its high efficiency, versatility, and low cost, CAI is finding increased applications in the analysis of ionic compounds.

For the separation of anions, an electro-osmotic flow (EOF) modifier, typically consisting of a cationic surfactant containing an aliphatic quaternary amine, is added to the electrolyte.[2] Through electrostatic interaction with silanol groups, surfactants first adsorb onto the inner wall of the capillary by neutralizing the surface. Additionally, hydrophobic interactions between tails of surfactant will introduce a second layer of surfactant onto the wall. As a result, a "bi-layer" structure of surfactant is formed on the capillary wall so that the wall becomes positively charged.[3] A positively charged capillary wall causes the EOF to be reversed, moving toward the anode independent of electrolyte pH. By reversing the polarity of the power supply, anions will migrate in the same direction as EOF toward the detector.

Since the majority of small ions have minimal chromophores in the UV/visible region, indirect detection is often employed. The background electrolyte (BGE) consists of a high UV-absorbing species, often called co-ion, that has the same polarity as the analyte. An optimum wavelength is chosen so that the difference of extinction coefficient between the co-ion and analyte is maximized, yet the co-ion does not saturate the detector.[4,5] The displacement of co-ion by analyte at the detector results in a negative peak. The background electrolyte containing co-ion must possess a mobility similar to that of analyte in order to obtain a symmetric peak shape. If the mobility of the analyte is faster than the co-ion, the electrophoretic peak fronts. Conversely, when the mobility of an analyte is slower than co-ion, the electrophoretic peak tails.[2,6]

Indinavir sulfate is the active ingredient of Crixivan™, a potent inhibitor of HIV protease.[7,8] A solution of sulfuric acid H_2SO_4 in ethanol is used to convert indinavir freebase monohydrate to its sulfate salt form. The ethanolic solution of 1 molar sulfuric acid is kept below -10°C during the indinavir sulfate salt formation. It has been known that at near ambient temperatures, mixtures of primary or secondary alcohols and sulfuric acid give rise to mono-alkyl sulfate esters.[9,10] Specifically, sulfuric acid may react with ethanol to form monoethylsulfuric acid (MES). Previous studies also indicated that the possibility of a second ethanol molecule further reacting with MES to form diethylsulfate was minimal.[9,11] Accordingly, the possible esterification reaction between sulfuric acid and ethanol could result

in MES as a potential impurity in the indinavir sulfate drug substance. To evaluate the impact of possible formation of MES upon the quality of indinavir sulfate processing, it was imperative to determine the rate of esterification as a function of temperature.

A literature survey reveals that existing analytical methods for measuring monoethylsulfate predominantly employ indirect titrations which are insensitive and time consuming.[12-14] A non-aqueous titration, employing diphenylguanidine as titrant in a 1:1 ethylene glycol:acetone matrix, took advantage of the fact that the sulfuric acid and monoethylsulfuric acid could be differentiated based on their behavior at the first and second degrees of acid dissociation in acetone.[13] Additionally, an ion chromatography method has been employed in analyzing the level of monoethylsulfate extracted from atmosphere samples.[15,16]

Other approaches that have been reported included radioisotope, conductance, and turbidimetric techniques.[17-19] More recently, a related application employing indirect detection CZE has been reported for the separation of ethoxylated alcohol sulfates.[20]

A CZE method for the analysis of monoethylsulfate is described below. The assay is fast, simple, specific, and sensitive. The esterification of sulfuric acid by ethanol and the formation of MES were monitored and kinetic data was obtained as a function of temperature.

MATERIALS

Reagents

Ethanol 200 proof was purchased from Quantum Chemical Co. (Anaheim, CA). Concentrated sulfuric acid (97%) and potassium chromate were obtained from J.T. Baker (Phillipsburg, NJ). Potassium monoethylsulfate standard was from Pfaltz & Bauer (Waterbury, CT). Sodium sulfate was purchased from EM Science (Cherry Hill, NJ). The additive used in CZE background electrolyte CIA-Pak™ OFM Anion-BT was bought from Waters (Milford, MA). Benzoic acid and diphenylguanidine were from Aldrich (St. Louis, MO). Potassium hydrogen phthalate was obtained from Acros Organics (Pittsburg, PA). Sodium hydroxide was from Fisher Scientific (Fair Lawn, NJ).

Instrumentation

The capillary electrophoresis was carried out with a HP^{3D} CE system (Hewlett-Packard, Germany). The fused silica capillary employed has a total length of 65 cm, an effective length of 56 cm, and an inner diameter of 75 μm (Hewlett-Packard, Germany). The polarity of the electrodes was reversed and the voltage applied was - 20 kV which typically yielded a current of - 20 μA. All injections were performed under pressure mode at 50 mbar for 5 seconds.

The preparation of background electrolyte, containing 0.5 mM CIA-Pak™ OFM Anion-BT, followed the recipe developed by Waters.[2] The chromate BGE was prepared at 5 mM concentration and pH adjusted to 8.0 with sulfuric acid. The phthalate BGE was also at 5 mM concentration and its pH was adjusted to 5.6 with sodium hydroxide NaOH. The benzoate BGE, in which 5% (v/v) methanol was added to help solubilizing benzoate, had a concentration of 20 mM and its pH was adjusted to 6.0 with NaOH. Electrolyte was filtered through a 0.45 μm Millipore membrane prior to use and replenished after every three injections. Signals were detected at 270, 230, and 225 nm for chromate, phthalate, and benzoate BGE, respectively. The reference wavelength and sample detection wavelength were reversed to provide positive peaks for analysis. The capillary temperature was maintained at 30°C.

The data were collected and analyzed by PE Nelson AccessChrom system. All results were the average of three injections.

METHODS

Determination of Purity of the Potassium Monoethylsulfate Standard

Commercial grade potassium monoethylsulfate ($KEtSO_4$) was dried at 40°C overnight prior to use as a standard. The purity was assessed for residual solvents by thermogravimetry, and for the presence of carbonate and bicarbonate by titration with sodium hydroxide or hydrochloric acid. For the specific lot of $KEtSO_4$ used in this study, the $KHCO_3$ level was determined to be 14.3 wt%, K_2CO_3 was 0.8 wt%, and residual solvents were 0.05 wt%. An impurity profile of the standard material by CZE showed no other impurity anions. Thus the purity of the standard was assigned to be 84.9%.

Sulfuric Acid/Ethanol Solution Make-up and Incubation

Absolute ethanol (189 mL) was chilled in an Erlenmeyer flask on an ice bath for 30 minutes. Sulfuric acid (11 mL) was then added to the cold ethanol drop-wise with constant stirring, yielding a concentration of 1 molar H_2SO_4 ethanolic solution. This solution was immediately allocated into scintillation vials and incubated under selected temperature conditions. The temperatures were controlled by a RTE-111 water bath (Neslab Instruments, Protsmouth, NH).

Determination of Sulfate and Monoethylsulfate Ions by Non-Aqueous Titration

Approximately 1 g of H_2SO_4/EtOH mixture was weighed out and diluted into a 1:1 ethylene glycol:acetone mixture. The sample was titrated coulometrically with 0.1 N diphenylguanidine in the same solvent. The titrant was standardized with a standard solution of HCl (approximately 0.1 N). Upon titration of the sample, two breaks of approximately 12 mL each were obtained. The first break corresponded to the neutralization of the monoethylsulfuric acid and the first proton of sulfuric acid, while the second one corresponded to the neutralization of the second proton of sulfuric acid.[13] The weight % of monoethylsulfate and sulfate was calculated as per reference.[13]

Determination of Sulfate and Monoethylsulfate Ions by CZE

One milliliter of H_2SO_4/ethanol mixture was accurately weighed (around 0.85 g) into a 25 mL volumetric flask and immediately diluted to volume with DI water. This solution was used for determination of the amount of monoethylsulfate formed. After further 100-fold dilution, the solution was analyzed for the level of sulfate ion, and the level of monoethylsulfate ion in the cases that samples were at ambient or above for over 6 hours. A calibration curve ranging from 0.05 to 2 mM was generated for the calculation of SO_4^{2-} and $EtSO_4^-$ concentrations in the millimolar range.

After correction for dilutions, the concentrations of each ion in millimoles per gram or weight percents were reported. The standards were kept in a refrigerator while not in use.

Calculations

Calculation of the magnitude of EOF (μ_{EOF}) and effective mobility (μ_{eff}) are given in the following equations:

$$\mu_{EOF} = \frac{\ell L}{V t_{EOF}} \quad (1)$$

$$\mu_{eff} = \left(\frac{1}{t} - \frac{1}{t_{EOF}}\right)\frac{\ell L}{V} \quad (2)$$

where ℓ is the effective capillary length, L is the total length, t is the migration time, and V is the applied voltage.[21] The magnitude of t_{EOF} in different BGE system could be obtained by the use of a neutral marker such as methanol.

The calculation of ionic strength is given by $\frac{1}{2}\ \Sigma C_i Z_i^2$, where C_i is the molar concentration of ion species i, and Z_i is the valence of i.

The efficiency of a specific peak N is calculated using equation

$$N = 5.54\ (T_R/W_{1/2})^2 \quad (3)$$

where T_R is the migration time of the analyte, and $W_{1/2}$ is the peak width at half height.

RESULTS AND DISCUSSION

Method Development of Capillary Ion Analysis

Due to the differences in the electrophoretic mobility between sulfate and monoethylsulfate, several BGE systems were evaluated to optimize the peak shape and efficiency for both peaks. Three electrolyte systems developed by Waters for the analysis of anions of different mobility were employed.[2] Each containing 0.5 mM CIA-Pak™ OFM Anion-BT osmotic flow modifier, chromate, phthalate, and benzoate electrolytes were recommended to be used for high, intermediate, and low mobility ions, respectively.

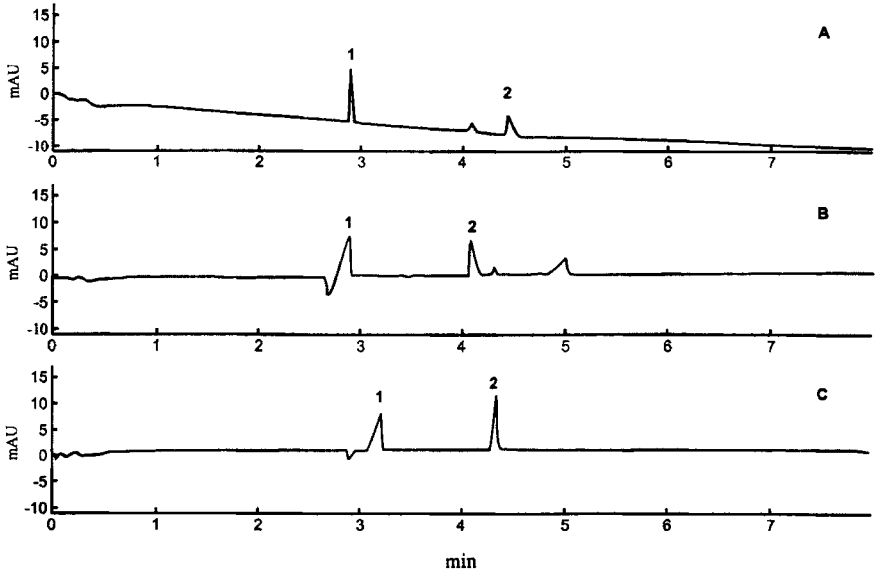

Figure 1. Electropherograms of sulfate and monoethylsulfate in different BGE systems. (A): 5 mM chromate + 0.5 mM OFM Anion-BT, pH 8.0, signals monitored at 270 nm. (B): 5 mM phthalate + 0.5 mM OFM Anion-BT, pH 5.6, monitored at 230 nm. (C): 20 mM benzoate + 0.5 mM OFM Anion-BT, pH 6.0, monitored at 225 nm. Peak 1: sulfate SO_4^{2-}, peak 2: monoethylsulfate $EtSO_4^-$.

By examining the peak shapes of the analytes shown in Figure 1, we concluded that the mobility of chromate BGE was similar to that of sulfate, the mobility of phthalate BGE was higher than MES but lower than sulfate, and the mobility of benzoate BGE was close to MES.

Considering the detection of low levels of MES was of most interest, the benzoate + OFM Anion-BT composition was chosen as the working electrolyte because it resulted in the highest efficiency for $EtSO_4^-$ peak, see Table 1.

The data in Table 2 include the EOF and the effective mobility of sulfate and MES in different BGE systems. Generally, with the same electric field, temperature, and modifier concentration, the magnitude of EOF on a bare silica capillary mainly depends on ionic strength. This is because increased ionic strength results in double-layer compression, decreased zeta potential, and reduced EOF.[21]

Table 1

Comparison of Peak Efficiencies Using Different BGE Systems

		Chromate BGE	Phthalate BGE	Benzoate BGE
Co-ion conc. (mM)		5	5	20
OFM Anion-BT conc. (mM)		0.5	0.5	0.5
pH		8.0	5.6	6.0
Resulting Peak Efficiency N ($x10^4$)	SO_4^{2-}	7.0	0.6	1.2
	$EtSO_4^-$	2.6	3.7	9.4

Table 2

Comparison of μ_{EOF} and μ_{eff} in Different BGE Systems

		Chromate BGE	Phthalate BGE	Benzoate BGE
Calculated Ion Strength $\frac{1}{2}\Sigma C_i Z_i^2$		15	13	21
μ_{EOF} ($x10^{-4} cm^2 V^{-1} S^{-1}$)		2.3	3.1	3.2
μ_{eff} ($x10^{-4} cm^2 V^{-1} S^{-1}$)	SO_4^{2-}	8.2	7.4	6.2
	$EtSO_4^-$	4.6	4.3	3.8

However, with the use of cationic surfactant, the trends in changes of reversed EOF are far more complicated due to the "bi-layer" structure of surfactant adsorbed on the inner capillary wall.[3] When electrolyte concentration goes up initially, electrostatic repulsion between the surfactant head groups decreases, resulting in increased adsorption of surfactant forming the second layer on the capillary wall and consequently increased EOF. As the buffer concentration increases further, EOF starts declining because the shrinking of double layer becomes dominant.

Table 3

Comparison of the Weight Percent Results Obtained by Titration and CZE

Storage Condition	Time Point (hr)	wt% SO_4^{2-} by Titration	wt% SO_4^{2-} by CZE	wt% $EtSO_4^-$ by Titration	wt% $EtSO_4^-$ by CZE
	Initial	11.1	12.1	<LOQ	0.27
Freezer	24	11.1	11.7	<LOQ	0.28
Ambient	2	10.8	11.3	0.34	0.70
	6	10.1	10.4	1.26	1.38
	24	8.1	8.3	3.93	3.93

In our experiments, chromate and phthalate were both at 5 mM concentration. Chromate has two charges while the second hydrogen of phthalate at pH 5.6 (pK_a = 5.5) is partially deprotonated. As a result, chromate BGE has higher ionic strength and lower EOF than phthalate. The benzoate BGE, however, contained 20 mM concentration of co-ion as well as 5% (v/v) MeOH. The difference in composition of benzoate BGE makes its comparison with the other two BGE not straight forward. Typically, organic modifiers increase electrolyte viscosity and thus slow down EOF.[21] For the benzoate BGE studied, we observed a μ_{EOF} value similar to that of phthalate BGE but higher than chromate, as exhibited in Table 2.

Method Validation

As displayed in Table 3, under freezer conditions (~ -17°C), no significant amount of $EtSO_4^-$ formation was observed within 24 hours. The initial low level of monoethylsulfate is believed to be due to a local heating effect during the sulfuric acid/ethanol mixing. However, within the same period of time under ambient conditions, the amount of $EtSO_4^-$ increased to 3.9 wt%. The accuracy of the CZE method was assessed by comparison with the data obtained from the non-aqueous titration. The discrepancy at low levels of $EtSO_4^-$ is because the CZE assay demonstrated a 25 fold increase in sensitivity for the monoethylsulfate ion. The limit of detection LOD (S/N = 3) of the CZE method for MES was lower than 1.40 μg/mL, or 0.004 wt% of sample solution, and the LOQ (S/N = 10) was lower than 2.80 μg/mL or 0.008 wt%. The non-aqueous titration had an optimum LOQ of

approximately 0.2 wt%. The injection precision of the CZE method was evaluated from six consecutive injections of a 0.5 mM standard. The %RSD for sulfate and MES peaks, respectively, were 1.1 and 1.4 for area counts, and 0.3 and 0.6 for migration times. The detector response was linear to both ions within the concentration range evaluated, i.e., 1.96 μg/mL to 196 μg/mL for SO_4^{2-} and 2.14 μg/mL to 214 μg/mL for $EtSO_4^-$. Quenching of the reaction mixture by 25 fold dilution in water proved to be sufficient to stop the esterification reaction, as the same sample preparation was injected the next day showing no change in peak areas. The quenched solution was stored under ambient conditions.

Kinetic Studies of Esterification of Sulfuric Acid by Ethanol at Selected Temperatures

In general, the reaction between sulfuric acid and alcohol shows a second order kinetics.[22] The value of the rate constant depends on the quantities of water present.[10,14,22] In the presence of a large excess of alcohol or sulfuric acid, the reaction demonstrates a pseudo-first-order behavior.[17,22] In this study, the molar concentration of ethanol was about 15 fold excess compared to sulfuric acid, therefore, its concentration was considered constant throughout the reaction.[23]

For a pseudo-first-order reaction, the relationship between reactant concentration [A] and time t is expressed as

$$\ln ([A]/[A]_0) = - kt \quad (4)$$

where k is the rate constant. Considering at time t, $[P] + [A] = [A]_0$, where [P] represents the concentration of product formed. Equation (4) hence can be rewritten as

$$\ln ([A]_0 - [P]) = - kt + \ln [A]_0 \quad (5)$$

Rate constant k is obtained from the slope of plot $\ln ([A]_0 - [P])$ verses time at different temperatures. Using Arrhenius equation

$$\ln k = - E_a/RT + \text{constant} \quad (6)$$

a plot of ln k versus 1/T should yield a straight line and the activation energy E_a can be obtained from the slope.

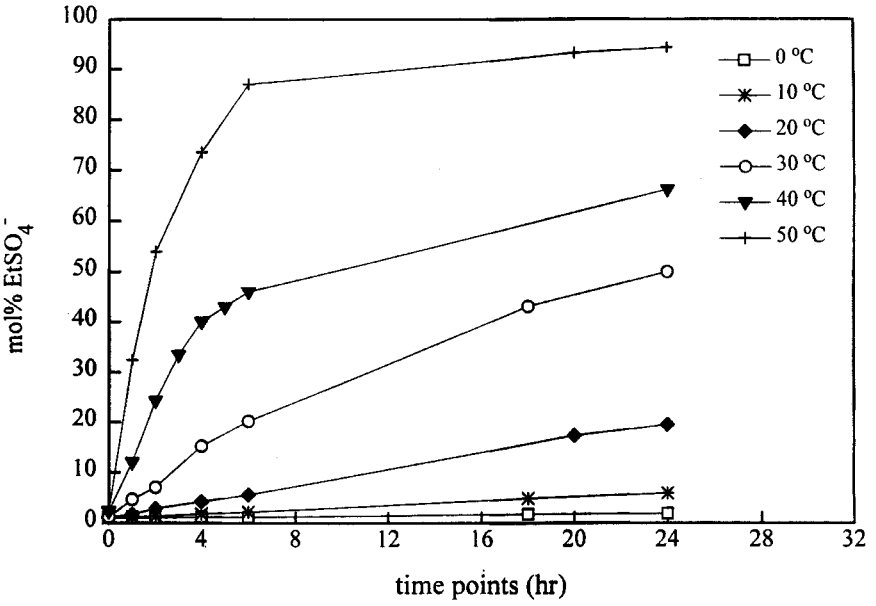

Figure 2. Formation of Monoethylsulfate in 1 Molar H_2SO_4 /Ethanol Solution. mol% $EtSO_4^-$ formed = $[EtSO_4^-]_t$ / $[SO_4^{2-}]_0$ (mmol/g).

To address the kinetics of the esterification of 1 molar sulfuric acid in ethanol and the formation of monoethylsulfate, experiments were carried out at selected temperatures. As displayed in Figure 2, the stability of H_2SO_4/EtOH solution is highly temperature dependent.

After 24 hours storage at 0°C, the increase in the amount of MES was minimal - only about 0.8 mol%. At 50°C, on the other hand, the reaction reached equilibrium in the same period of time with about 90% of sulfuric acid converted to MES. This result was consistent with the literature data that at the given H_2SO_4/EtOH ratio, when reaching equilibrium the percentage of sulfuric acid reacted with ethanol was about 85%.[10] To obtain the rate constants, a method of "initial rates" was applied.[23] The method takes into consideration that in the early stage of a reaction the starting material concentrations have only changed slightly while the back reaction can be ignored. Using this approximation, performing a direct calculation of the rate d[A]/dt will result in rate constants that are approximately equal to the true values.

Table 4

Rate Constants of the Esterification of 1M Sulfuric Acid by Ethanol

Temperature (°C)	Rate Constant ($x10^4$ min^{-1})	Standard Deviation ($x10^4$ min^{-1})	Linear Correlation Coefficient
0	0.063	0.005	0.986
10	0.34	0.01	0.999
20	1.50	0.07	0.995
30	6.01	0.28	0.997
40	20.6	1.4	0.995
50	76.2	2.1	0.999

By plotting [$EtSO_4^-$] versus time at each temperature, the linear portion of the curve was first selected. These data points were considered representative of the initial stage of the reaction and hence were used in determining the esterification rates. The data were fitted into equation (5) for first-order kinetics, and the results are summarized in Table 4. Note that although MES and sulfate concentrations can both be determined directly, equation (5) along with measured [$EtSO_4^-$] were employed instead of [SO_4^{2-}] being used in equation (4) because at low temperatures the decrease in sulfate concentration was insignificant. Previously unreported, the activation energy was calculated to be 24.9 kcal/mol from this study with a correlation coefficient of 0.9997 according to equation.[6] This activation energy corresponded to a 4 fold increase in the reaction rate with a 10°C temperature increase, which was also in agreement with literature data.[11] Since for most reactions the rate coefficient increase factor for every 10^0C temperature change is between 1.8 to 4.1,[24] this result further illustrated that the esterification reaction rate studied was highly temperature dependent. Because of the significant temperature dependence of the rate constant, it is critical to maintain processing temperature below 0°C during indinavir sulfate formation.

CONCLUSIONS

The capillary ion analysis method described is sensitive and specific to monitor the esterification of H_2SO_4 by ethanol and the formation of monoethylsulfate. The electrophoretic conditions were optimized for optimal efficiency and sensitivity.

The pseudo-first-order rate constants of the esterification reaction were determined as a function of temperature and the activation energy was calculated. The results provided insights into the indinavir sulfate salt formation and ensured a robust process.

REFERENCES

1. P. Jandik, W. R. Jones, A. Weston, P. R. Brown, LC-GC, **9**, 634-641 (1991).

2. W. R. Jones, **Handbook of Capillary Electrophoresis**, J. P. Landers, ed., Chapter 9, CRC Press, Inc., FL, 1993.

3. C. A. Lucy, R. S. Underhill, Anal. Chem., **68**, 300-305 (1996).

4. D. R. Heiger, R. Weinberger, "Determination of Small Ions by Capillary Zone Electrophoresis with Indirect Photometric Detection", Application Note, Hewlett-Packard, Germany, 1994.

5. H. Small, **Ion Chromatography**, Chapter 8, Plenum Press, New York, 1989.

6. F. E. P. Mikkers, F. M. Everaerts, P. E. M. Verheggen, J. Chromatography, **169**, 1-10 (1979).

7. J. P. Vacca, B. D. Dorsey, W. A. Schlief, R. B. Levin, S. L. McDaniel, P. L. Darke, J. Zugay, J. C. Quintero, O. M. Blahy, E. Roth, V. V. Sardana, A. J. Schlabach, P. I. Graham, J. H. Condra, L. Gotlib, M. K. Holloway, J. Lin, I.-W. Chen, K. Vastag, D. Ostovic, P. S. Anderson, E. A. Emini, J. R. Huff, Proc. Natl. Acad. Sci. USA, **91**, 4096-4100 (1994).

8. D. Askin, K. Eng, K. Rossen, R. Purick, K. Wells, R. Volante, P. Reider, Tetrahedron Lett., **35**, 673-676 (1994).

9. G. Oddo, E. Scandola, Gazz. Chim. Ital., **39 (II)**, 1-21 (1909), translated from Italian.

10. R. Page, **Ethylene**, S. A. Miller, ed., Chapter 9 (II), Ernest Benn Limited, London, 1969.

11. R. Kremann, Monatsh., **31**, 245-74 (1910), translated from German.

12. C. M. Suter, E. Oberg, J. Am. Chem. Soc., **56**, 677-679 (1934).

13. T. V. Rozhkova, L. G. Aleksandrova, L. G.; Khim. Prom-st., Ser.: Metody Anal. Kontrolya Kach. Prod. Khim. Prom-sti., 7, 40-43 (1980), translated from Russian.

14. D. J. Clark, G. Williams, J. Chem. Soc., **1957**, 4218-4221 (1957).

15. D.J. Eatough, M. L. Lee, D. W. Later, B. E. Richter, N. L. Eatough, L. D. Hansen, Environ. Sci. Technol., **15**, 1502-1506 (1981).

16. T. Smith-Palmer, B. R. Wentzell, L. D. Hanson, C. D. MacPherson, The Sci. of the Total Environ., **83**, 185-190 (1989).

17. R. E. Robertson, Can. J. Chem., **33**, 1536-43 (1955).

18. M. I. Vinnik, I. S. Kislina, A. N. Kitaizorodskii, A. T. Nikitaev, Izv. Akad. Nauk. SSSR, Ser. Khim., **12**, 2671-2677 (1986), translated form Russian.

19. I. Sperber, J. Biol. Chem., **172**, 441-444 (1948).

20. L. K. Goebel, H. M. McNair, J. Microcol. Sep., **5**, 47-50 (1993).

21. D. N. Heiger, **High Performance Electrophoresis - An Introduction**, Chapter 2, Hewlett-Packard, German, 1994.

22. N. C. Deno, M. S. Newman, J. Am. Chem. Soc., **72**, 3852-3855 (1950).

23. J. H. Espenson, **Chemical Kinetics and Reaction Mechanisms**, J. Ricci, J. W. Bradley eds., Chapters 1 and 2, McGraw-Hill Book Co., New York, 1981.

24. P. W. Atkins, **Physical Chemistry, 3rd Ed.**, Chapter 28, W. H. Freeman and Company, New York, 1986.

Received July 20, 1997
Accepted September 16, 1997
Manuscript 4539