injection were used, concentrations of acid on the order of 2×10^{-12} M are measurable. From Figure 2, noting the recorder sensitivity etc., it is clear that the 4-hydroxybenzoic acid could easily be detected in 10^{-11} M solution as its *p*-bromophenacyl derivative. Also, there is no reason the quantities of solutions used in the reaction could not be reduced below 1 mL by use of small vials and appropriate stirrers. Jordi (*12*) has indicated that the level of detectability for *p*-bromophenacyl derivatives lies in the range of 10^{-14} mol but clearly, in this work, limits of detectability lie in the range of 10^{-17} mol. This is perhaps a reflection of the improvements in instrumentation over the last few years and is consistent with the results obtained by Barcelona et al. (*8*).

Morozowich and Douglas (*13*) have also reported a fast derivatization method for *p*-nitrophenacyl esters of prostaglandins using *N,N*-diisopropylethylamine. The authors in this paper indicate a limit of detection of 1 ng which, again, may be a reflection of the state of the art in 1975. *p*-Nitrophenacyl bromide is also much less readily available than the reagent used in this work.

Thus we have a derivatization technique which, when combined with a modern HPLC and the intense phenacyl chromophore, permits subpicogram detection and analysis of carboxylic acids.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Shriner, R. L.; Fuson, R. C.; Curtin, D. Y. "The Systematic Identification of Organic Compounds", 5th ed.; Wiley: New York, 1964; p 235.
(2) Pokras, H. H.; Bernstein, H. I. *J. Am. Chem. Soc.* **1943**, *65*, 2096.
(3) Borch, R. F. *Anal. Chem.* **1975**, *47*, 2437.
(4) Hullett, D. A.; Eisenreich, S. J. *Anal. Chem.* **1979**, *51*, 1953.
(5) Durst, H. D. *Tetrahedron Lett.* **1974**, 2421.
(6) Durst, H. D.; Milano, M.; Kikta, E. J.; Connelly, S. A.; Grushka, E. *Anal. Chem.* **1975**, *47*, 1797.
(7) Takagama, K.; Qureshi, N.; Jordi, H. C.; Schnoes, H. K. *J. Liq. Chromatogr.* **1979**, *2*, 861.
(8) Barcelona, M. J.; Liljestrand, H. M.; Morgan, J. J. *Anal. Chem.* **1980**, *52*, 321.
(9) Clark, J. H.; Miller, J. M. *Tetrahedron Lett.* **1977**, 599.
(10) Nagels, L.; De Beuf, C.; Esmans, E. *J. Chromatogr.* **1980**, *190*, 411.
(11) Giraldi, P. N. *Farmaco, Ed. Sci.* **1959**, *14*, 90; *Chem. Abstr.* **1960**, *54*, 3299.
(12) Jordi, H. C. *J. Liq. Chromatogr.* **1978**, *1*, 215.
(13) Morozowich, W.; Douglas, S. L. *Prostaglandins* **1975**, *10*, 19.

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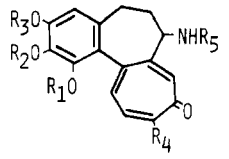
Determination of Colchicine and Colchiceine in Microbial Cultures by High-Performance Liquid Chromatography

Allan E. Klein and Patrick J. Davis*

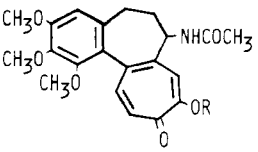
Division of Pharmaceutical Chemistry, College of Pharmacy, The University of Texas at Austin, Austin, Texas 78712

Colchicine (**1**), the major alkaloid of *Colchicum* species, has traditionally been employed for the treatment of gout (*1*). The antineoplastic activity of colchicine and its derivatives have recently increased interest in their potential use as chemotherapeutic agents (*2*). While colchicine itself may prove too toxic for use in this regard (*3*, *4*), there is ample evidence to indicate that derivatives of colchicine exhibit a higher therapeutic index and, hence, hold more promise for clinical application (*5*, *6*). For this reason, we are examining the use of microorganisms to metabolically prepare derivatives of colchicine and related alkaloids (*7*). Colchiceine (**2**) is a reported mammalian metabolite of colchicine (*8*) and is a potential microbial metabolite as well. The suggestion has been made (*9*) that the O-dealkylated product of colchicine with *Streptomyces griseus* as reported by Velluz et al (*10*, *11*) may, in fact, be colchiceine, although this still appears to be open to question.

A problem with such studies is that the analytical techniques reported thus far for colchicine have lacked specificity for the determination of the parent compound in the presence of its various metabolites or derivatives. Thin-layer chromatography has been successfully used for the qualitative evaluation of some colchicine derivatives (*12*, *13*). Normal-phase (*14*) as well as reverse-phase (*15–17*) high-performance liquid chromatographic (HPLC) systems have been employed for colchicine derivatives, and we recently reported the successful separation of six colchicine derivatives on a reverse-phase system (*18*). Colchiceine (**2**) has not been previously determined in the presence of colchicine and related compounds. Thin-layer chromatography has been described for this acidic metabolite (*13*). However, we have observed poor mobility in diethylamine-containing solvent systems. The compound typically is strongly adsorbed by silica gel and shows little or no mobility in a variety of solvent systems. In addition, the chromatographic analysis is complicated by the vinylagous acid functionality which favors the formation of



1. $R_1=R_2=R_3=CH_3$; $R_4=OCH_3$; $R_5=COCH_3$
2. $R_1=R_2=R_3=CH_3$; $R_4=OH$; $R_5=COCH_3$
4. $R_1=R_2=R_3=CH_3$; $R_4=OCH_2CH_3$; $R_5=COCH_3$
5. $R_1=R_2=R_3=CH_3$; $R_4=OCH_3$; $R_5=CH_3$
6. $R_1=R_2=R_3=CH_3$; $R_4=NHCH_3$; $R_5=COCH_3$
8. $R_1=H$; $R_2=R_3=CH_3$; $R_4=OCH_3$; $R_5=COCH_3$
9. $R_2=H$; $R_1=R_3=CH_3$; $R_4=OCH_3$; $R_5=COCH_3$
10. $R_3=H$; $R_1=R_2=CH_3$; $R_4=OCH_3$; $R_5=COCH_3$



3. $R=CH_2CH_3$
7. $R=CH_3$

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two products upon derivatization to the corresponding vinylagous esters such as **3** and **4** (*19*).

In the present work we have developed an analytical scheme for the determination of colchiceine in the presence of colchicine which utilizes a rapid derivatization step. Rather than attempt to form a single product from colchiceine, we have determined the metabolite as the sum of the two isomeric ethyl esters. The utility of the method for application to microbial systems was demonstrated by double-blind analysis of spiked microbial samples.

EXPERIMENTAL SECTION

Apparatus. A Model 950 pump and 970A variable-wavelength detector (Tracor, Austin, TX) and a Model 7120 100-μL loop injector (Rheodyne, Berkeley, CA) were employed for all HPLC analyses. Detection was at 350 nm, and a Model HP-3380A reporting integrator (Hewlett-Packard, Palo Alto, CA) at an input sensitivity of 0.1 V/AU and a slope sensitivity of 1 mV/min was used for peak area measurement and chromatogram recording. Flow rates were either 3.0 mL/min (2800 psi) for colchicine or 4.0 mL/min (3800 psi) for colchiceine derivatives on a 10 cm × 4.1 mm i.d. Lichrosorb RP-18, 10-μm particle size column (Alltech Assoc., Arlington Heights, IL). Dead time (t_0) was measured by the pressure fluctuation observed on the base line after an injection of mobile phase. Analyses were performed with acetonitrile:methanol:phosphate buffer pH 6.0 μ = 0.05 (16:5:79) as the mobile phase.

NMR spectra were obtained in $CDCl_3$ solution with a Model HA-100, 100-MHz spectrometer (Varian, Palo Alto, CA) using Me_4Si as an internal standard.

Reagents. Organic solvents used in the mobile phase were chromatographic quality (Lichrosorb) obtained from MCB Manufacturing Chemists (Cincinnatti, OH). Water was deionized and doubly distilled in glass. Iodoethane was obtained from Aldrich Chemical Co. (Milwaukee, WI). Anhydrous ethanol (USI Chemicals Co., Tuscola, IL) was reagent quality and used for all derivatizations. Silver oxide was used as the purified fine powder (Fisher Scientific Co., Fair Lawn, NJ). All other reagents were certified ACS grade.

Mobile phases were prepared by filtering individual solvents through glass fiber pads, GF/F grade (Whatman, Clifton, NJ), mixing, and degassing by sonication prior to use.

Standard Compounds. Colchicine (**1**) and *N*-methylcolchiceinamide (**6**) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Demecolcine (**5**) was obtained from the National Cancer Institute, Bethesda, MD. Colchiceine (**2**) was prepared by the mild acid treatment of colchicine according to the method of Zeisel (*20*). The product was recrystallized from diethyl ether–chloroform (40:1) as white crystals (mp 178–179 °C) [reported (*20*) 178–179 °C] and was identical with a sample provided by T. J. Fitzgerald of Florida A&M University (Tallahassee, FL).

Ethyl isocolchicinate (**3**) and ethyl colchicinate (**4**) were prepared from 100 mg of colchiceine (**2**) in 20 mL of ethanol by the addition of 8 mL of iodoethane and 150 mg of silver oxide. The mixture was stirred continuously at 40 °C. The reaction was stopped after 21 h when colchiceine was no longer detected at the origin by TLC using benzene–ethyl acetate–diethylamine–methanol (50:40:10:8). The ethyl isomers were separated with this solvent system using 1 mm thick Silica Gel GF_{254}, 20 × 20 cm, plates (Brinkmann) on which the isoethyl isomer (R_f = 0.31–0.42 bandwidth) demonstrated lower mobility than the *n*-ethyl isomer (R_f = 0.50–0.56 bandwidth). Each of the corresponding TLC zones was scraped from the plates and shaken with 40 mL of methanol, which was then filtered and reduced to a small volume on a rotary evaporator. The individual isomers were crystallized as light yellow powders by the addition of an excess of dry diethyl ether to produce approximately equal amounts. After recrystallization from methanol–diethyl ether the products were collected in yields of approximately 80% theoretical, mp **4** 152–155 °C and **3** 184–189 °C. Spectral data for **3** and **4** were consistent with literature reports, including ultraviolet spectra (*19*), proton nuclear magnetic resonance spectra (*21*, *22*), and electron-impact mass spectra (*22–24*). In addition, the presence of the ethyl group in either spectrum was confirmed by NMR decoupling experiments.

All standard compounds were homogeneous as determined by TLC or HPLC.

Samples. Standard compounds dissolved in methanol were added to dry, silylated tubes which were then gently heated under a nitrogen stream to remove the solvent. The residue was taken up in 1 mL of a microbial culture of *Streptomyces spectabilis*, which had been grown by a two-stage fermentation procedure (*25*). The final concentrations were in the range of 10–200 μg/mL. For absolute recovery experiments on compounds **1** and **2**, standard colchicine or standard ethyl colchicinate solutions in HPLC mobile phase were used as reference samples. Recoveries were calculated by comparison of peak area ratios (ratio of standard compounds to the appropriate internal standard, as described in the Procedure section) obtained by extraction of microbial culture samples vs. those obtained from direct injection of standard solutions dissolved in the mobile phase. All glassware in this and subsequent procedures was silanized with 2% trimethylsilyl chloride in toluene.

Routine Procedure. For the analysis of colchicine (**1**) and colchiceine (**2**) in the microbial culture, unknown samples of 1.0 mL of microbial culture containing from 20 to 200 μg/mL of each compound were analzyed in duplicate. A 20-μL portion of demecolcine (**5**) 550 μg/mL in 10% methanol–water, was added to each sample as an internal standard for colchicine, followed by 120 μL of 1 N NaOH (final pH 12.1–12.4). The samples were mixed, and 2 mL of chloroform was added. After the samples were rocked for 5 min followed by centrifugation for 5 min at 817*g*, a 1-mL chloroform aliquot was transferred from the lower phase of each sample to a Silli-vial where it was evaporated to dryness at 60 °C under a dry nitrogen stream. The residues were reconstituted with 100-μL portions of the HPLC mobile phase and 20-μL aliquots taken for sample injection to determine colchicine.

The upper aqueous phase from the alkaline extraction of each sample was saved for the determination of colchiceine. An 800-μL aliquot of this aqueous solution was mixed with a 210-μL portion of *N*-methylcolchiceinamide (**6**), the internal standard used for colchiceine, 125 μg/mL in 10% methanol–water. The solution was acidified with 125 μL of 1 N HCl (to a final pH of approximately 3.0), and 2 mL of chloroform was added. After the mixture was rocked for 5 min and then centrifuged as previously described, a 1.5-mL aliquot of the chloroform layer was transferred to a second Silli-vial. This chloroform layer was evaporated to dryness as described earlier, and then reconstituted with 200-μL of ethanol. A 5-mg portion of silver oxide was then added to each sample, and the derivatization was initiated by the addition of 200 μL of iodoethane. The sample was capped and placed in a heating block at 40 °C where it was stirred continuously for 1 h. A 200-μL portion of the mixture was then withdrawn and quickly filtered through a GF/F glass fiber pad under vacuum, the pad was washed with 200-μL of the HPLC mobile phase, and the washings were combined with the original filtrate in a small vial. The biphasic mixture was vortexed for 10 min, and, after the mixture settled, a 60-μL portion of the upper aqueous phase was analyzed by HPLC for the ethyl derivatives of colchiceine.

Standards containing 23, 46, 69, 115, and 230 μg of colchicine/mL and 22, 45, 67, 112 and 224 μg of colchiceine/mL of culture were treated in the same manner as the unknowns.

Samples were used to determine loss of colchiceine due to the initial alkaline extraction. These separate samples were made alkaline with 1 N NaOH (to pH >12), extracted with 2 mL of chloroform, and then acidified to pH 3 and worked up according to the described method.

Additional colchiceine samples including the internal standard were prepared in absolute ethanol, and appropriate aliquots were taken and derivatized according to the described procedure. These samples were used as references to determine the colchiceine loss due to the acid extraction step.

Calculations. Peak area ratios were obtained for colchicine by dividing the peak area for the compound by the peak area for demecolcine, while for colchiceine they were calculated by first summing the peak areas for the iso- and *n*-ethyl colchicinates and then dividing this by the peak area for *N*-methylcolchiceinamide. Calibration curves were prepared by using least-squares regression to determine the best fit line for the data obtained from the standards and were expressed as the peak area ratios from the standards vs. the concentration of either colchicine or colchiceine in the original sample.

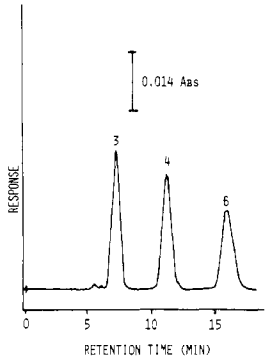

Figure 1. HPLC analysis of colchiceine (**2**) from microbial samples following derivatization to yield isoethyl colchicinate (**3**) and ethyl colchicinate (**4**) at a flow rate of 4 mL/min. *N*-Methylcolchiceinamide (**6**) was used as an internal standard.

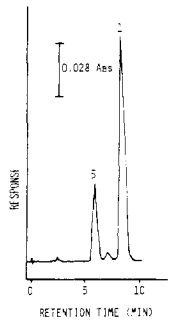

Figure 2. HPLC analysis of colchicine (**1**) from microbial samples at a flow rate of 3 mL/min. Demecolcine (**5**) was used as the internal standard.

RESULTS AND DISCUSSION

The analysis of colchicine (**1**) and colchiceine (**2**) is initiated by a simple acid–base partition step to separate the two components in the biological sample. The esterification of colchiceine (**2**) with iodoethane to yield the ethyl derivatives **3** and **4** was chosen for this HPLC procedure because of the inability to elute the underivatized vinylagous acid (**2**) on a variety of reverse-phase and normal-phase columns. Conversion to ethyl derivatives was chosen to avoid any ambiguity with residual colchicine in biological samples, since conversion to methyl derivatives would yield a mixture of additional colchicine (**1**) and isocolchicine (**7**) (*19*). The use of silver oxide as a catalyst allowed for a rapid reaction at low temperature with the derivatization reaching completion in 1 h at 40 °C for concentrations of up to 200 μg/mL colchiceine. This use of silver oxide as a catalyst for esterification is analogous to that described for iodomethane to form methyl esters (*26*), although the experimental conditions are closer to those described for O-methylation in methanol (*27*). The relative amounts of the individual isomers formed during the derivatization were approximately equal, with the *n*-ethyl colchicinate typically predominating to a small extent. By summation of the peak areas of the isomers, this variation had a negligible effect on the quantitation. The internal standard *N*-methylcolchiceinamide (**6**) was unreactive over the 1-h derivatization period.

The use of a 10-cm Lichrosorb RP-18 column was sufficient to separate the isoethyl colchicinate (**3**), ethyl colchicinate (**4**), and the internal standard *N*-methylcolchiceinamide (**6**) within 16 min at a flow rate of 4 mL/min (Figure 1). As shown in Figure 2, this same column was conveniently used to analyze for colchicine (**1**) using demecolcine (**5**) as an internal standard by simply reducing the flow to 3 mL/min. It should be mentioned, however, that general screening for other metabolites of colchicine would be facilitated by using a 25-cm column as described in our previous publication (*18*). This increase in column length would be necessary to resolve potential phenolic (**8–10**) and other metabolites of colchicine.

Recovery experiments for colchicine (**1**) from microbial samples were designed to minimize any possible loss of colchiceine (**2**) in the initial alkaline extraction. It was found that significant amounts of colchiceine were extracted into chloroform at a sample pH of 10, while less than 5% of a 150 μg/mL colchiceine sample was extracted with chloroform at a pH of 12.1. The samples were consequently adjusted to pH greater than 12 prior to the first extraction step. The mean absolute recovery over the colchicine concentration range from 10 to 60 μg/mL (n = 4) was 82.5 ± 3.0%. The accuracy and precision of the colchicine determinations were evaluated by blind sample analyses. The percentage accuracy ranged from 0.3% to 5.8% (mean = 2.5%) for the determination of six samples at different levels of colchicine between 20 and 200 μg/mL, and the mean relative standard deviation was 1.9% (n = 3) for values determined in duplicate within the set. Consistent linear calibration curves were obtained for the colchicine (**1**)/demecolcine (**5**) area ratio in the concentration range of 10–200 μg/mL (slope = 0.093, y intercept = –0.578, n = 6, r = 0.998).

The mean absolute recovery of colchiceine (**2**) determined as the ethyl derivatives, over the concentration range from 20 to 200 μg/mL (n = 4), was 77.4 ± 3.0%. This value is close to the amount found (75%) after a sample extracted as described above was compared for sample loss with a sample dissolved in ethanol. These results indicate that the majority of sample loss is occurring before the derivitization step. The accuracy and precision of the colchiceine determinations were measured by a set of blind sample analyses, analogous to that used for colchicine. The percentage accuracy ranged from 0.55% to 9.20% (mean = 5.4%) for the determination of six samples at different levels of colchiceine between 23 and 200 μg/mL. The mean relative standard deviation was 5.5% (n = 3) for values determined in duplicate within the set. Linear calibration curves were obtained for the sum of the ethyl esters to *N*-methylcolchiceinamide area ratio in the concentration range of 10–230 μg/mL (slope = 0.013, y intercept = –0.024, n = 8, r = 0.996).

Potential interference in the colchiceine derivatization by the ethylation of phenolic colchicines (**8–10**) present in the acidic extract was investigated. Only the ethylated 2-demethylcolchicine (**9**) had a similar retention time on the 10-cm Lichrosorb RP-18 column with the *n*-ethyl colchicinate (**4**) and was a possible source of interference. The presence of the phenol can be determined by a separate sample extraction at pH 8.6 followed by an analysis according to that described for colchicine. If the phenol is present, an initial cleanup step, such as a silica gel column or TLC separation (for example, the TLC system described in the Experimental Section could be used) may be necessary if its level is significant compared to that for colchiceine. The overall method, as described, is otherwise free of interference from the investigated colchicine derivatives and provides a rapid and accurate analysis of colchicine as well as an efficient derivatization procedure for an almost equally accurate analysis of colchiceine, which has otherwise proven to be unanalyzable. The alkyl iodide esterification in the presence of silver oxide may prove useful for the derivatizaiton of other vinylagous acids which are difficult to analyze.

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LITERATURE CITED

(1) Woodbury, D. N. In "The Pharmacological Basis of Therapeutics"; 4th ed.; Goodman, L. S., Gilman, A., Eds.: MacMillan: New York, 1970; p 339.
(2) Sartorelli, A. C.; Creasey, W. A. *Annu. Rev. Pharmacol.* **1969**, *9*, 51.
(3) Leither, J.; Downing, V.; Hartwell, J. L.; Shear, M. S. *J. Natl. Cancer Inst. (U.S.)* **1952**, *13*, 379.
(4) Stolinsky, P. C.; Jacobs, E. M.; Irwin, L. E.; Pajak, TH. F.; Bateman, J. R. *Oncology* **1976**, *3*, 151.
(5) Leiter, J.; Hartwell, J. L.; Kline, I.; Nadkarni, M. V.; Shear, M. J. *J. Natl. Cancer Inst. (U.S.)* **1952**, *13*, 731.
(6) Hunter, A. L.; Klassen, C. D. *J. Pharmacol. Exp. Ther.* **1975**, *192*, 605.
(7) Davis, P. J., submitted to *Appl. Environ. Microbiol.*
(8) Schonharting, M.; Mende, G.; Siebert, G. *Hoppe-Seyler's Z. Physiol. Chem.* **1974**, *355*, 1391.
(9) Kieslich, K. "Microbial Transformations of Non-Steroid Cyclic Compounds"; G. Thieme: Stuttgart, 1976; p 225.
(10) Velluz, L.; Bellet, P. *C.R. Hebd. Seances Acad. Sci., Ser. C* **1959**, *248*, 3453.
(11) Roussel-UCLAF, British Patent, 923 421, 1963; *Chem. Abstr.* **1963**, 13320a.
(12) Hufford, C. D.; Collins, C. C.; Clark, A. M. *J. Pharm. Sci.* **1979**, *68*, 1239.
(13) *Potesilova*, H.; Hrbek, J., Jr.; Santavy, F. *Collect. Czech. Chem. Commun.* **1967**, *32*, 141.
(14) Jarvie, D.; Park, J.; Stewart, M. J. *Clin. Toxicol.* **1979**, *14*, 375.
(15) Petitjean, P.; Van Kerckhoven, L.; Pesez, M.; Bellet, P. *Ann. Pharm. Fr.* **1978**, *36*, 555.
(16) Forni, G.; Massarani, G. *J. Chromatogr.* **1977**, *131*, 444.
(17) Soczewinski, E.; Dzido, T. *J. Liquid Chromatogr.* **1979**, *2*, 511.
(18) Davis, P. J.; Klein A. E. *J. Chromatogr.* **1980**, *188*, 280.
(19) Horowitz, R. M.; Ullyot, G. E. *J. Am. Chem. Soc.* **1952**, *74*, 587.
(20) Zeisel, S. *Monatsh. Chem.* **1888**, *9*, 1.
(21) Cross, A. D.; El-Hamidi, A.; Pijewska, L.; Santavy, F. *Collect. Czech. Chem. Commun.* **1966**, *31*, 374.
(22) Wildman, W. C.; Pursey, B. A. In "The Alkaloids, XIII"; Manske, R., Ed.; Academic Press: New York, 1973; pp 418, 422.
(23) Wilson, J. M.; Ohashi, M.; Budzikiewicz, H.; Santavy, F.; Djerassi, C. *Tetrahedron*, **1963**, *19*, 2225.
(24) Blade-Font, A. *Tetrahedron Lett.*, in press.
(25) Davis, P. J.; Weiss, D.; Rosazza, J. P. *J. Chem. Soc., Perkin Trans. 1* **1977**, 1.
(26) Olsen, R. K. *J. Org. Chem.* **1970**, *35*, 1912.
(27) Wilds, A. L.; Shunk, C. H. *J. Am. Chem. Soc.* **1943**, *65*, 469.

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Kinetics with Microsecond Mixing of Liquid Reactants

Paul Davidovits* and Shu-Chi Chao

Department of Chemistry, Boston College, Chestnut Hill, Massachusetts 02167

Jet techniques have been used previously for rapid freezing of liquids (*1*). However the potential of jets for fast mixing of liquid reactants has not been explored. We describe here a new technique for rapid kinetic studies in which jets are used to mix two reactants on a time scale of about 5 μs. This is 2 orders of magnitude faster than the mixing time provided by current stopped flow techniques (*2–4*). The apparatus is shown schematically in Figure 1. Some of the techniques used here were developed for high-speed ink-jet printers (*5, 6*). One of the reactant fluids is contained in a stainless steel cylinder pressurized to about 200 psi. The liquid is forced through a glass nozzle 80 μm in diameter which forms a jet traveling at about 40 m/s. The type of jets used in these experiments can be obtained from Siemens Corp. They can also be formed relatively simply by drawing out a capillary from a glass tube. Connections to the reservoir were provided by Altex Teflon tubing. A Millipore Corp. filter was used to prevent clogging. The second reactant is formed into a thin sheet by passing it through a fan-shaped nozzle made by flattening glass tubing ($^1/_4$ in.). The thickness of the emerging sheet is about 150 μm. The liquid is circulated by a pump and its flow is smoothed out by an integrator. The technique for forming such sheets was developed for circulating dye lasers (*7*).

When the jet is passed through the sheet a certain amount of sheet fluid is incorporated into the jet. (See Figure 2.) The amount of sheet fluid picked up by the jet is reproducible and depends primarily on the thickness of the sheet and the size and speed of the jet. Typically the sheet fluid is about 35% of the final jet volume. As can be seen in Figure 2, the integrity of the jet is not disturbed substantially by the passage through the sheet. The flow rate is obtained by measuring the amount of liquid collected in a given time. Distances are measured with a calibrated microscope and the jet velocity (v) is calculated from the measured flow rate and jet diameter. The reaction time (t) corresponding to a downstream distance (x) along the jet is obtained by $t = x/v$. (Here $x = 0$ corresponds to the position of the sheet.)

The mixing time is measured by using phenolphthalein indicator to monitor an acid–base reaciton. The jet fluid is a red solution of 0.25 M sodium hydroxide and 0.063 M phenolphthalein. The sheet fluid contains an excess of hydrochloric acid. The acid–base and the phenolphthalein reactions are very rapid (diffusion limited) (*8*) and therefore the change of the jet from red to colorless after it passes through the sheet is determined by the speed of mixing of the two liquid reactants. The extent of the red color past the sheet which is measured photographically or with a microscope is typically 0.2 mm. With a jet velocity of 40 m/s this corresponds to a mixing time of 5 μs. The mixing time depends on the thickness of the sheet and the flow velocities of the reactants. Mixing times as short as 3 μs have been measured.

The appartus was tested with the reaction

$$Fe^{3+} + SCN^- \rightarrow FeSCN^{2+} \qquad (1)$$

for which the rate has been previously measured (*9*). At present we are not equipped in our laboratory to perform the type of rapid kinetics studies which would explore the microsecond rate measuring potential of the system. The concentration of reactants was chosen accordingly to provide reaction times on the order of 10^{-4} s. The sheet was formed by a 0.09 M^{-1} solution of ferric nitrate [$Fe(NO_3)_3 \cdot 9H_2O$] which provided the Fe^{3+} ion. The jet contained 0.41 M of sodium thiocyanate (NaSCN). The concentrations of Fe^{3+} and SCN^- in the mixed jet at the sheet were 0.032 and 0.27 M, respectively. Because of the large excess of SCN^- the reaction was pseudo first order. The sheet also contained 0.20 M acid (HNO_3) which suppresses unwanted reactions of the Fe^{3+} ion.

In the experiment one observes that the jet is colorless immediately after the sheet and then as the reaction proceeds it gradually turns red downstream due to the product $FeSCN^{2+}$ ion. The density of this ion in the jet is obtained by color matching with a solution of known $FeSCN^{2+}$ concentration. Thus for example, with a 6.8×10^{-4} mol L^{-1} concentration of $FeSCN^{2+}$ best color match was obtained at

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