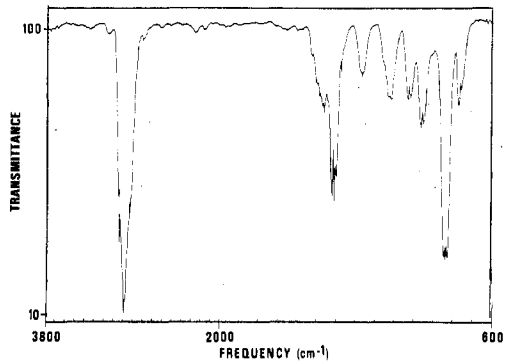

Figure 6. Gas phase spectrum of propylene oxide (50 scans, cell pressure is 30 Torr)

to allow for acceptable variations in ethylene oxide concentration.

The results of our study on difference spectrometry indicate that the method can be developed as a quality control technique. However, computer programs will have to be written to automate the data manipulation before it can become a viable technique.

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Enhancement of the Fluorescence Intensity of Derivatives of Amino Acids in Mixed Solvent Systems

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A number of reagents are known to react with amino acids to form fluorescent derivatives and it has been shown that the fluorescence intensity of the derivatives can be increased by consideration of the solvent system used for its formation. The use of mixed solvent systems such as DMSO/water in place of water for the formation and detection of *o*-phthaldehyde (opt) derivatives of amino acids was found to raise the fluorescence intensity significantly (e.g., 64% for tryptophan–opt in 17% DMSO (v/v) compared to the case where DMSO was absent). Similar observations were found with dansyl amino acids and fluorescamine amino acids.

In an analytical procedure where the detection is performed by fluorometry, the ultimate sensitivity is directly related to the fluorescence quantum yield of the analyte (or of a derivative directly related to the analyte). The fluorescence quantum yield of many compounds is very sensitive to the environment of the excited state (*1*). It has been determined, for example, that water is capable of interacting with the excited state of indole (*2*), tryptophan and its metabolites (*3*), and tryrosine (*4*) to form an excited state complex (exciplex). The formation of an exciplex is competitive with fluorescence; hence the fluorescence quantum yield is reduced, thus raising the minimum detection limit of an analytical procedure. A reasonable approach to the problem of exciplex formation in fluorometric analyses might be to perform the analysis under nonaqueous conditions. In theory, this would serve the analyst quite nicely; however, the removal of water may be time consuming. In those cases, the removal of water is not a viable procedure for the analysis.

Table I. Relative Fluorescence Intensity of *o*-Phthaldehyde Derivatives of Amino Acids in Mixed DMSO[a]

	% DMSO						
Amino acid	0	7	10	13.5	17	20	27
Alanine	1.00	1.00	1.34	1.72	1.91	1.57	1.66
Phenylalanine	1.00	1.11	1.15	1.20	1.23	1.34	1.40
Histidine	1.00	1.50	1.70	2.15	2.20	2.45	
Tryptophan	1.00	1.40	1.69	1.63	1.64	1.58	1.7

[a] All data relative to the fluorescence of the derivative in 0% DMSO.

We have recently shown that the fluorescence of various indoles, tryptophan metabolites, and tyrosine metabolites (*3*) is considerably higher in mixed DMSO/water or ethanol/water than in neat water; this indicates that it may be very useful to consider the overall solvent system used in the analysis of these compounds.

A common tactic in analytical chemistry is the formation of a derivative of the compound of interest. The final solvent system in such an analysis is a composite of the original solvent of the sample, solvents used to isolate the compound of interest from the matrix, and solvents used in the formation of

Table II. Relative Fluorescence Intensity of Dansyl Chloride Derivatives of Amino Acids in Mixed Solvent Systems[a]

	% DMSO								
Amino acid	0	4	8	12	16	20	24	28	32
Alanine	1.00	1.70	2.00	2.20	2.42	2.46	2.54	2.68	2.58
Tryptophan	1.00	1.65	2.03	2.23	2.41	2.61	2.85	3.00	3.08
Lysine	1.00	1.04	1.85	2.00	2.15	2.30	2.30	2.52	2.48
Histidine	1.00	1.34	1.44	1.56	1.75	1.81	2.06	2.12	2.12

[a] All data relative to the fluorescence intensity of the derivative in 0% DMSO.

the derivative. This strongly suggests that a consideration of the solvents used in various steps of such an analysis may lead to an enhancement of the fluorescence intensity of the derivative.

We have studied two analytical procedures which involve the formation of fluorescent derivatives of amino acids and find that the use of DMSO in the place of water or acetone in the formation process may be quite useful in improving the luminescent intensity of the derivative.

EXPERIMENTAL

Salts used to prepare buffers (ACS grade or better) and DMSO (certified ACS grade) were received from Fisher Scientific Co. (Fairlawn, N.J.). Amino acids were obtained from Sigma Chemical Co. (St. Louis, Mo.). *o*-Phthaldehyde, *N,N*-dimethylaminonaphthalenesulfonyl chloride (dansyl chloride) and 2-mercaptoethanol were received from Eastman Organic Chemicals (Rochester, N.Y.). All reagents were used as received. Distilled water was doubly distilled from a glass still and used on the day of distillation.

Fluorometric measurements were made on an Aminco-Bowman Spectrophotofluorometer (American Instrument Co., Silver Spring, Md.) equipped with a Hamamatsu 1P21 photomultiplier, a Hanovia 901C Mercury–Xenon arc and an ellipsoidal condensing system.

Preparation of *o*-Phthaldehyde Derivatives. The *o*-phthaldehyde derivatives were made via a modification of Roth's procedure (*5*). A borate buffer (approximately 0.05 M sodium tetraborate) was prepared and the pH was adjusted to 9.5 with concentrated NaOH. A solution of 2-mercaptoethanol (5 mg/mL) in ethanol and a solution of *o*-phthaldehyde (10 mg/mL) in ethanol were prepared daily. The derivatives were prepared by mixing 0.166 mL of the *o*-phthaldehyde solution, 0.166 mL of the 2-mercaptoethanol solution, 4.40 mL of a solution of the amino acid (1×10^{-5} M in water), and 10.00 mL of a mixture of the borate buffer and DMSO. After a 5-min incubation period, the fluorescence was read at 455 nm, upon excitation at 340 nm.

Preparation of Dansyl Chloride Derivatives. One-tenth mL of a solution of the amino acid was mixed with 1.5 mL of a solution of dansyl chloride (1 mg/mL in acetone) and 0.1 mL of 20% Na_2CO_3. A total of 0.8 mL of DMSO and acetone was added to change the % DMSO in the final mixture. The solution was heated for 2.5 h at 35 °C. The fluorescence was measured at 514 nm, upon excitation at 335 nm.

RESULTS AND DISCUSSION

The relative fluorescence of the *o*-phthaldehyde derivatives of several amino acids may be seen in Table I. The corresponding data for the dansyl chloride derivatives appear in Table II. We observe a definite enhancement in fluorescence as the fraction of DMSO in the solvent system increases.

A small red shift in the λ_{max} for fluorescence was observed for the dansyl amino acids as the DMSO concentration was increased. A blue shift was observed for the fluorescence maxima for the *o*-phthaldehyde derivatives as the % DMSO was increased. These observations are consistent with Lippert's (*6*) theory relating the wavelength of maximum fluorescence intensity with the dielectric constant of the solvent. All fluorometric measurements for a given derivative (e.g., all the dansyl amino acids) were taken at the same wavelength. The error that is introduced by the shift in luminescence maxima will be small (ca. 2–4%) as the maximum shifted only 2–4 nm in 30% DMSO/70% water (or 30% DMSO/70% acetone) and this will not affect the results of this work.

Table III. Comparison of the Fluorescence Enhancement from 30% DMSO with Various Fluorescent Derivatives[c]

	Fluorescamine	Dansyl chloride[a]	*o*-Phthaldehyde[b]
Alanine	1.38	2.63	1.7
Tryptophan	1.98	3.04	1.8

[a] Average from 28 and 32% taken from Table II.
[b] Extrapolation from 27% DMSO shown on Table I.
[c] Each entry is the relative fluorescence of that derivative in 30% DMSO, compared to 0% DMSO.

Our data clearly indicate that the optimum sensitivity of fluorometric assays may be improved by careful consideration of the overall solvent system. This work, in conjunction with our recent investigation on the effect of using mixed solvent systems to enhance the luminescence of fluorescamine derivatives (*7*) shows that DMSO is a useful solvent for assays which involve the measurement of the fluorescence of a derivative. The greatest enhancement by DMSO is for the dansyl derivatives, as indicated in Table III. Chen (*8*) has indicated that the intense fluorescence of the dansyl derivatives leads to extremely low minimum detectable limits, and the use of DMSO should lead to an enhancement of sensitivity.

We observe that an upper limit to the level of DMSO present in the final solvent system does exist. In the case of the *o*-phthaldehyde derivatives, buffer salts begin to precipitate out at approximately 40% DMSO. The fluorescence of the dansyl derivatives and the fluorescamine derivatives increases with the addition of DMSO but a plateau in the intensity may be observed. In some cases, a decrease in the intensity may be observed (e.g., the fluorescamine derivative of alanine (*7*)). This suggests that the analyst should determine the optimum fraction of DMSO for the derivative of interest.

The enhancement of fluorescence by the use of mixed solvent systems is very useful for analytical work. For example, the fluorescence of a solution of the dansyl chloride derivative of tryptophan which has been prepared using 8% DMSO is double that of a solution of the derivative with no DMSO. If the derivative is made by a different procedure than we have outlined, it may still be useful to add DMSO to enhance the fluorescence. As an example, if 9% (by volume) DMSO is added to a solution of dansyltryptophan in acetone, the effect of the added DMSO will raise the fluorescence by 85% (not the 100% indicated in Table II as the dilution will have a small effect; the data in Tables I and II are for equivolume solutions).

Our work strongly suggests that it should be very useful for the analyst to carefully consider the overall solvent system used in fluorometric analysis, as a small change in solvent composition may lead to a very useful enhancement in sensitivity.

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Multichannel Pipet for Parallel Aliquoting of Samples and Reagents into Centrifugal Analyzer Minidiscs

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A multichannel pipet system has been developed for the parallel dispensing of samples and reagents into centrifugal analyzer minidiscs. The present pipet has eight channels; four channels for sample solutions and four channels for composite reagents. Each channel has a nominal volume of 67 μL and the reproducibility of delivery is within 0.5% RSD. The multichannel nature of the pipet allows the sample solutions and composite reagents to be simultaneously aliquoted and delivered within 30 s. A microprocessor (8080A, Intel Corporation) is used to control the hardware associated with the pipet, to control the timing necessary for proper operation and sequencing, and to monitor the operation of the pipet. The concepts, design, advantages, and disadvantages of the multichannel pipet are discussed and evaluation data are presented.

Minidisc Centrifugal Analyzers utilize centrifugal force in spinning disc to gain several analytical advantages. These include the parallel transfer and mixing of micro samples with composite reagent into multiple observation cells, the use of a single optical channel for encoding the quantitative chemical information for all samples, and the automatic degassing of the solutions. The measurements for all samples on the minidisc can be considered to be done in parallel because the sequential measurements are very rapid and, typically, measurements from several rotations of the minidisc are averaged to obtain the absorbance value for each sample. To maintain the high throughput advantage of essentially simultaneous measurements of many samples, a multichannel pipet has been developed for the parallel aliquoting and delivery of solutions into the minidiscs. The introduction of this parallel loading system can improve the overall sample throughput of the minidisc centrifugal analyzer.

Sample-Handling Considerations. Sample throughput is limited for centrifugal analyzers by the time required to load the sample discs. To improve throughput, this loading time must be reduced.

The minidisc used in this work, similar in size and volume capacity to that developed at Oak Ridge (*1*), is shown in Figure 1. This minidisc consists of three 3.49-in. diameter pieces, a $^3/_{16}$-in. Plexiglas central body and two $^1/_{10}$-in. UVT Plexiglas windows, sealed together using Dow Corning 3145 RTV Adhesive/Sealtant (Dow Corning Corp., Midland, Mich.). It contains eight measurement cuvettes which have optical entrance and exit ports formed along an outer ring of 1.50-in. radius. Associated with each measurement cuvette are the sample and reagent cuvettes which are 0.125-in. deep depressions machined into the central body. This 8-position minidisc is more compatible with the multichannel pipet reported here and its design helped ease the complexity of the initial design, construction, and implementation of the pipet.

A new X-Y mixer configuration has been incorporated into this minidisc by machining $^3/_{64}$-in. deep channels from the sample and reagent cuvettes to the measurement cuvettes. This geometry provides immediate mixing of sample and reagent as they travel out to the measurement cuvettes.

The sample-handling problem of the minidisc centrifugal analyzer is the filling of the sample and reagent cuvettes (nominal 67 μL each) before an analysis. Access to these cuvettes is through a 3-mm opening in the top plate of the minidisc (see Figure 1). For accurate and precise results, it is necessary to deliver 67-μL aliquots of solution through these small openings. Further, to improve the sample throughput, the entire minidisc, i.e., all the sample and reagent cuvettes, must be filled rapidly. Thus, in designing any delivery system for centrifugal analyzer minidiscs, it is necessary to provide reproducible delivery of small volumes either in rapid sequence or simultaneously to load the entire minidisc in a short period of time.

Some laboratories which have centrifugal analyzers use various types of manual pipets. A Pipetmen 200-μL adjustable pipet (Rainin Instrument Co., Brighton, Mass.) has often been used in our laboratory. Although these pipets are sometimes convenient, they do not solve the sample-handling problems for high throughput. The reproducibility and time required to manually load minidiscs are greatly dependent on the technician.

To eliminate the dependence upon a given technician for reliable results, various mechanized delivery systems have been reported. These include "Gemeni, the Miniature Centrifugal Analyzer" (Electro-Nucleonics, Inc., 368 Passaic Ave., Fairfield, N.J. 07006), "Automatic Gemsaec Fast Analyzer" (Electro-Nucleonics, Inc.), "Rotochem II, Parallel Fast Analyzer" (Aminco, American Instrument Co., Silver Spring, Md.), "Centrifichem" (Clinical Diagnostics, Union Carbide, Tar-

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