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HIGH-SPEED ANALYTICAL SENSOR FOR IN-LINE MONITORING OF DISSOLVED ANALYTES FLOWING IN A TUBE EMPLOYING A COMBINATION OF LIMITED DIFFUSION, LAMINAR FLOW AND PLUG SOLVENT INJECTIONS

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SUMMARY

A fast molecular weight sensor is described in which pure solvent is introduced as a plug into an analyte-containing mobile phase undergoing laminar flow within a capillary tube. If diffusion of the mobile phase analyte is limited, a complex concentration *vs.* time profile of capillary effluent is observed. Molecular weight and quantitative information regarding the analyte dissolved in the mobile phase is obtained from such profiles in a minute or less. Anomalous results due to analyte adsorption are avoided in this technique by cleaning the injection system with organic as well as aqueous solvents.

INTRODUCTION

Previous papers[1,2] have described a rapid method for determining molecular weights of single components[1] and polymers and mixtures of polymers[2] dissolved in a liquid phase. Our original method relies on injection of a liquid plug of analyte solution into a capillary containing a mobile phase which is flowing with a laminar flow profile. Concentrations *vs.* time profiles of the effluent from this capillary reveal two peaks or one peak and a shoulder, even for a single component, when radial diffusion of the analyte is limited in comparison with axial transport.

For a given temperature, the unique shapes of these concentration *vs.* time profiles of the analyte effluent measured at the exit of the capillary are dependent upon the inner diameter of the capillary, the flow-rate, the length of the capillary, and the diffusion coefficient(s) of the analyte(s) contained in the injected liquid plug. The anticipated profile shapes have been calculated by Atwood and Golay[3] and others (see refs. 4 and 5 and the references cited in refs. 1 and 2) as two component peaks or peak plus shoulder when there is limited radial diffusion in comparison with the axial transport of the injected analyte. Our method relies on the smoothly changing ratio of analyte effluent concentrations representing these two concentration *vs.* time profile components as the diffusion coefficient of the analyte changes, when all the other experimental variables are held constant.

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Analysis of the shapes of capillary effluent analyte concentration *vs.* time profiles in terms of a ratio (R) of concentrations at two characteristic times following injection of the analyte allows the empirical determination of molecular weights on the basis of a standard curve relating this concentration ratio R to molecular weight. Interpolation of R value data for a material of unknown molecular weight on the resulting standard curve can be used to yield molecular weights of biopolymers[1] or number-average molecular weights of synthetic polymers[2] in a minute or less. The type of analyte which can be investigated in this manner is limited only by its ability to undergo diffusion without physical entanglement or molecular aggregation in a liquid phase under laminar flow conditions, by the absence of significant adsorption of analyte components on the walls of the capillary, and by the existence of an appropriate standard curve for the analyte.

We report in this paper a new application of the same basic technique as described above, except that in the work reported here, we *employ the analyte solution as the mobile phase and inject a plug of pure solvent.* Such an experiment allows a quantitative determination of both the concentration and an empirical determination of the molecular weight of the analyte contained in the mobile phase. Thus, the analyte concentration *vs.* time profiles obtained in this system consist of *negative* rather than positive concentration *vs.* time traces, since the injected, initially analyte-free, pure solvent plug can only have an analyte concentration lower than that of the mobile phase as it emerges from the capillary and passes through the concentration detector.

The success of this new method depends on the absence of significant analyte adsorption to the inner surface of the injection system capillary surface. In this "negative profile mode" of our empirical molecular weight method, there are significant complications owing to analyte adsorption, even with low-molecular-weight compounds. This adsorption is especially important in the injection system of the apparatus. We have shown that such adsorption problems in the injection system can be eliminated or at least minimized in order to achieve reproducible results by a thorough cleaning of the injection system before loading[6]. This cleaning is accomplished by a combination of organic and aqueous solvents, the organic solvent flush being a critical phase of the cleaning process.

EXPERIMENTAL

The apparatus employed is essentially the same as reported previously[1,2] except for the use in some experiments of either a Harvard Model 909 or an ISCO μLC 500 syringe pump as the mobile phase pump. The system is essentially a conventional liquid chromatographic arrangement, except that the packed column is replaced with an untreated length (30–100 cm) of stainless-steel capillary (0.25–0.75 mm I.D.). Chemicals were reagent grade and were used without further purification. The Valco injection valve was flushed between injections with approximately 20 ml each of 2-propanol, methanol and Milli-Q water, in that order. If only water was used as the wash, different often irreproducible results were obtained, and positive peaks were obtained where negative peaks were expected[6]. We tentatively attribute these positive peaks to flow sensitive, metastable absorbed analyte in the injection system which is released under the zero flow condition while the injection system is loaded[6]. Further studies of these phenomena are needed.

RESULTS AND DISCUSSION

Concentration vs. time profiles of low-molecular-weight compounds in the positive profile mode

Our method[1,2] involving injection of a liquid analyte into a liquid mobile phase containing no analyte and yielding positive analyte concentration *vs.* time profiles, will hereafter be referred to as the "positive profile mode". Previous investigations, involving synthetic[2] and biopolymers[1] and employing the positive profile mode, demonstrated concentration *vs.* time profiles of the type predicted by Atwood and Golay[3]. In our previous studies no low-molecular-weight species were investigated.

In this paper, we demonstrate similar positive mode profiles, as seen in Fig. 1, for three different low-molecular-weight compounds at three different flow-rates. Fig. 1 illustrates the method used previously[1,2] for empirical determinations of molecular weights with one modification. For low-molecular-weight compounds, the ratio R is defined as

$$R = \frac{h_{1.35t_b}}{h_{2.0t_b}}$$

where h represents the positive (or negative) height above (or below) the baseline value of the concentration *vs.* time profile at either 1.35 or 2.0 times the breakthrough time, t_b, defined as the time interval between injection and first compound from the injection emerging from the capillary at the detector. The above definition of R is slightly different from that previously reported in that an empirical value of 1.35 is used in the numerator rather than the previously employed value[1] of 1.2 t_b. This higher value is an empirical fitting coefficient used to locate the top of the first sharp peaks on the left sides of the two component profiles illustrated in Fig. 1. This empirical coefficient is apparently different for very-high- and very-low-molecular-weight compounds. The constant 2.0 in the denominator of the above expression is theoretical in origin, since it can be used to locate the time of the maximum in a gaussian absorbance *vs.* time curve which represents the case where radial diffusion is rapid in comparison with axial transport.

Fig. 2 illustrates the dependence on molecular weight and mobile phase flow-rate of R values calculated as indicated above from the data of Fig. 1. In agreement with our previous work, R values are approximately linear with flow-rate for a single compound[2] and smoothly increasing functions of molecular weight[1,2]. These studies, in combination with our earlier observations with polymers, serve to emphasize the wide dynamic molecular weight range of the positive profile mode of our empirical molecular weight method since the same type of quantitative behavior of R values is observed over more than three orders of magnitude in molecular weight. This would be expected if the R value is primarily a reflection of the diffusion properties of the dissolved analyte(s).

Pure solvent injection into mobile phase containing analyte —the negative profile mode

When pure solvent is injected as a plug into an analyte-containing mobile phase in laminar flow in a capillary, there is diffusion of the analyte into the pure solvent plug as it assumes a laminar profile and is transported through the capillary. The shape of

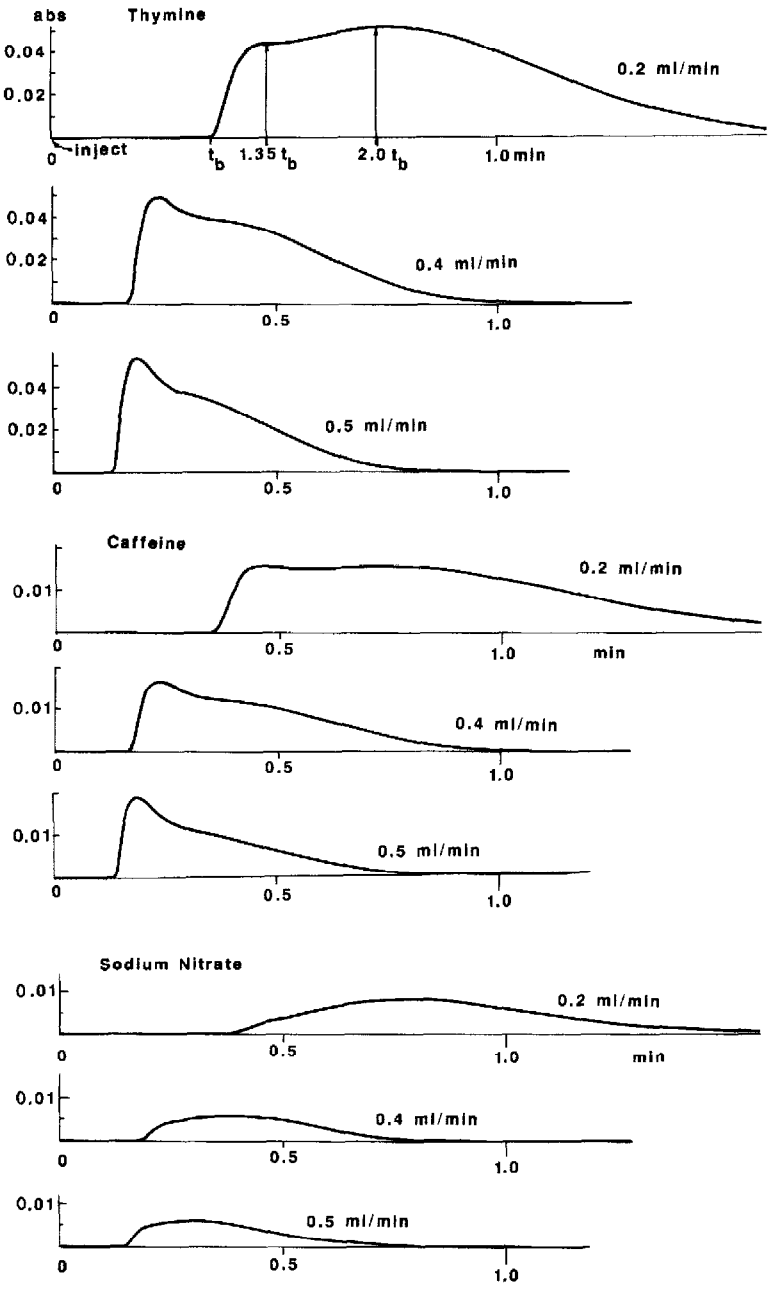

Fig. 1. Effect of molecular weight and flow-rate on concentration *vs.* time profiles from solutions of low-molecular-weight compounds injected into water mobile phase and passed through a capillary tube under laminar flow conditions where radial diffusion is limited in comparison with axial transport through the tube (90 cm × 0.5 mm I.D.). An ISCO μLC 500 syringe pump was used. Solutions concentrations: caffeine, 0.24 m*M*; thymine, 0.056 m*M*; $NaNO_3$, 0.090 m*M*. Absorbance monitored at 254 nm.

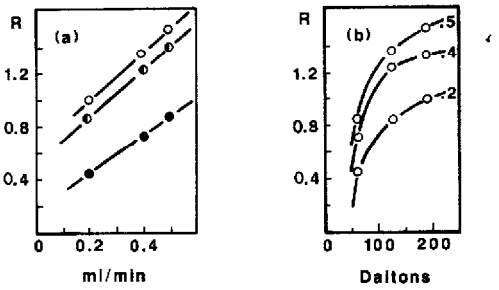

Fig. 2. Variation of R values (ratios of capillary effluent analyte concentration at two selected times after a plug injection of an analyte solution) with: (a) molecular weight; (b) flow-rate (values shown in ml/min on the right). ○ = Caffeine; ◐ = thymine; ● = $NaNO_3$. Data taken from Fig. 1.

the resulting negative concentration *vs.* time profile of the effluent should be dependent on the same experimental parameters as with the positive profile mode. This type of experiment will be designated as the "negative profile mode" of our empirical molecular weight method.

Fig. 3 illustrates the results of switching caffeine solution and pure solvent (water) as the injected phase and mobile phases in positive and negative mode experiments. The positive profile is obtained by injecting a plug of 8 μl of the caffeine solution into water mobile phase. The negative profile is obtained by injecting an 8-μl volume of pure water into a caffeine solution mobile phase with the same caffeine concentration used to obtain the positive profile under otherwise identical conditions of flow-rate and temperature, employing the same stainless-steel capillary and experimental system.

Within experimental error, the positive and negative peaks are scaled mirror images of one another, except near the last third of the profile. Fig. 3 illustrates this point by plotting the inverted negative profile (dashed line) on the same coordinates with the positive profile. A crossing of the curves is noted in the right-hand portion of the profiles. Calculated R values, using either the 1.2 or the 1.35 numerator coefficient, are within experimental error of one another for the positive and the negative profiles, indicating quantitative peak scaling in the region of the profiles used in R value calculations. The lack of quantitative peak scaling in the trailing edge of the profiles may be due to wall adsorption effects, since molecules in the tail segment have a higher probability of colliding with the wall.

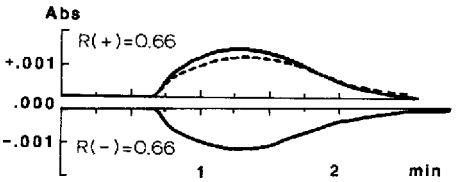

Fig. 3. (Solid lines) Comparison of positive profile mode (analyte solution injected into water mobile phase) and negative profile mode (water injected into mobile phase solution containing analyte) employing the same analyte, a 0.24 mM caffeine solution. (Dashed line) Inversion of negative mode profile for direct comparison with positive profile mode result. Capillary: 100 cm × 0.75 mm I.D.; flow-rate: 0.3 ml/min; ISCO μLC 500 syringe pump.

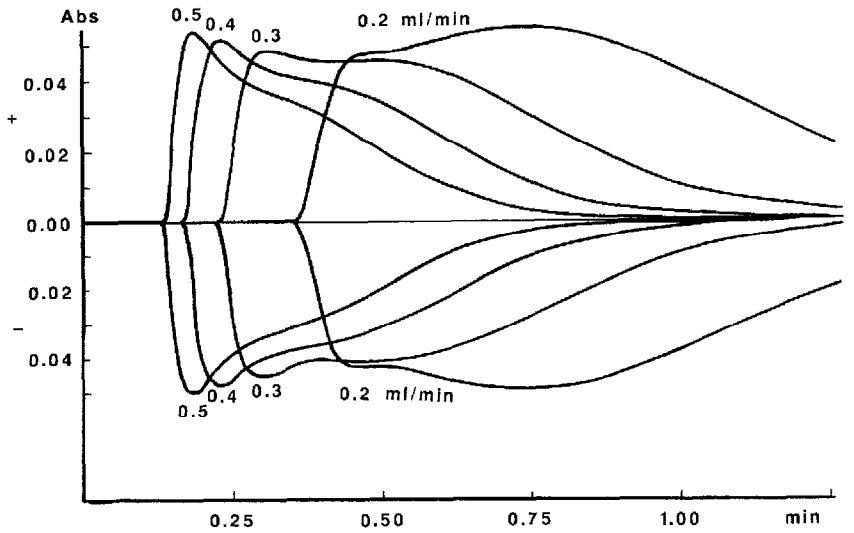

Fig. 4. Effect of flow-rate on positive and negative profile mode results from the injection of 10 μl of a 0.05 m*M* thymine solution into water (or 10 μl water into 0.05 m*M* thymine mobile phase) flowing in a 30 cm × 0.5 mm I.D. capillary. Flow-rates indicated; Altex 100A pump.

Results similar to those in Fig. 3 are observed in Fig. 4 in which a thymine solution is analyzed in the positive and negative profile modes at different flow-rates. Again, the early portions of the negative profiles are almost exact scaled mirror images of the positive profiles. In Fig. 5, the negative peaks from Fig. 4 are inverted (dashed line) and compared directly with the positive peaks. Once more, there is a non-quantitative scaling of the negative and positive profiles at later times as is the case with Fig. 3. The slight quantitative differences between absolute absorbances in the positive and negative profiles for a given flow-rate may arise because of adsorption problems which have been encountered[6]. However, *R* values calculated from the above data comparing positive and corresponding negative profiles are again within experimental error, as illustrated in Table I. Thus, the ability to switch injected and mobile phases without

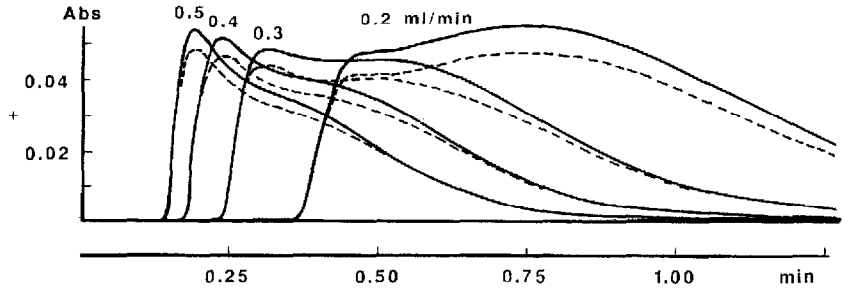

Fig. 5. Inverted negative profile mode results from Fig. 4 (dashed line) compared with positive profile mode results from Fig. 4.

TABLE I

COMPARISON OF *R* VALUES FROM POSITIVE AND NEGATIVE PROFILE MODES

Data taken from Fig. 4.

Flow-rate (ml/min)	*R (positive mode)*	*R (negative mode)*
0.2	0.86	0.88
0.3	1.11	1.16
0.4	1.21	1.24
0.5	1.43	1.39

changing the *R* value apparently does not depend on flow-rate for low molecular weight samples in the molecular weight range and flow-rate ranges reported.

Molecular weight dependence of R values —the negative profile mode

As might be expected from the above data and our previous experience with the positive profile mode results, the negative profile mode should be useful in providing empirical determinations of molecular weights employing the *R* value concept. Fig. 6 illustrates the dependence of *R* values, calculated from a large number of data taken

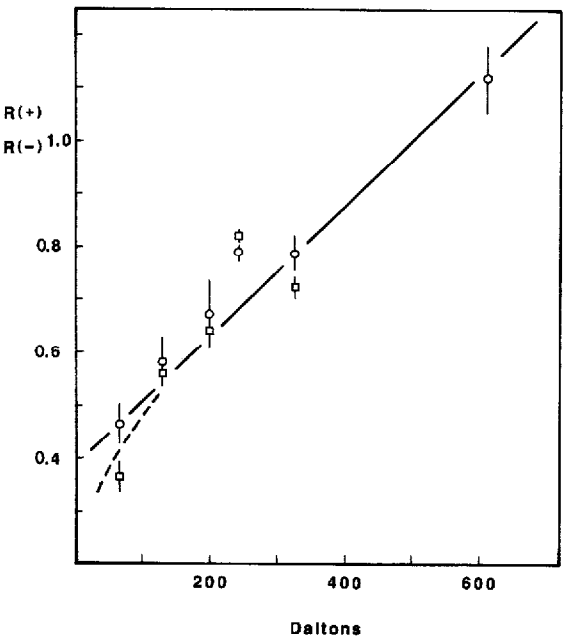

Fig. 6. Effect of molecular weight on positive (○) and negative (□) profile mode *R* values. Average values taken from different runs made on different days with the same 90 cm × 0.75 mm I.D. capillary. Solution concentrations varied over a factor of 4–8 with no apparent correlations of *R vs.* concentration. Altex pump; flow-rate, 0.3 ml/min; injections, 10 μl; solvent, water.

from parallel positive and negative profile mode experiments, done on the same equipment and at the same flow-rate, on molecular weight for a number of low-molecular-weight substances. The data in Fig. 6 were obtained over an extended period of time but employed the same capillary tubing. Smaller experimental error in obtaining a standard curve for either the positive or the negative mode would be expected than that shown in Fig. 6 if the standard curve were prepared and compared with the unknown within a shorter time span. Both molecular weight discrimination and day-to-day reproducibility of R values obtained with this method are on the order of 10–15% with the system employed. These two values could be reduced by the use of a more reliable pump than the one used to gather the data in Fig. 6.

The positive and negative mode R values shown in Fig. 6 are nearly within experimental error of one another. The near linear dependence of R on molecular weight is reminiscent of our previous data on biopolymers[1]. The reason for the relatively large deviation of 2′-deoxyadenine (molecular weight 269) from the linear portion of the figure may arise from the severe adsorption problems encountered with this compound in other experiments in which careful washing of the injection system was not carried out. The wide scatter noted for nitrate data can be traced to the near-Gausssian shape of the nitrate profiles, with which R value calculations are difficult because of the uncertainty in determination of breakthrough times. Gaussian or near-Gaussian peaks should not be used for analysis in this method and can be avoided by increasing the flow-rate. A shift to a higher flow-rate would increase all R values in Fig. 6 but the near-linear relationship may not be retained[1,2]. Values for R at a single flow-rate were independent of analyte concentration over a factor of 4 to 8 for a number of different compounds. These points were included in calculating the experimental scatter.

Peak shapes in the positive profile mode of our technique have been shown in all cases not involving adsorption problems to give those shapes calculated from the assumption of limited diffusion and laminar flow. These positive profile mode peak shapes have been independent of molecular weight, provided the flow-rate is adjusted to maintain the same, limited degree of diffusion, *i.e.* slow flow for high-molecular-weight substances and faster flow for lower-molecular-weight substances. In this paper, we have demonstrated that the profiles obtained for the same analyte in the positive and negative profile modes are, for the most part *scaled*, mirror images of one another, especially in the early portions of the profiles. Deviations from an exact mirror image relationship between positive and negative profiles may be due to adsorption problems, even for low-molecular-weight compounds. Thus, in the absence of significant wall adsorption, we predict that our negative profile mode technique, described and illustrated in this paper, can be used to quickly characterize *any* molecules or particles of *any* molecular weight where those species are capable of free diffusion in a liquid undergoing laminar flow. However, because absorption problems may be more serious with increased molecular weight[7], each individual molecular system would have to be investigated in both the positive and negative modes to determine the extent of adsorption problems encountered when continuously passing the analyte through the capillary system, including the injection port region. These added exploratory investigations may be worth the extra effort, if the reward is an analysis system which requires no sample to be withdrawn from a stream and allows *in situ* analysis.

Even though all of the examples we have shown in this paper are for single molecular species, we believe that both positive and negative profile methods can be used to characterize or monitor mixtures of molecules by measuring or monitoring profile shapes. Since the technique assumes free diffusion of molecular species, profiles should be linearly dependent on analyte concentration, providing the absorbance is in the Beer's law range, and individual profiles for each analyte should be additive for mixtures of analytes. Analyte solutions could be monitored for impurities, provided the diffusion and extinction coefficients of the impurity or impurities allow such an analysis to be sensitive enough to detect the impurity through changes in the positive or negative profile shape.

CONCLUSIONS

We conclude from the above results that the same type of empirically determined molecular weight information may be obtained in very short times through both the positive and the negative profile modes of our capillary method, *i.e.*, by either injecting analyte solution into pure solvent mobile phase or by injecting pure solvent into analyte solution employed as the mobile phase. Discrimination of small differences (less than about 15%) in molecular weight cannot easily be accomplished with either the positive or the nagative profile modes of this method. The method is employed to greatest advantage when there are significant changes in molecular weights to be quickly characterized.

The experimental information obtained from either mode contains two different pieces of information. First, with proper calibration, the integrated area of the positive or negative profile gives quantitative information on the amount of material present. Secondly, the shape of the positive or negative profile reflects the diffusion characteristics and therefore also the molecular weight of the dissolved species, provided shape and solvent interaction characteristics of a series of compounds in the standard curve are similar.

POSSIBLE APPLICATIONS OF THE METHOD

The methods described above are most useful in a situation in which the composition of a solution is to be quickly and repetitively monitored for *changes in composition or concentration* over an extended period of time. Given the low cost, the wide dynamic range, and the speed of the positive and negative profile methods, we suggest the following as logical applications of these empirical molecular weight methods.

A side stream from a pipe or a continuously recycling sampling stream from a stirred reactor could be fed through either the loop of an injector or through the system capillary. Since it has been shown[2] that our capillary method yields number average molecular weights, it is anticipated that the method can be used as a fast sensor for *changes* in number-average molecular weight of a polymer solution. The method could use a capillary shunt which continuously draws off material for sampling from a process container or pipeline, with periodic injections of the appropriate solvent. Fast computer feedback from the concentration detector could be sent to an alarm device when any deviation from a standard profile is observed and standard analytical

methods could be employed for pinpointing specifically what chemical changes triggered the profile difference-based alarm.

Water from a municipal water supply or waste water from an industrial source can be monitored for purity by continuing to pass this water through either the injection loop or through the capillary. A mobile phase of pure water could be passed through the capillary system or through the loop and a periodic injection could be made to check for positive or negative profiles which quickly characterizes the number-average molecular weight of an impurity in addition to providing quantitative information regarding the amount of impurity present.

When there are solubility problems in which there can be drastic changes in the average molecular weight of a substance beause of changing experimental variables, the positive or negative profile methods would provide a valuable means of quickly observing the changing average molecular weights which might precede precipitation or colloid formation. We believe with proper development this technique is capable of determining molecular weights in the order of tens of seconds.

Such complex biological fluids as blood and urine often need to be monitored for sudden changes in composition. For example, kidney failure, in which high-molecular-weight biopolymers are suddenly found in the urine could be be monitored with either the positive or negative profile methods. Monitoring of dialysis fluids also would appear to be another possible application. Blood monitoring may be possible but also may present special problems. The limits of our method at the very high end of the moleular weight range have yet to be tested. Preliminary experiments in our laboratory with very high molecular weight biological systems appear to be quite promising.

Thus, many applications of this new method can be envisioned for a wide range of molecular weight compounds including industrial polymers and waste water, biopolymers and clinical samples, provided there are not significant problems of wall adsorption. For higher-molecular-weight compounds, these problems may be minimized by increasing the capillary tube diameter and decreasing the flow-rate.

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