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COMPUTER-ASSISTED OPTIMIZATION OF ELUENT CONCENTRATION AND pH IN ION CHROMATOGRAPHY

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SUMMARY

A retention relationship applicable to ion chromatography using low capacity ion-exchange columns is derived and evaluated for a number of monovalent and divalent inorganic anions. A linear dependence of $\log k'$ on $\log [\text{eluent}]$ is observed and this is used as the basis of a mixture design computer-assisted procedure for selection of the optimum eluent concentration in single column ion chromatography. The same approach is then successfully applied to the optimization of eluent pH and it is shown that this method yields a similar optimal mobile phase to that obtained when eluent concentration is optimized for the same chromatographic system.

INTRODUCTION

In recent years, several procedures have been described for computer-assisted selection of the optimum mobile phase composition in high-performance liquid chromatography (HPLC)¹⁻⁵. In these procedures, a large number of mobile phase compositions are evaluated by the computer for their suitability to effect a desired separation, and the optimum mobile phase is then selected. The advantages of these procedures over conventional empirical search methods are that the optimum mobile phase is generally determined more rapidly, and many possible mobile phases can be considered in the calculations.

Knowledge of solute retention data for the range of mobile phase compositions to be searched provides the basis for evaluation of each possible mobile phase and subsequent selection of the optimum. These retention data may be acquired in two distinct ways⁶. The first method, which is described as a "mixture design" approach, involves the measurement of solute retention times for a limited number of mobile phase compositions and the subsequent use of these data to predict retention times for further mobile phase compositions. This prediction is based on a theoretical or empirical knowledge of retention behaviour under varying mobile phase compositions. The second method, described as a "factorial design" approach, involves the measurement of retention data for a relatively large number of mobile phase compositions selected to cover the range of mobile phases to be searched. In this case,

prediction of retention data for those mobile phases not actually tested is achieved by extrapolation of measured data, rather than on the basis of theoretical knowledge of retention behaviour. The essential difference between mixture and factorial design approaches is that the former method requires considerably less initial retention data than the latter method.

Applications of computer-assisted optimization procedures have generally been confined to reversed-phase HPLC. Primary mobile phase variables, such as the percentages of organic modifiers, have been optimized using mixture design procedures for binary, ternary and quaternary solvent mixtures^{1,2,4,5,7,8}. On the other hand, secondary mobile phase variables such as pH have generally been optimized using the factorial design approach⁹⁻¹² however a mixture design method for optimization of mobile phase pH in reversed-phase HPLC has recently been described¹³.

For some time, we have been investigating the determination of inorganic anions by HPLC. One of the most promising approaches to this determination is ion chromatography using columns of low ion-exchange capacities, together with eluents comprising dilute solutions of aromatic acids^{14,15}. This approach is typified by the separation of common inorganic anions on a low capacity silica-based ion-exchanger, using phthalate as eluent. Retention of ions can be manipulated by changing either the concentration or pH of the eluent. Although many excellent separations have been achieved using ion chromatography, no systematic search procedure for selection of the optimum mobile phase composition appears to have been developed. In this paper, we report the results of a study of the application of computer-assisted optimization procedures to single column ion chromatography, with emphasis on mixture design methods.

THEORY

In order to develop a mixture design optimization procedure for ion chromatography, a theoretical knowledge of retention behaviour is required. For a solute ion A^{y-} and an eluent ion B^{x-} , the following ion-exchange equilibrium applies:



where the subscripts m and s refer to mobile and stationary phases, respectively. The selectivity coefficient $K_{A,B}$ is given by:

$$K_{A,B} = \frac{(a_{A_s^{y-}})^x (a_{B_m^{x-}})^y}{(a_{A_m^{y-}})^x (a_{B_s^{x-}})^y} \quad (2)$$

where a is the activity of the species.

In analytical applications, the amount of solute injected onto the column is generally small, so the concentrations of A^{y-} in the mobile and stationary phases can be assumed to be low. Similarly, the stationary phase concentration of the eluent ion B^{x-} is small, since this is governed by the ion-exchange capacity of the column which, in the case under consideration, is low. In addition, the mobile phase concen-

tration of the eluent ion B^{x-} is small (*ca.* 10^{-3} M) in this form of ion chromatography. Eqn. 2 can therefore be rewritten as:

$$K_{A,B} = \left(\frac{[A^{y-}]_s}{[A^{y-}]_m} \right)^x \left(\frac{[B^{x-}]_m}{[B^{x-}]_s} \right)^y \quad (3)$$

The partition ratio, K , for solute A^{y-} is given by:

$$K = \frac{[A^{y-}]_s}{[A^{y-}]_m} = k' \frac{V_m}{V_s} \quad (4)$$

where k' is the capacity factor for solute A^{y-} , and V_m and V_s are the volumes of the mobile and stationary phases, respectively. Combination of eqns. 3 and 4 gives:

$$K_{A,B} = \left(k' \frac{V_m}{V_s} \right)^x \left(\frac{[B^{x-}]_m}{[B^{x-}]_s} \right)^y \quad (5)$$

Since $K_{A,B}$, V_m , V_s and $[B^{x-}]_s$ are constant at a given column temperature, then eqn. 5 can be written as:

$$\log k' = \text{constant} - \frac{y}{x} \log [B^{x-}]_m \quad (6)$$

EXPERIMENTAL

Materials and apparatus

The HPLC system consisted of a Waters Assoc. (Milford, MA, U.S.A.) M45 pump and U6K injector, and an Erma Optical Works (Tokyo, Japan) Model 7510 refractive index detector, connected to a Houston Instruments (Austin, TX, U.S.A.) Omniscrite recorder. The column used was a Vydac (The Separations Group, Hesperia, CA, U.S.A.) 302 IC silica-based ion-exchanger (0.1 mequiv./g), 250×4.6 mm I.D. Computer calculations were made on a VAX 11-780 computer with a VMS operating system, using software based on the optimization procedure described by Schoenmakers *et al.*¹.

Water used for preparation of the mobile phases was purified on a Millipore (Bedford, MA, U.S.A.) Milli-Q system and was degassed and filtered through a $0.45\text{-}\mu\text{m}$ filter before use. All reagents used were analytical grade.

Procedures

Eluents were prepared daily by dissolving an appropriate amount of phthalic acid or potassium hydrogen phthalate in water and adjusting the pH where necessary with sodium hydroxide, using a calibrated pH meter.

Stock solutions (1000 ppm) of the anions were prepared in pure water, using

appropriate amounts of the sodium salts. These solutions were stored in polythene containers. Mixtures of ions were prepared at the concentrations specified in the figure captions.

The column and detector were allowed to equilibrate with each eluent and small injection volumes (1–10 μl) were used to determine retention times with each mobile phase, using a flow-rate of 2.0 ml/min. Under these conditions, the void volume of the column was measured to be 2.9 ml. The eluted ions were detected by negative changes in the background refractive index of the eluent. The principles of this mode of detection have been described elsewhere^{14,16}.

RESULTS AND DISCUSSION

Optimization of eluent concentration

The linear relationship between $\log k'$ and $\log [\text{eluent}]$ predicted in eqn. 6 was tested for a number of univalent and divalent inorganic anions using various concentrations of phthalic acid (H_2P) at pH 5.3. This pH was selected because phthalic acid is virtually completely ionised and exists as an approximately equal mixture of the singly and doubly charged forms (47.6% as HP^- and 52.2% as P^{2-}) at this pH. The data obtained are illustrated in Fig. 1, which shows the variation in $\log k'$ with $\log [\text{eluent}]$ for seven ions, and a "system" peak. This peak elutes at long retention times and its size is dependent on a variety of experimental parameters, including the type and concentration of the sample ion, the injection volume and the pH of the sample and eluent¹⁷. Clearly, this peak must be considered in selection of the optimum mobile phase, since it may interfere with some solute ions.

The plots in Fig. 1 show excellent linearity, as predicted by eqn. 6. Moreover,

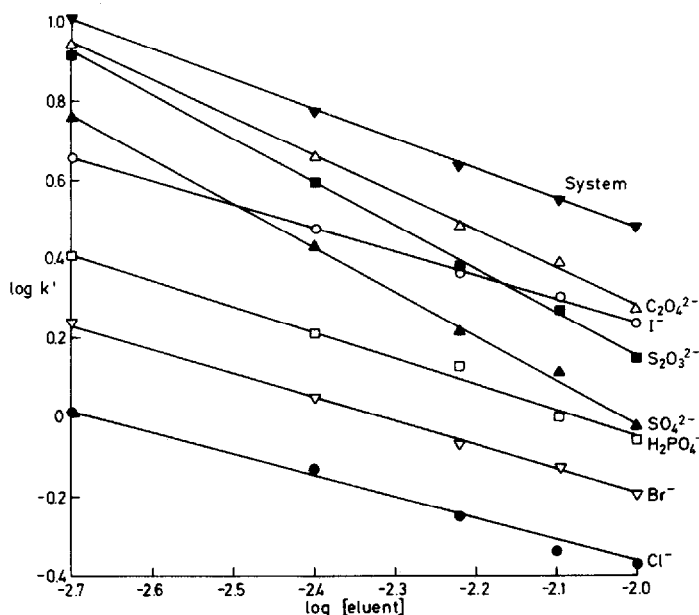


Fig. 1. Retention data for inorganic anions obtained with 2.0–10.0 mM phthalate eluents at pH 5.3.

the slopes of these plots can be used to deduce which form of the eluent (*i.e.* HP^- or P^{2-}) is predominantly responsible for elution of the sample ions. These slopes are listed in Table I, together with theoretical slopes (according to eqn. 6) calculated by assuming the charge on the eluent ion to be -1 , -2 or -1.52 . The latter value was calculated using the actual proportions of HP^- and P^{2-} existing at pH 5.3. The agreement between theoretical and observed slopes in Table I for monovalent anions using the actual eluent charge of -1.52 suggests that elution of these ions is achieved by both HP^- and P^{2-} , in proportion to their concentrations.

TABLE I

OBSERVED AND PREDICTED SLOPES OF $\log k'$ VERSUS \log [ELUENT] PLOTS

The predicted slopes were calculated by assuming that the active form of the eluent was singly charged ($x = 1$), doubly charged ($x = 2$) or the actual composition at pH 5.3 ($x = 1.52$).

Ion	Slope			
	Observed	Predicted		
		$x = 1$	$x = 1.52$	$x = 2$
Cl^-	-0.63	-1.0	-0.66	-0.5
Br^-	-0.63	-1.0	-0.66	-0.5
H_2PO_4^-	-0.67	-1.0	-0.66	-0.5
SO_4^{2-}	-1.13	-2.0	-1.33	-1.0
$\text{S}_2\text{O}_3^{2-}$	-1.10	-2.0	-1.33	-1.0
I^-	-0.60	-1.0	-0.66	-0.5
$\text{C}_2\text{O}_4^{2-}$	-0.98	-2.0	-1.33	-1.0
System	-0.75	-	-	-

A different picture emerges when the divalent anions are considered. Sulphate and thiosulphate, which are both fully ionised at pH 5.3, show slopes slightly greater than unity, suggesting that the P^{2-} form of the eluent predominantly controls their retention. The measured slopes indicate however that HP^- also partially contributes to the elution of these ions. The remaining divalent anion, oxalate, shows a slope of almost unity, however when it is considered that at pH 5.3, oxalate exists as both the divalent ion (92.8%) and the protonated monovalent ion (7.2%), the reduced total charge on the anion would explain a decreased slope in comparison to sulphate and thiosulphate. The above conclusion regarding the form of the eluent predominantly responsible for control of retention can therefore apply equally well to oxalate.

This aspect was further examined by constructing plots of $\log k'$ versus \log [eluent] at pH 2.9 since at this pH phthalate exists as H_2P (53.3%) and HP^- (46.7%). These plots were linear for chloride and nitrate and showed slopes of -0.95 and -0.91 respectively, which are in approximate agreement with the theoretical slope of -1.00 for singly charged eluent and solute ions. The above results differ from those observed for succinate eluents using higher capacity ion-exchangers, where it was found that only the doubly charged form of the eluent was involved in the elution of both singly and doubly charged solute ions¹⁸.

Selection of an appropriate mixture design optimization procedure for appli-

cation to ion chromatography is greatly facilitated by the existence of a linear retention relationship, such as that given in eqn. 6. The procedure developed by Schoenmakers *et al.*¹ for selection of the optimum ternary mobile phase in reversed-phase HPLC appears to be ideally suited to ion chromatography. In this approach, retention data are obtained for three binary mobile phases of water with methanol, acetonitrile or tetrahydrofuran. Linear plots of $\ln k'$ versus percentage of organic modifier are then constructed over the entire range of ternary mixtures intermediate between the limiting binary mixtures. An appropriate criterion based on the product of resolution (R_s) of adjacent peaks is then used to select the optimum mobile phase, using interpolated retention data obtained from the abovementioned linear plots as a basis for calculation. The sample is then chromatographed with the predicted optimal composition, the experimental retention data so obtained are used to refine the plots of $\ln k'$ versus percentage modifier, and the optimization calculations are repeated. This process continues until there is little or no change in the optimal composition. As a result of the criterion used, the optimal chromatogram will tend to show an even distribution of peaks.

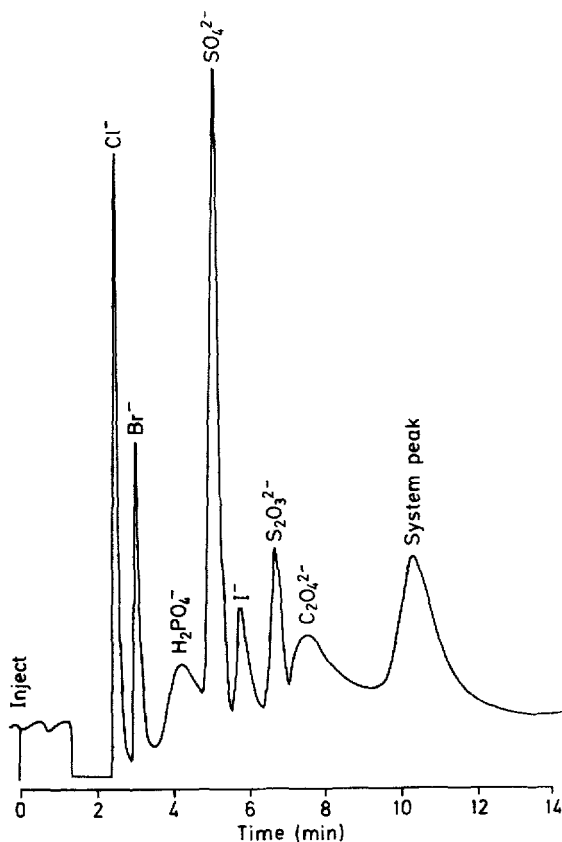


Fig. 2. Chromatogram obtained at the optimum eluent concentration of 4.7 mM. Conditions: column, Vydac 302 IC; eluent, 4.7 mM potassium hydrogenphthalate at pH 5.3; flow-rate, 2.0 ml/min; injection volume, 2.0 μ l; solute concentrations, 1000–1500 ppm; detection, indirect refractive index.

This procedure was applied to the separation of the ions listed in Table I. Retention data obtained at two eluent concentrations (2.0 and 10.0 mM) were used as initial input to define the linear plots of $\log k'$ versus $\log [\text{eluent}]$ and the optimal eluent concentration was determined using the iterative procedure described above. After two iterations, the optimum eluent concentration was found to be 4.7 mM. A chromatogram obtained with this eluent is shown as Fig. 2. In this chromatogram, all peaks are resolved and are evenly distributed, in accordance with the criterion used to assess the suitability of each mobile phase composition.

In principle, any criterion based on peak separation can be used as the basis of the separation search. Different criteria may be required for particular separations such as the case illustrated in Fig. 3. Here the ions to be separated comprise an early eluting group (Cl^- , NO_2^- , Br^- and NO_3^-) and two later eluting ions (I^- and SO_4^{2-}), as well as the system peak. This separation could not be satisfactorily optimized using the resolution product criterion employed in Fig. 2, since an even distribution of peaks over the chromatogram is not attainable without poor resolution of the early eluting ions. Instead, an alternative resolution criterion based on the value of R_s for the least resolved peak pair was used. The eluent pH was maintained at 4.0 and the retention data measured at eluent concentrations of 1.0 and 10.0 mM. These data were used to initiate the optimization procedure and after two iterations, the optimum eluent concentration was found to be 3.0 mM. Fig. 3 illustrates the chromatogram obtained with this eluent and represents the best compromise between

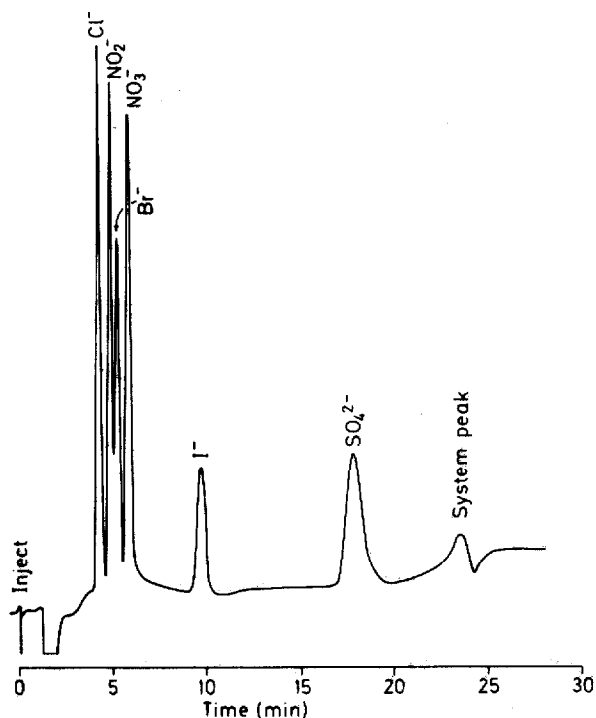


Fig. 3. Optimized chromatogram for the separation of some inorganic anions. The criterion used was R_s of the least resolved peak pair. Conditions: eluent, 3.0 mM potassium hydrogenphthalate at pH 4.0; solute concentrations, 590–1500 ppm. Other conditions as in Fig. 2.

fully resolving nitrite and bromide without causing loss of resolution between sulphate and the system peak.

Optimization of eluent pH

The discussion so far has concerned only selection of the optimal eluent concentration at a given eluent pH. An alternative approach is to vary the eluent pH whilst maintaining eluent concentration at a constant value. Successful application of the mixture design optimization procedure used in the previous section will be dependent on the shapes of plots of retention data *versus* eluent pH. Table II shows retention data obtained for 5.0 mM phthalate eluents over the pH range 4.3–6.0, and Fig. 4 shows plots of $\log k'$ *versus* eluent pH for the same ions as in Fig. 1. Fig. 4 shows that only some of the ions gave a linear retention relationship, whilst others gave curved plots. The curvature can be explained in terms of protonation or deprotonation reactions for anions of weak acids (*e.g.* oxalate and dihydrogenphosphate) which cause a change in the predominant form of the anion existing in solution at different pH values.

One of the features of the optimization procedure used in this paper is that it can be successfully applied in cases where a non-linear retention relationship exists. In such cases, the retention relationship is approximated by a series of straight lines which interconnect known data points. Thus as the iterative optimization process advances, more retention data are successively obtained and are used to more closely approximate the true retention relationship. Indeed, termination of the iterative process can only occur when the true retention relationship has been satisfactorily approximated, at least in the range of mobile phase compositions surrounding the optimal composition. In view of this, the optimization procedure should be applicable to the location of the optimal eluent pH in ion chromatography.

TABLE II

RETENTION DATA FOR INORGANIC ANIONS USING 5.0 mM PHTHALATE ELUENTS AT THE INDICATED pH VALUES

The mobile phase flow-rate was 2.0 ml/min.

Ion	Retention time (sec)					
	pH 4.3	pH 4.7	pH 5.0	pH 5.3	pH 5.7	pH 6.0
H ₂ PO ₄ ⁻	174	174	174	183	210	204
IO ₃ ⁻	174	138	132	123	120	120
BrO ₃ ⁻	177	171	156	150	144	129
CN ⁻	181	174	168	138	132	132
Cl ⁻	185	177	162	160	147	146
NO ₂ ⁻	228	219	198	190	183	180
Br ⁻	243	237	213	210	198	195
ClO ₃ ⁻	255	243	225	219	213	210
NO ₃ ⁻	285	276	246	240	234	228
SO ₄ ²⁻	486	396	336	290	246	233
SO ₃ ²⁻	486	396	330	282	246	201
I ⁻	576	564	516	512	510	498
C ₂ O ₄ ⁻	624	632	528	444	366	309
S ₂ O ₃ ²⁻	684	588	474	408	354	330
System	766	725	658	684	901	1026

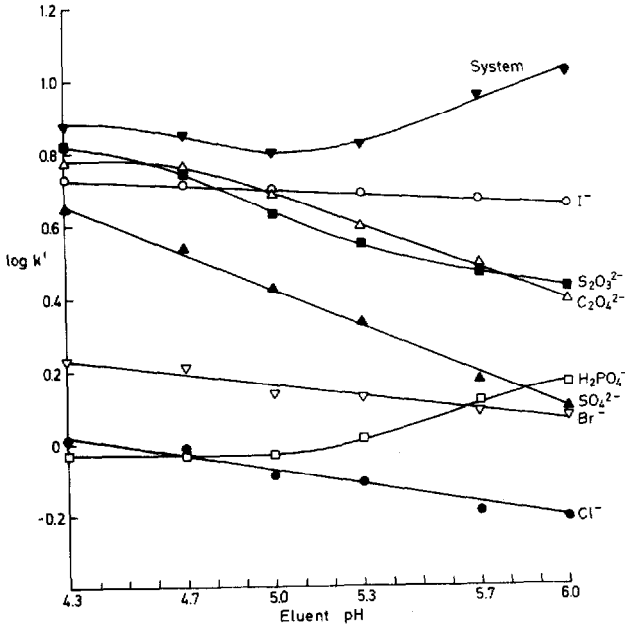


Fig. 4. Retention data for inorganic anions obtained with 5.0 mM phthalate eluents in the pH range 4.3-6.0.

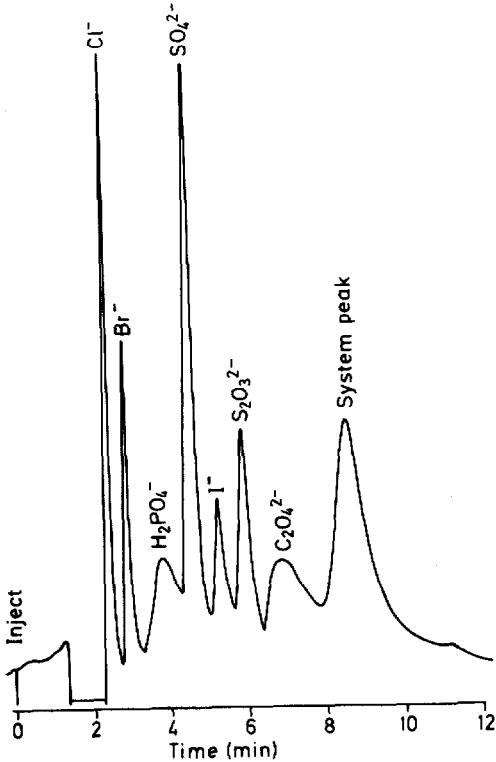


Fig. 5. Chromatogram obtained at the optimum eluent pH of 5.3. Conditions: eluent, 5.0 mM potassium hydrogenphthalate at pH 5.3. Other conditions as in Fig. 2.

To investigate this, the retention data obtained at pH 4.3 and pH 6.0 were used as initial input data, and the optimum pH calculated using the iterative process. The pH value selected after four iterations was pH 5.3. A chromatogram obtained at this pH is shown in Fig. 5. Clearly, the optimum mobile phase obtained using pH optimization (*i.e.* 5.0 mM, pH 5.3) should agree with that obtained by optimization of the eluent concentration (*i.e.* 4.7 mM, pH 5.3) for the same chromatographic system. The slight difference between these optima can be attributed to errors in approximation of the non-linear retention relationships observed for some ions when the eluent pH was varied.

CONCLUSIONS

This study has shown that the linear relationship existing between $\log k'$ and $\log [\text{eluent}]$ in single column ion chromatography can be used as the basis of a mixture design procedure for selection of the optimal eluent concentration. Similarly, the eluent pH may be separately optimized, although more iterations of the optimization procedure are required to account for the curvature of plots of $\log k'$ versus eluent pH for some anions.

The above procedures provide for systematic selection of eluent concentration (at a fixed pH value) or eluent pH (at a fixed eluent concentration) in ion chromatography. An investigation into the simultaneous optimization of both eluent concentration and pH is currently in progress in this laboratory.

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