DIONEX

Technical Note

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Gradient Elution In Ion Chromatography: Anion Exchange With Conductivity Detection

Gradient elution is a powerful technique in ion chromatography. By varying the concentration of the eluant, ions with widely differing affinities for the separator resin can be eluted in one run. However, just as in other forms of chromatography, gradient elution places certain restrictions on the choice of eluant. In particular, it is important to use eluants that produce a minimum shift in baseline conductivity during the run, as well as a fast equilibration time from one run to the next. In this Technical Note, guidelines will be presented to help develop successful analyses using gradient elution. In addition, a simple gradient program which produces a good separation of many common inorganic and organic anions will be described. Although this discussion will focus on anion exchange, most of the concepts are applicable to cation exchange chromatography.

How is gradient elution accomplished?

Eluants used in anion exchange contain an anionic compound in high concentration which competes with the analyte ions for sites on the resin. Gradient elution is best accomplished by increasing the concentration of the eluant anion during the run, i. e. by performing a concentration gradient. In addition, a pH gradient can be used, in which a fixed concentration of a weak acid is mixed with an increasing concentration of a strong base such as sodium hydroxide. pH gradients are essentially concentration gradients, as the purpose of increasing the pH during an anion exchange run is to increase the concentration of the dissociated form of the weak acid eluant.

Other types of gradients are more difficult to use. For example, a composition gradient can be performed by changing from a weakly retained eluant ion to a much more strongly retained ion. There is a wide difference in affinity for the resin between weakly and strongly retained analytes, such as monovalent acetate and trivalent citrate. To elute the more strongly retained citrate, ions from the stronger eluant must completely displace the weakly retained eluant ions from the resin. Depending on the ratio of column ion exchange capacity to eluant concentration, it may take a long time for the resin to be converted from the weak eluant to the strong eluant form. (This effect is similar to solvent demixing in reversed phase HPLC.) Also, to repeat the run, the resin form must be converted back to the weak eluant. This can only be done by pumping a high concentration of the weak eluant, thus increasing the equilibration time between runs.

Concentration gradients avoid these problems by always keeping the form of the resin the same.

With gradient elution, changing the concentration of the eluant results in changes in background conductivity. By using chemical eluant suppression to reduce the highest background conductivity to less than 10 µS, baseline changes during a run can be limited to only a few µS. This is accomplished using an appropriate eluant and an Anion MicroMembrane Suppressor. In addition, baseline changes of up to 20 µS can be compensated by a technique described later in this technical note.

What types of eluants can be used?

There are two types of eluants which can be suppressed to produce background conductivities of only a few µS. One type is the salt of a weak acid. The weakest acid in aqueous solution capable of forming a salt is water itself, with the salt being a strong base such as sodium hydroxide. It is an excellent choice as an eluant for gradient elution as it is converted in the suppressor to water regardless of its concentration. There are other salts of weak acids which can be suppressed to produce low background conductivities. These include borate and many phenates such as p-cyanophenate. In general, the salts of weak acids with pK_a's greater than 7 are acceptable. As pK_a decreases, the extent of dissociation following suppression increases, resulting in increased background conductivity and increased baseline shift during gradient elution. The other type of eluant which can be used includes those anions which are anionic at high pH but are

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converted to zwitterions or cations following suppression. Amino acids such as glycine are good choices because they are primarily converted to their cationic form and removed by the suppressor.

Is sodium hydroxide the best choice for an eluant?

Because sodium hydroxide is converted to water in the suppressor, it is the best choice for an eluant. As long as the capacity of the suppressor is not exceeded, the eluant hydroxide concentration has little effect on background conductivity. The suppression capacity of the AMMS usually exceeds 100 mM NaOH at a flow rate of 1.0 mL/min. A gradient run could begin with a few mM NaOH and end at 100 mM. Examples of gradient elution using sodium hydroxide are shown in Figures 1 and 2. In Figure 1, the HPIC-AS5 column is used. This column can elute anions with charges ranging from monovalent for chloride to hexavalent for tetrapolyphosphate. In Figure 2, the HPIC-AS5A (5μ) column is used to elute a large number of anions in one run. The initial eluant is weak enough to retain fluoride well out of the void volume and separate several weakly retained monoprotic organic acids, while the final eluant concentration is capable of eluting triprotic phosphate and citrate. As shown in Figure 2, 36 ions eluted in 30 minutes illustrates the power of gradient elution.

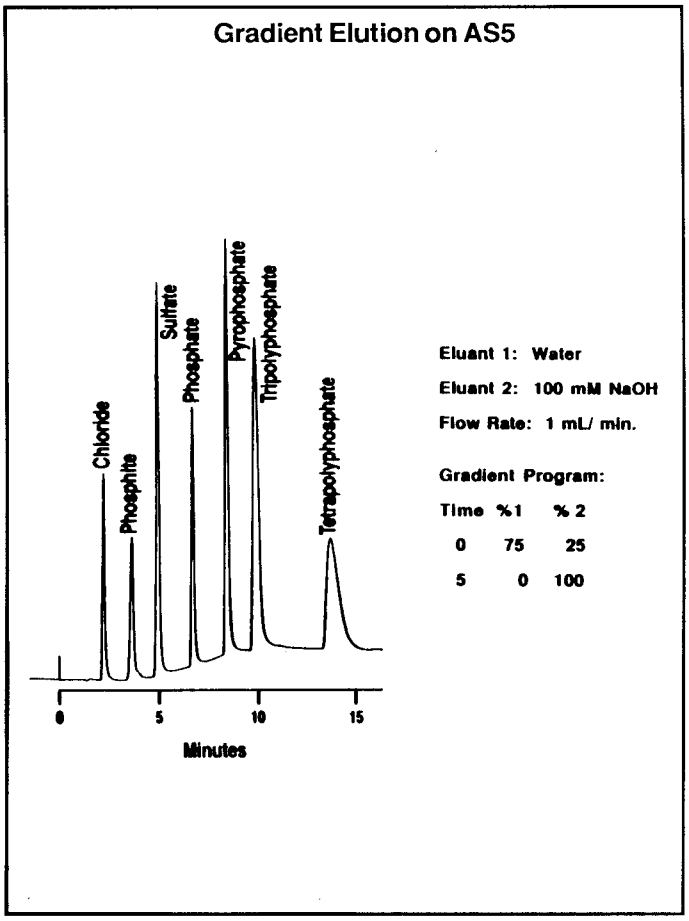

Figure 1

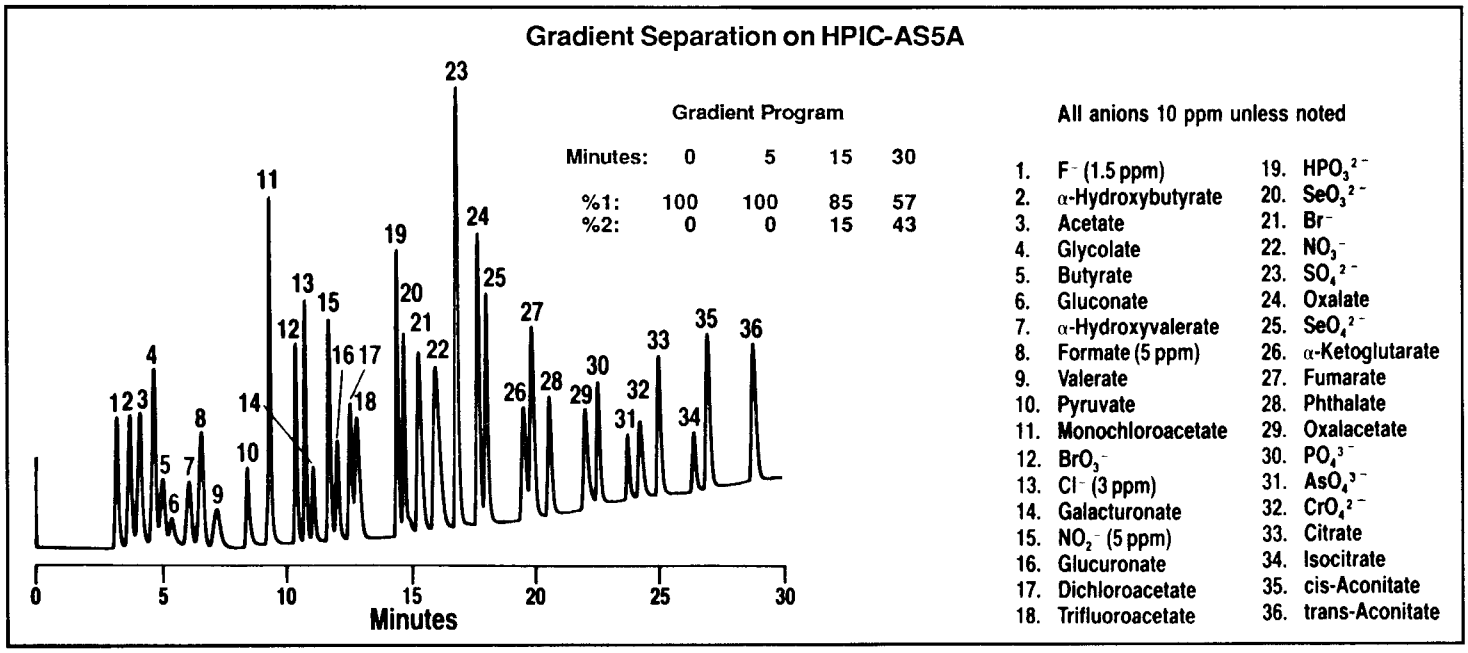

Figure 2

How should sodium hydroxide eluant be prepared?

Carbon dioxide readily dissolves in basic solutions, producing carbonate. Carbonate contamination in sodium hydroxide eluants can greatly increase the baseline shift during a gradient run. Eluants should be prepared from 50% solutions of sodium hydroxide, as they contain lower concentrations of carbonate than the solid. 18 meg-Ω deionized water should be used and degassed prior to addition of sodium hydroxide. Eluants should be maintained under an inert atmosphere during use. The Dionex Eluant Degas Module (P/N 37124) and Eluant Container Set (P/N 38752) provide a convenient method for ensuring that the eluants are properly degassed and free from carbonate contamination.

When should p-cyanophenate be used?

p-Cyanophenate is a more powerful eluant than hydroxide and should be used with higher capacity separators. The HPIC-AS6 provides excellent selectivity of diprotic organic acids. Due to its higher capacity (approximately ten times that of the AS5A), the stronger eluant ion p-cyanophenate is necessary to elute di- and trivalent ions. An example of gradient elution on the HPIC-AS6 using p-cyanophenate is shown in Figure 3. In this run, the concentration of p-cyanophenate in the eluant is increased from 2.4 mM at the beginning of the run to 35 mM at the end.

Can carbonate – bicarbonate buffers be used for gradient elution?

Carbonate containing eluants are generally not acceptable for gradient elution. Following suppression, carbonic acid is formed. With a relatively low pK_a of 6.2, the baseline drift is too severe. Although salts of weak acids with pK_a's as low as 5 can be used for isocratic elution, due to their higher background conductivity following suppression, they are generally not acceptable for gradient elution.

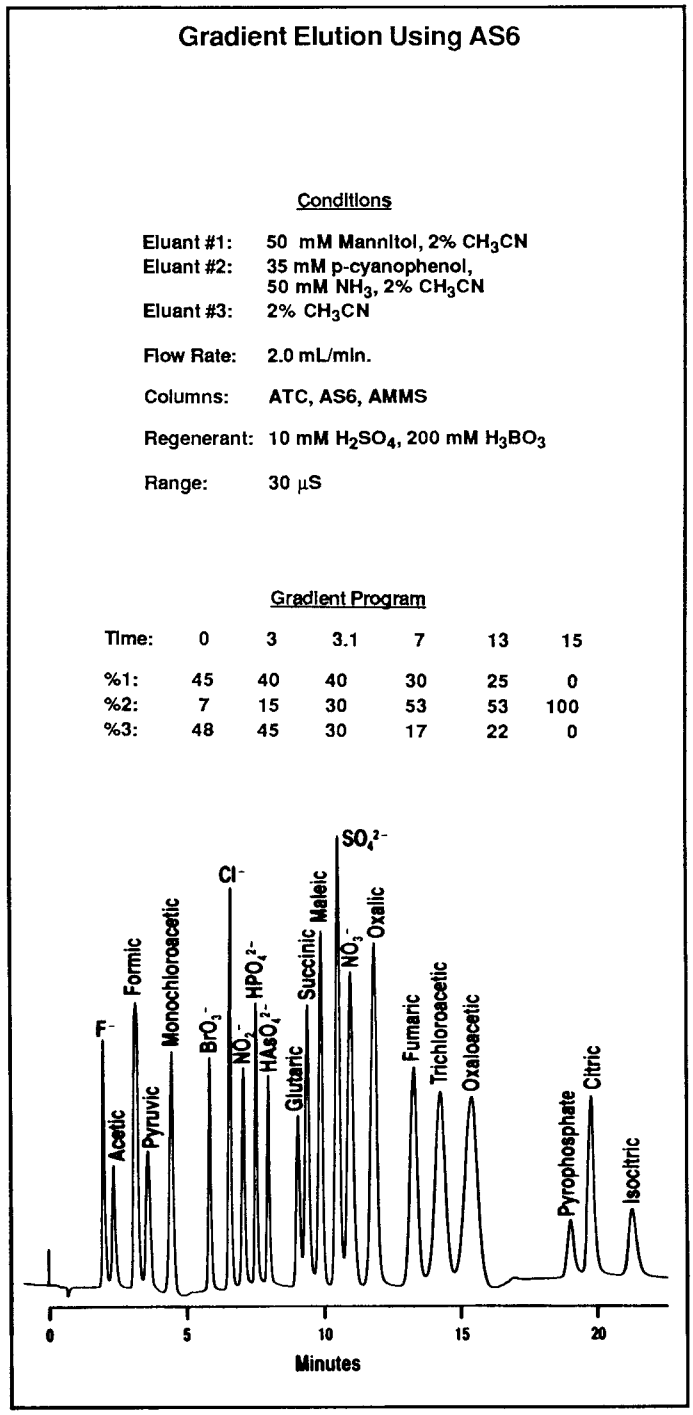

Figure 3

How does changing the eluant concentration affect elution?

The relationship between elution volume and gradient steepness (i. e. how rapidly the eluant concentration is being increased) is described by equation [1] and shown graphically in Figure 4. In this equation, elution volume is used instead of retention time; the two are related by the eluant flow rate.

$$\log \frac{V_r - V_m}{V_m} = \frac{A}{A+E} \log R + \log \text{Const.} \qquad [1]$$

V_r is the retention volume, V_m is the mobile phase volume (column void volume), A is the analyte ion's charge, E is the eluant ion's charge, R is the gradient ramp steepness in mM of eluant concentration per mL of eluant pumped, and log Const. is the Y intercept of the log/log plot. This equation predicts that an increase in gradient steepness will decrease the retention time of divalent ions more than monovalent ions. An advantage monovalent eluants have over divalent (or other polyvalent) eluants is that a given change in eluant concentration produces a greater change in analyte retention time. Compared to a monovalent eluant, during a gradient run, the concentration of a divalent eluant must be changed by a much larger amount to elute ions of widely differing retention.

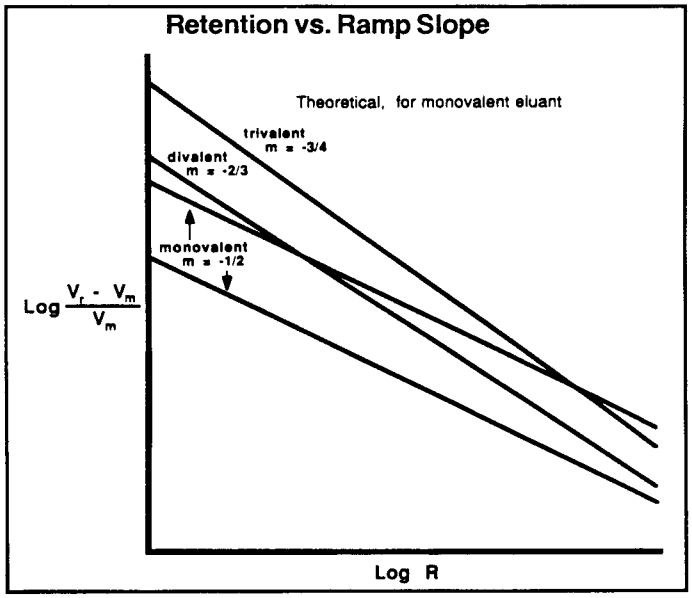

Figure 4

Can selectivity be changed by changing the gradient?

For ions of different charge, the elution order, and therefore the selectivity, can be changed by adjusting the gradient. For example, if two ions with different charge coelute, an increase in gradient steepness will separate the two, causing the ion of higher charge to elute first. In theory, one could obtain the optimum conditions for a gradient elution from a plot such as that shown in Figure 4. In practice, this is not straightforward because equation [1] assumes that a single linear gradient is used with the initial eluant concentration equal to zero. Faster gradient runs can be obtained by starting the eluant concentration at a higher value. Also, the best combination of speed and selectivity is often the result of combining several different gradients, often with isocratic sections and step changes. With more complicated gradient programs, the retention equation becomes unwieldy.

Equation [1] can be easily used to predict trends. This is demonstrated in Figure 5. In all three chromatograms, the initial eluant concentration is 2.5 mM p-cyanophenate. This concentration is increased between 2 and 7 min. to 18.5 mM in (a) (where sulfate and nitrate co-elute) to 22.5 mM in (b), resulting in baseline separation. In (c), the eluant concentration is increased as in (a) to 18.5 mM, but in 5 min. instead of 9 min. The effect is similar in that the divalent anions elute earlier, but because of the long period holding at 18.5 mM, the separation between the divalent anions is not compressed as in (b), but is instead similar to (a). The chromatogram in Figure 3 of anions on the HPIC-AS6 was developed with this method and uses a combination of step changes, linear gradients, and isocratic sections.

How can baseline slope be minimized?

When eluants other than sodium hydoxide are used, it is often necessary to minimize the baseline slope which results as the eluant concentration is increased. In anion exchange, this can be accomplished chemically by adding mannitol to the weak eluant and boric acid to the regenerant. Although the reaction between boric acid and mannitol is not well understood, they do combine to produce an acid considerably stronger than either of

the two by themselves. As a neutral polyalcohol, mannitol has little effect on either separation or detection. Boric acid in the dilute sulfuric acid regenerant is neutral, and can diffuse through the cation exchange membranes of the AMMS. The two then combine in the suppressor, producing a conductive species. By decreasing the mannitol concentration in approximately inverse proportion to the increase in eluant concentration, the baseline changes can be counteracted. This method is demonstrated in Figure 6, in which mannitol was used to balance the baseline in (b), but not in (a).

Another method of baseline compemsation is computer baseline subtraction. For this method, a blank run with no injection is stored in the computor's memory and subtracted point by point from succeding runs. This method works well as long as the baseline profile is reproducible.

How are contaminants in the eluant removed?

A problem encountered in gradient elution is the presence of extraneous peaks in the chromatogram caused by trace impurities in the eluant. In anion exchange, the most common impurities are chloride, sulfate, and carbonate. In the early part of a gradient run, while the eluant is weak, these ions are concentrated at the front of the separator. As the eluant strength increases, they are eventually eluted, and appear as interfering peaks at the expected retention times. Carbonate can be minimized by using properly deionized water and low carbonate sodium hydroxide. The effect of contaminants can be minimized by placing a small column, called the Anion Trap Column (ATC), in front of the injection valve. The ATC is filled with high capacity, low efficiency anion exchange resin. It successfully prevents trace anionic contaminants from reaching the separator column during the early part of the run. Later in the run, the eluant strength may be high enough to elute the contaminants. The low efficiency of the resin spreads out the contaminant peaks so that they do not interfere with the chromatography. A consequence of the use of an ATC is that if a composition gradient is used, the time needed to convert the form of the resin in the ATC must be considered.

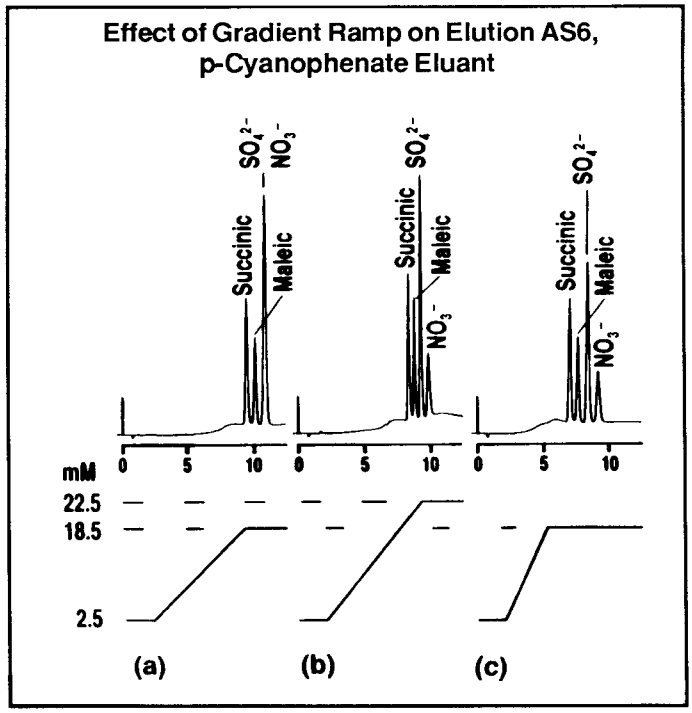

Figure 5

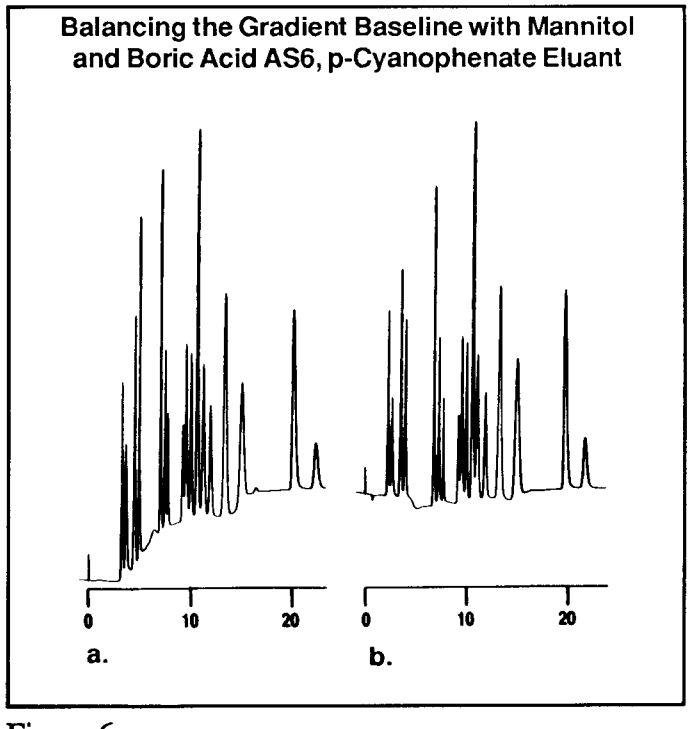

Figure 6

An alternate approach is to use a higher efficiency trap column which is regenerated at the end of each gradient run with a high concentration eluant, removing any accumulated ionic contaminants. The HPIC-AG8 guard column can perform as a trap column because of its high affinity for carbonate, the primary contaminant in sodium hydroxide eluants. An example of the use of an AG8 as a trap column is the separation of anions in coffee shown in Figure 7. For this chromatogram, carbonate and other contaminants on the trap column were removed with a strong borate eluant. The sample is then injected before the sodium hydroxide eluant has removed all of the borate from the trap column. The small amount of borate left forms a complex with quinate, increasing its retention from coelution with acetate to elution between glycolate and formate.

How are gradients used with MPIC?

Mobile Phase Ion Chromatography (MPIC) is a form of reversed phase ion pair chromatography in which chemically suppressed conductivity detection is used. As in reversed phase HPLC, gradient elution is accomplished by varying the percentage of organic solvent in the eluant during the run.

Although the ionic strength of the eluant may remain the same throughout the run, the background conductivity can decrease due to the changing dielectric constant. These baseline changes can be compensated either by chemical means or by computer baseline subtraction. Often, MPIC is used to elute ions which are very strongly retained. When this is the case, the baseline conductivity change can be minimized without affecting the separation by adding very weakly retained ions directly to the stronger eluant (higher percentage of organic solvent). Using gradient MPIC, the separation of five alkyl sulfonates and sulfates is shown in Figure 8. The AMMS-MPIC suppressor (P/N 37106) should be used.

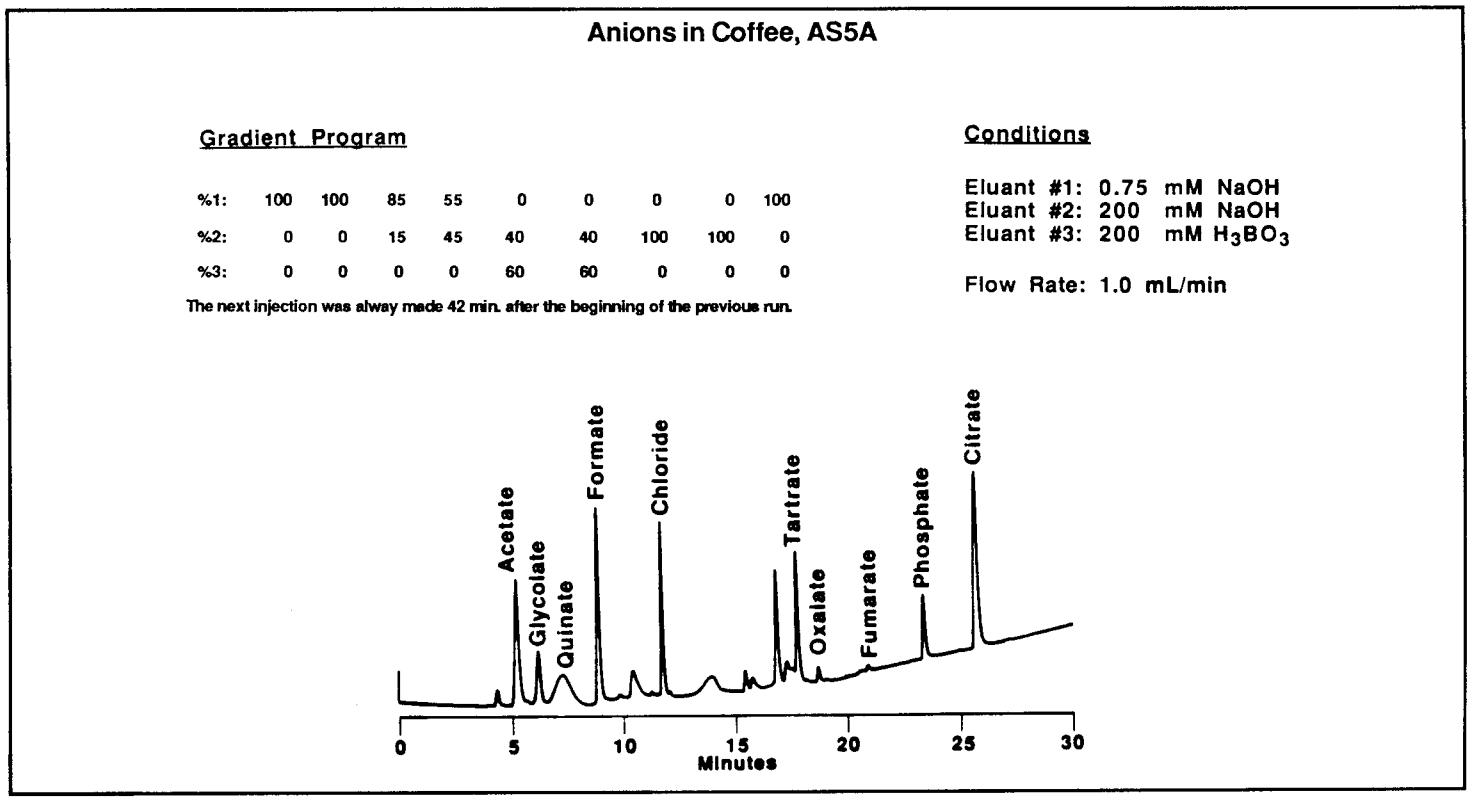

Figure 7

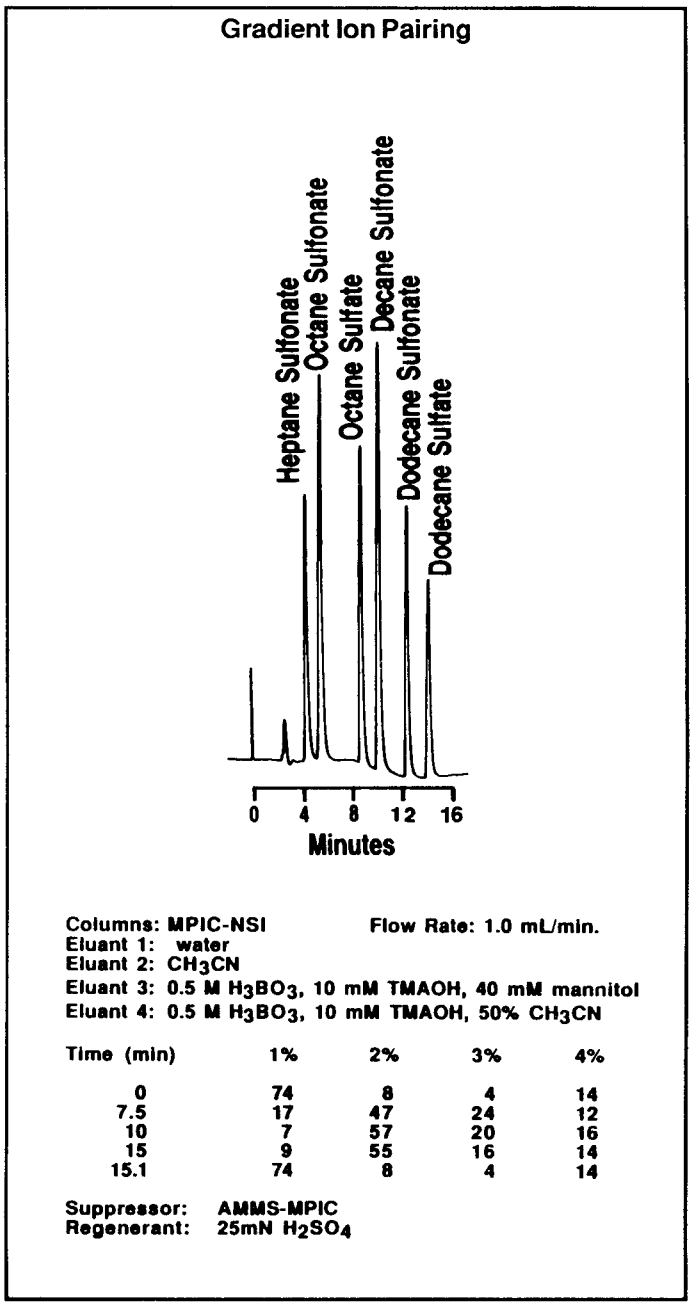

Figure 8

What conditions does Dionex recommend for gradient elution?

Although many columns and eluants can be used for gradient elution, the conditions used in Figure 2 are recommended as a good starting point. The HPIC-AS5A (5μ) separator with sodium hydroxide eluant provides the optimum combination of efficiency, selectivity, and speed without an unacceptable baseline slope. If fewer ions than the 36 shown in Figure 2 need to be separated, the gradient steepness can be increased to reduce the run time. Remember that changing the gradient will affect the elution order of ions of different charge. In particular, increasing the gradient ramp slope will cause sulfate and other divalent ions to elute earlier than nitrate. When the considerations discussed in this technical note are followed, gradient elution can be used to solve many chemical analysis problems that are difficult to solve using isocratic elution.

Ion chromatograph:	Dionex series 4000i or IC with GPM
Trap column:	ATC (P/N 37151) or HPIC-AG8 (P/N 37023)
Guard column:	HPIC-AG5A (P/N 37134)
Separator column:	HPIC-AS5A (P/N 37131)
Eluant #1:	0.75 mM sodium hydroxide
Eluant #2:	200 mM sodium hydroxide
Eluant flow rate:	1.0 mL/min
Suppressor:	Anion MicroMembrane, AMMS (P/N 38019)
Regenerant:	25 mN H_2SO_4
Regenerant flow rate:	10 mL/min