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HIGH PERFORMANCE CHELATION ION CHROMATOGRPHY: A NEW SEPARATION TECHNIQUE

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Abstract:

High performance Chelation Ion Chromatography (HPCIC) has been applied successfully to a number of transition and post-transition metals. This technique is based on the exploitation of the chelation effect of stationary phases in the presence of comparatively reduced electrostatic ion exchange interactions. The common ways to suppress ion exchange include increasing the ionic strength and pH of the eluent and the column temperature. The main features of HPCIC are the much greater flexibility in selectivity control compared to ion exchange and the relative insensitivity to high ionic strengths.

Key Words: Chelation ion chromatography, Chelating agents, Metal cations, Pre-concentration, γ-Aminohydroxamate resin.

Introduction:

High Performance Chelation Ion Chromatography is a technique which exploits complexation for the separation and detection of an analyte. HPCIC is an efficient chelation ion chromatography wherein the analyte forming complex with the stationary phase which is eluted by using mobile phase. Correct choice of chelating functionality is a critical factor for the efficient functioning of separating system.

To distinguish the use of high-efficiency chelating ion-exchange columns for analytical determinations from the more general use of low efficiency batch separation columns, called this technique "high performance chelation ion chromatography, HPCIC".

Unlike the majority of low-efficiency chelation exchangers that are used as preconcentration columns for batch separations, HPCIC can be used for high efficiency analytical separations of trace metals[1].

Principle:

The main principle of High Performance Chelation Ion Chromatography is Chelation. Some electrostatic interactions may also be responsible for separation. These can be reduced by increasing the ionic strength and based on the solubility and stability of complex formed, the analyte distributes between the stationary and mobile phase.

Factors influencing HPCIC:

A) Influence of temperature on the retention of metal cations and on the selectivity of separation:

Temperature has a significant effect on retention in chelation exchange.[2,3] Increasing the temperature of a chromatographic column can improve the selectivity of separation and increases the efficiency. The dependence of retention ($\ln k^1$) on the column's temp can be expressed by the following equation[4].

$$\ln k^1 = - \Delta H^0/RT + \Delta S^0/R + \ln\phi$$

Where k^1 = Capacity factor

ΔH= Enthalpy of sorption

ΔS^0 = entropy

ϕ = phase ratio.

B) Influence of Organic solvents**:**

Small addition of organic solvents to the eluent changes its dielectric constant and can influence secondary equilibria in the chromatographic system, such as solvation of metal ions and solubility of metal complexes in the mobile phase if organic complexing reagents are present in the eluent.

C) Ionic strength:

An increase in ionic strength will increase the ratio of chelation to ion exchange and at very high salt concentrations, ion exchange will become very small (or) insignificant. This relatively insensitive response to high ionic strength is the main property exploited for the preconcentration and batch separation of trace metals from complex matrices. Although the variation in the ratio of ion exchange to chelation exchange is not particularly important for these batch separation procedures, it is very important for HPCIC[5].

The most effective way to increase the ionic strength of the eluent is by adding common electrolytes. These salts should be pure and should have a good solubility in aqueous solution, low complexing ability towards analytes and should not produce any non-soluble products in the column. Alkali metals and ammonium salts such as nitrates, chlorides and perchlorates are suitable for this purpose[6].

D) Effect of eluent pH:

Effect of an increase in pH on retention will take place when any ion exchange sorption is "swamped" or "surpressed" by using a high ionic strength eluent. A steady increase in retention will occur over the whole pH range. The presence of a complexing acid in the eluent will still have an influence, but only to slow the rate of increase of retention[7]. For example chelating anion exchangers representing a majority of all known chelating sorbents (8-hydroxy quinoline, polyamines, hydrazones etc), the increase in the pH of the eluent means that retention occurs only through chelation on the surface.

Commonly used Stationary phases, Chelating ligands and mobile phases in HPCIC:

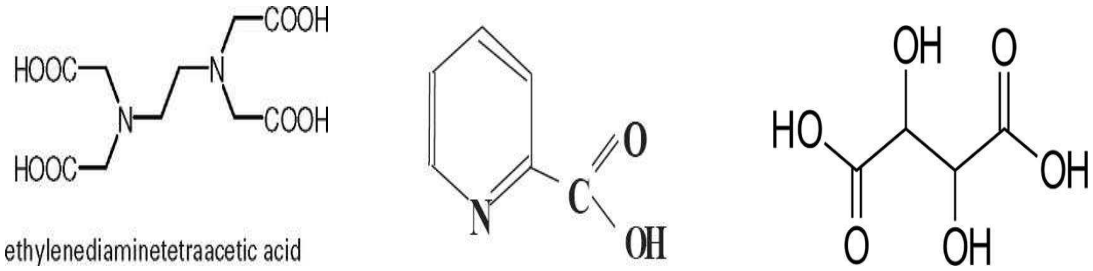
Impregnated (or) chemically modified supports such as silica, titanica, alumina, calcium carbonate are used as stationary phases.

Frequently used chelating ligands are carbamates, β-diketone diamines, iminodiacetate, aminoacids, azophenyl methane dyes and triphenyl methane dyes. Generally 2-3 dentate chelates provide better separation. Some other chelating ligands are 8-hydroxy quinoline bonded to silica gel, polysaccharide cross-linked glycide methacrylate gel, cross-linked co-polymer acrlonitrile, divinyl benzene (DVB), polystyrene-divinyl benzene (PS-DVB) resins.

Chelating ligands are immobilized either by simple adsorption or by covalent bonding onto a variety of substrates such as silica[8] and styrene-divinyl benzene resin[9-16] or anion exchangers[17], but the bulk of the chelating phases involve the covalent attachment of ligand either directly or via an organic spacer to the solid matrix. A series of silica-based sorbents with different surface concentrations of bonded 8-hydroxyquinoline appear to be the most effective in resolution and speed of separation[18].

Stationary phases produced by impregnating chelating dye stuffs can be used both for separation and detection.

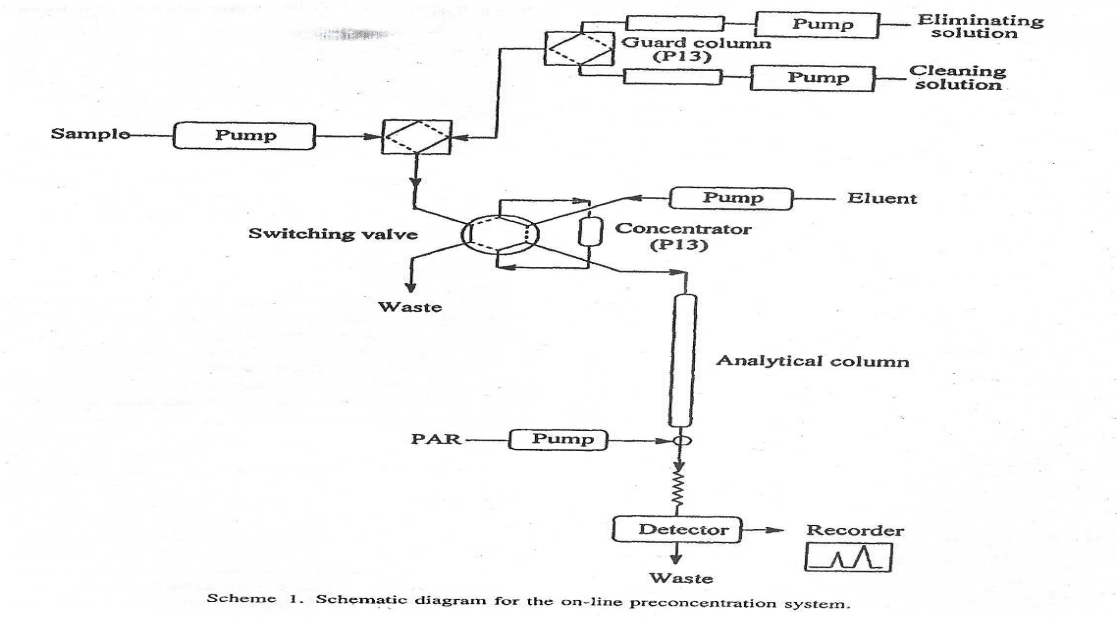
Mobile phases used in HPCIC are tartaric acid, mandilic acid, oxalic acid, lactic acid, picolinic acid, dipicolinic acid, methanol, EDTA, tartrate buffer, borate buffer.



Picolinic acid Tartaric acid

Fig 1: Some commonly used mobile phases in HPCIC.

Instrumentation of HPCIC



Scheme 1. Schematic diagram for the on-line preconcentration system.

Components of high performance chelation ion chromatography include[19]:

1. Pumps
2. Solvent reservoir
3. Columns
4. Detectors
5. Recorder

1. Pumps:

The two major functions of the pump in HPCIC are, namely:

(i) To pass the mobile phase through the column at high pressure, and

(ii) At a constant controlled flow rate.

HPCIC makes use of two types of pumps. They are:

(i) Gradient Pump

(ii) Eluent Pump

In gradient elution technique, the polarity of the solvent is gradually increased and hence the solvent composition has to be changed. Hence these pumps are used when two or more solvent pumps are used for such separations.

2**.** Solvent reservoir:

Solvent reservoir consists of a glass bottle having a lid and polytetrafluoroethylene tube to pass the mobile phase from the reservoir to the pump. Any liquid entering the pump should be free from dust and particulate matter, otherwise these foreign substances may give rise to irregular pumping action, damage valves, irregular behaviour of column owing to its contamination and ultimate blockade of column.

3. Columns:

Column plays an important role in HPCIC which decides the separation efficiency.

Size: 20-300nm length

1-7.5mm diameter

Particle size: 5-20um

Columns used are long capillary and available in packed manner called monolithic or packed columns.

Columns used in HPCIC are:

(i) Pre-concentration column

(ii) Isolation column

(iii) Analytical column

Fig 2: JPP Chromatography HPCIC Column.

HPCIC columns are packed with high quality spherical silica particles chemically bonded with metal chelating groups. To ensure optimum efficiency throughout the life of the column the following points should be noted.

1. All eluents should be filtered to 0.45 µm or lower using the appropriate filter system.

2. As the stationary phase is silica based, alkaline eluents should not be used under any circumstances, even for short periods. It is recommended that eluent pH should kept within the range 1 to 6.

3. After a period of time, depending on the type of eluent using, the column can become partially saturated with strongly adsorbed metals such as copper and iron. By this there will be a significant shortening of the retention times. It depends not only on the type of eluent, but also on the quality of the components used in making up the eluent. If this problem occurs, a column clean-up procedure should be adopted.

To carry out the column clean-up, pump 10mM dipicolinic acid in water through the column for an hour at a flow rate between 0.8 and 1ml per minute. After this, switch back to normal eluent. If there is a large difference in pH between the dipicolinic acid solution and the eluent it can take a while for the column to re-equilibrate, for one or

two hours. Then check when equilibration is re-established by injecting some analyte standards until retention times stabilise.

4. Detectors:

Detection is done by using chemical reagents like metallochromic ligands, fluorescent reagents, chemiluminescent reagents.

Ex: Arsenazo-I,III, 4-(2-pyridylazo resorcinol), elemental analysers.

HPCIC includes UV detector and variable wave length detectors[20.]

UV detector**:**

This detector is based on the principle of absorption of UV visible light from the effluent emerging out of the column passed through a photo cell placed in the radiation beam.

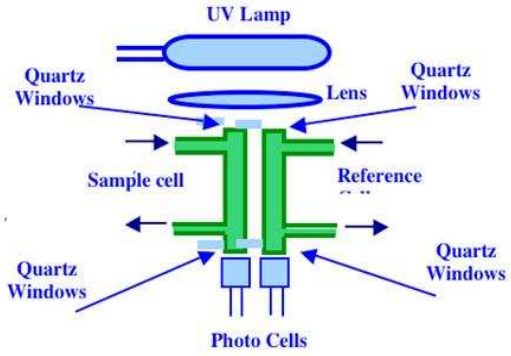


Fig 3: UV detector

Variable wave length detector:

This type of detectors spread over wide range between 210-800nm capable of performing more selective detection possible.

Fig 4: Variable Wave length detector

5. Recorders and Integrators:

Recorders are used to record the responses obtained from detectors after amplification, if necessary. They record the base line and all the peaks obtained with respect to time. Retention time for all the peaks can be found out from such readings, but the area of individual peaks cannot be known.

Integrators are improved versions of recorders with some data processing capabilities. They can record the individual peaks with retention time, height and width of peaks, peak area, percentage of area, etc. Integrators provide more information on peaks than recorders.

Advantages:

HPCIC includes the following merits:

(i) Reduction in analysis time.

(ii) Ability to determine ionic analyses in trace levels.

(iii) It has been used for preconcentration and batch separation of metal cations in complex matrices.

(iv) It is used for the trace analysis of complex samples such as sea water, saturated brines etc.

(v) It is used for the separation and determination of alkaline-earth and transition metal ions[21,22].

Disadvantages:

HPCIC includes the following demerits:

(i) Degraded detection limit and accuracy.

(ii) Kinetics of chelation exchange were found to be slower than those of ion exchange.

(iii) The chelation-ion exchange column is used as pre-concentration column only but it is not involved in the analytical separation of metal ions.

(iv) Increase in the temperature of the eluent does not produce a significant change in the selectivity of separation.

(v) It requires gradient elution to avoid long retention times[23].

APPLICATIONS OF HPCIC

The range of applications of chelating exchangers used for the separation and determination of alkaline-earth and transition metal ions by HPCIC include:

1. γ-Aminobutyrohydroxamate resin or its derivative, N-methyl-γ-aminobutyrohydroxamate in a column is used to concentrate and separate lanthanides from the alkali and alkaline-earth metals and other matrix components[19].
2. Determination of actinides in environmental and biological samples using HPCIC coupled to sector-field inductively coupled plasma mass spectrometry[24].
3. Isocratic separation of lanthanides and yttrium by high performance chelation ion chromatography on iminodiacetic acid bonded to silica[25].
4. HPCIC using high efficiency chelating substrates with rapid exchange kinetics will produce fast analytical separations.
5. The special nature of HPCIC and its ability to analyze very concentrated samples, it is the determination of alkaline-earth metals in saturated brines.
6. Silica is usually used for the attachment of chelating groups as acidic conditions are mainly used for the separation of transition metal ions to avoid hydrolysis.
7. Chelation ion chromatography is a technique that combines selective chelation concentration with analytical separation and selective detection.
8. Chelation ion chromatography allows aluminium to be preconcentrated from matrices which is high in salts, acids, (or) bases and selective concentration is eluted to a conventional cation exchange columnfor separation from other transition metals.
9. The determination of selected transition metals in tomato leaves and vitamin tablets showed good aggrement with certified values.

10. New developments in PCR detection have also occurred and when combined with these HPCIC columns can give loss on drying for some metals rivailing those of the most sensitive AAS techniques[26] **.**

Summary and Conclusions

Chromatographers have used chelating or complexing reagents only as additives in the eluent to increase the speed and selectivity of the separation of metal ions in ion chromatography. To achieve efficient and selective separation using immobilized chelating ligands has met the problem of obtaining a "pure" chelating effect. Based on the correct realization of the chelating and ion exchange properties of substrates through optimization of ionic strength, temperature and pH of the eluent, allows not only to solve this problem, but also to achieve some unique selectivities in the preconcentration separation of trace metal ions and to perform difficult analyses of samples having complex matrices.

FUTURE DIRECTIONS

The existing HPCIC instrument has more benefits by developing methods[26].

Methods for improving Signal/Noise (S/N) ratios:

The detection limit:

The Limit of detection (LOD) is a very important parameter in any quantitative trace analytical method. The most common way of obtaining an LOD, which is usually defined in chromatography as an analyte signal twice the peak-to-peak noise level of a continuously monitored base line, relies on S/N calculations. Since most modern instrumental techniques require conversion of detector responses to electrical signals, noise is normally revealed as random voltage (or) current fluctuations in the base line before and after an analyte passes through the detector. The lowest concentration at which one can assert with a certain degree of confidence that a particular analyte is present in the sample, i.e., a "definite response" above the noise, is the LOD.

Characterization of base line noise:

Baseline noise is divided into three categories, namely,

1.Short term noise:

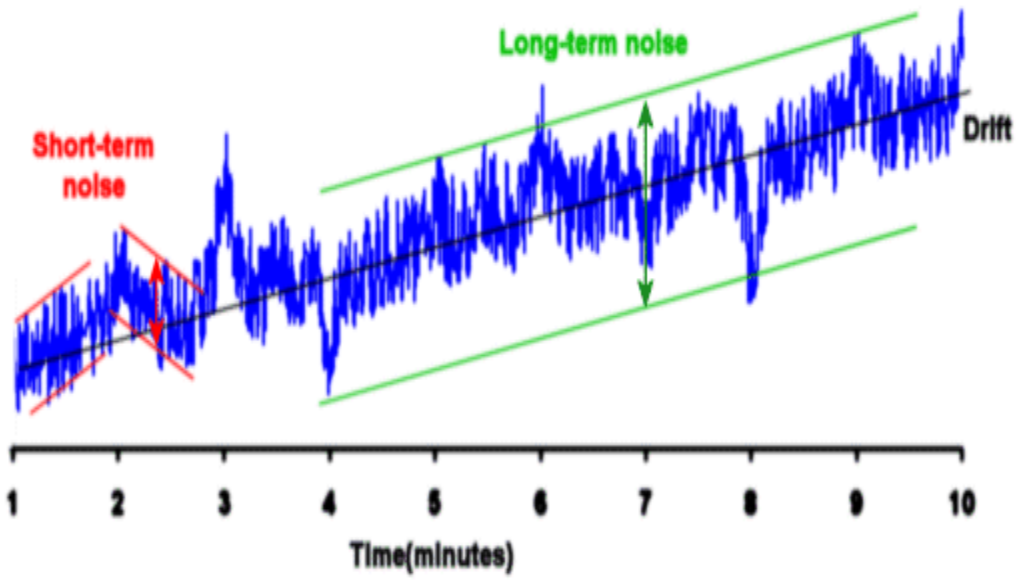
It is generated by relatively fast, fairly even fluctuations in the base line where the time between fluctuations is much shorter than the analyte peak base width. Short-term noise, having a time constant measured in seconds or less.

2.Long term noise:

It develops from slower random variations in the back ground, where the time between the major fluctuations is of the same order as the base width of the analyte peak. This type of noise is also referred to as base line wandering. Long-term noise, having a time constant ranging from a few seconds to a few minutes.

3.Drift:

A slow unidirectional change in the base line, which is more (or) less linear, though can be curved. It is a steady movement of the baseline, having a time constant from several minutes to hours.



Pulse dampeners:

The construction of dampeners depends on the level of pressure at which pulsations take place. HPLC dampeners are incorporated in line between the pump and the injector. The energy of the pump pulses is absorbed

and released out of phase with the frequency of the pump strokes, thus reducing (or) dampening the amplitude of the pulses.

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