ION SELECTIVE ELECTRODES IN ION CHROMATOGRAPHY

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ABSTRACT

The number of applications of potentiometric detection in ion chromatography is increasing in the use of ion selective electrodes for which the response is not limited to a few number of ions.

In this research, membrane electrodes, for a number of ions, based on PVC were prepared to examine selectivity, detection limit and reproducibility for chromatographic and flow-injection measurements via mixed solution method and flow-injection technique. The selectivity sequence of anion selective electrodes for single charged inorganic anions was $F^- < Cl^- < Br^- < NO_2^- < NO_3^- < I^-$. This was $Li^+ < NH_4^+ < Cs^+ < Na^+ < K^+$ for cation selective electrodes. The detection limits for most of anions and cations were found to be at the nanogram or picogram levels.

Response times of bromide and chloride electrodes were established to concentration and flow-rate changes in a flowing stream. When flow-rate was increased, response time of electrode decreased, but response volume increased. Response time was decreased for low activities rather than high activities of solute ion when flow-rate increased. At high flow-rates, response time was independent of the activity of the solute ion. Also a new approach to the definition of the response time in flowing conditions was purposed in order to be able to indicate their performances.

An evaluation of the influence and contribution of the sample dispersion on the sensitivity of tubular liquid membrane bromide selective electrode based on PVC was examined with a new, easy reliable approach in a flow-injection system for chromatographic measurements. Using water as carrier, it was observed that the dispersion influence was completely dependent on the response time of electrodes. When sample in the carrier passes through the electrode surface just within the electrode response time, better peak shape and sensitivity were obtained at each flow-rate, whilst shorter remaining time of sample caused tailing and decreasing of the sensitivity, longer remaining time caused peak broadening.

An all solid-state tubular PVC-matrix membrane electrode as detector in non-suppressed ion chromatography was employed for detection of some monovalent common anions including I^- and SCN^- at sub-ppb levels. Non-suppressed separation of halides and strongly retained anions I^- and SCN^- in one run was achieved using Phosphate solution as a new efficient eluent over Dionex IonPac-AS4A and AG4A columns. Potentiometric detection of eleven monovalent inorganic and organic anions with the use of all solid-state contact tubular membrane electrode (cell volume 2 µl) as detector in non-suppressed ion chromatography was demonstrated using phosphate and hydrogen phosphate as eluents at low concentrations. Theoretical and practical considerations were discussed, and in particular, sensitivity, linearity, detection limit and dynamic behaviour were presented. Applications to river, drinking and rain water samples were described.

In any liquid chromatographic technique, the eluent composition provides greatest flexibility for manipulating the retention of solutes in order to achieve a desired separation. Utilizations of a new composition of the HCO_3^-/CO_3^{-2} buffer solution or phosphate solution as eluents were demonstrated for the separation of twelve inorganic and organic anions with good resolution in six minutes using Dionex-100 ion chromatographic system.

A simple and selective method for single ion chromatographic

separation (in seven minutes) and potentiometric detection (at sub-ppb levels) of Na^+, NH_4^+, K^+, Rb^+, TMA^+, Cs^+ and Tl^+ cations was established using copper and magnesium salts as eluents, with Dionex IonPac-CS3 analytical and guard columns and all solid-state contact tubular membrane potassium selective electrode as detector. The application of the method for drinking, river, spring, sea water samples and orange juice, urine and saliva samples were illustrated. As the detector was highly selective and sensitive to only monovalent cations, no interference from other cations, the method was easily applied to many sample types examples including the determination of Na^+ and K^+, on the surface of the glassware adsorbed during the fabrication stage, and in many inorganic and organic chemicals were given.

It might be the over-all efficiency of ion chromatographic procedures that allows the routine separation and detection of inorganic and organic anions and cations at low levels in a simultaneous system. A simple, selective, sensitive reproducible and rapid method needing only 8 minutes or less to complete a simultaneous potentiometric detection and independent separation of a group of fourteen inorganic and organic monovalent common anions and cations was developed using copper or magnesium sulphate salt as eluents with Dionex IonPac-AS4A and -CS3 anion and cation-exchange columns in tandem. The only difference of the method from other simple chromatographic applications was just one more chromatographic column and one more potentiometric detector. The method was flexible, as the anions will not interfere with the detection of cations or cations will not interfere with the detection of anions, that detectors can be used at the end of the two column in tandem or one can be used after first column. The first column can be anion or cation-exchange.

A method for simultaneous determination of sodium, potassium and chloride in bovine serum albumin plasma was established. It involved independent ion chromatographic separation on anion and cation- exchange columns and simultaneous potentiometric detection by anion and cation selective electrodes. The concentrations of monovalent cations and chloride was increased with the increasing of dilution of non-filtered sample, there was no significant changes in the concentrations determined in the sample which was filtered.

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CHAPTER 1

1. SURVEY OF PREVIOUS INVESTIGATIONS

1.1 INTRODUCTION

Efficiency and quality in analytical laboratories and industrial processes involves chemical analysis. The ever present need for reliable and less time consuming methods of chemical analysis has stimulated rapid advances in the field of chromatography. Efforts on the development of efficient stationary phases and more sensitive methods for detection of solute ions, have enabled the determination of ion content in many sample matrices in industry, analytical laboratory, clinical environment, etc.

In 1975, since the introduction of ion chromatography by Small,[1] ion chromatographic systems incorporating not only conductivity detection, but also spectrophotometric, amperometric, voltammetric, and potentiometric detection, have been used.

The conductivity detection is the most widely used detection method for ion chromatographic applications as problems with sensitivity and reliability have hindered extensive use of other detection methods. The applicability of a limited number of eluents and the expense of the suppressor columns have created interest in other means of detection. Consequently, many inorganic and organic ions have been conveniently monitored by potentiometry using a variety of ISEs.[11-77]

In parallel with the development of ion chromatography, there has been the development of the technology of ISE fabrication, resulting in micro construction, reliable, high performance ISEs manufactured at low cost.

High selectivity and availability of suitable designs of ISE flow cells to minimize dead space and carry-over of sample solutions, allowing more rapid sample throughput, have led the ISEs to extensive use in flow injection analysis.[2]

When ISEs are coupled with an ion chromatographic separation technique, the resulting high selectivity response to a limited

number of ions is the main disadvantage of ISEs as detectors. Nevertheless, some of these electrodes, however, have more general response and have been used as detectors in ion chromatographic systems. Therefore, the number of applications of potentiometric detection in ion chromatography is increasing with the use of ISEs of which the response is not limited to a small number of ions.
Manz et al.[3] compiled a literature with 25 references in 1985. Since then, no extensive study has been made to bring together the literature on the use of potentiometric detection with ISEs in liquid chromatography, There are, however, some general reviews,[4-8] on the use of electrochemical detection in ion chromatography and on the applications of ISEs, which have partly included the use of ISEs as detectors in ion chromatography.
Also, recently, Tarter[9] and Haddad[10] have written books in which potentiometric detection in ion chromatography is discussed in one chapter.
This review chapter is an attempt to bring together work in which ISEs have been coupled with ion chromatography techniques as detectors. For convenience, some important applications of ISEs are briefly discussed. Also a comprehensive list of applications of ISEs, according to membrane type, and applied to chromatographic technique, is given in table 1.

1.2 APPLICATIONS OF ISEs IN LIQUID CHROMATOGRAPHY

Interest in the use of ISEs as detectors in liquid chromatography has been gradually increasing since the use of a miniaturized silver/silver chloride electrode for direct potentiometric detection of halide ions in the presence of other inorganic anions.[12]
Solid-state membrane electrodes have some advantages over other membrane types, such as fast response, long-life time, and also the possibility of use in organic solvents. Hence, many types of solutes have been monitored using solid-state type electrodes as detectors in ion chromatographic techniques.
Halide electrodes based on mixtures of Ag_2S or sparingly soluble silver halides have been used for sensitive and selective

detection of halides[39] and iodide, thiosulphate and thiocyanate.[40] Hershcovitz et al.[29] carried out indirect detection of halides and thiocyanate using a silver wire electrode coated with silver salicylate. It is reported that the detector was more sensitive than a conductometric detector for ion chromatography of halides and thiocyanate. In fact, gradient elution in ion chromatography has been found to be quite problematic when conductivity or spectrophotometric detection is employed. A silver/silver chloride electrode was reported to be suitable for programming the eluent gradient run, because of little or no response to ions in eluent, for efficient separation and detection of halide ions and pseudohalides.[53]

Loscombe et al.[18] and Wada et al.[52] used copper selective membrane electrodes for potentiometric detection of amino acids. Detection was achieved by measuring the drop in the free copper ion concentration resulting from the complexation, in methanol-water mixture used as eluent, between amino acids and added copper ion solution.

Dorey[23] who suggested a cupric ion selective membrane electrode which was sensitive to many rare earth metal cations, used copper(II) ethylenediaminetetraacetic acid as post-column reagent. Metal ions in the eluent displaced copper(II) from the post-column reagent, the increase of free copper(II) was then measured by the electrode. No chromatographic data were reported.

Commercial bromide and fluoride selective electrodes, with limited applicability to one ion at a time, have been used at high pumping speed to maintain fast determinations of anions. Up to 18 samples per hour were reported to be analyzed for chloride, nitrate and sulfate.[27]

Haddad, Alexander and Trojanowicz described the use of metallic copper electrode based on active copper surface, for the measurement of many types of solutes. Potentiometric detection with metallic copper electrode is versatile, and based on change in the concentration of cuprous or cupric ions at the electrode surface, or oxidation or reduction of electrode surface by solutes providing a potential which is measured by metallic copper

electrode.[56] Using this phenomenon, many inorganic anions,[45] alkaline earth metals,[42] amino acids,[26] carboxylic acids,[56] transition metal ions,[44,47] ascorbate, hydrazine, hydroxylamine[51] and free nitriloacetic acid[60] have been successfully monitored by ion chromatography with a metallic copper electrode as the detector.

Liquid membrane electrodes are less selective if compared to counter parts, and hence could be more suited for more general potentiometric detection of ions in ion chromatography. Schultz and Mathis[13] first described the use of a commercial liquid membrane nitrate anion selective electrode to determine nitrite, nitrate and various phthalate ion isomers by ion chromatography. A commercial liquid membrane chloride anion selective electrode with a micro membrane cell has been used in gel permeation chromatography for potentiometric detection of fluoride, sulfate, acetate, citrate, iodide and thiocyanate.[16] The detector cell was divided into two compartments by an ion-exchange membrane column. Eluate passes through one side of the cell and pure eluent through the other side. The different compositions of the two solutions bring about a change in the membrane potential which is used to detect the ionic species.

Dielder et al.[25] reported a flow-through cell with a non-selective ion-exchange liquid membrane electrode for potentiometric detection of anions and cations in ion chromatography, but the electrode suffered from poor sensitivity.

A platinum wire electrode coated with PVC, employing a mixture of neutral carrier ligands, has been used for potentiometric detection of alkali cations and NH_4^+.[34] With this type of arrangement, detection limits varied with the selectivity of each neutral carrier ligand toward the individual ions, possibly due to the response of the electrode to the components present in the eluent exhibited poor sensitivity.

Perchlorate, nitrate and p-toluenesulphonate anion selective electrodes based on an olephilic anion-exchange resin have been described by Ishibashi et al.[36] for use in ion chromatography as detectors. Electrodes were reported to have a life time up to one

year without significant loss in performances. Koizumi et al.[57] also reported the use of anion selective electrodes based on an olephilic anion-exchange resin membrane as detector in ion chromatography of oxyanions, such as, IO_3^-, BrO_3^-, ClO_3^- and NO_3^-.

A PVC tubular membrane electrode has been successfully used for the determination of Cl^-, Br^-, NO_2^- and NO_3^- in environmental samples by ion chromatography.[62]

A non-selective membrane electrode based on conducting polymers has been developed by Campanella et al.[58] for nonspecific potentiometric detection of some anions in ion chromatography.

An interesting application of a highly selective liquid membrane electrode is described by Trojanowicz and Meyerhoff[65] who used a wall-jet type valinomycin-based potassium selective electrode for detection of anions and cations in replacement ion chromatography. The electrode in replacement-type detection arrangement is reported to have the capability of sensitive and selective determination of a wide range of ions, and to be a rival of conventional conductivity detection of separated ions. Trojanowicz and Meyerhoff[63] also determined anions and cations using flow-through wall-jet type polymeric pH electrodes in suppressed ion chromatography.

Recently, Kolycheva and Muller[73] reported the use a solid contact flow-through calcium selective electrode for selective potentiometric detection of trace calcium in the presence of other alkali metals and magnesium ions. Glass electrodes have also been used as detectors in ion chromatography. Egashira[20] described a method for the indirect detection of carboxylic acids with a glass membrane pH electrode by monitoring small changes in the pH of post-column reagent buffer continuously added to the eluent.

Georges and Khalil[49] proposed the use of a capillary flow-through glass pH electrode as detector for measurement of pH shifts caused by cation exchange phenomena in ion chromatography.

Nomura et al.[59,60] successfully used alkali metal-free phosphate glasses containing silver oxide as sensing materials for the direct detection of various anions[59] and determination of iodide in sea water.[60] The preparation of glasses is reported to be by

replacement of aluminium in the sodium aluminium silicate glasses by phosphorus.

Since the change of potential at the surface of electrode in potentiometric cells does not depend on the surface area of the electrode membrane, very small volume detectors and microelectrodes can be designed for use at the end of the separation column.

Simon et al.[33,35,46,54,72] described liquid membrane microelectrodes as detectors of extremely small dead volume in open tubular column liquid chromatography. A micro-liquid membrane electrode has been used for potentiometric detection of alkali metals and quaternary ammonium compounds as they are separated on capillary columns.[33]

A liquid membrane ion selective microelectrode with a tip diameter of about 1 μm has been applied to the detection of iodide at levels about 7.6×10^{-16} g by bringing the electrode tip into contact with the eluent stream at the column end.[54]

Recently, detection of ca. 10^{-8} mol/l concentrations of alkali and alkaline earth metals has been carried out using a potentiometric microelectrode based on an end-column detector in capillary zone electrophoresis.[72]

Ion chromatography requires detectors with minimal dead volumes and suitable geometry of the flow channels. Therefore, various flow-through cells and miniaturized electrodes have been designed suitable for obtaining lower detection limits. Apart from microelectrodes, so far a cell with detection volume about 0.3 μl has been reported.[31] Watanabe et al.[71] applied an anion sensitive field effect transistor as a detector for ion chromatography of alkali metal cations. Sodium and potassium cations in human serum have been determined using a miniature cation-exchange column. A micro detection cell arrangement made 1 μl sample injections possible.

1.3 AIMS OF WORK

Experiments with ISEs and HPLC apparatus were carried out in this research with the aims of:

i. developing a range of ISEs for a number of monovalent ions using PVC-matrix electroactive membranes
ii. evaluating performances of ISEs by means of calibration, interference and reproducibility studies
iii. assessing the general applicability of the electrodes for detection of ions in flowing streams
iv. establishing response times of ISEs to concentration and flow-rate changes under flowing conditions that could be used as indicative of their performances
v. evaluating the contribution and influence of the sample dispersion on the sensitivity of electrodes
vi. employing ISEs as detectors in chromatographic analysis of ions vii. establishing efficient separations of inorganic and organic monovalent anions on commercially available columns used in ion chromatography by developing suitable eluents which allow sensitive determinations with ISEs as detectors
viii. determining monovalent anions in various sample matrices
ix. establishing simple and sensitive separation and detection methods for monovalent cations using suitable eluents in ion chromatography with ISEs
x. determining monovalent cations in various sample matrices
xi. developing simultaneous and independent detection and separation methods for monovalent inorganic and organic anions and cations using an electrode array as detectors in ion chromatography
xii. employing the method to analyse various sample matrices
Overall the aim was a practical and critical assessment of ISEs as detectors in ion chromatography of anions and cations in order to establish the advantages and disadvantages of a potentiometric detection system with ISEs over existing detection systems and separation systems.

Table 1. a comprehensive survey of potentiometric detectors used in liquid chromatography

(a)

type of membrane	chromatogr. method	eluent	solutes detected	refs
liquid	ion-exchange	HCl	Na ,K	11
"	"	$KH_2PO_4+Na_2SO_4$	NO_3^-,NO_2^- and phthalate isomers	13
"	"	NaCl	inorganic and organic anions	16
"	ion-exchange and RP-HPIC	NaCl, phosphate buffer and 1-dodecenesulphate	cations and anions	25
"	ion-exchange	———	anions	31
"		HNO_3	monovalent cations	34
"	"	Na_2SO_4	ClO_4^-,NO_3^-	36
"	"	CH_3COONa	anions	37
"	"	Na_2SO_4	oxyacid anions	57
"	"	$CO_3^{=}/HCO_3^-$	anions	58
"	"	———	anions	62
"	suppressed ion-exchange	HNO_3 and NaOH	anions and cations	67
"	capillary zone electrophoresis	Na-acetate	alkali and alkali earth metals	72
"	ion-exchange	ethylenediamine chloride($EDAH_2Cl_2$	alkali and alkali earth metals	73
"	"	———	Ca^{2+}	74

(b)

type of membrane	chromatogr. method	eluent	solutes detected	refs
solid state	ion-exchange	acetate	halides	12
"	"	———	halides and SCN^-	15
"	"	———	Cu^{2+}	17
"	RP-HPIC	water-methanol containing KNO_3	amino acids	18
"	ion-exchange	———	Cl^-	19
"	"	$NaNO_3$	Cl^-,Br^-	21
"	"	———	halogenides	22
"	"	CuEDTA	transition and rare earth metals	23
"	"	———	F^-	24
"	RP-HPIC	NaH_2PO_4+NaOH+ formaldehyde	amino acids	26
"	suppressed ion-exchange	$CO_3^=/HCO_3^-$	anions	27
"	ion-exchange	salicylic acid	anions	29
"	"	$NaNO_3$	halides	30
"	RP-HPIC	TBA-SO_4+H_2SO_4	Cl^-,SCN^-	32
"	ion-exchange	diacetic acid	anions	38
"	"	H-phthalate	anions	39
"	"	$HPO_4^{-2}/H_2PO_4^-$	anions	40
"	"	phosphate,citrate and phthalate	aliphatic and aromatic acids	41
"	"	tartrate and di-ethylenetriamine	Ca^{+2},Sr^{+2},Ba^+	42
"	"	KNO_3,phthalate and salicylate	halogenides, SCN^-	43
"	"	citric acid,EDTA, EDA and TETA	divalent cations	44

(b) continued

"	"	tartrate and orthophosphate	anions	45
"	"	citric, tartaric and oxalic acid	transition metals	47
"	"	tartrate and orthophosphate	anions	48
"	"	salicylic acid	anions	50
"	"	tartrate, citrate and EDA	reducing species	51
"	"	ethanol-water	aminoacids	52
"	"	Na-perchlorate	halides and pseudohalides	53
"	"	H-phthalate and orthophosphoric acid	carboxylic acids	56
"	suppressed ion-exchange	$CO_3^=/HCO_3^-$	anions	61
"	replacement ion-exchange	perchlorate and HNO_3	anions and cations	66
"	suppressed ion-exchange	$CO_3^=/HCO_3^-$	F^-, PO_4^{-3}	68
"	ion-exchange	HNO_3	nitriloaceticac.	70
"	RP-HPIC	TBA-Cl	metal complexes	75
"	ion-exchange	perchlorate and phthalate	anions	76
"	"	camphorosulphonic acid	aliphatic acids	77

(c)

type of membrane	chromatogr. method	eluent	solutes detected	refs
micro electr.	open tubular ion chromatogr.	NH_4NO_3	alkali metals	33
"	"	NaCl and NaOCl	NO_3,SCN	35
"	"	NH_4NO_3	anions and cations	46
"	"	LiOAc	Li^+,Na^+,K^+	54
glass electr.	ion-exchange	KCl+KOH	aliphatic acids	20
"	suppressed ion-exchange	$CO_3^=$ and OH^- gradient	anions	55
"	ion-exchange	tartrate and citrate	anions	59
"	"	citrate and	anions	60
"	"	TBA-OH	anions	69
capillary glass el.	——	water	perchlorate	49
ISFET	ion-exchange	HNO_3	monovalent cations	71

1.4 REFERENCES

1. Small H., Stevens T.S and Bauman W.C., *Anal. Chem.*, 1975, 47, 1801.

2. Arnold M.E. and Meyerhoff M.E., *Anal. Chem.*, 1984, 56, 20R.

3. Manz A., Frobe Z. and Simon W., "*Microcolumn Separations*", Novotony M. and Ishii D. Eds., Elsevier, Amsterdam, 1985, p 297.

4. White P.C., *Analyst*, 1984, 109, 677.

5. Haddad P.R., *Chromatographia*, 1987, 24, 217.

6. Horvai G. and Pungor E., *Critical Reviews in Anal. Chem.*, 1989, 21, 1.

7. Koryta J., *Anal. Chim. Acta*, 1990, 233, 1.

8. Rocklin R.D., *J. Chromatogr.*, 1991, 546, 175.

9. Haddad P.R., "*Ion Chromatography*", Tarter J.G. Ed., Dekker, New York, 1987.

10. Haddad P.R. and Jackson P.E., "*Ion Chromatography*", Elsevier, Amsterdam, 1990.

11. Spencer H.G. and Lindstrom F., *Anal. Chim. Acta*, 1962, 27, 573.

12. Franks M.C. and Pullen D.L., *Analyst*, 1974, 99, 573.

13. Schultz F.A. and Mathis D.E., *Anal. Chem.*, 1974, 46, 2253.

14. Campanalla L., Angelis G.De, Gozzi D. and Ferri T., *Analyst*, 1977, 102, 723.

15. Bardvin V.V., Ivanov Yu M. and Shartukov O.F., *Zh. Anal. Khim.*, 1978, 33, 1732.

16. Deguchi T., Kuma T. and Nagai H., *J. Chromatogr.*, 1978, 152, 349.

17. Ishibashi N. and Jyo A., *Asahi Garasu Kogyo Gigutsu Shoreikai Kenkyu Hokoku*, 1978, 33, 47.

18. Loscombe C.R., Cox G.B. and Dalziel J.A.W., *J. Chromatogr.*, 1978, 166, 403.

19. Akaiwa H., Kawamoto H. and Hasegawa T., *Talanta*, 1979, 26, 1027

20. Egashira S., *J. Chromatogr.*, 1980, 202, 37.

21. Akaiwa H., Kawamoto H. and Hasegawa T., *Talanta*, 1980, 27, 909.

22. Midorikawa M., Patent, *Japan Kokai Tokyo Koho*, JP 8 031 931, 1980.

23. Dorey R.C., *Diss. Abs. Int.*, 1980, 41, 558.

24. Kokubu N., Hayasida Y., Kobayasi T. and Yamasaki A., *Denki Tsushin Daigaku Gakuho*, 1980, 31, 113.
25. Deelder R.S., Linnsen H.A.J., Koen J.G. and Beeren A.J.B., *J. Chromatogr.*, 1981, 203, 153.
26. Alexander P.W., Haddad P.R., Low G.K.C. and Maitra C., *J. Chromatogr.*, 1981, 209, 29.
27. Slanina J., Bakker F.P., Jongejan P.A.C., Van Lansen L. and Mois J.J., *Anal. Chim. Acta*, 1981, 130, 1.
28. Buchanan D.C. and Thoene J.G., *J. Liq. Chromatogr.*, 1981, 4, 1587.
29. Hershcivitz H., Yarnitzky C. and Schmuckler G., *J. Chromatogr.*, 1982, 252, 113.
30. Akaiwa H., Kawamoto H. and Osumi M., *Talanta*, 1982, 29, 689.
31. Suzuki K., Ishiwada H., Inoue H. and Shirai T., *Presented at Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy*, Atlantic City, NJ, 1982, Abstract no:332.
32. Jyo A., Mori K. and Ishibashi N., *Bull. Chem. Soc. Jpn.*, 1983, 56, 3507.
33. Manz A. and Simon W., *J. Chromatogr. Sci.*, 1983, 21, 326.
34. Suzuki K., Aruga H. and Shirai T., *Anal. Chem.*, 1983, 55, 2011.
35. Frobe Z., Richon K. and Simon W., *Chromatographia*, 1983, 17, 467.
36. Ishibashi N., Jyo A. and Imato T., *Anal. Chem. Symp. Ser.*, 1983, 17(Chem. Sens.), 570.
37. Ishibashi N., Jyo A. and Imato T., *Kagaku no Ryoiki Zokan*, 1983, 138, 107.
38. Suzuki K., Aruga H., Ishiwada H., Oshima T., Inoue H. and Shirai T., *Bunseki Kagaku*, 1983, 32, 585.
39. Wang W., Chen Y. and Wu M., *Analyst*, 1984, 109, 281.
40. Butler E.C.V. and Gershey R.M., *Anal. Chim. Acta*, 1984, 164, 153.
41. Haddad P.R., Alexander P.W. and Trojanowicz M., *J. Chromatogr.*, 1984, 315, 261.
42. Haddad P.R., Alexander P.W. and Trojanowicz M., *J. Chromatogr.*, 1984, 294, 397.
43. Muller H. and Scholz R., *4th Symposium on Ion Selective*

Electrodes, Matrafured, 1984, P:553, Ed. E. Pungor.

44. Alexander P.W., Haddad P.R. and Trojanowicz M., *Anal. Chim. Acta*, 1985, 177, 183.

45. Haddad P.R., Alexander P.W. and Trojanowicz M., *J. Chromatogr.*, 1985, 321, 363.

46. Manz A., Frobe Z. and Simon W., *J. Chromatogr.*, 1985, 30, 297.

47. Haddad P.R., Alexander P.W. and Trojanowicz M., *J. Chromatogr.*, 1985, 324, 319.

48. Alexander P.W., Haddad P.R. and Trojanowicz M., *Chromatographia*, 1985, 20, 179.

49. Georges J. and Khalil M., *Anal. Chim. Acta*, 1986,182, 281.

50. Andreis V.E., Kienzle A. and Schreck H., *Pharm. Ind.*, 1986, 58, 1063.

51. Haddad P.R., Alexander P.W. and Trojanowicz M., *J. Liq. Chromatogr.*, 1986, 9, 777.

52. Wada H., Ohtsuka C. and Nakagawa G., *Hagoya Kogyo Daigaku Gakuho*, 1986, 38, 113.

53. Lockridge J.E., Fortier N.E., Schmuckler G. and Fritz J.S., *Anal. Chim. Acta*, 1987, 192, 41.

54. Manz A. and Simon W., *Anal. Chem.*, 1987, 59, 74.

55. Shintani H. and Dasgupta P.K., *Anal. Chem.*, 1987, 59, 802.

56. Haddad P.R., Alexander P.W., Croft M.Y. and Hilton D.F., *Chromatographia*, 1987, 24, 487.

57. Koizumi S., Imato T. and Ishibashi N., *Anal. Sci.*, 1987, 3, 319.

58. Campanella L., Ferri T., Magone M., Mihic T., Russo M.V. and Salvi A.M., *Pap. Int. Conf. Ion Exch. Processes*, 1987, 315.

59. Nomura T. and Nakagawa G., *Bull. Chem. Soc. Jpn.*, 1987, 60, 2864.

60. Nomura T., Hikichi Y. and Nakagawa G., *Bull. Chem. Soc. Jpn.*, 1988, 61, 2995.

61. Keuken M.P., Slanina J., Jongejan P.A.C. and Bakker E.P., *J. Chromatogr.*, 1988, 439, 13.

62. Lizhu Z., Jinglan C. and Jinyao Y., *Fenxi Huaxue*, 1988, 735.

63. Trojanowicz M. and Meyerhoff M.E., *Anal Chem.*, 1989, 61, 787.

64. Yamaguchi T., *Kagaku to Kogyo (Tokyo)*, 1989, 42, 255.

65. Trojanowicz M. and Meyerhoff M.E., *Anal. Chim. Acta*, 1989, 222, 95.

66. Trojanowicz M., Pobozy E. and Meyerhoff M.E., *Anal.Chim. Acta*, 1989, 222, 109.

67. Trojanowicz M. and Meyerhoff M.E., *Z. Anal. Chem.*, 1989, 334, 691.

68. Talasek R.T., *J. Chromatogr.*, 1989, 465, 1.

69. Slais K., *J. Chromatogr.*, 1991, 540, 41.

70. Buchberger W., Haddad P.R. and Alexander P.W., *J. Chromatogr.*, 1991, 546, 311.

71. Watanabe K., Tohda K., Sugimoto H., Eitoku F., Inoue H. and Suzuki K., *J. Chromatogr.*, 1991, 566, 109.

72. Haber C., Silvestri I., Roosli S. and Simon W., *Chimia*, 1991, 45, 117.

73. Kolycheva N. and Muller H., *Anal. Chim. Acta*, 1991, 242, 65.

74. Kolycheva N. and Muller H., *Russ. J. Anal. Chem.*, 1991, 46, 1626.

75. Buchberger W., Haddad P.R. and Alexander P.W., *J. Chromatogr.*, 1991, 558, 181.

76. Alexander P.W., Glod B.K. and Haddad P.R., *J. Chromatogr.*, 1992, 589, 201.

77. Glod B.K. and Haddad P.R. and Alexander P.W., *J. Chromatogr.*, 1992, 589, 209.

CHAPTER 2

2. ION SELECTIVE ELECTRODES

2.1 INTRODUCTION

An ion selective electrode, ISE, is an electrode which displays selectivity towards a particular ion in solution. The best known example is the glass electrode, which responds to hydrogen ions in solution. Other ISEs have been developed which are selective towards other species such as F^-, K^+, $Ca^{2+}$and NO_3^-. It is important to note that all such electrodes are prone to interference.

Since the publication of Rechnitz's article in 1967[1] and later that of Covington in 1969,[2] several thousands of articles concerning the application of these amazing tools in various branches of chemistry, such as, clinical, environmental, etc., have emerged. The introduction of new and reliable ISEs for various cations, anions and neutral species has been a subject of interest to many scientists in different fields.

ISEs are membrane electrodes comprising an internal reference system interfaced with a thin layer which is either solid or liquid, and might be conducting or non-conducting in presence of the analyte ions. The function and the mechanism of the electrical conduction of ISEs is mainly related to the active material and the nature of the compounds used in the preparation of the membrane.

A logical and acceptable classification of ISE, based on the nature of the active materials, has been presented by Covington.[3]

a) **Glass:** the glass ISE responds to such cations as H^+, but a similar response is made towards Na^+, K^+ and NH_4^+ by altering the composition of the glass.

b) **Insoluble inorganic salts:** this deals with solid-state membranes, which comprise a compacted disc or a single crystal of sparingly soluble inorganic salt such as silver chloride.

c) Organic materials: in this group, the active materials are long chain ion-exchangers such as alkylammonium and complexing agents, and also include cyclic and acyclic neutral compounds.

All these materials in the different groups possess an ion-exchange capacity to a certain extent.
The construction and operation of various ISEs have been subject of many books,[4-7] reviews.[2,8-15] Another source of information is the journal, "Ion Selective Electrode Reviews".
Here a general outline of principles and components will be given.

2.2 PRINCIPLES OF OPERATION

ISEs are membrane electrodes, and typically direct electrochemical sensors. Conventional ISEs consist of an internal reference electrode, and an external reference electrode. The internal reference electrode is immersed in an aqueous solution in contact with the inner side of the membrane. The electric potential difference developed across the membrane depends on the difference in ion activity of solutions on the two sides. The generation of potential differs in accordance with the type of membrane. The potential difference generated are measured relative to the external reference electrode whose potentials are assumed to be invariant. The potential difference of the ISE cell, can be represented as:

internal reference electrode	internal solution	ISE membrane	test solution	external reference electrode

or

int. ref. el. e.g. copper wire	support material	ISE membrane	test solution	ext. ref. el.

The potential of the ISE is given by the Nernst equation.

$$E = const. + \left(\frac{2.303RT}{zF} \right) \log a_i$$

where E = potential of ISE

R = gas constant

T = absolute temperature

a_i = activity of *primary ion*

F = Faraday constant

z = the charge on the ion sensed.

A calibration curve, plotting voltage versus the negative log of activity of concentration for two or more standards, can then be used to determine the activity of the solute in a sample.
The fundamental basis of potential measurements is considered here but more comprehensive discussions can be found elsewhere.[5,16,17]

2.3 SELECTIVITY AND CALIBRATION

Ideally ISEs should respond exclusively to the species of interest. However, it is important to appreciate that in all instances electrodes are selective but not necessarily specific, to the ion of interest. The difficulty in distinguishing different ions, particularly those of the same charge, is an important parameter which limits the use of those devices where selectivity is desired. Considering this, the response of an electrode may deteriorate in the presence of other ions and the effect of this deterioration on the measured potential is accounted for by the simplified Nicolsky-Eisenman equation which is modified from the Nernst equation:

$$E = Eo + \left(\frac{2.303RT}{zF}\right) \log \left[a_i + K_{ij}(a_j)^{z_i/z_j}\right]$$

where a_j = activity of the interfering species in the sample,
a_i = activity of the ion of interest,
K_{ij} = selectivity coefficient of the electrode which gives fundamental information on how the interferent affects the response of the electrode. The smaller the value of K_{ij},. the less

the effect of the interferent, the opposite being true as K_{ij} is increased. The selectivity coefficients depend upon the composition of the membrane, varying widely from one type to another,[18] and also depend upon the composition of the sample and method of evaluation.

2.4 METHODS OF DETERMINING K_{ij}

According to the IUPAC[19] definition, the selectivity coefficient determines the capacity of the ISE to discriminate between different ions in solution. There have been various methods described[20-22] for the determination of selectivity coefficients, K_{ij}, which are based on potential measurements either in separate solutions or in mixed solutions, containing primary and interfering ions.

2.4.1 SEPARATE SOLUTION METHOD

The K_{ij} of an electrode is calculated from measurements of potential difference due to ions i and j in separate solutions. The potential of the electrode is determined in a solution containing only the primary ion i, and then the interfering ion j only. Then for $a_i = a_j$;

$$E_i - E_j = + \left(\frac{2.303RT}{zF}\right) \log K_{ij}$$

where E_i = potential in the solution containing i only,
E_j = potential in the solution containing j only.
This method has been criticized by Buck[23] for not relating to a real situation.

2.4.2 MIXED SOLUTION METHODS

These methods determine the selectivity coefficient in the presence of both the primary ion and interferent ions in the same solution and this method is recommended by IUPAC.[19]
The potential differences of the ISE are taken, either in solutions where the primary ion, i, concentration is kept constant

and the interferent ion, j, concentration is varied, or more usually with interfering ion kept constant while the primary ion concentration is varied. A plot of a_i versus E is constructed from which the value of K_{ij} is calculated.[24]
From the Nicolsky-Eisenman equation, the selectivity coefficient of the electrode is;

$$K_{ij} = a_{i(j)}/a_j,$$

where $a_{i(j)}$ = activities of ion of interest and interferents in the sample.

2.4.3 CONSTANT VOLUME, CONTINUOUS DILUTION METHOD

The method was first introduced by Horvai et al.[25] in 1986, and allows continuous measurements to be made for calibrating and determining the selectivity of ISEs. If a stirred flow-through cell of a constant volume is filled with a solution containing a concentration C_o of an ion A, and this is diluted by passing through a solution which does not contain the ion A, then the concentration of A in the cell will be at time t C_t:

$$C_t = C_0 \, e^{-Ft/V_r},$$

where v_r denotes the constant volume of solution in the cell, t is time from the start of dilution and F is the flow-rate. The equation is only valid if the flow-rate F is kept constant. The continuous dilution method is versatile, resulting in simple and reliable calibration and selectivity measurements. It is an easy method but it shows one drawback in that calibration is only possible by decreasing solution concentrations. The rate of dilution must be lower than the rate of response of the ISEs.
A comprehensive evaluation of this method has been given by Pungor et al.[26]

2.5 DETECTION LIMIT

Many different definitions of detection limit for ISEs have been suggested. IUPAC,[19] recommended the use of "the practical limit of detection", defined the detection limit as the activity of the primary ion "i" at which the electrode potential deviates by 18 mV from the extrapolated linear portion of the calibration curve.
The limit of detection for each electrode determines its measuring range. The detection limit depends both on the properties of the electrode and the conditions of measurements. Therefore, it is important that the conditions of measurements are stated.

2.6 RESPONSE AND LIFE TIME

Rapid response time and long life time are essential parameters for ISEs. A rapid response becomes more important when especially ISEs are used in continuous measurements as the range of response time varies[27,28] from 10^{-3}s to several hours. Therefore it is necessary to know the response time of an electrode if it is to be used in a flow system such as, chromatography and flow injection analysis etc. IUPAC[29] defined the response time as the time taken for the potential at the cell containing the electrode to reach a value 1 mV from the final equilibrium potential after a ten fold change in the activity of ion of interest. Nevertheless, $T_{1/2}$[30] and $T_{95\%}$[31] parameters have been defined as response times.
In general, the response time depends upon the membrane type, the magnitude and the direction of concentration change, the presence of interferents,[32] temperature, age and condition of electrode[33] and the thickness of diffusion layer at the membrane surface.[31]
The life time is the time period during which a given electrode operates in a reliable way without membrane renewal or cleaning. ISEs based on polymer membranes have short life times compared to the solid-state or glass electrodes, due to leakage and dissolution of active material and plasticizer. However, the life time of all electrodes, and in particular of PVC based electrodes, depends on the nature of active material,[34,35] plasticizer[36] and individual membrane components,[37] and is influenced by the analytical method applied. The measuring conditions, e.g. the

aggressive substances present in the sample solution, substances adhering to the electrode surface, and substances dissolving the electroactive material from the surface layer, can generally shorten the life time of the electrode.
On the other hand, the short life time for the polymer membrane electrodes is not a disadvantage because of the ease and inexpensiveness of the membrane replacement.

2.7 REFERENCE ELECTRODES

As discussed above, potentiometric measurements require a pair of electrodes: an indicator electrode that responds to the analyte and a reference electrode that maintains a fixed known potential. In principle, any electrode could serve as a reference electrode, but it must satisfy the following criteria: stability, low temperature sensitivity, reproducibility and non-polarizibility.[38] The most commonly used reference electrodes are the calomel and silver/silver chloride electrodes. But both of them are prone to interference by sulphide, iodide and bromide ions.[39] The calomel electrode is also affected by the presence of hydroxide ions, as is the silver/silver chloride electrode at pH>10. A drift in the observed response combined with an increase in the resistance of the electrode, is normally indicative of interference. Further information about reference electrodes can be found elsewhere.[40,41]

2.7.1 CALOMEL ELECTRODE

This may be represented by

$$Hg \mid Hg_2Cl_2 \mid KCl$$

for which the cell reaction is

$$2\ Hg + 2\ Cl^- \rightleftharpoons Hg_2Cl_2 + 2\ e^-$$

A saturated potassium chloride solution is normally used and the electrode is referred to as a saturated calomel electrode, SCE. This has[42] a standard potential of +0.244 V at 25 °C.

A pool of mercury covered by a layer of calomel (Hg_2Cl_2) is in contact with a reference solution containing chloride ions and saturated with calomel. The preparation of calomel electrodes is well documented elsewhere.[43] Calomel electrodes, however, are not stable when miniaturized, currents of less than 0.5 μA are sufficient to polarize the electrode.

2.7.2 SILVER/SILVER CHLORIDE ELECTRODE

This may be represented by

Ag | AgCl | KCl

and half cell reaction is

$$Ag_{(s)} + Cl^- \rightleftharpoons AgCl + e^-$$

The standard potential of a 3.5 mol dm^{-3} potassium chloride silver/silver chloride electrode is +0.205 V at 25 °C.[42] In its simplest form, the electrode consists of a silver wire or plate coated with silver chloride and in contact with a solution containing chloride ions. It is often used as an internal reference electrode in pH electrodes and ISEs. Several methods can be used for preparation of silver/silver chloride electrodes as described by Ives.[38] They are mostly prepared by the thermal-electrolytic method, as described by Bates.[39] It is more rugged than the calomel electrode and easy to miniaturize, therefore, it is used extensively in biological applications.

2.8 POLYMER MEMBRANE ELECTRODES

Polymer membrane refers to a type of membrane, in which an electroactive material is dispersed into a polymer with an organic substance as a plasticizer. Membranes may contain anionic or cationic functional groups, or be neutral. Selectivity is generally on the basis of stability constant of resulting complex between the ion and active material.

The trend to utilize a membrane matrix to give strength, life

time, selectivity and reduce the amount of active material in the membrane dates back more than fifty years. The early work of Tendeloo[44] on the fabrication of a calcium electrode using calcium in paraffin wax, paved the way for further developments in utilizing support materials in membranes. In 1958, Parsons[45] reported the preparation of solid disc membranes by moulding a mixture of fine particles of ion-exchangers and polymer under pressure. In 1970, the contribution of Moody, Oke and Thomas,[46] to the development of early polymer membranes, is significant. An interesting configuration of the polymeric electrode is the coated-wire ISE as presented in 1971. In this modification platinum or silver wire, coated with a polymeric matrix containing various active materials, is used.

The first plasticized PVC based membrane was reported by Bloch et al.[47] in 1966 and Shatkay[48] in 1967. Since then an impressive number of papers have appeared dealing primarily with those of plasticized membranes of PVC in ISEs and a number of commercial electrodes based on PVC were introduced, such as Ca^{2+}, K^{+}, NO_3^{-}, etc.

The developments of successful membranes became possible after some progress in understanding better the nature of the membrane, and discovery of effective ion-exchangers as active materials. Four different classes of polymer based electrodes have been described for conventional ISE construction and use.[49] Within these classes, PVC remains the most significant support polymer of choice as described by Thomas.[50,51] Many types of polymers have been utilized for the construction of polymer membrane ISEs. Many of these polymers are discussed in a review by Moody.[52] Especially with the advent of PVC membranes, electrode life times and sensitivities increased tremendously. It has now been established that in order to obtain a functional electrode i.e. showing a Nernstian response, PVC membranes must be plasticized and that the nature of plasticizer influences the selectivity.[53] In recent years, a large number of reviews[11-15] became available covering all aspects of polymer matrix membranes.

2.9 ELECTROACTIVE MATERIALS (IONOPHORES)

Ionophores are one of the fundamental components of ion selective membranes. The first criterion for the selection of an ionophore is its selective character towards the species in the solution, and its capability of dissolving a liphophilic ion and transporting that ion through a liphophilic membrane phase. Generally, ionophores are organic compounds with cyclic structure. However, there are also some acyclic compounds which possess similar properties.

Numerous ion-exchanger systems responsive either to cations or anions of varying charge, have been utilized as active species in membranes. Phosphate esters,[54,55] tetraphenylborate,[56,57] oxine[58] systems and neutral organic compounds[59-63] for cation selective, and quaternary ammonium salts,[64,65] quaternary phosphonium salts[66,67] and many other compounds[68,69] for anion selective, electrodes have been employed. Although there has been a vast amount of literature on ionophores, Ovchinikov,[70] Koryta[7] and Edmonds[71] have written books on many aspects of naturally occurring ionophores and the properties of ionophores respectively. Izatt and Christensen[72,73] have edited two books which serve as a good introduction to synthetic ionophores. In recent years a number of reviews[74-82] on all aspects of synthetic ionophores have appeared.

2.10 PLASTICIZERS

Usually, plasticizers are used to induce flexibility and reduce the glass transition temperature (Tg) of PVC. The temperature at which the rubbery state of the polymer changes to a glassy state is called the glass transition temperature. The nature of the monomer plays an important role in determining this temperature.[83] The value of the glass temperature varies over a large range (-120°C to +130°C).[83,84] The main parameters which effect the Tg are: the flexibility of the chains and the intermolecular interaction between substituent groups. More flexibility and higher molecular weight of chains increases the Tg. The plasticizers are organic compounds with high boiling points. The

asset of plasticizers is their ability to soften the PVC, however, a good plasticizer should also possess a combination of the following properties: chemical inertness, low volatility, low solubility and good compatibility with the matrix. Perry et al.[85] reported that the addition of active carrier to an ISE membrane only improves the selectivity of the electrode. For a functional ISE membrane, the plasticizer must show a response to some extent. The influence of the dielectric constant of the medium on the selectivity of the membrane has been investigated.[86] Membranes with neutral carriers in low dielectric media prefer monovalent cations to divalent cations. The discrimination of between cations within a given group in the periodic table was said to be insignificant.

Depending on the usage of the PVC, plasticizers are classified into four general groups[87]: primary, secondary, polymeric and other plasticizers. The primary and secondary plasticizers are used, when low temperature and flexibility are of prime importance, e.g. di-(ethylhexyl) sebatate, di-(phenyl) phthalate. The amount and the type of plasticizer is very important and critical in the functioning of the PVC membrane. Membranes without plasticizer are rigid and brittle, whilst membranes with too much plasticizer (more than 70%) are jelly-like.[88]

2.11 SOLVENT

The other fundamental component in the membranes is the solvent. The solvent must be compatible with the other components. The solvent should dissolve as much active material as possible, and should have low vapour pressure and viscosity.[89] The effect of the solvent on the response range and the selectivity of the membrane has been classified by the comprehensive work of Garbett and Torrance.[90]

2.12 ION-EXCHANGE

Advances in synthetic ionophore production and application of such compounds to a broader range of electrodes have increased dramatically over the past two decades. In addition to the

studies on the ionophore production and their application, the chemical recognition of specific ions by ionophores has also been examined extensively. In comparison to the covalent bond, a complex results from combinations of relatively weak intermolecular interactions, such as electrostatic forces, hydrogen bonding, Van der Waals' forces and the steric effects, and ion-dipole electrostatic attractions. The understanding of the response mechanism of polymer membrane electrodes begins with an understanding of the ion-exchange process that occur within the membrane itself.

2.13 CATION-EXCHANGE

The work of Pedersen[91] who reported the first neutral synthetic organic crown ether which complexed alkali and alkaline earth metal cations, spirred interest in uncharged hydrophobic ion-extracting and complex forming compounds. The relationship between structure and cation selectivity has been intensively examined by the systematic variation of the number of oxygen atoms linked to the ether, ring size, length of (CH_2) bridge and introduction of aromatic or heteroaromatic systems in the ring.

In liphophilic media, crown ethers possess a hydrophilic cavity consisting of hydrophilic structural elements such as oxygen and nitrogen. The resulting electronegative cavity size is suitable for alkali and alkaline earth metal cations according to their size.[92] The lower limit of cavity size is determined by steric interference as well as by electrostatic repulsion between the hydrophilic groups. The upper limit, however, depends on conformational flexibility of the binding sites within the molecule. In hydrophobic media, the polarization is reversed and they possess a hydrophobic cavity.[93] The selectivity may depend on the relationship between the stability constant of the complex and the cation/cavity radius size.[94]

The size of the hydrophilic cavity of an 18-crown-6 compared with the radii of some alkali metal cations is shown in table 1.

Table 1. comparison of cation / and cavity radii

cation	radius° A	crown ether	cavity radius
Li^+	0.76	18-crown-6	1.34-1.43
Na^+	1.02		
K^+	1.38		
Cs^+	1.67		

From the table, it can be seen that the radius of Li^+ is much too small, of Na^+ too small, of K^+ just right and of Cs^+ too large to fit into the hydrophilic cavity of an 18-crown-6 molecule. The better fit of the K^+ cation into the hydrophobic cavity of the molecule is also confirmed by the experimentally determined stability constant of complex (K_s), the better fit the larger K_s.[95]

It is substantiated by structural studies that, when the cation radius is very much larger than hydrophilic cavity radius, sandwich complexes of 2:1 molecule:cation stoichiometry result.[96] Conversely when the cation radius is very much lower than the hydrophilic cavity radius, binuclear 1:2 molecule:cation stoichiometrical complexes result.[97] Therefore, the cavity model has limited usefulness for predicting relative binding capacities of metal cations with large crown compounds. As the number of ring atoms increases, the flexibility of the molecule increases and it becomes difficult to define the cavity diameter. For example, dibenzo 30-crown-10 forms a very strong complex with K^+.[98] Numerous other factors, including charge on the cation, the solvation enthalpies and entropies of the cation, and the rigidity or flexibility of the molecule, can influence K_s.[95]

Selectivity of crown ether complexation can be defined as the ability of the crown molecule to discriminate and selectively bind a particular metal cation in the presence of other cationic species. The selectivity, S, of a crown ether towards two different cations M_1 and M_2 can be expressed by the ratio of the stability constants, Ks, of the complexes LM_1 and LM_2:

$$S = \frac{K_s(LM_1)}{K_s(LM_2)}$$

where, L is the crown ether ligand. Numerically high factors correspond to high selectivity, low factors mean low discrimination. A crown ether can exhibit high selectivity for several metal cations of similar size or charge but much lower selectivity for another group of ions. For example, 18-crown-6 which exhibits low selectivity for K^+/Na^+, Rb^+ and Cs^+ but high selectivity for K^+/divalent cations. This phenomenon is related to the donor atom type (N,S,O) on the crown ether molecule. The type of donor atom at the binding site alters the nature of the interactions and can lead to quite substantial changes in complexation selectivity.[95] For example, whilst oxygen as the donor atom in 18-crown-6 enhances the complexation of alkali metal cations, sulphur as the donor atom enhances complexation of transition metal ions and reduces that of alkali metal cations. More detailed discussions on the thermodynamics of cation/molecule interactions can be found in an extensive review.[99]

2.14 ANION-EXCHANGE

Many anion selective electrodes are based on classical exchangers, complexes of long chain organic bases, such as quaternary ammonium or phosphonium salts and metalloporphyrin-based membrane systems. Some quaternary ammonium or phosphonium salts possess the required selectivity and can be utilized in PVC based ISEs. At the membrane interface, a rapid anion-exchange process takes place between the free anions in the aqueous phase and the anions bound to the organic site groups. How effective an electrode is depends basically on the selectivity of this anion-exchange process. When the selectivity is too high towards an anion, the organic anion-exchanger forms a more stable complex with the particular ion in aqueous phase than with any other. Since quaternary ammonium compounds function as dissociated anion-exchangers, equilibrium anion extraction into the membrane is based solely on the difference between the free energy of solvation of the anions in the aqueous sample and in the organic membrane phases. When lipophilic tetraalkylammonium salt type anion-exchange compounds

are incorporated into polymer membranes the Hofmeister pattern is almost always observed (i.e., selectivity to ClO_4^- >IO_4^- >SCN^- >NO_3^- >I^- >Br^- >Cl^- >HCO_3^- ≥ $H_2PO_4^-$.[100]). Long hydrocarbon chains are used to give insolubility in the aqueous phase. Sensitivity and life time might be expected to improve on lengthening the alkyl chain of the active material. However, in terms of selectivity, very little can be gained by using active materials with alkyl chains >C_{12}. Interest in nitrate selective electrodes has led to the utilization of the tetradodecylammonium nitrate (TDD-NO_3^-) salt as selective exchanger in PVC matrix membranes. In general, one can say that only quaternary phosphonium and ammonium compounds have so far shown lower selectivity towards one ion in the presence others.

In case of the metalloporphyrin-based membrane systems, unique potentiometric anion selectivity has been attributed to the preferred axial site coordination of given anions with the metal centre. The observed potentiometric response to anions has been shown to be dependent on the nature of the metal centre, its oxidation state, and the exact structure of the porphine ring surrounding the metal cation.[101] When such compounds are incorporated into polymer membranes, the potentiometric response to anions deviates significantly from the classical Hofmeister selectivity sequence.

2.15 REFERENCES

1. Rechnitz G.A., *Chem. Eng. News*, 1967, 45(25), 146.

2. Covington A.K., *CRC Crit. Rev. Anal. Chem.*, 1974, 3(4), 355.

3. Covington A.K., *Chem. Brit.*, 1969, 5, 388.

4. Bailey P.L., "*Analysis with Ion Selective Electrodes*", Heydon, London, 1976.

5. Covington A.K., "*Ion Selective Electrode Methodology*", (Covington A.K. Ed.) CRC Press, Boca Raton, Florida, 1979.

6. Freiser H., "*Ion Selective Electrodes in Analytical Chemistry*" Vol.1, Plenum Press, New York, 1980.

7. Koryta J., "*Ion Selective Electrodes*", 2nd Ed., Cambridge University Press, London, 1983.

8. Koryta J., *Anal. Chim. Acta*, 1982, 139, 1.

9. Koryta J. *Anal. Chim. Acta*, 1984, 159, 1.

10. Koryta J., *Ann. Rev. Mater. Sci.*, 1986, 16, 13.

11. Arnold M.A. and Meyerhoff M.E., *Anal. Chem.*, 1984, 56, 20R.

12. Moody G.J. and Thomas J.D.R., *Ion Sel. Elec. Rev.*, 1986, 8, 209.

13. Solsky R.L., *Anal. Chem.*, 1988, 60, 106R.

14. Koryta J., *Anal. Chim. Acta*, 1990, 233, 1.

15. Solsky R.L., *Anal. Chem.*, 1990, 62, 21R.

16. Plambeck J.A., "*Electroanalytical Chemistry Basic Principles and Applications*", John Wiley, New York, 1982.

17. Sarjeant E.P., "*Potentiometry and Potentiometric Titrations*", John Wiley, New York, 1984.

18. Pungor E., Toth K. and Hrabeczy-Pall A., *Pure Appl. Chem.*, 1979, 51, 1980.

19. IUPAC, "Recommendation for Nomenclature of Ion Selective Electrodes", *Pure Appl. Chem.*, 1976, 48, 127.

20. Pungor E. and Toth K., *Anal. Chim. Acta*, 1969, 47, 21.

21. Srivasan K. and Rechnitz G.A., *Anal. Chem.*, 1969, 41, 1203.

22. Moody G.J. and Thomas J.D.R., "*Selective Ion Sensitive Electrodes*", Merrow, Watford, 1973.

23. Buck R.P., *Anal. Chem.*, 1972, 44, 2708.

24. Hiiro K., Wakida S. and Yamane M., *Anal. Sci.*, 1988, 4, 149.

25. Horvai G., Toth K. and Pungor E., *Anal. Chim. Acta*, 1976, 82, 45.

26. Pungor E., Nagy G. and Toth K., "*Ion Selective Electrode Methodology*", (Covington A.K. Ed.), Vol.2, CRC Press, Boca Raton, Florida, 1979.

27. Blaedel W.J. and Dinwiddie D.E., *Anal. Chem.*, 1975, 47, 1070.

28. Shatkay A., *Anal. Chem.*, 1976, 48, 1039.

29. Lindner E., Toth K. and Pungor E., *Pure Appl. Chem.*, 1986, 58, 469.

30. Rechnitz G.A. and Kresz M.R., *Anal. Chem.*, 1966, 38, 1786.

31. Fleet B., Ryan T.H. and Brand M.J.D., Anal. Chem., 1974, 46, 12.

32. Marcovic P.L. and Osburn J.O., *Am. Inst. Chem. Eng. J.*, 1973, 9, 504.

33. Morf W.E., Lindner E. and Simon W., *Anal. Chem.*, 1975, 47, 1596.

34. Keil L., Moody G.J. and Thomas J.D.R., *Analyst*, 1977, 102, 274.

35. Oesch U. and Simon W., *Anal. Chem.*, 1980, 52, 629.

36. Attivat A.S., Christian G.D., Hallman J.L. and Bertsch R.A., *Talanta*, 1988, 35(10), 789.

37. Hodinar A. and Jyo A., *Anal. Chem.*, 1989, 61, 1169.

38. Ives D.J.G. and Janz G.J., "*Reference Electrodes*", Academic Press, New York, 1961.

39. Bates R.G., "*Determination of pH:Theory and Practice*", 2nd Ed., John Wiley, New York, 1973.

40. Newman J.S., "*Electrochemical Systems*", 2nd Ed., Prestice-Hall, Inc., Englewood Cliffs, New Jersey, 1991.

41. Durst R.A., "*Ion Selective Electrodes*", Proceedings of a Meeting Held at NBS, Gaithersburg, Maryland, NBS Special Publication 314, 1969.

42. Bard A.J., Parsons R. and Jordan J., "*Standard Potentials in Aqueous Solutions*", Marcel Dekker Inc., New York, 1985.

43. Hills J.G. and Ives D.J.G., *J. Chem. Soc.*, 1951, 311.

44. Tendeloo H.J.C., *J. Biol. Chem.*, 1936, 113, 333.

45. Parsons J.S., *Anal. Chem.*, 1958, 30, 1262.

46. Moody G.J., Oke R.B. and Thomas J.D.R., *Analyst*, 1970, 95, 910.

47. Bloch R., Shatkay A. and Saroff H.A., *Biophys. J.*, 1967, 7, 865.

48. Shatkay A., *Anal. Chem.*, 1967, 39, 1056.

49. Meares P., *NATO ASI Ser., Ser C*, 1986, 181.

50. Thomas J.D.R., *Anal. Chim. Acta*, 1986, 180, 289.

51. Thomas J.D.R., *J. Chem. Soc.*, 1986, 82, 1135.

52. Moody G.J., Saad B.B. and Thomas J.D.R., *Ion Sel. Elec. Rev.*, 1988, 10, 71.

53. Baum G. and Lynn M., *Anal. Chim. Acta*, 1973, 65, 393.

54. Moody G.J., Nassory N.S. and Thomas J.D.R., *Analyst*, 1978, 103, 86.

55. Awathi S.P., Kulkarni V.T. and Sunderasen M., *J. Electrochem. Soc.*, India, 1988, 37(4), 309.

56. Davies J.E.W., Moody G.J., Price W.M. and Thomas J.D.R., *Lab. Prac.*, 1973, 72, 20.

57. Jaber A.M.Y., Moody G.J. and Thomas J.D.R., *Analyst*, 1988, 113(9), 1409.

58. Malone T. and Christian G., *Anal. Lett.*, 1974, 7, 33.

59. Stefanac Z. and Simon W., *Chimia*, 1966, 52, 562.

60. Ammann D., Guggi M., Pretsch E. and Simon W., *Anal. Lett.*, 1975, 8, 709.

61. Ryba O. and Petranek J., *J. Electroanal. Chem.*, 1976, 67, 321.

62. Zhou X., Luo Y., Wu C., Zou Z. and Hu Q., *Anal. Chim. acta*, 1988, 212, 325.

63. Luk'yanenko N.G., Nazarova N.Y, Karpinchik O.S. and Mel'nik O.T., *Anal. Chim. Acta*, 1988, 215, 289.

64. Fabiani C., *Anal. Chem.*, 1976, 48, 865.

65. Guo D., Ji C., He P. and Gao H., *Anal. Proc.*, 1987, 24, 343.

66. Wolso A. and Pool K., *Talanta*, 1976, 23, 387.

67. Oki A., Yamura M., Kummamato.S., Maeda S., Takashita T. and Takagi M., *Chem. Lett.*, 1989, 1, 95.

68. Ishibashi N. and Kohara H., *Anal. Lett.*, 1971, 4, 785.

69. Martin C.R. and Freiser H., *Anal. Chem.*, 1980, 52, 562.

70. Ovchinikov Yu A., Ivanov V.T. and Shkrob A.M., "*Membrane Active Complexones*", Elsevier, Amsterdam, 1974.

71. Edmonds T.E., Chapter 1-3, "*Chemical Sensors*", Blackie, Edinburgh, 1988.

72. Izatt R.M. and Christensen J.J., "*Progress in Macrocyclic Chemistry*", Vol.1, Wiley, New York, 1979.

73. Izatt R.M. and Christensen J.J., "*Synthetic Multidentate Mycrocyclic Compounds*", Academic Press, New York, 1978.

74. Lehn J.M., *Pure Appl. Chem.*, 1978, 50, 821.

75. Kothoff I.M., *Anal. Chem. Rev.*, 1979, 79, 385.

76. Lehn J.M., *Pure Appl. Chem.*, 1980, 52, 1441.

77. Ammann D., Lanter F., Steiner R.A., Schulthess P., Shijo Y. and Simon W., *Anal. Chem.*, 1981, 53, 2267.

78. Ammann D., Morf W.E., Anker P., Meier P.C., Pretsch E. and Simon W., *Ion Sel. Elec. Rev.*, 1983, 5, 3.

79. Oggenfuss P., Morg W.E., Oesch U., Ammann D., Pretsch E. and Simon W., *Anal. Chim. Acta*, 1986, 180, 299.

80. Lockhart J.C., *J. Chem. Soc.*, 1986, 82, 1161.

81. Morf W.E. and Simon W., *GBF Monogr. Ser.*, 1987, 10, 13.

82. Pretsch E., Badertscher M., Welti M., Maruizumi T., Morf W.E. and Simon W., *Pure Appl. Chem.*, 1988, 60(4), 567.

83. Sorrenson W.R. and Campbell T.W., "*Preparative Method of Polymer Chemistry*", Wiley, Interscience N.Y., 1960.

84. Nielsen L.E., "*Mechanical Properties of Polymers*", Reinhold N.Y., 1960.

85. Perry M., Lobel E. and Bloch K., *Memb. Sci.*, 1976, 1, 233.

86. Simon W., Morf W.E. and Meier P., "*Structure and Bonding*, 1973, 16, 113.

87. Breon PVC Resins, *BP*, 1973.

88. Arami M., *PhD Thesis, University of Newcastle upon Tyne*, 1984.

89. Covington A.K. and Davison P., "*Ion Selective Electrode Methodology*" (Covington A.K. Ed.), Vol.1, Chapter 6, CRC Press, 1979.

90. Garbett K. and Torrance K., *Central Electricity Research Laboratories*, Report, 1974, 1975.

91. Pedersen G.J., *J. Amer. Chem. Soc.*, 1967, 89, 7017.

92. Lehn J.M., "*Structure and Bonding*", Springer Verlag, Berlin, 1973.

93. Weber E. and Vogtle F., *Top Curr. Chem.*, 1981, 98, 1.

94. Lockhart J.C., *J. Chem. Soc.*, 1986, 82, 1161.

95. Izatt R.M. Eatough D.J. and Christensen J.J., "*Structure and Bonding*", Springer Verlag, Berlin., 1973, 161.

96. Mallinson P.R. and Truter M.R., *J. Chem. Soc. Perkin Trans.*, 1972, 2, 1818.

97. Hughes D.L., *J. Chem. Soc. Dalton Trans*, 1975, 2374.

98. Frensdorf H.K., *J. Amer. Chem. Soc.*, 1971, 93, 600.

99. Izatt R.M., Bradshaw J.S., Nielsen S.A., Lamb J.D. and Christensen J.J., *Chem. Rev.*, 1985, 85, 271.

100. Hofmeister F., *Arch. Exp. Pathol. Parmakol.*, 1988, 24, 247.

101. Park S.B., Matuszewski W., Meyerhoff M.E., Liu Y.H.and Kadish K.M., *Electroanalysis*, 1991, 3, 909.

CHAPTER 3

3. ION CHROMATOGRAPHY

3.1 INTRODUCTION

Liquid chromatography, LC, derived from conventional column chromatography, includes all chromatographic techniques in which the mobile phase is a liquid. The development of very efficient separators having packing materials with very small particle sizes requires the use of high pressure systems. Hence, LC can be called high pressure (or performance) liquid chromatography (HPLC). The introduction of HPLC in the early 1970s has been dependent on fundamental studies by Horvarth,[1] Knox,[2] Scott[3] and Synder.[4] HPLC defines the experimental technique, but does not indicate anything about the phase system. Therefore all distribution mechanisms e.g. size exclusion, reverse and normal phase adsorption, liquid-liquid partition and so called ion chromatography (IC) can be used in the HPLC mode.[5]

The term *ion chromatography* was first introduced in 1975 by Small, Stevens and Bauman.[6] They described a novel ion-exchange chromatographic method for the separation and conductometric detection of anionic and cationic species. The method employed the additional second column after the separator column and conductivity detector. This second column, which has been called the *suppressor*, removes the background conductance of the eluent, and often enhances the detectability of the ionic species. The eluent is pumped through the injection valve, the column(s) and conductivity detector. The samples are introduced into the eluent via the injection valve. The most important part of the system is the analytical column which determines the quality of analysis.

In 1979, a number of ion chromatographic techniques[7,8] were developed which eliminated the use of suppressor column in the original technique developed by Small et al.[6] They used an ion-exchange column coupled directly to a conductivity detector. This method became known under a variety of names; *single column*

ion chromatography, direct conductometric detection or *non-suppressed ion chromatography* the term used through the thesis as it is commonly used elsewhere. That may be the reason that a perfect definition of IC could not be made fulfilling all its requirements. Nevertheless, a definition given by Rocklin[9] is chosen here that IC is simply HPLC of ions on low capacity ion-exchange columns with conductometric detection, but implying that any separation and detection method can be used and still be called IC. Therefore IC can be based on several separation mechanisms, not only ion-exchange. And each mechanism has its particular advantages and disadvantages and applied to different sample types.

3.1.1 ION-EXCHANGE CHROMATOGRAPHY

The essential principle of this technique is of an ion-exchange process between the eluent and the exchange groups covalently bound to the stationary phase. Both inorganic and organic anions and cations can be determined using ion-exchange chromatography. More detailed discussion of this technique is given in section 3.4.

3.1.2 ION-PAIR CHROMATOGRAPHY

In the mid 1970s, the term *ion-pair chromatography* was first introduced by Haney,[10] Water Associates[11] and Knox.[12] They found that adding lipophilic ions (ion-pair reagents) to the mobile phase, such as alkylsulphonic acids or quaternary aliphatic amines, permits solute ions with opposite charges to be separated on known reversed phase columns. The separation mechanism involved is mainly ion-interaction, in which ion-pair formation in the mobile phase and adsorbtion of the ion-pair reagent on to the non-polar surface of the reversed-phase column are combined. The method has been applied to determine inorganic (particularly large) ions,[13-15] although it is best suited for determination of large organic ions, such as alkaloids and surfactants.[5] It is also described under a variety of names: soap chromatography,[16]

surfactant chromatography[17] and ion-interaction chromatography[18,19] etc.

3.1.3 ION-EXCLUSION CHROMATOGRAPHY

This method was first introduced by Rich et al. of Dionex Corporation[20] and is an HPLC technique using strong ion-exchange resin columns. Relatively weakly ionic anions or non-ionics are separated on strong cation-exchangers, and weakly ionic cations or non-ionics are separated on strong anion-exchangers.

Ion-exclusion has limited flexibility in selectivity, and is particularly suitable for the separation of carboxylic acids,[21,22] aminoacids[23] and alcohols.[24] But it has also been applied to the separation of some inorganic and organometallic ions.[25]

3.1.4 MISCELLANEOUS SEPARATION METHODS

Some of these are; ligand-exchange chromatography[26] and reversed-phase chromatography.

3.2 DETECTION TECHNIQUES

The requirements were not only for the development of fast and efficient separation techniques but also that they are coupled with detectors, which are capable of precise measurement of species over a broad range of sensitivities. Many efforts have been made in this direction. Each of the above separation techniques can be applied with one or more of the following detection methods.

3.2.1 CONDUCTIVITY DETECTION

This has been regarded as the most used detection method in IC. It is commonly applied to detection of inorganic cations such as alkali and alkaline earth metals[27] and inorganic common anions.[28]

The detection principle is based on the conductance of a typical eluent prior to, and during the elution of the solute(s). The detector response observed in a flow-through cell is proportional to the solute concentration and to the difference in equivalent conductances between the eluent and the solute(s). The difference

can be positive or negative, depending on the background conductivity of the eluent. If the eluent contains high conductivity ions, such as hydrogen for cation-exchange and hydroxide for anion-exchange, the selectivity will decrease as the equivalent conductivity of the solute(s) is decreased. This is commonly referred to as *indirect conductivity* detection. If the eluent contains low conductivity ions, the sensitivity will increase on elution of solute(s) with high equivalent conductivity.

3.2.2 AMPEROMETRIC DETECTION

This detection method is based on the oxidation or reduction of solute molecules at the surface of an indicator electrode in a detector cell. For detection, any solute molecule can be oxidized and reduced but must be electrolysed at a lower potential than the components of the eluent. The method can be applied to very sensitive and selective determination, especially of catecholamines, phenols,[29] and carbohydrates.[30]

3.2.3 SPECTROPHOTOMETRIC DETECTION

This is based on direct measurement of the amount of ultraviolet or visible light absorbance by the solute ions. The detector is a very sensitive one for highly light absorbing solute ions, and offers advantages compared to the other detection methods. It is limited to those compounds which absorb ultraviolet or visible radiation. The major application of the detection method is the determination of aromatic and heterocyclic acids and amines, which can be applied to the determination of some inorganic cations[31] and anions.[32]

3.2.4 POST-COLUMN REACTION DETECTION METHODS

These method involve a chemical reaction of the solute ions with a suitable reagent, which is generally added to the eluent prior to the detector cell as the solutes are eluted from the column. This detection method can be applied to the determination of some inorganic anions[33] and cations.[34]

3.2.5 POTENTIOMETRIC DETECTION

A potentiometric detector measures the potential difference of an electrode in equilibrium with contacting solution and a reference electrode. The potential difference is related to the concentration of ion(s) in the solution in accordance with Nernst equation. The measurement of these potential changes provides the method of determining ion concentrations.

More detailed discussion of potentiometric detection in IC is given in section 3.6.

IC has become a rapid and sensitive technique for the analysis of many sample matrices. Several reviews[35-42] and books[43-50] on IC have been published, including the development and the use of its components, and the potential of this technique as an analytical tool.

3.3 SOME CHROMATOGRAPHIC FUNDAMENTAL PARAMETERS

In this section a brief overview of some of the important definitions applicable to liquid chromatography is provided. For further background information on chromatographic parameters, numerous books,[48-50] dissertations[51,52] and articles[53-55] are available.

In a typical chromatographic operation, the eluent is pumped through the injection valve, the column(s) and the detector(s). A diagram of basic components of IC is shown in figure 1. The sample to be separated is injected into the flowing eluent via the injection valve. The most important part of the system is the analytical column, in which separation is accomplished via the dynamic competition between ionic species and ionic components of the eluent, and which determines the quality of analysis. The detector(s) detect the ionic specie(s) as they are separated. The response from such a detector has generally the appearance similar to that shown in figure 2. If the injected sample contains just two species in addition to the components of the eluent, now, for the first component within the dynamic equilibrium between the eluent and stationary phases, can be written

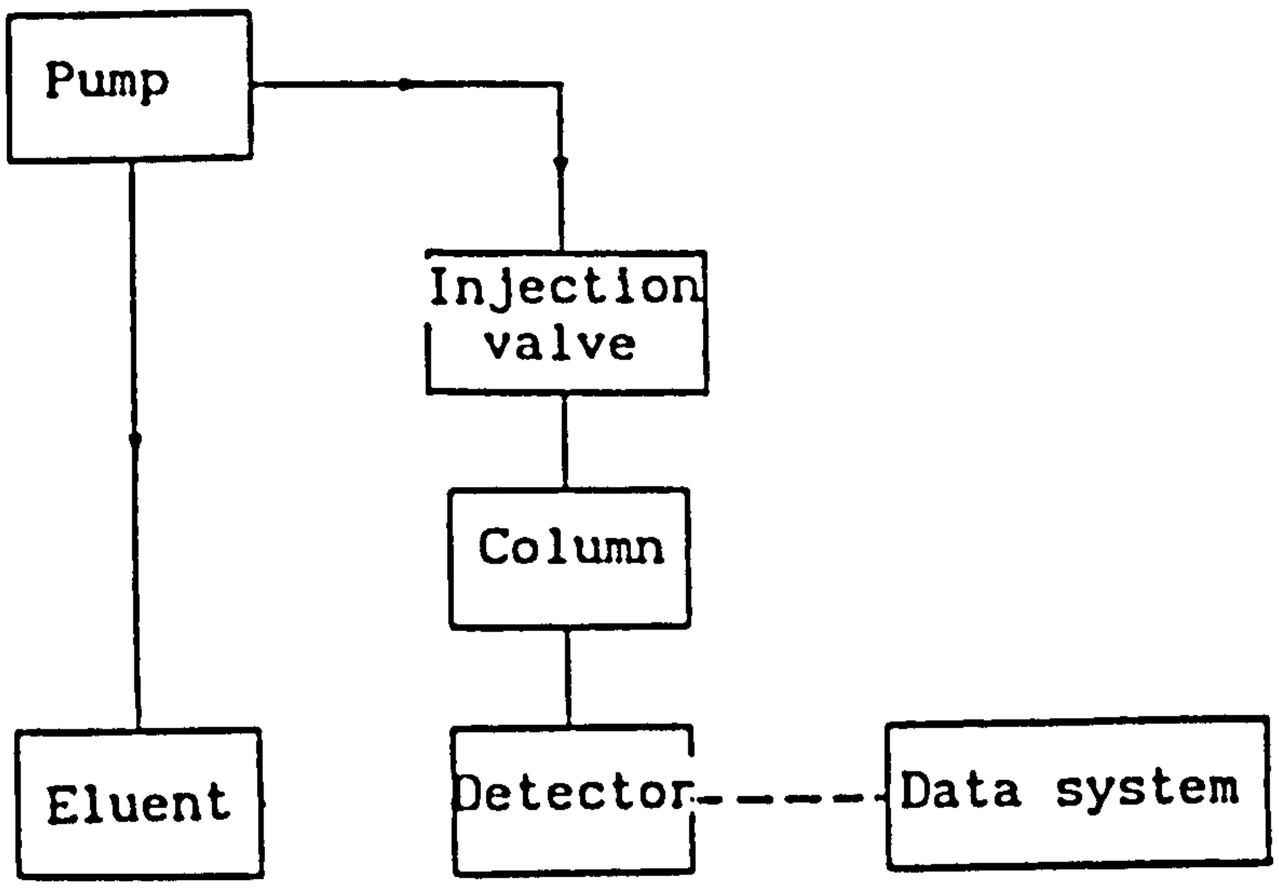


Figure 1. components of ion chromatography

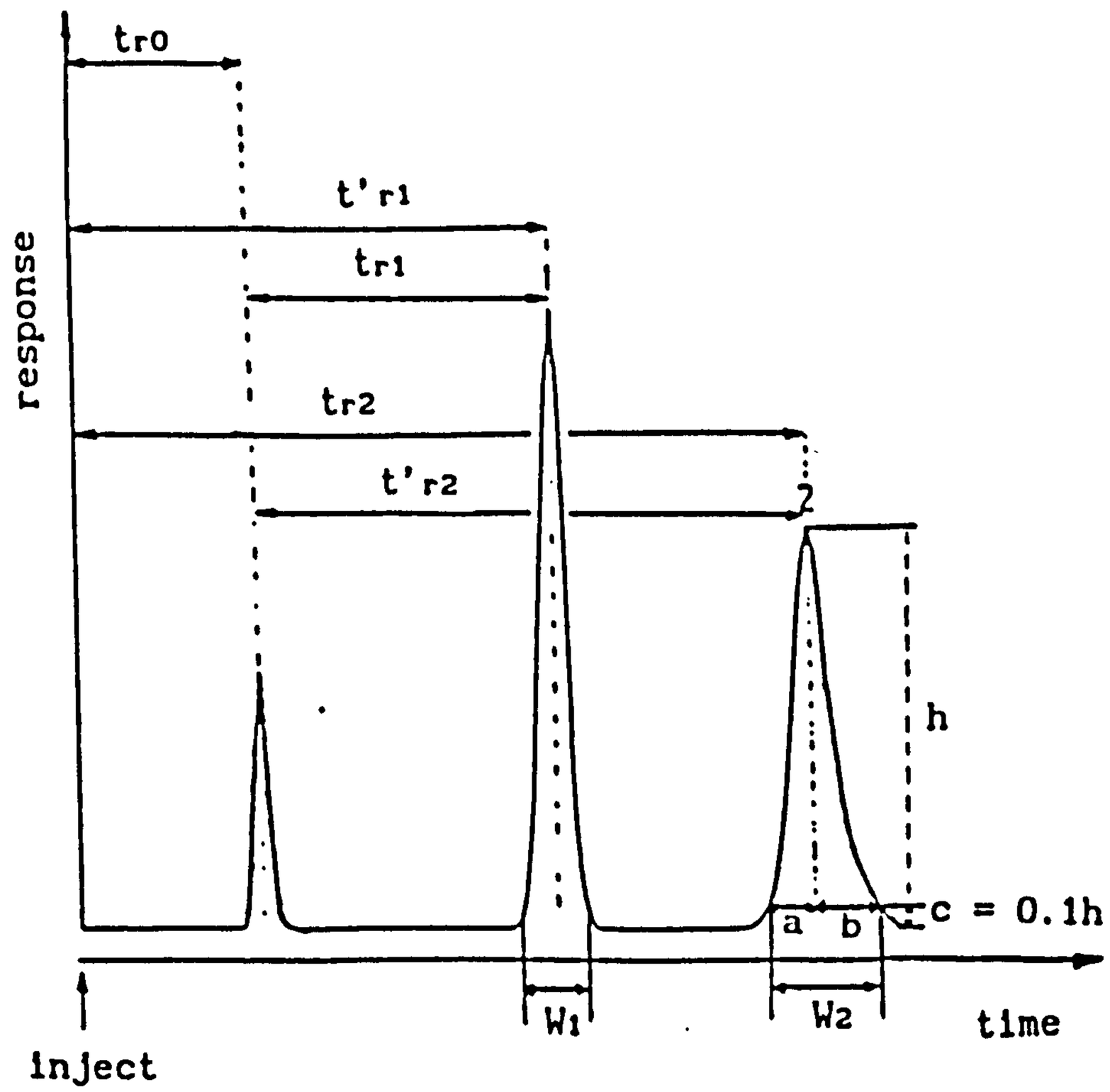


Figure 2. A typical appearance of detector response when two sample components are injected. Parameters for assessing the resolution, retention, selectivity and tailing effect.

$$Xm \rightleftharpoons Xs$$

where the subscript "m" refers to the eluent phase and "s" refers to the stationary phase. The distribution coefficient of component X between two phases can be expressed by:

$$Dx = \frac{[X]s}{[X]m}$$

The distribution coefficient, Dx, is dependent on the size of the population of molecules of component X in two phases. The only factor which affects the retention of one component over the another is the distribution ratio, provided that each component has a unique value of the distribution ratio. Hence, each component would have different time of travel through the stationary phase and a characteristic retention time.

The capacity factor, k, is given by the volume ratio of eluent and stationary phases and distribution coefficient:

$$k = Dx\ (\frac{Vs}{Vm})$$

where V_s is the volume of stationary phase and V_m is the volume of eluent in the column.

3.3.1 RETENTION

A convenient and useful measure of the retention of species is given by the adjusted or net retention time, t'_{r1},:

$$t'_{r1} = t_{r1} - t_{r0}$$

where t_{r1} is the gross retention time, the time from injection to the peak maximum, and t_{r0} is the dead time, the time required for a compound to migrate in the column without any interaction. The retention factor "k_r" is defined as the time the species spends in the stationary phase relative to the time it spends in the eluent and is given by:

$$k_r = \frac{t'_{r1}}{t_{r0}}$$

the values t'_{r1} and t_{r0} are dependent on the flow-rate of the eluent, the response characteristics of the detector, and the physical dimensions of the column and connecting tubing(s).

3.3.2 RESOLUTION

In chromatography, the resolution, Rs, is defined as the difference in the adjusted retention time of two peaks, $(t'_{r2}-t'_{r1})$, in terms of their average peak width and is given by:

$$Rs = \frac{t'_{r2}-t'_{r1}}{(W_2-W_1)/2}$$

where Rs is peak resolution, t'_{r2} and t'_{r1} are the net retention times of peaks 2 and 1, and W_2 and W_1 are the base widths of peaks 2 and 1 respectively. The resolution gives the fraction of the sample component in the eluent, and is related to the retention factor, k_r:

$$Rs = \frac{1}{1+k_r}$$

For two adjacent peaks $W_1=W_2$, the average value can be substituted by the width of the second eluted peak.

$$Rs = \frac{t'_{r2}-t'_{r1}}{W_2} = \frac{\Delta t'_r}{W_2}$$

where the resolution is 1, the retention time difference of the two peaks is equal to the width of the second eluted peak. It should be noted that all the above relationships are valid only for Gaussian (symmetrical) peaks, but they are usually applied also to non-Gaussian peaks.

3.3.3 SELECTIVITY

The selectivity is given by the ratio of the adjusted retention

times of two peaks and is a measure of their relative separation. It can be expressed as:

$$S = \frac{tr2-tr0}{tr1-tr0} = \frac{t'r2}{t'r1}$$

3.3.4 EFFICIENCY

As the sample species traverse the chromatographic column, the width of the peaks increases. The increase of the peak width is related to the species migration in the column and explained by the *Plate theory*. The *plate theory* divides a chromatographic column into theoretical plates, in which it is considered that the column consists of a series of thin sections. Within each section, thermodynamic equilibrium of the sample molecules between the eluent and stationary phase is realized. The number of theoretical plates, N, is related to the height equivalent to one theoretical plate, HETP, and the length of the column, L, and is given by:

$$N = \frac{L}{HETP}$$

The value HETP is defined as the ratio of the peak width and the gross retention time, and for a column, it can be calculated from a chromatogram:

$$HETP = \frac{L}{16} \left(\frac{W}{tr1}\right)^2$$

And the expression can be derived for the number of theoretical plates:

$$N = 16 \left(\frac{tr1}{W}\right)^2$$

A more sophisticated expression gives the possibility of calculation of the resolution for a column from the parameters: selectivity, capacity and the number of theoretical plates:[51]

$$Rs = \underset{a}{1/4\ N} \quad \underset{b}{\left(\frac{S-1}{S}\right)} \quad \underset{c}{\left(\frac{k}{k+1}\right)}$$

Term *a* refers to the column efficiency, term *b* refers to the column selectivity and term *c* refers to the column capacity.

3.3.5 TAILING AND FRONTING

The appearance of the signal by elution of a species from a column is usually asymmetric (non-Gaussian) and this phenomenon is called "tailing" as seen in figure 2. The tailing factor (the degree of asymmetry) is given by:

$$T = \frac{b}{a}$$

The tailing effect can be attributed to adsorption processes in the column and slow response of the detector. The opposite asymmetry is termed *fronting*, and often occurs when some of the sample species, responsible for the signal, cannot find free sorption sites. Fronting can often indicate column overload.[56]

3.4 ION CHROMATOGRAPHY EMPLOYING ION-EXCHANGE TECHNIQUE

In recent times, this technique has been improved using high efficiency ion-exchange materials combined with flow through detection. Separations can be performed on columns which are of a much smaller size. The size of the column is dependent on a number of factors including the chromatographic efficiency of the packing and its mechanical stability. Typical columns are 15-25 cm in length, with an internal diameter of 2-5 mm. Ion-exchangers of new and improved substances are made with particles of uniform size packed into a column housing constructed of rigid material. The particles of packing material are about 3.5 μm across and are generally very much smaller than those used for classical column chromatography. The flow-rate of the mobile phase can be precisely controlled and a very small amount of the

sample matrix may be introduced into the eluent via the injection port. Information on the type of the resin used and columns available from manufacturers is summarized by Johnson[27] and Haddad.[46]

Special continuous flow-through detectors are available capable of handling high flow-rates and detecting very small amounts. Finally automatic instrumentation has been developed capable of rapid analysis and high resolution of the eluted components.[56] Further information about ion-exchange techniques can be found in books.[45-47]

3.5 CLASSIFICATION OF ION CHROMATOGRAPHIC METHODS EMPLOYING ION-EXCHANGE SEPARATION

Ion chromatographic methods employing ion-exchange separation may be divided into two main groups of non-suppressed and suppressed ion chromatography.

3.5.1 NON-SUPPRESSED ION CHROMATOGRAPHY

Non-suppressed ion chromatographic methods comprise all those methods in which an ion-exchange column is used to separate a mixture of ions with the separated solutes being passed directly to the detector. The basis of these methods parallels the traditional high performance liquid chromatography approach in which the chromatographic column is coupled directly to the detector. More information can be found elsewhere.[46,57]

3.5.2 SUPPRESSED ION CHROMATOGRAPHY

Suppressed ion chromatographic methods consist of those in which an additional device, called a "suppressor", is inserted between the ion-exchange separator column and the conductivity detector. The function of the suppressor is to modify both the eluent and the solute in order to improve the detectability of the solutes with the conductivity detector.[6] The suppressor requires a regenerant solution to enable it to operate for extended periods. Some books[47,48,57] give further background information.

3.5.3 DIFFERENCES BETWEEN NON-SUPPRESSED AND SUPPRESSED ION CHROMATOGRAPHY [47,57]

With the exception of the suppressor itself, the only distinction between suppressed and non-suppressed methods is the small difference in column capacities and the use of a specialised group of the eluents.

The non-suppressed method requires columns with relatively higher capacities, and the eluents to give maximum conductivity changes or to have a low background conductivity in order to allow a sensitive conductivity detection of the sample ions.

The suppressed method requires columns with relatively higher capacities, and the eluents from which the products in the suppressor reaction must have very low conductivities. The non-suppressed method is simpler and requires less equipment, the second method provides greater sensitivity. However, greater sensitivity might be obtained by alternative detection methods applicable to ion chromatography, which do not require the use of a suppressor.

3.6 POTENTIOMETRIC DETECTION IN ION CHROMATOGRAPHY

Potentiometric detectors measure potential difference at the surface of an electrode in equilibrium with contacting solution with respect to a reference electrode. The potential of the electrode is related to the concentration of ion(s) in the solution in accordance with the Nernst equation. The measurement these potential changes provides a method of determining ion concentrations. A potentiometric cell incorporates the indicator electrode which monitors the activity of the species and a reference electrode that provides a constant potential relative to the indicator electrode potential.

Several kinds of indicator electrode have been used with the liquid chromatography system and many species have been monitored with a number of ISEs,[59-64] but the high selectivity, slow response and non-availability of commercial detectors are the main drawbacks with detectors employing ISEs. Further drawbacks which exist with some indicator electrodes in flowing solutions are

non-linear response and poor baseline stability. This instability arises because the electrode is used under conditions where the concentration of the active solute in the flowing solution is zero. It is often advantageous if a low background level of an electroactive solute is added to the eluent in order to give a stable baseline electrode potential. Whilst this can be achieved by adding the solute ion to the eluent, it brings a number of disadvantages: samples with levels of the solute less than that present in the eluent will provide negative detector signals, whilst those with concentrations similar to this level cannot be detected at all.[39]

Nevertheless, potentiometric detection with ion selective electrodes might be associated with the following characteristics: i) simplicity of the instrumentation and cell design. ii) the electrode potential is not highly affected by eluent concentration and flow-rate. iii) with liquid membrane electrodes, baseline stability is good and response time is short. iv) the existence of a resistance between indicator electrode and reference electrode is not critical, the reference electrode can be placed remotely from the indicator electrode, because the electrical contact between the two electrodes is maintained via the flowing solution.[46] v) they are relatively cheap, simple to operate, very sensitive and suitable for extreme miniaturization and hence on-column detection in very narrow columns.[65]

3.7 PRINCIPLES OF POTENTIOMETRIC DETECTION IN ION CHROMATOGRAPHY

The response of the indicator electrode is given by the Nernst equation.

$$E = const. + \left(\frac{2.303RT}{zF}\right) \log c$$

where E is the potential of the indicator electrode, and R, T and F have their usual meanings, and c is the concentration (strictly the activity) of the solute ion sensed by the electrode. The symbol "z" refers to the equivalents of charge of per mole of analyte for a membrane electrode. The potential change (ΔE)

accompanying a change in the concentration of the solute ion from c_1 to c_2 is given by:

$$\Delta E = + \left(\frac{2.303\ RT}{zF} \right) \log \frac{C2}{C1}$$

The equation implies a logarithmic electrode response profile as the concentration of the solute ion is increased. Linear response can also be found in the low concentration ranges typically encountered in chromatographic analysis.

3.8 CALIBRATION AND RESPONSE CHARACTERISTICS OF POTENTIOMETRIC DETECTORS IN ION CHROMATOGRAPHY

In chromatography, calibrations of the electrode potential versus solute concentration show either a linear or logarithmic relationship, depending on the concentration range studied. When the total analyte concentration at the electrode surface is very low, a log dependence of the electrode potential might not be observed. That is, the detector is operating in sub-Nernstian region.

A silver/silver electrode has been used with the acetate eluent, the response was reported to be linear in sub-ppm range, based on amounts injected.[66] On the other hand, the response of silver/silver chloride electrode has been reported to be neither linear or logarithmic.[67]

Detectors based on liquid membrane ISEs show peak shape characteristics which are dependent on the equilibrium of the ion-exchange membrane at the electrode surface. The peaks will be influenced by the concentration of the analyte injected and also by the nature of the eluent used. A liquid membrane electrode has been employed for detection of nanomole amounts of nitrite and nitrate, and a linear response over a range of 0-10 nanomol for nitrate and 0-3 nanomol for nitrite was reported.[61]

3.9 FLOW-CELLS

For successful potentiometric detection, a suitable flow-cell must be designed to accommodate the indicator and reference electrodes.

The performance of a detector can be assessed by the volume constant or cell volume, in which a solute remains for a time. Considering the dependence on the dispersion, the sensitivity of the indicator electrode can be greatly decreased with large volume cells and connector tube(s) that are part of the detector.
For chromatographic efficiency, it is necessary that the cell is placed as close as possible to the analytical column outlet whilst keeping the internal volume of the cell as low as possible to minimize the solute dispersion.[46]

A variety of flow-cells has been designed for efficient potentiometric detection of ions of interest in chromatography. A simple flow-cell configuration has been reported using flow-through cap over the end of the electrode producing a volume of about 5 µl.[61]

A cylindrical ISE has been accommodated in a flow-cell to obtain a volume of about 6 µl.[68] It is reported that the cell permits the reference electrode to be placed in close proximity to the indicator electrode, and it is possible to obtain reliable potentiometric readings.[69]

A cell design reported for the copper electrode is housed in a flow-cell of about 2 µl volume.[63]

3.10 FLOW INJECTION ANALYSIS

This analytical technique was introduced in 1975 by Ruzicka and Hansen.[70] Since then, it has found wide applications and a considerable literature has been accumulated.

The principle of the flow injection analysis (FIA) is based on the injection of a definite volume of a sample solution into a non-segmented carrier stream of a suitable solution. The injected sample forms a "zone" which begins to disperse and react with the carrier as it is transported towards a detector. The magnitude of the dispersion in a practical system is dependent on the operating parameters, including sample volume, tubing diameter and length, and flow-rate. By changing these parameters, the dispersion may be controlled, and this allows optimization of a flow-injection system for many diverse applications.

Limited dispersion is appropriate when FIA systems are used with ISEs as detectors. Sample dispersion decreases with decreasing flow-rate, and thus increases the sensitivity of the determination.

The basic components of an FIA system are a pump, a sample injection valve, an analytical manifold, and a detector and readout system. A simple FIA assembly is shown schematically in figure 3.

FIA has the following characteristics: i) it is very considerably faster than other analysis methods, e.g., chromatography. The output peaks are usually obtained within 30 s of sample injection. One of the significant parameters to control precision in FIA is the timing. This can be achieved using precise volumes of samples and reproducible flow-rates of pumping. ii) It consumes less reagent and normally less sample. iii) It is ready for use almost immediately after start-up, no time is required for the baseline to stabilize.

FIA is based on a combination of the following features: 1) sample injection, 2) controlled dispersion of the injected sample "zone" during its transport from the point of injection to the point of detection, which can be manipulated to suit exactly a given analytical procedure and 3) reproducible timing of all events within each assay cycle. This approach yields a highly reproducible readout even when the mixing is incomplete, the chemistry does not reach equilibrium, and the signal is transient. A more detailed discussion of FIA theory and practice may be found.[71,72]

figure 3.

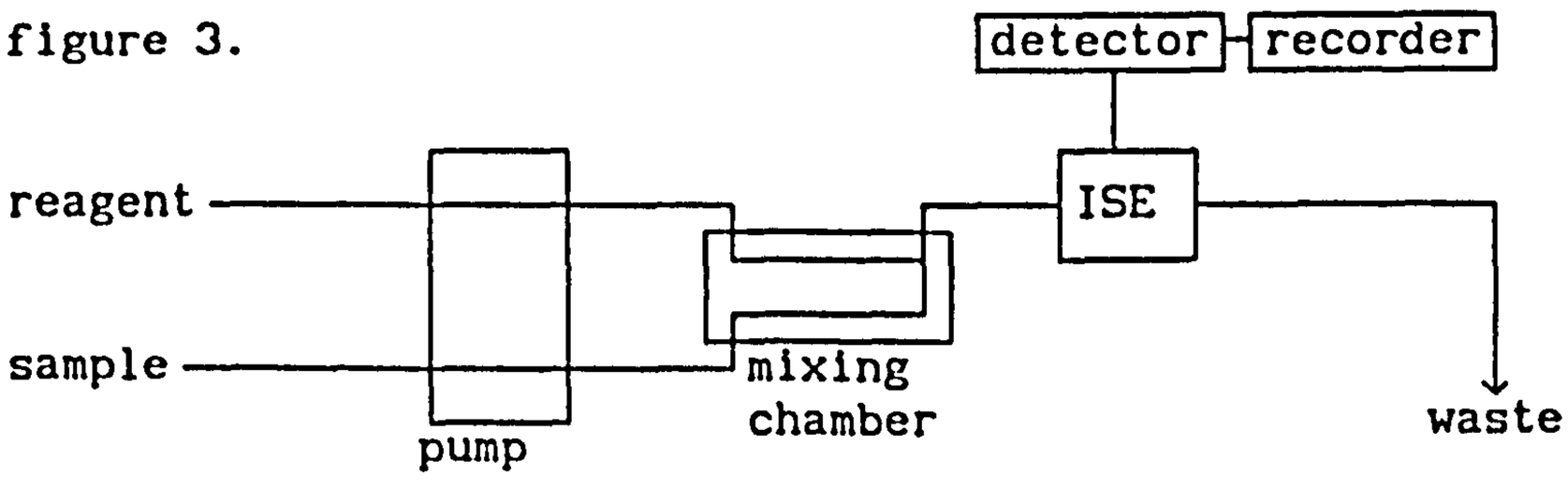


Figure 3. A basic diagram of a flow-injection system incorporating ISE.

3.11 REFERENCES

1. Horvarth C., Melonder W. and Molnar I., *J. Chromatogr.*, 1976, 125, 129.

2. Knox J.H., "*Practical High Performance Liquid Chromatography*" Simpson C.F. Ed., Heyden and Son Ltd., 1976.

3. Scott R.P.W., "*Practical High Performance Liquid Chromatography*", Simpson C.F. Ed., Heydon and Son Ltd., 1976.

4. Synder L.R., *Chromatogr. Rev.*, 1965, 7, 1.

5. Heftmann E., "*Chromatography*", 5th Ed., Elsevier, Amsterdam, 1992.

6. Small H., Stevensen T.S. and Baumann W.C., *Anal. Chem.*, 1975, 47, 1801.

7. Harrison K. and Burge D., *Abstracts Pittsburgh Conference on Analytical Chemistry*, 1979, 301.

8. Gjerde D.T., Fritz J.J. and Schmuckler G., *J. Chromatogr.*, 1979, 186, 509.

9. Rocklin R.D., *J. Chromatogr.*, 1991, 546, 175.

10. Wittmer D.P., Nuessle N.O. and Haney Jr W.G., *Anal. Chem.*, 1975, 47, 1422.

11. Water Associates, "*Paired Ion Chromatography; an Alternative to Ion-Exchange*", Milford, Mass, 1975.

12. Knox J.H and Laird G.R., *J. Chromatogr.*, 1976, 122, 17.

13. Cassidy R.M. and Elchuk S., *Anal. Chem.*, 1982, 54, 1558.

14. Barkley D.J., Blanchette M., Cassidy R.M. and Elchuk S., *Anal. Chem.*, 1986, 58, 2222.

15. Papp E. and Fehervari A., *J. Chromatogr.*, 1988, 447, 325.

16. Knox J.H. and Laird G.R., *J. Chromatogr.*, 1976, 122, 17.

17. Tomlinson E., Jefferies T.M. and Riley C.M., *J. Chromatogr.*, 1978, 159, 315.

18. Bidlingmeyer B.A., Deming S.N., Price W.P., Sachok B. and Petrusek M., *J. Chromatogr.*, 1979, 186, 419.

19. Rotsch T.D. and Pietrzyk D.J., *J. Chromatogr. Sci.*, 1981, 19, 88.

20. Rich W., Smith F., McNeil L. and Sidebottom T., "*Ion Chromatographic analysis of Environmental Pollutants*" (Mulik J.D. and Sawicki E. Eds.), Ann Arbor Science, Ann Arbor, Michigan, 1979.
21. Pohl ,C. A. and Johnson E.L., *J. Chromatogr. Sci.*, 1980, 18, 442.
22. Rich W., Johnson E., Lois L., Stafford B., Kabra P. and Marton L., "*Liquid Chromatography in Clinical Analysis*" (Marton L and Kabra P. Eds.), Humana Press, Clifton, NJ, 1981.
23. Dionex Corp. Sunnyvale, CA, "*The Capabilities Edge in Ion Analysis*", LPN 32213, 1982.
24. Jupille T., Gray M., Black B. and Gould M., *Amer. Lab.*, 1981, 13, 80.
25. Taraszewski W.J., Pitluck M.R., Haworth D.T. and Pollard B.D., *Abstracts, Pittsburgh Conference*, Atlantic City, New Jersey, Paper No:851, 1983.
26. Helfferich F., *Nature*, London, 1961, 189, 1001.
27. Johnson E.L, in "*Ion Chromatography*" (Tarter J.G. Ed.), Marcel Dekker Inc., New York, 1987.
28. Stillian J., *LC*, 1985, 3, 802.
29. Roston D.E. and Kissinger P.T., *Anal. Chem.*, 1982, 54, 429.
30. Dionex Technical Note, on "*Carbonhydrates by Dionex Ion Chromatography*", LPN 0320846, 11/1987.
31. Haddad P.R. and Foley R.C., *Anal. Chem.*, 1989, 61, 1435.
32. Jackson P.E. and Haddad P.R., *J. Chromatogr.*, 1986, 335, 87.
33. Imanari T., Tanabe S., Toida T. and Kawanishi T., *J Chromatogr.*, 1982, 250, 55.
34. Smith D.L. and Fritz J.S., *Anal. Chim. Acta*, 1988, 204, 87.
35. MacDonald J.C., *Amer. Lab.*, 1979, 11, 45 36. Pohl C.A.and Johnson E.L., *J. Chromatogr. Sci.*, 1980, 18, 442.
37. Smith Jr. F.C. and Chang R.C., *CRC Crit. Rev. Anal. Chem.*, 1980, 9, 197.
38. Jupille T., Burge D. and Togami D., *Res. Dev.*, 1981, 13, 80, 83.
39. Schwarzenbach R., *J. Chromatogr.*, 1982, 251, 339.
40. Fritz J.S., *LC*, 1984, 2, 446.
41. Fritz J.S., *Anal. Chem.*, 1987, 59, 335A.

42. Dasgupta P.K., *Anal. Chem.*, 1992, 64, 775A.
43. Smith F.C.Jr and Chang R.C., "*The Practice of Ion Chromatography*", Wiley, New York, 1982.
44. Mulik J.D. and Sawicki E., in "*Ion Chromatographic Analysis of Environmental Pollutants*", Ann Arbor Science, Ann Arbor, Michigan, 1978.
45. Tarter J.G., "*Ion Chromatography*", Marcel Dekker, New York, 1987.
46. Haddad P.R. and Jackson P.E., "*Ion Chromatography*", Elsevier, Amsterdam, 1990.
47. Small H., "*Ion Chromatography*", Plenum Press, New York, 1989.
48. Frank C.S. and Richard C.C., "*The Practice of Ion Chromatography*", Wiley, New York, 1983.
49. Fritz J.S., Gjerde D.J. and Pohlandt C., "*Ion Chromatography*", Huethig, Heidelberg, 1982.
50. Jonsson J.K., "*Chromatographic Theory and Basic Principles*", Dekker, New York, 1987.
51. Jenke D. R., *Diss. Abs. Int. [B]*, 1983, 44(1).
52. Iskandarni Z., *Diss. Abs. Int. [B]*, 1982, 43(4), 1088.
53. Sandra P., *J. High Res. Chromatogr.*, 1989, 12, 82.
54. Sandra P., *J. High Res. Chromatogr.*, 1989, 12, 275.
55. Jenke D.R., *Anal Chem.*, 1984, 56, 2674.
56. Weis J., "*Handbook of Ion Chromatography*", Dionex Corp., Sunnyvale, California, 1986.
57. Jupille T., "*Ion Chromatography*" (Tarter J.G. Ed.), Marcel Dekker, New York, 1987.
58. Tarter J.G., Johnson E.L. and Jupille T., in "*Ion Chromatography*" (Tarter J.G. Ed.), Marcel Dekker, New York, 1987.
59. Butler E.C.V. and Gershey R.M., *Anal. Chim. Acta*, 1984, 164, 153.
60. Muller H. and Scholz R., in *Ion Selective Electrodes, ACS Anal. Chem. Symp. Series*, 1984, 22, 4.
61. Schultz F.A. and Mathis D.E., *Anal. Chem.*, 1974, 46, 2253.
62. Suzuki K., Aruga A. and Shirai T., *Anal. Chem.*, 1983, 55, 2011.

63. Alexander P.W., Trojanowicz M. and Haddad P.R., *Anal. Lett.*, 1984, 17, 309.

64. Lockridge J.E., Fortier N.E., Schmuckler G. and Fritz J.S., *Anal. Chim. Acta*, 1987, 192, 41.

65. Manz A. and Simon W., *Anal. Chem.*, 1987, 59, 74.

66. Franks M.C. and Pullen D.L., *Analyst*, 1974, 99, 503.

67. Lockridge J.E., Fortier N.E., Schmuckler G. and Fritz J.S., *Anal. Chim. Acta*, 1987, 192, 41.

68. Keuken M.P., Slanina J., Jongejan P.A.C. and Baker F.F., *J. Chromatogr.*, 1988, 439, 13.

69. Shintani H. and Dasgupta P.K., *Anal. Chem.*, 1987, 59, 802.

70. Ruzicka J. and Hansen E.H., *Anal. Chim. Acta*, 1975, 78, 145.

71. Macdonald A.M.G., Pardue H.L., Townshend A. and Clerc J.T., *Anal. Chim. Acta*, 1986, 179(special issue).

72. Ruzicka J. and Hansen E.H., *Anal. Chim. Acta*, 1988, 214, 1.

4 INSTRUMENTATION AND TECHNICAL CONSIDERATION

4.1 INTRODUCTION

This chapter deals with experimental features common to all studies carried out, and apparatus used in potentiometric measurements, methods of calibration and chromatographic analysis.

4.2 CHEMICAL REAGENTS AND GLASSWARE

All experimental solutions were prepared using analytical grade chemicals and unless otherwise stated in deionized water. Normal cleaning procedures were adopted for the glassware to guarantee their cleanliness in the experiments. Specialized items of glassware used in the experiments will be described in the appropriate section..

4.3 CELL DESIGN

The potential of a single electrode cannot be measured, therefore one must consider not the ISE potential, but the potential difference of a cell containing the reference electrode, sample and ISE. The general electrochemical scheme is shown below.

Reference electrode||sample solution||ISE

Ion chromatography requires detectors with minimal dead volumes and suitable geometry of the flow channels. For a successful potentiometric detection, a suitable flow cell must be designed to accommodate the indicator and reference electrodes. The performance of a detector can be assessed by the volume constant, or cell volume, in which a solute remains for a time. The above considerations were taken into account for the design of potentiometric cells which will be described in the appropriate section.

4.4 ION SELECTIVE ELECTRODES

ISEs used in this research were home made and either flow-through or dip type. The construction and the use of the electrodes is described where appropriate. The composition of all membranes for each electrode used was similar to the following:

component	% composition
active material	4
PVC	67
plasticizer	28
potassium tetraphenyl borate(KTPB)	1

Tetrahydrofuran (THF) was used as solvent for preparation of all membranes. See appendix I for the preparation.

4.5 REFERENCE ELECTRODES

Two types of electrodes were used to supply a stable reference potential, the calomel and the silver/silver chloride (Ag/AgCl) electrodes.

4.5.1 THE CALOMEL ELECTRODE

For all experiments, the reference electrode was a porous plug double junction calomel electrode (Russell, Scot.). The second junction was filled with 3 mol dm^{-3} tetramethylammonium chloride (TMA-Cl) instead of KCl solution. The use of TMA-Cl solution as the source of Cl^- ions is appropriate because it gives rise to no leaking of KCl at the electrode assembly liquid junction with the flowing stream.

4.5.2 THE SILVER/SILVER CHLORIDE ELECTRODE

It is very reproducible and easy to make. It was employed as an inner reference electrode instead of the calomel electrode. The electrode has several advantages and has found extensive

applications in cells without liquid junctions. The Ag/AgCl electrodes used were prepared in this laboratory. See appendix II for the preparation.

4.6 BUFFER AMPLIFIERS

The membrane resistance of ISEs is very high, of the order of 10^4 megohm. It was therefore necessary for high impedance input instruments to be designed for general laboratory use. The input impedance of the buffer amplifier should be greater than that between the test solution and ISE. A circuit diagram for the single impedance buffer amplifier, manufactured in the Department of Chemistry workshops, University of Newcastle Upon Tyne is shown in figure 1. In a single impedance buffer amplifier, the membrane potential is dependent on the concentration of the primary ion, interferents and the activity effects e.g. changes in activity of the primary ion arising from changes in concentration of other ions. The latter may be avoided in flowing conditions by working with stream solutions at medium ionic strength e.g. using an eluent as an ionic strength adjuster which incorporates a salt showing small affinity toward the membrane electrode.

Since two working electrodes are operated in a simultaneous detection system, the buffer amplifier was designed with dual high impedance circuitry, which is shown in figure 2. The amplifier included a controllable offset so that it was possible to make measurements over a large range.

High impedance devices are very susceptible to electrostatic pick-up[1] and therefore it was necessary for good shielding of electrode systems; covering electrode leads with aluminium foil, and ensuring the operator does not wear nylon or woollen clothing.

4.7 PRETREATMENT OF ELECTRODES

All electrodes were conditioned by pretreatment in dilute solutions of primary ions prior to use as this has been shown to reduce the response time of membranes. Then they were generally used without any further treatment for months.

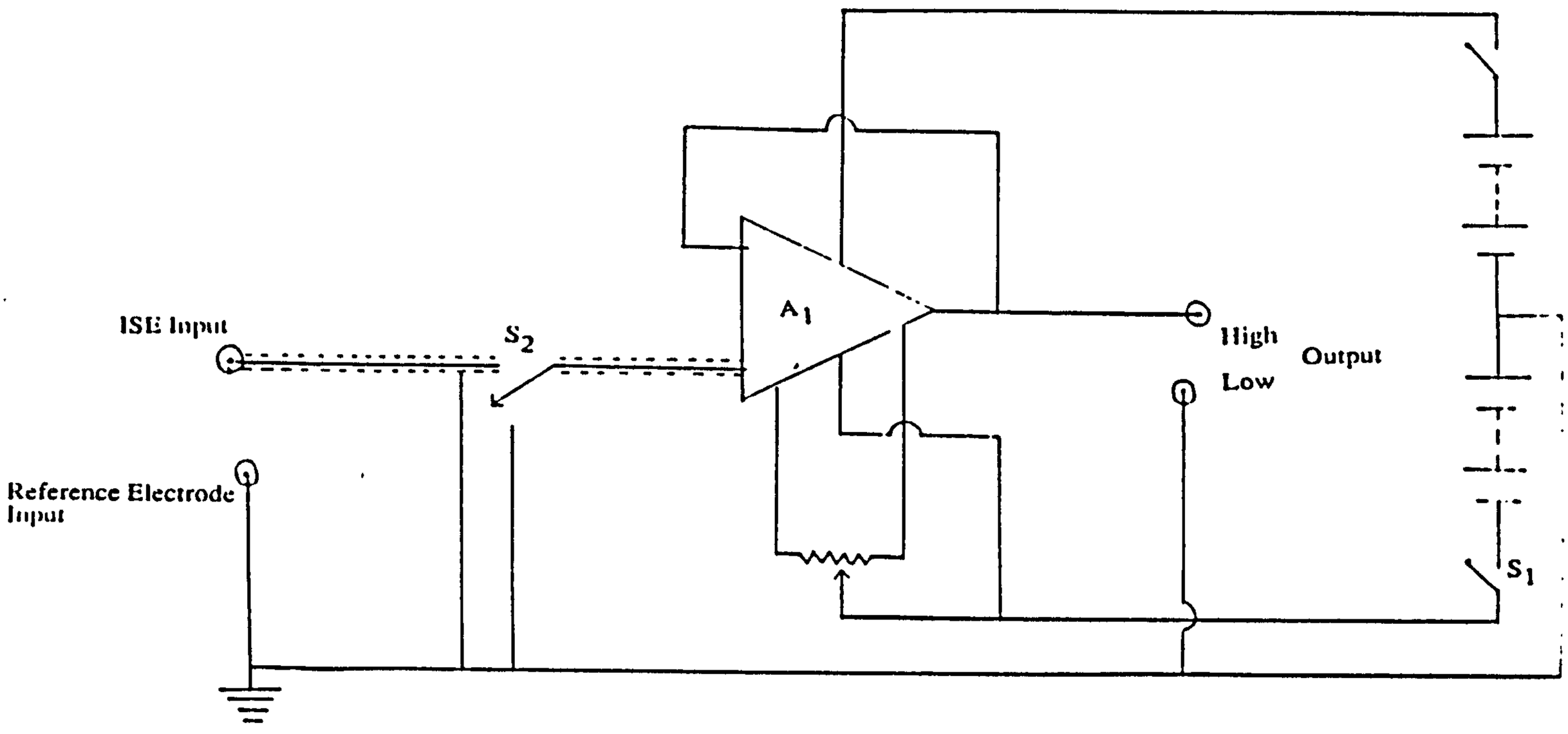


Figure 1. Circuit diagram of a single input high impedance buffer amplifier.

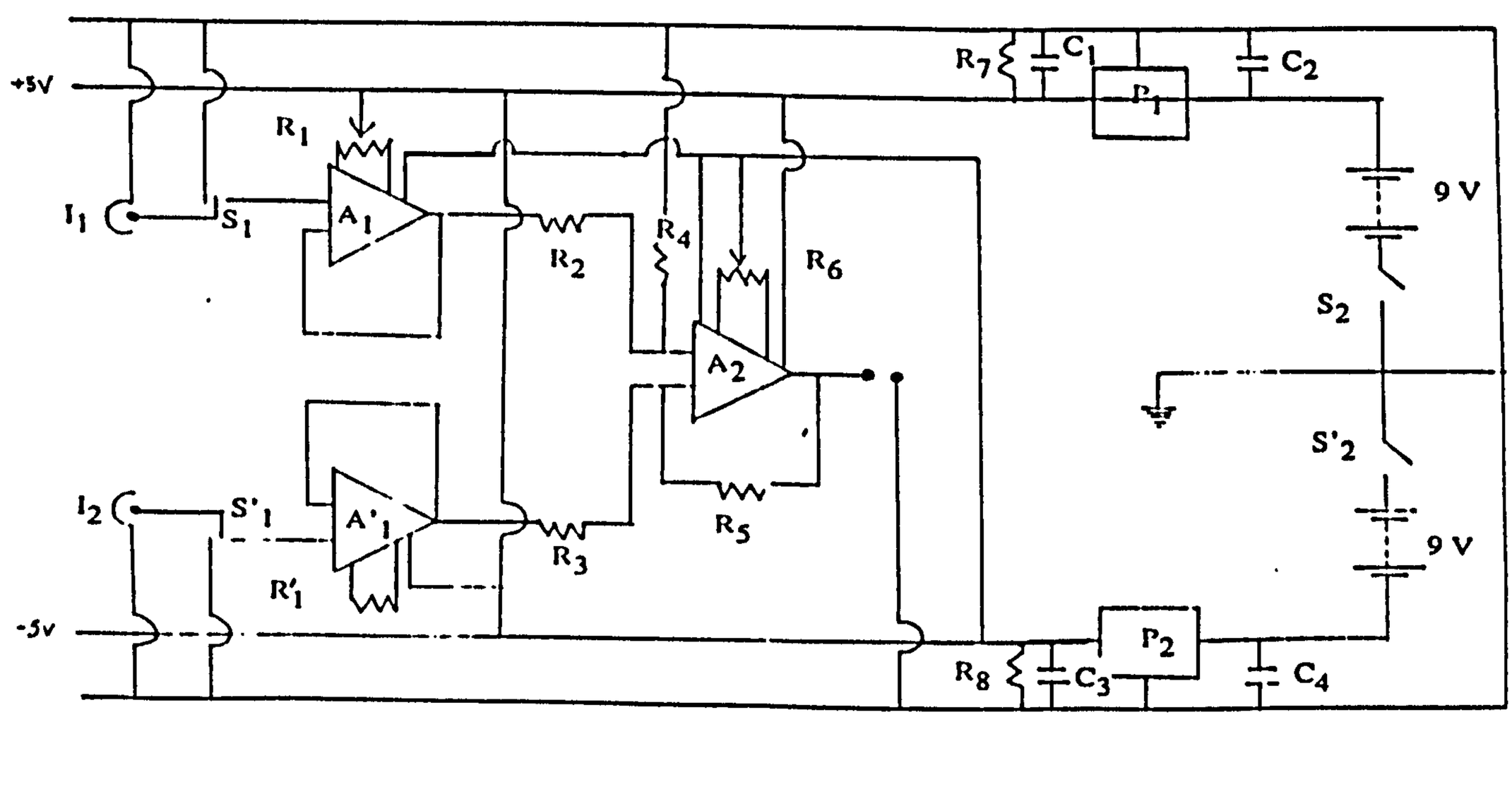


Figure 2. Circuit diagram of a dual input high impedance buffer amplifier.

Annotation for Figure 2.

The Circuit Diagram for a Double High Impedance Input Differential Amplifier.

I_1, I_2	Two high impedance inputs
S_1, S'_1	Double pole double throw toggle switch
S_2, S'_2	Double pole single throw toggle switch
A_1, A'_1	High performance dual F.E.T. amplifier (R.S. Component no. 305-939)
A_2	High performance single F.E.T. amplifier (R.S. Component no. 305-945)
P_1	Fixed Voltage - 100 mA regulator (R.S. Component 78L05, output voltage +5 V (+5%))
P_2	Fixed Voltage - 100 mA regulator (as above)
$R_1 = R_2$	20-turn cermet trimmer, 10 KΩ
$R_2 = R_3 = R_4 = R_5$	10 KΩ (matched to < 1%)
R_6	20-turn cermet trimmer, 10 KΩ
$R_7 = R_8$	4.7 KΩ
$C_1 = C_3$	0.47×10^{-6} Farad capacitor
$C_2 = C_4$	0.22×10^{-6} Farad capacitor

4.8 BEAKER TO BEAKER STUDIES

This is the most commonly used method for calibration and interference examination of ISEs. Most electrode evaluations described in the literature include calibration graphs produced from results with standard solutions prepared by serial dilution, occasionally to below 10^{6} mol dm^{-3}. Preliminary ISE calibration and detection limit studies were carried out with solutions 10^{-6} to 10^{-1} mol dm^{-3} of analytes. Serial analyses were carried out from least to most dilute solutions as ISE responses have been shown to be faster when the concentration jump occurs in this direction.[2] Interference studies were also performed by this method. The method is quick and simple and it was possible to reject a faulty electrode immediately.

4.9 FLOW METHODS

Ion selective electrodes are finding increasing application in continuous flow systems. A higher precision is normally obtainable with continuous flow than with dip methods, because of the greater standardization of the conditions in which the sample is presented to the electrode. Flow-injection and constant volume dilution methods were used in the studies on selectivity characteristics, response time and dispersion effects.

4.9.1 FLOW INJECTION SYSTEM

The dispersion process limits the spacing of samples and the resulting throughput rate in flow-injection, and results peak broadening in chromatographic analysis.

The response time of an electrode is one of the significant parameters in flow injection analysis and with non-selectivity in the chromatographic systems. Response times of the electrodes vary from one type to another depending on the membrane composition. It is, therefore, necessary to know such characteristics of the electrodes if they are to be used as detectors in an ion chromatography technique. Flow-injection is the most suitable technique to examine these characteristics,

which may allow predictions for some chromatographic requirements (i.e. flow-rate, resolution, separation time).

It is vital in FIA that a very reproducible flow-rate is maintained. Therefore, for flow-injection measurements, either a constant flow peristaltic pump (Crouse, Eng.), which was connected to a three-way valve and flow-through detector cell with PTFE tubing ca. 1 mm, or an HPLC pump (Perkin Elmer series 3) with a Rheodyne injection valve (Perkin Elmer 7125) were used. A schematic diagram of the system used is shown in figure 3. With the latter one, precise volumes of samples were introduced into the carrier stream via the injection valve using a Hamilton 100 µl injector. Ion selective electrodes fitted into a flow-through cell were incorporated into the continuous-flow system and the separate double junction calomel electrode was located in a reservoir downstream from the indicator electrode, so that the cell dead volume was kept as small as about 2 µl. All connection tubes were narrow PTFE (commonly 0.5-1.0 mm). If recorder tracings were noisy, all fittings and connections were checked to ensure that joints had been made properly.

It was a single line approach, where the injection valve and the flow-through detector are connected with a single channel. The single line mode technique is more practical and simpler to carry out because the mechanics of the flow system are simplified.[3] The set-up and the shut-down of the method are very rapid and reproducible detection at low concentrations was possible as the pump gave very reproducible flow-rates.

4.9.2 CONSTANT VOLUME DILUTION SYSTEM

The technique has been widely used as a calibration method. It is very simple and accurate. However, it is important that the values of experimental parameters are chosen carefully when using this method. That is, the magnitudes of volume dilution vessel used, the flow-rate of the solution and the stirring rate within the dilution vessel should be carefully considered.

The measuring system was set up as for a conventional constant volume dilution experiment replacing the peristaltic pump with the

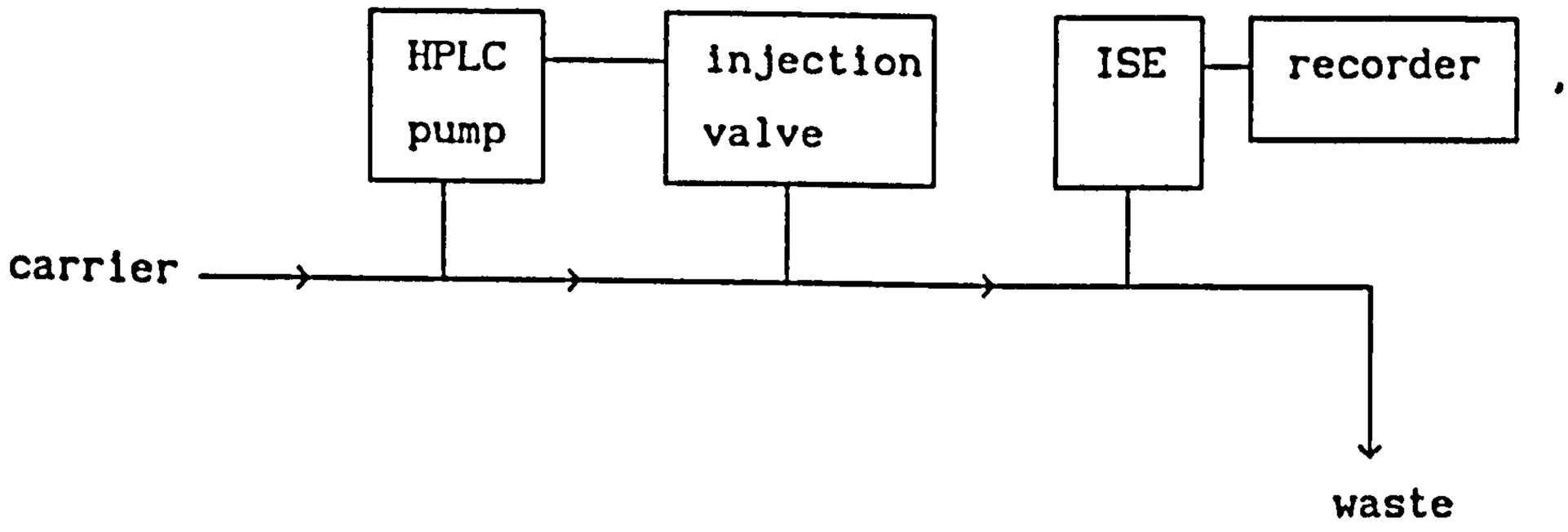


figure 3. Schematic diagram of the flow-injection system used.

HPLC pump (Perkin Elmer Series 3). The response of several ISEs with different flow cells was monitored on the buffer amplifier and recorded on the chart recorder (SE 120 BBC model). The constant volume dilution vessel was made from a water jacketed glass tube with a B19 ground glass joint and a diagram is shown in figure 4. The volume of the dilution vessel used was determined accurately by mass (using deionized water) and the flow-rate used was 6 ml min^{-1}. 10^{-1} mol dm^{-3} solutions of primary ions were used for all calibrations taken.

4.10 HPLC APPARATUS

The HPLC is the most used system for single ion chromatography techniques. In this research, the HPLC system used was a Perkin Elmer Series 3 pump module. The system contains two 6000 psi reciprocating pumps and a Rheodyne injection valve. Each pump was capable of pumping at a rate of from 0.1 to 30.0 ml min^{-1}. The pump performance specifications were as following: Flow-rate accuracy: ±2.5% at 1 ml min^{-1} at 3.45 MPa (500 psi). Pressure flow capability: 8 ml min^{-1} ±10% at 34.5 MPa (5000 psi). Pump materials in direct contact with solution stream: 316 stainless steel, PTFE, glass, gold/nickel alloy and sapphire. The Rheodyne injection valve used was a syringe loading injector valve module from Perkin Elmer (7125). The valve injects a desired volume of sample into the stream pumped. Samples were introduced directly by a Hamilton 100 µl sample injection syringe.

The flow-rate of each pump was controlled by a microprocessor incorporated in the instrument. Three modes of operation can be permitted: independent mode, flow program and solvent program. Through the research, independent mode was the only one used as all separations were performed by using a single eluent or a mixture of two eluents based on previous trials.

4.11 ION CHROMATOGRAPHY APPARATUS

Ion chromatography apparatus used in this research is described in **chapter 8.**

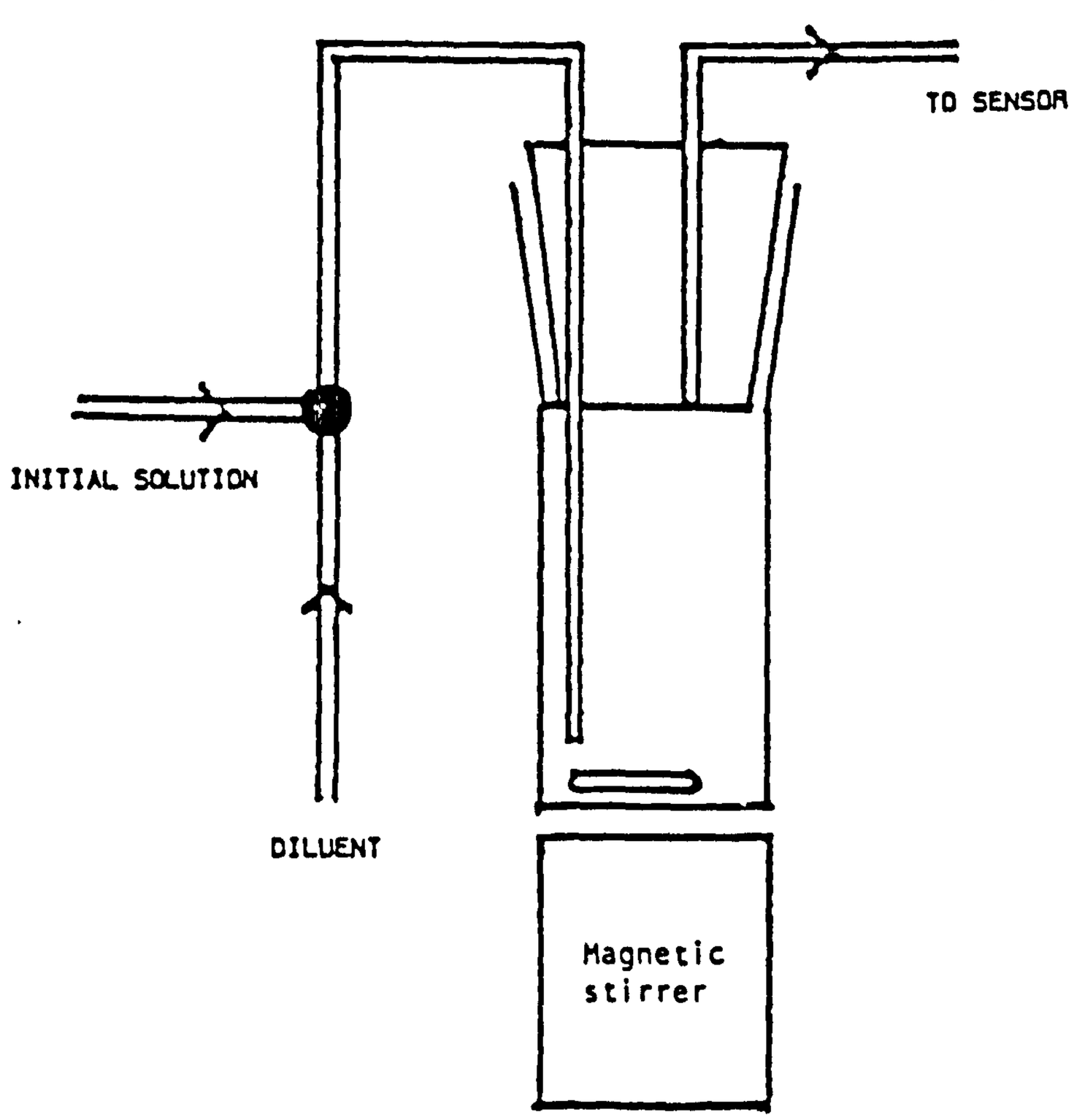


figure 4. Constant volume dilution cell

4.12 COLUMNS

The columns used through the chromatographic measurements were IonPac-AS4A (250 x 4 mm) analytical and AG4A (100 x 4 mm) guard anion-exchange, and IonPac-CS3 (250 x 4mm) analytical and (100 x 4 mm) guard cation-exchange columns from Dionex.

4.13 THE CHOICE OF SUITABLE COLUMN

The first point to be made is that the detection mode employed in ion chromatography is the chief factor which determines the type of eluent and column used. Conductivity detection gives excellent sensitivity when the conductance of the eluted solute ion is measured in an eluent of low background conductance. Similarly potentiometric detection gives excellent selectivity when the potential of the eluted ion is measured in an eluent of low background potential. This suggested that more diluted eluents should be preferred, and that in order for such eluents to act as effective competing ions, the ion-exchange capacity of the column should be low when conductivity or potentiometric detection are used. The stationary phases in HPLC are typically derived from silica substrates. The instability of silica in pH extremes is well known. Therefore the column should be stable throughout the entire pH range (0 to 14). This pH stability allows eluents of extreme pH values to be used, and samples of any pH to be injected directly without cleanup. For the sensitivity and the reproducibility of the detection method to be examined, the performance and the reproducibility of the column should be high. All these requirements can be met with Dionex HPIC-AS4A and HPIC-CS3 analytical and guard columns, and they were successfully employed for potentiometric detection of anions and cations in ion chromatography.

4.14 REFERENCES

1. Edmonds T., *"Chemical Sensors"*, Chapter 1, Blackie, Glasgow, 1987.

2. Tóth K., Gavaller I. and Pungor E., *Anal. Chim. Acta*, 1971, 52, 131.

3. Ruzička J. and Hansen E.H., *Anal. Chim. Acta*, 1988, 214, 1.

CHAPTER 5

5. SELECTIVITY AND DETECTION LIMIT CHARACTERISTICS OF PVC MEMBRANE ION SELECTIVE ELECTRODES EXAMINED FOR ION CHROMATOGRAPHY MEASUREMENTS

5.1 INTRODUCTION

Availability of flow-through design of ISE cells allowing more rapid sample throughput and high selectivity has led to extensive use of ISEs in FIA. However, high selectivity towards a small number of ions is a main drawback for their use as detectors in ion chromatographic measurements.

Nevertheless, several types of ISEs have already found wide applications as detectors in IC.[1-3] Moreever, the detection limits for inorganic anions and cations obtained potentiometrically are reported to be better than those possible by any other detection method.[4]

Ion chromatographic systems require the use of less selective and more sensitive electrodes as detectors. Selectivity and detection limits of PVC-matrix membrane ISEs vary from one type to another depending on membrane composition and preparation. Hence, before they can be used as detectors in ion chromatography, such charecteristics must be examined under suitable conditions to obtain useful information.

This study describes determination of the selectivity, detection limit and reproducibility characteristics of PVC-matrix membrane chloride, bromide, nitrite, nitrate, sulphite, and hydrogen phthalate selective electrodes based on tetradodecylammonium salts (TDDA-) for anions, and of sodium and potassium selective electrodes based on dibenzo and dicyclo-18-crown-6 compounds for cations, by the mixed solution and flow-injection methods.

5.2 EXPERIMENTAL

5.2.1 Measuring System and Reagents

A pH ion meter (Corning pH Ion Meter 155) with a glass electrode

(Russell, Scot.) were used during the dip test measurements. A double junction calomel electrode (Russell, Scot.), containing 3 mol dm^{-3} tetramethylammonium chloride internal solution, was used as a reference electrode for the flow injection measurements.

The flow system consisted of a constant flow peristaltic pump (Crouse, Engl.), which was connected to a three-way valve and flow-through detector cell with ca. 1 mm PTFE tubing.

Potentiometric response was measured with a digital voltmeter (Thandar TM 45), a high input impedance buffer amplifier, an off-set box and two noise filters (Lab. made). A BBC SE 120 model chart recorder was used for recording of potential difference.

Several flow-through potentiometric cells, designed for use in flowing conditions, are shown in figure 1.

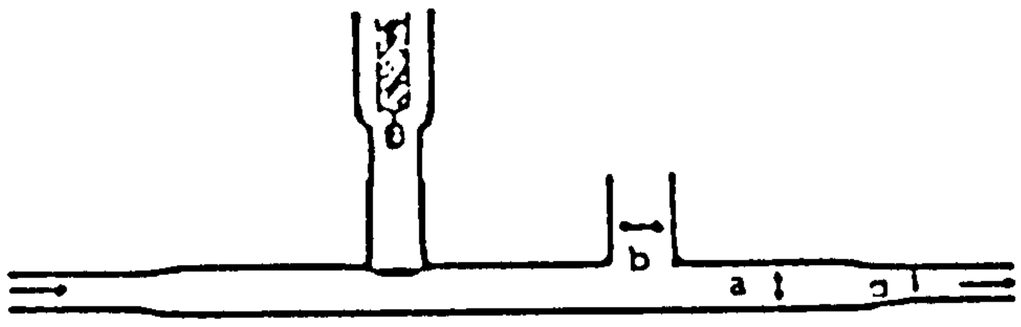
Potassium tetraphenylborate, TDDA-Br and PVC were from Fluka. Dibutyl phthalate (DBP) was from Aldrich and was used as plasticizer in anion selective membranes. Dioctyl sebacate (DOS) was from Sigma and was used as plasticizer in cation selective membranes. As ionophores, for anion selective membranes, TDDA-Cl, TDDA-NO_2, TDDA-NO_3, TDDA-SO_3 and TDDA-H-phthalate salts were obtained from TDDA-Br by repeated exchange with AnalaR grade inorganic salts in water-chloroform phases, and then recrystallised from ethanol. Dicyclo-18-crown-6-NaI and -KI, dibenzo 18-crown-6-NaI, -KI and -KBr were prepared and used as ionophores for cation selective electrode membranes.

Inorganic and organic anions used in the test mixtures were in the potassium and sodium forms and were AnalaR grade. Inorganic and organic cations used in the test mixtures were in the chloride and bromide forms and were AnalaR grade.

0.001 and 0.01 mol dm^{-3} solutions of sulphate and 0.005 mol dm^{-3} solution of TEA-Cl were used as carriers during anion and cation measurements respectively.

Individual standards were prepared in the carrier solutions to ensure the results were obtained with the response conditions as similar as possible to the ion chromatographic system.

The mixed solution method was applied for selectivity coefficient measurements.



Appearance from the side

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b:7mm
c:10mm
d:2mm

Appearance from above

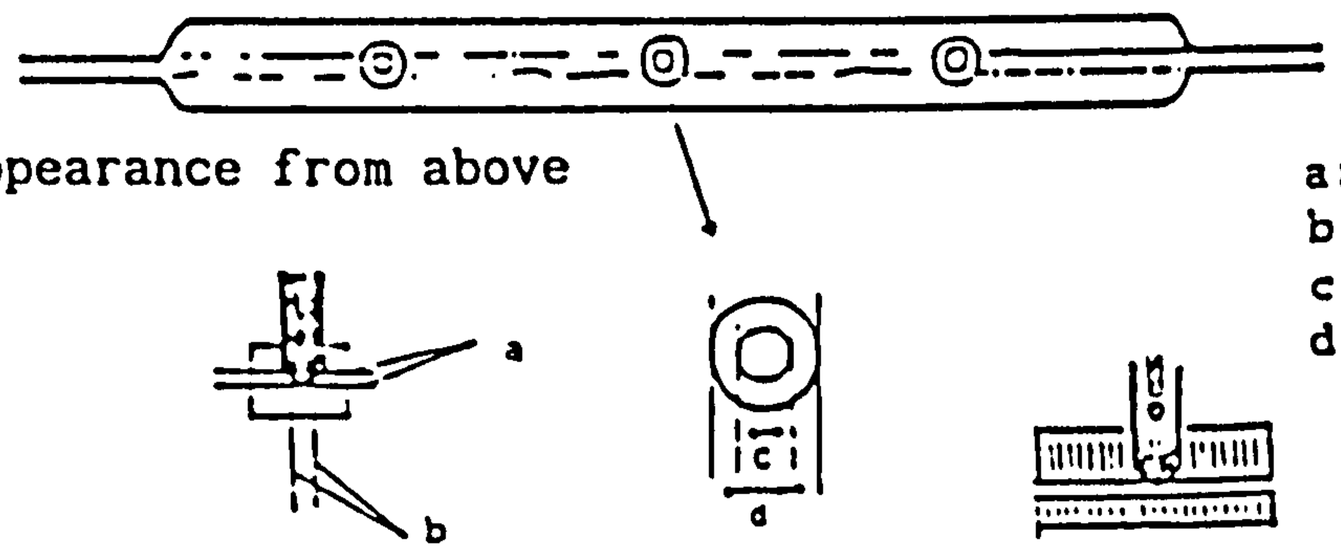


Figure 1. Potentiometric cells designed for use in flow injection and chromatographic systems.

5.2.2 Preparation of Electrodes

The electrodes were fabricated in a similar manner to that described by Griffiths, Moody and Thomas.[5] Accurately weighed high molecular weight PVC, plasticizer and ionophore were dissolved in freshly distilled tetrahydrofuran (THF) (see Appendix A for details of this distillation).

In general, 28 wt% PVC, 68 wt% plasticizer and 4 wt% ionophore were used. The mixture was shaken for a minimum of 12 h on a mechanical shaker, (Junka & Kunkel, Ika., Vibrax VXR, supplied by S.H. Scientific, Blyth Northumberland), before casting the membrane, by pipette, to ensure no air bubbles were introduced at this stage, into a circular PTFE mould. The diameter of the moulds was chosen so that the thickness of resulting membrane was ca. 1 mm. The THF solvent was allowed to evaporate slowly, taking approximately, 36 h, after which the PVC membrane was transfered from the mould using tweezers to a PTFE cutting plate where circular membrane discs of ca. 8 mm diameter were cut.

The electrode body consisted of a 6 mm glass tube with B9 Quickfit socket. A 3 cm section of 6 mm internal diameter PVC tubing was fixed to the glass tube using PVC cement, formed from PVC granules and THF. The end of the PVC tubing was made even by dipping the tube in THF and rotating the electrode body on a tile. This ensured a flat surface to which the membrane could be adhered. The membrane was attached to the PVC tube using PVC cement and then allowed to dry for 30 min. The seal of the electrode was tested. If the membrane seal was not airtight, then the membrane was removed, the PVC tube cleaned and the membrane re-fixed before repeating the procedure.

An internal filling solution, containing the species of interest, filled two-thirds of the assembly, and an internal silver/silver chloride reference electrode with B9 cone was placed in the electrode body (see Appendix B for the details for the preparation). The electrode was allowed to condition by dipping the membrane in a solution containing 10^{-2} mol dm^{-3} of the ion to be sensed for 12 h.

5.3 RESULTS AND DISCUSSION

Calibration curves obtained for six anion selective electrodes, based on PVC, are shown in figure 2. Detection limits and limit of Nernstian response of the electrodes are shown in table 1. Selectivity coefficients of five anion selective electrodes for inorganic anions are shown in table 2. Figure 3 shows peaks of several anions at low concentration levels, which were obtained with a bromide selective electrode in the flow injection system using 10 μl and 50 μl injection volumes of anion standards of interest. The anions were separately injected into 10^{-3} mol dm^{-3} solution of $SO_4^{=}$ as carrier at flow-rate a 1.5 ml min^{-1}. Anion peaks, by injection separately into the carrier, were obtained using a chloride selective electrode in the flow injection system and are shown in figure 4. Figure 5 shows the anion peaks obtained with a sulphide selective electrode in the flow injection system. Figure 6 shows the detection of anions with a nitrite selective electrode which was achieved at a higher flow-rate of 10^{-3} mol dm^{-3} solution carrier in the flow injection system. Samples with lower volumes can be detected with a bromide selective electrode in the flow injection system. The peaks obtained from injections of 5, 10 and 15 μl volumes of anion standard solution are shown in figure 7.

For capability and flexibility of the detection system, two groups of peaks of anions were obtained with two different membrane electrodes constructed in one potentiometric cell in the flow injection system as shown in figure 8. This sort of arrangement may also be possible for cation and anion detections simultaneously.

Figures 9 and 10 show calibration curve of seven cation selective electrodes based on neutral compounds. Detection limits and limits of Nernstian response of the electrodes are shown in table 3.

Detection of five cations, injected separately into $5x10^{-3}$ mol dm^{-3} TMA^{+} solution as carrier, was achieved using a sodium selective electrode in the flow injection system, as shown in

figure 11. Figure 12 shows five peaks of cations, injected separately in the carrier at flow-rate 5 ml min^{-1}, which were obtained using a sodium selective electrode based on dibenzo-18-crown-6-NaI in the flow injection system. Figure 13 shows the response of a sodium selective electrode based on dicyclohexyl-18-crown-6-NaI for cations injected separately into the carrier at flow-rate 3.5 ml min^{-1}.

The detection of cations in the flow injection system was achieved using a potassium selective electrode, based on a mixture of dicyclohexyl-18-crown-6-KI and dibenzo-18-crown-6-KI. Using this method, a more general response can be obtained in ion chromatography of anions and cations separately.

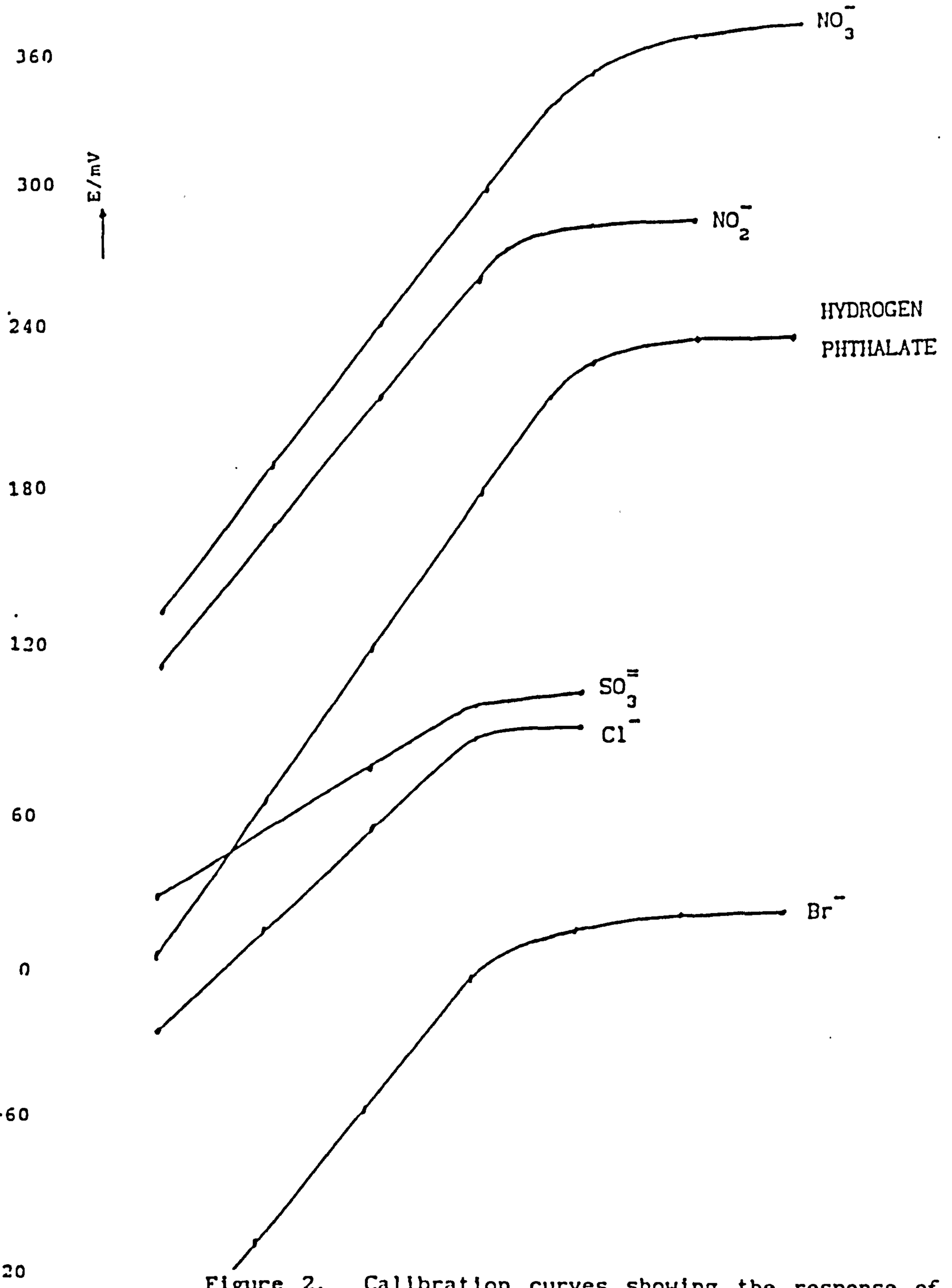


Figure 2. Calibration curves showing the response of anion selective electrodes based on PVC to varying concentrations of their primary ions.

selective electrodes based on PVC.[a]

determinant	active material	limit of Nernstian range (mol L^{-1})	detection limit[b]
Cl^-	TDDA-Cl	10^{-4}	$5x10^{-5}$
Br^-	TDDA-Br	$5x10^{-5}$	10^{-5}
NO_2^-	TDDA-NO2	$6x10^{-5}$	$2x10^{-5}$
NO_3^-	TDDA-NO3	$2x10^{-5}$	10^{-6}
$SO_3^{=}$	$TDDA_2-SO_3$	10^{-4}	$5x10^{-5}$
H-phthalate$^-$	TDDA-H-pht.	$2x10^{-5}$	10^{-6}

a) All measurements were made via the dip test.

b) The values were determined graphically.

Table 2. Selectivity coefficients of liquid membrane anion selective electrodes based on PVC.

electrode	interferent	concentration of interferent (mol L^{-1})	selectivity coefficient
bromide	Cl^-	10^{-3}	$0.2x10^{-5}$
	NO_2^-	10^{-3}	$0.8x10^{-4}$
	NO_3^-	10^{-4}	$0.5x10^{-3}$
	F^-	10^{-2}	$0.2x10^{-5}$
	$SO_4^{=}$	10^{-1}	$0.3x10^{-4}$
	$CO_3^{=}$	10^{-1}	10^{-5}
chloride	Br^-	10^{-3}	$0.5x10^{-5}$
	NO_2^-	10^{-3}	$0.8x10^{-2}$
	NO_3^-	10^{-4}	0.2
	F^-	10^{-1}	$0.2x10^{-3}$
	$SO_4^{=}$	10^{-1}	$0.2x10^{-4}$
	$CO_3^{=}$	10^{-1}	10^{-5}
Nitrite	Cl^-	10^{-3}	$0.5x10^{-3}$
	Br^-	10^{-3}	$1.2x10^{-3}$
	NO_3^-	10^{-4}	$1.5x10^{-1}$
	I^-	10^{-5}	$1.2x10^{-1}$
	$SO_4^{=}$	10^{-1}	$0.2x10^{-3}$

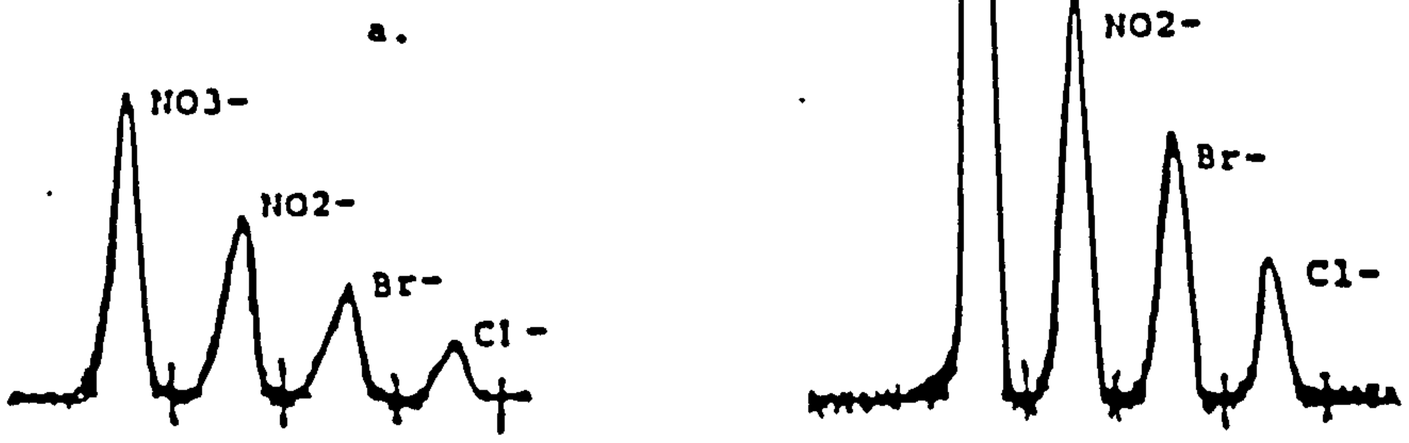


Figure 3. Peaks of anions from separate injections, obtained using the bromide selective electrode in the flow injection system with 10^{-3} mol dm^{-3} $SO_4^{=}$ solution as carrier at flow-rate 1.5 ml min^{-1}. Injections: a) 10 µl b) 50 µl of 0.1 mmol dm^{-3} standard solution of each anion.

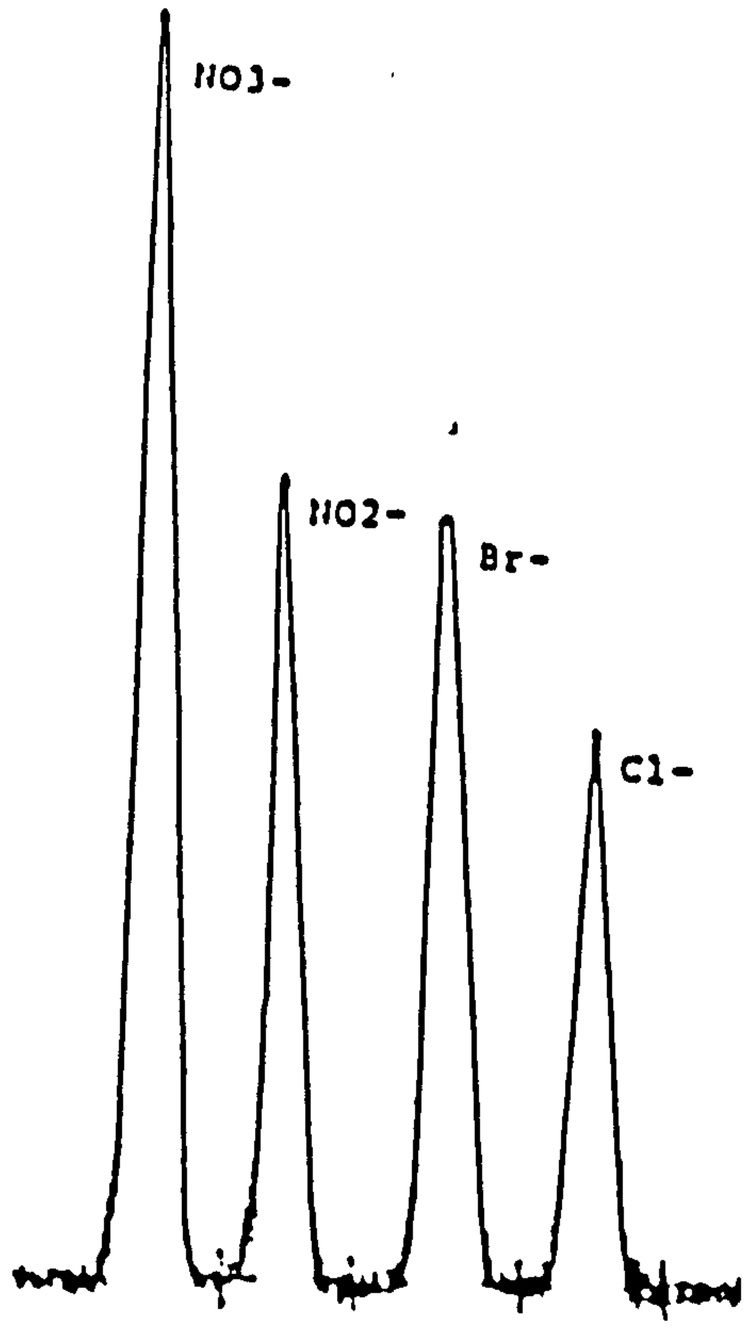


Figure 4. Anion peaks obtained using the chloride selective electrode in the flow injection system with 10^{-3} mol dm^{-3} $SO_4^{=}$ solution as carrier at flow-rate 1.3 ml min^{-1}. Injections: 10 µl of 10^{-4} mol dm^{-3} standard solution of each anion.

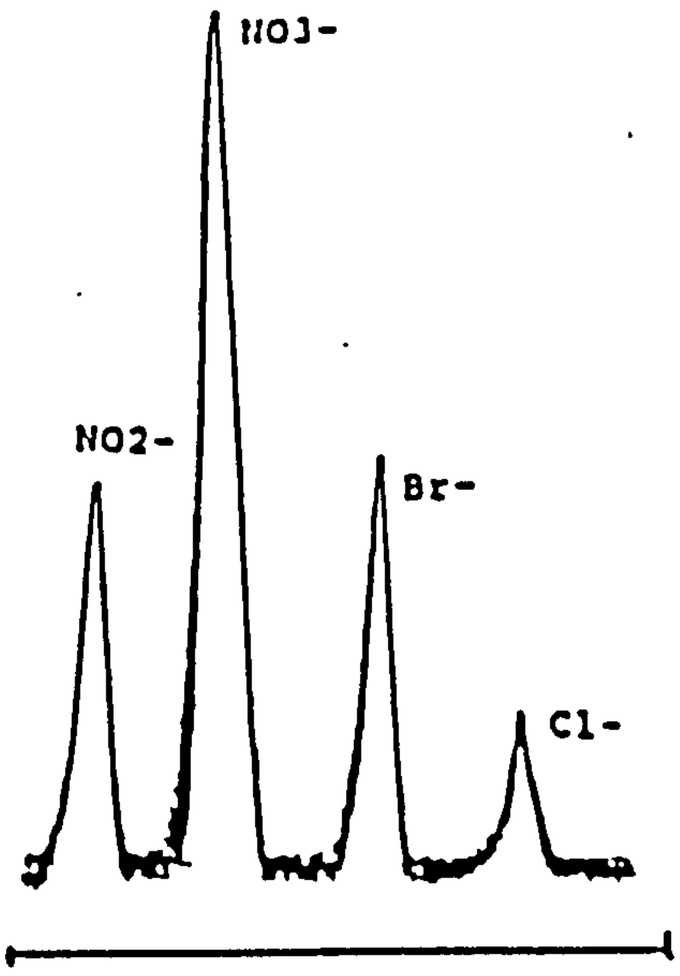


Figure 5. Anion peaks obtained using the sulphite selective electrode in the flow injection system with 10^{-2} mol dm^{-3} $SO_4^{=}$ solution as carrier at flow-rate 1.9 ml min^{-1}. Injections: 100 µl of 10^{-4} mol dm^{-3} standard solution of each anion.

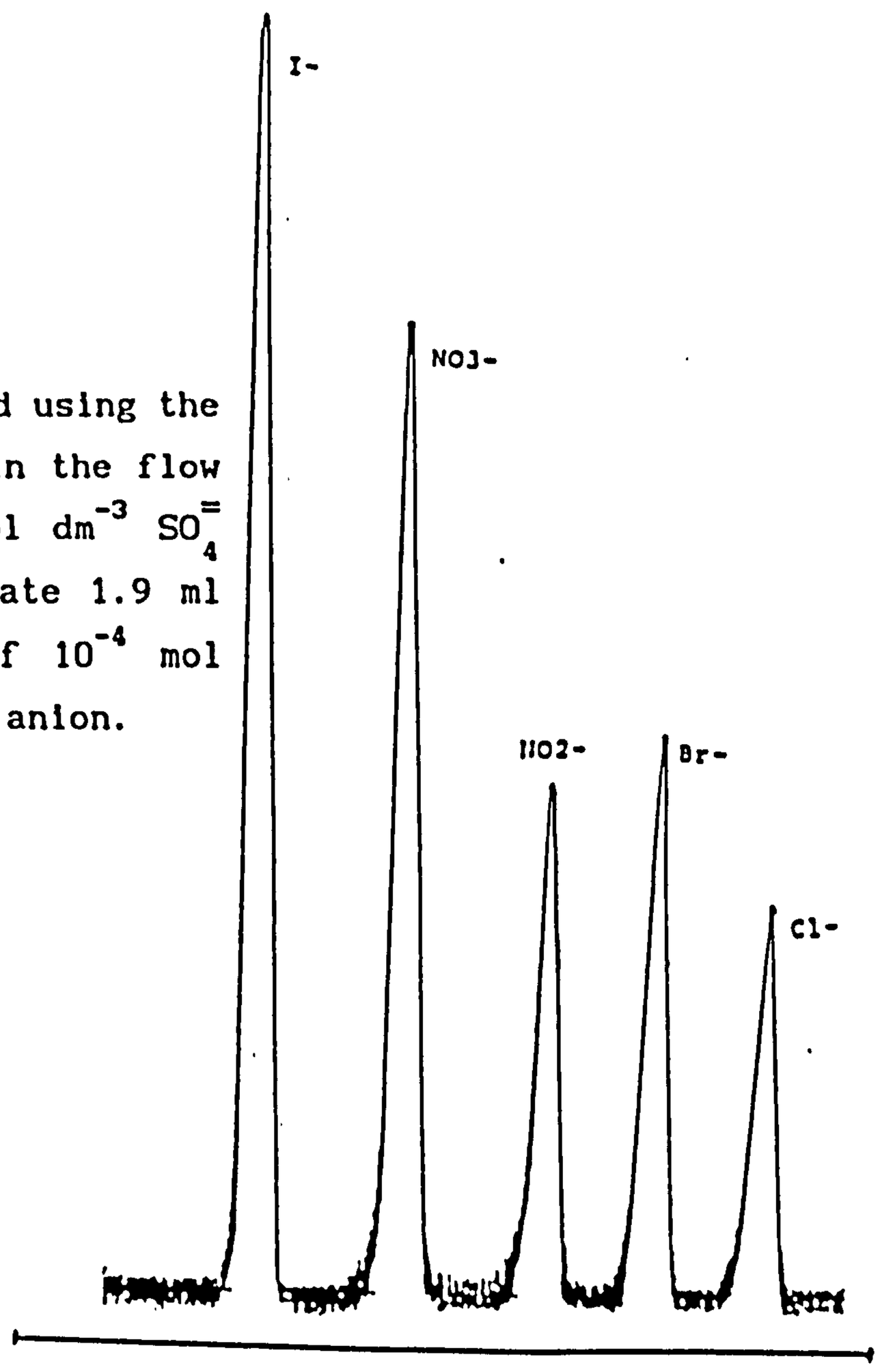


Figure 6. Anion peaks obtained using the nitrite selective electrode in the flow injection system with 10^{-2} mol dm^{-3} $SO_4^{=}$ solution as carrier at flow-rate 5 ml min^{-1}. Injections: 100 µl of 10^{-4} mol dm^{-3} standard solution of each anion.

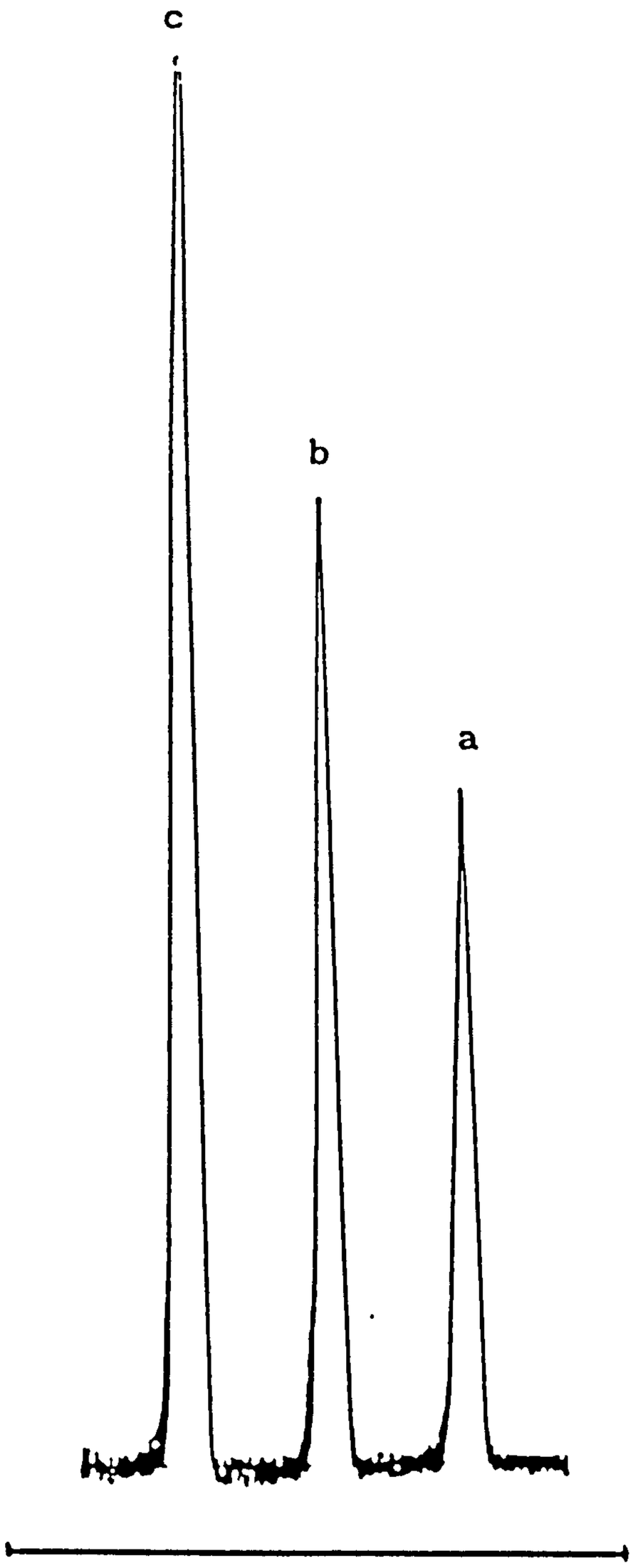


Figure 7. Potentiometric response of the bromide selective electrode at low volumes of anion standard solutions, carrier: 10^{-3} mol dm^{-3} solution of $SO_4^{=}$, flow-rate: 1.5 ml min^{-1}, injections: a) 5 µl, b) 10 µl, c) 15 µl of 10^{-3} mol dm^{-3} solution of bromide.

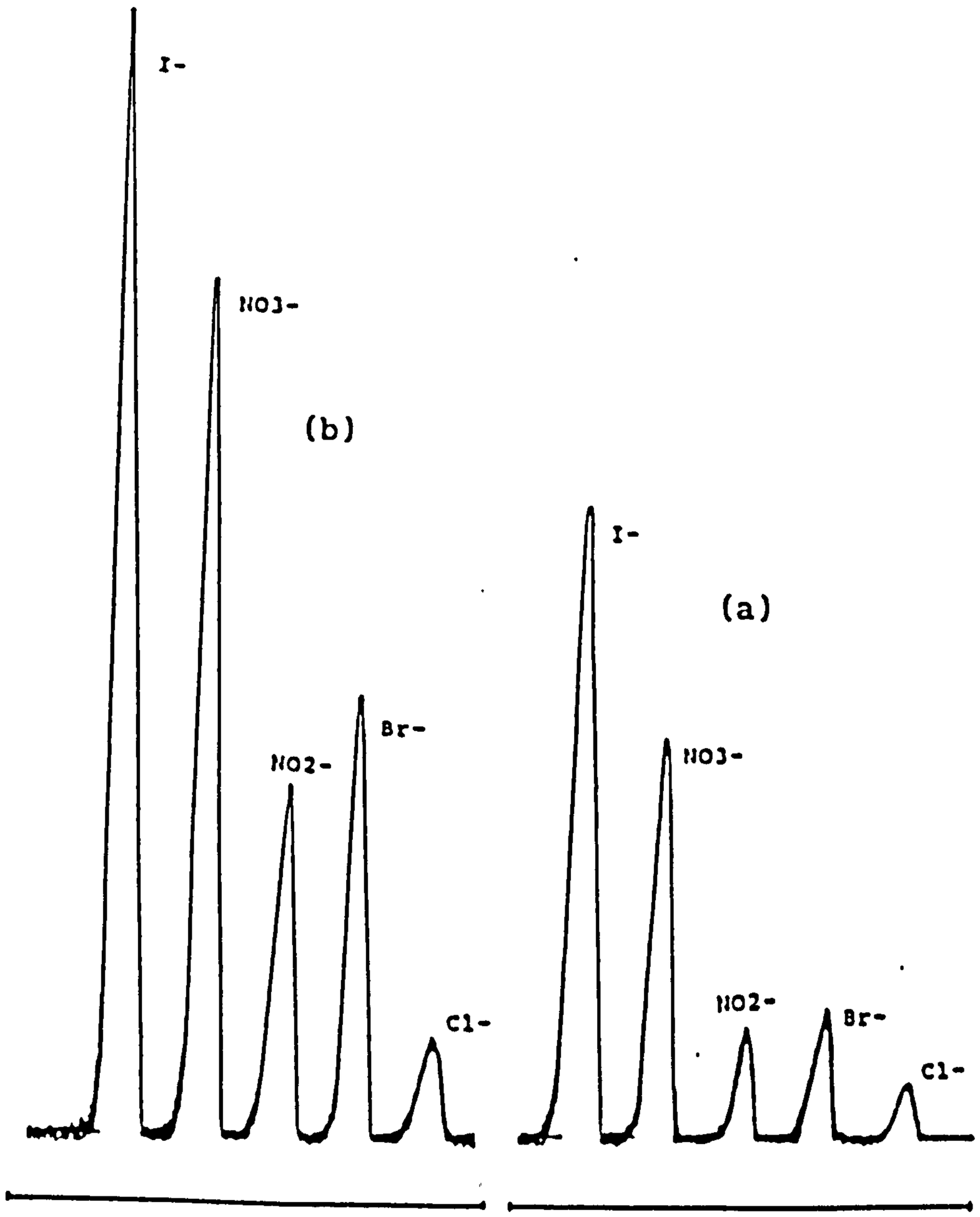


Figure 8. Two peak groups of anions obtained using the bromide (a) and the chloride (b) selective electrodes in one detection cell, carrier: 10^{-2} mol dm^{-3} solution of $SO_4^{=}$, injections: 200 µl of 0.05 mol dm^{-3} standard solution of each anion.

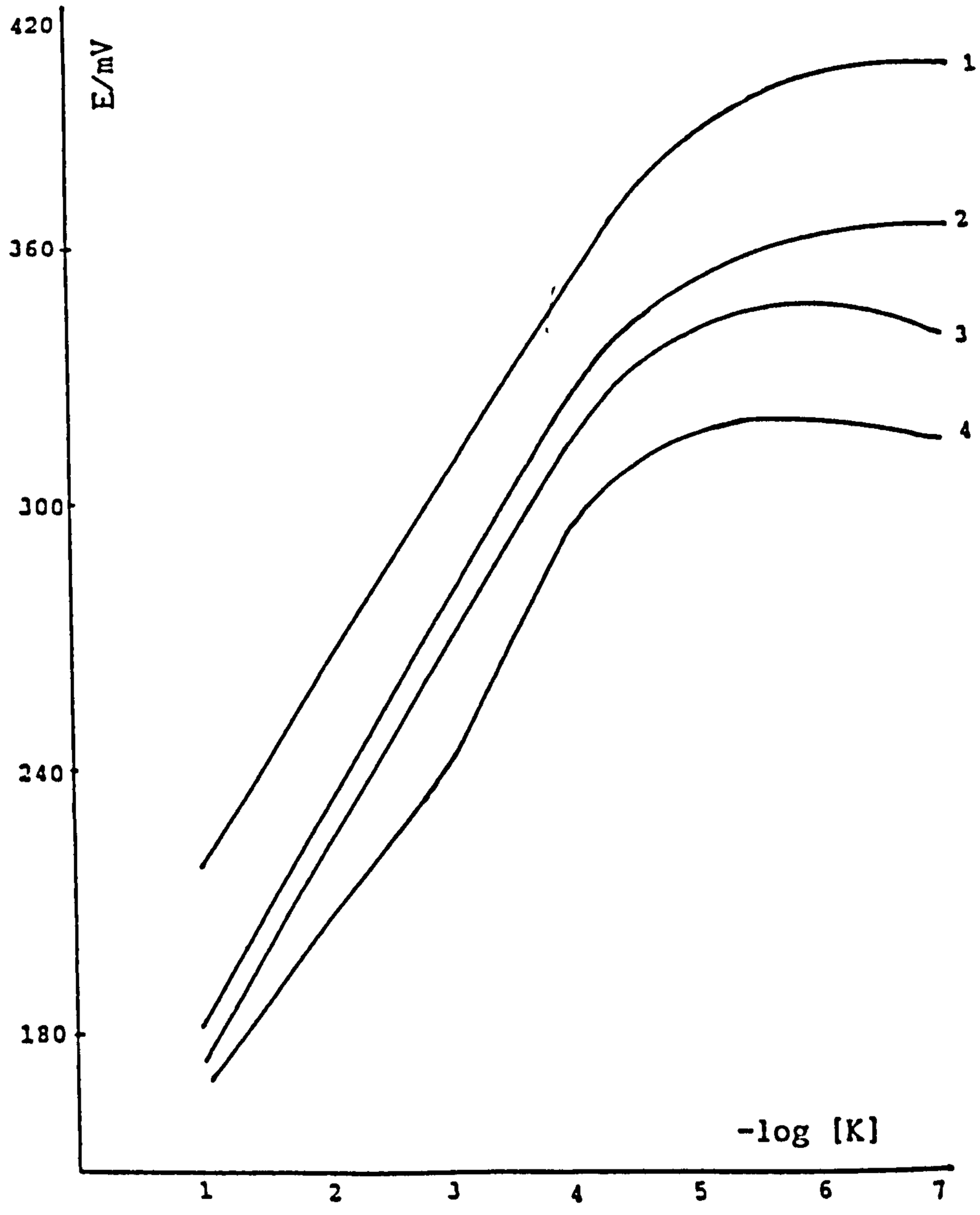


Figure 9. Calibration curves showing the response of four potassium selective electrodes to varying concentrations of standard potassium solution.

1. Dibenzo-18-crown-6-KBr
2. Dibenzo-18-crown-6-KI
3. Dibenzo-18-crown-6-KI and dicyclohexyl-18-crown-6-KI
4. Dicyclohexyl-18-crown-6-KI

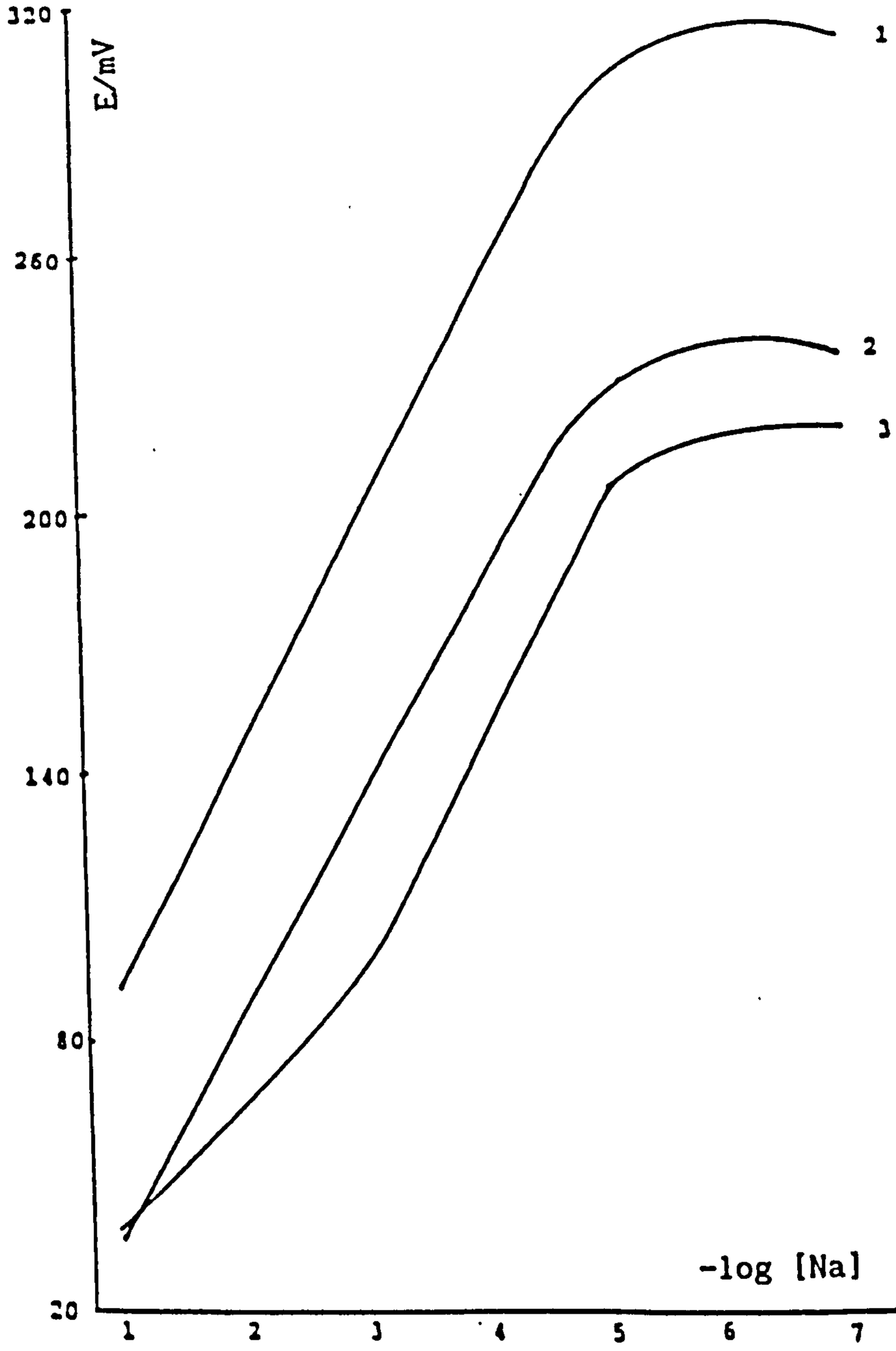


Figure 10. Calibration curves showing the response of three sodium selective electrodes to varying concentrations of standard sodium solution.

1. Dibenzo-18-crown-6-NaI
2. Dibenzo-18-crown-6-NaI and dicyclohexyl-18-crown-6-NaI
3. Dicyclohexyl-18-crown-6-NaI

Table 3. Response charecteristics of liquid membrane cation selective electrodes based on PVC.[a,b]

determinant	active material	limit of Nerns. range (mol L^{-1})	detection limit[c]
Na^+	dibenzo-18-crown-6-NaI	$2x10^{-5}$	$3x10^{-6}$
	dicyclohexyl-18-crown-6-NaI	10^{-2}-10^{-5}	$5x10^{-6}$
	dicyclohexyl-18-crown-6-NaI and dibenzo-18-crown-6-NaI	10^{-4}	$5x10^{-5}$
K^+	dibenzo-18-crown-6-KBr	$2x10^{-5}$	$2x10^{-6}$
	dibenzo-18-crown-6-KI	10^{-4}	$2x10^{-5}$
	dicyclohexyl-18-crown-6-KI	10^{-2}-$5x10^{-5}$	$2x10^{-5}$
	dibenzo-18-crown-6-KI and dicyclohexyl-18-crown-6-KI	10^{-4}	$5x10^{-6}$

a) The plasticizer was dioctyl sebacate and potassium tetrakis (4-chlorophenyl)borate was added to the membrane to increase the conductivity.

b) All measurements were made by the dip tests.

c) The values were determined graphically.

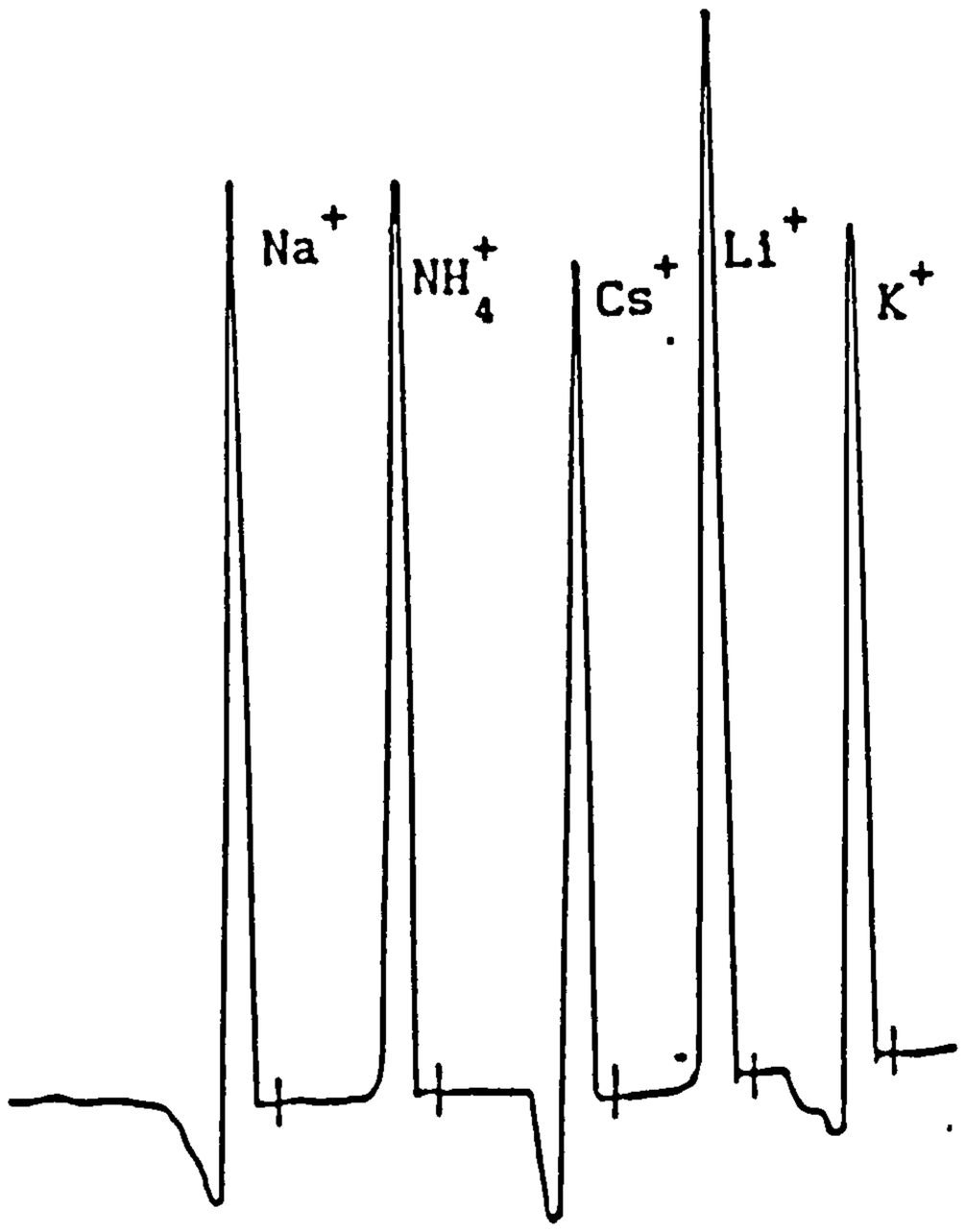


Figure 11. Cation peaks obtained using the sodium selective electrode based on dicyclohexyl-18-crown-6-NaI in the flow injection system, carrier: 5×10^{-3} mol dm^{-3} solution of TEA^+, flow-rate: 3 ml min^{-1}, injections: 75 µl of 10^{-4} mol dm^{-3} standard solution of each cation.

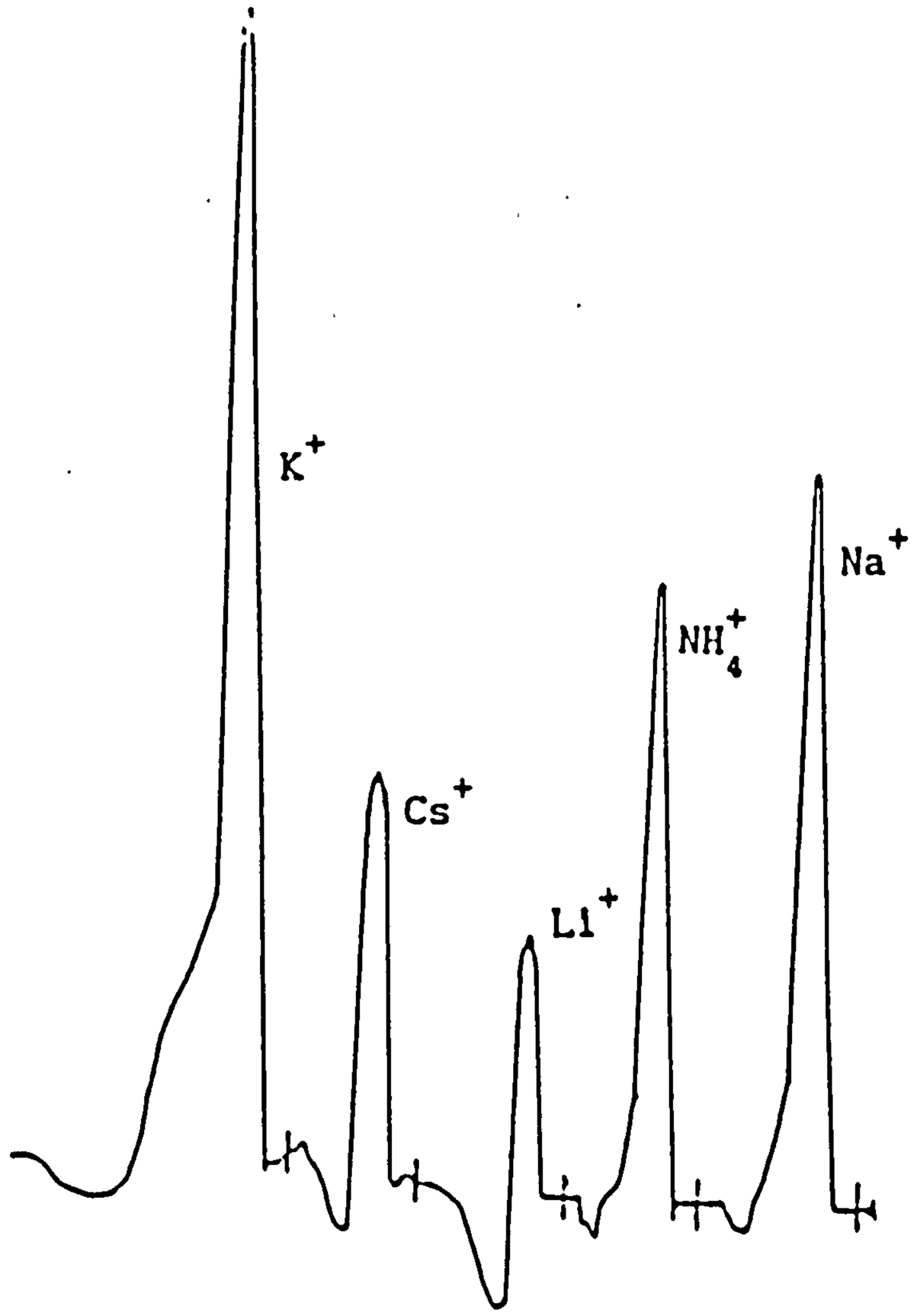


Figure 12. Cation peaks obtained using the sodium selective electrode based on dibenzo-18-crown-6-NaI in the flow injection system, carrier: $5x10^{-3}$ mol dm^{-3} solution of TEA^+, flow-rate: 5 ml min^{-1}, injections: 75 µl of 10^{-4} mol dm^{-3} standard solution of each cation.

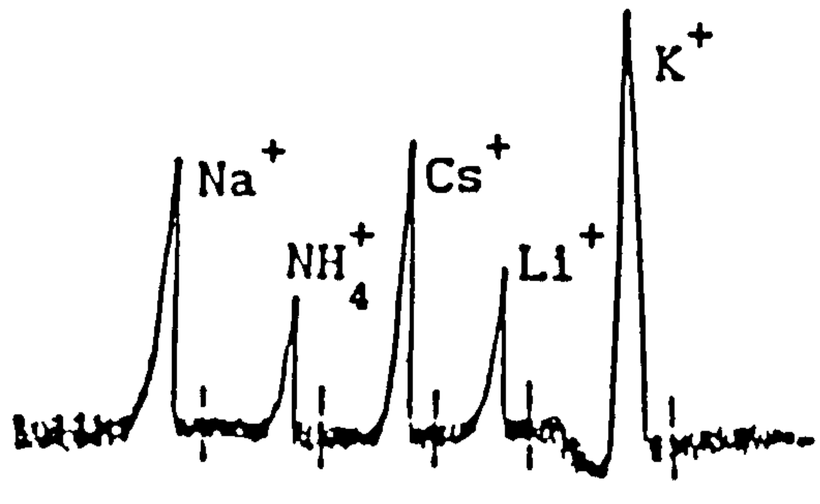


Figure 13. Cation peaks obtained using the sodium selective electrode based on dicyclohexyl-18-crown-6-NaI in the flow injection system, carrier: $5x10^{-3}$ mol dm^{-3} solution of TEA^+, flow-rate: 3.5 ml min^{-1}, injections: 5 µl of 10^{-4} mol dm^{-3} standard solution of each cation.

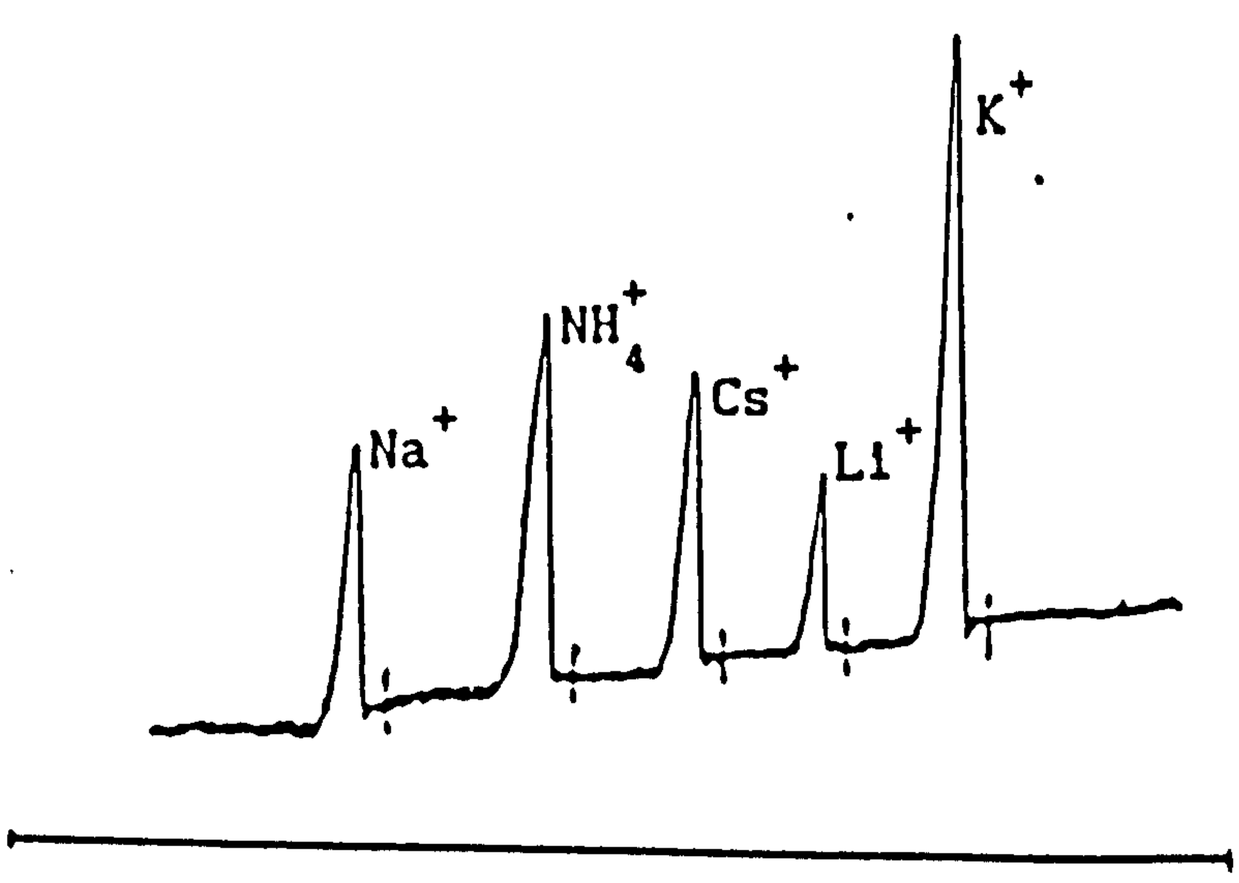


Figure 14. Cation peaks obtained using the potassium selective electrode based on dicyclohexyl-18-crown-6-KI in the flow injection system, carrier: $5x10^{-3}$ mol dm^{-3} solution of TEA^+, flow-rate: 3.5 ml min^{-1}, injections: 5 µl of 10^{-3} mol dm^{-3} standard solutions of K^+ and Na^+, 25 µl of 10^{-3} mol dm^{-3} solution of the others respectively.

5.4 REFERENCES

1. Schultz F.A. and Mathis D.E., *Anal. Chem.*, 1974, 46, 2253.

2. Suzuki K., Aruga H. and Shirai T., *Anal. Chem.*, 1983, 55, 2011.

3. Trojanowicz M. and Meyerhoff M.E., Z. *Anal. Chem.*, 1989, 334, 691.

4. Trojanowicz M. and Meyerhoff M.E., *Anal. Chim. Acta*, 1989, 222, 95.

5. Griffiths J., Moody G.J. and Thomas J.D.R., *Analyst*, 1972, 97, 420.

CHAPTER 6

6.1 RESPONSE TIMES OF LIQUID MEMBRANE ANION SELECTIVE ELECTRODES BASED ON PVC

6.2 INTRODUCTION

Analytical applications require that ISEs take a minimum time to reach a steady potential and a maximum stability of potential. Therefore, the response time is one of most significant parameters to characterize the nature of ISEs. Typical response times of membrane electrodes vary from seconds to several minutes depending not only on properties of ISEs, but also on the experimental conditions selected, the magnitude and direction of the concentration change, mode of electrode use, concentration of interfering ion(s), the design of electrochemical cell, and also on the time dependence of other factors, such as streaming potential, diffusion potential and electronics used for recording the transient signal.

If an indicator electrode response function is much larger than that of the other parts of the electrochemical cell, then the influence of the other sources becomes negligible. ISEs have been increasingly used as detectors with many kinds of analysis systems in which a flowing stream is used as an eluent.[1] There has been no study of the determination of the response time in such conditions. Also, there is no universally accepted definition of the response time available since it is greatly dependent on the methods used for the determination, and on other factors such as selectivity, detection limit, life time, etc. In chapter 5, selectivity and detection limits of the electrodes used were determined by using a flowing stream in a system. In order to be able to compare the response time, experimental methods should be carefully selected. Therefore, the aim of this study was the determination of the response time and an evaluation of the the factors affecting the determination in a flowing stream. Also, a new approach to the definition will be suggested to obtain

analytically useful parameters, which permit comparison of performance characteristics of a different type of ISEs and which might be used as detectors in chromatography and flow injection analysis(FIA).

6.3 ATTEMPTS TOWARDS DEFINITION OF THE RESPONSE TIME

According to the IUPAC recommendation,[2] the definition of response time is: "The length of the time which elapses between the instant at which the concentration of the ion of interest in a solution in contact with the ISE and a reference electrode and the first instant at which the potential of the cell becomes equal to its steady value within 1 mV." The experimental conditions used should be stated, i.e. mode of the electrode use, the history and preconditioning of the electrode, and the composition of the solution on to which the electrode was exposed prior to the measurement and temperature.

Some authors,[3-5] however, consider the response time of an ISE as the time taken by the potential to change from its initial value to within a given limit of the "final" value, the latter referring to a shift of 50% of the difference between the two potentials (t_{50}),[3,4] or a shift of 95% (t_{95}).[5]

In some cases different kinds of response curves have been reported[6,7] which cannot be described, nor the ISE response represented, by a single equation. In this case, even response time, $t\alpha$, might lose its theoretical significance as was suggested by Uemasu and Umezawa,[8] who also proposed a new definition of the response time, the quantity $t(\Delta t/\Delta E)$, based on the fact that the size of the potential change of ISEs generally decreases as a function of time. Uemasu and Umezawa's proposal has some advantages, as it could give a more realistic measure of the practical performance of the electrode. It can be applied to ISEs whose response speed is slow so that the final value is not readily determined. Also, it can be determined without knowing beforehand $E\alpha$ or $t\alpha$ values. This was also recommended by Linder.[9]

6.4 A NEW APPROACH TO THE DEFINITION OF THE RESPONSE TIME

On the basis of such attempts towards the definition of response time, a quantity $\Delta t\alpha$ as a response time can also be defined especially for ISEs to be used as detectors in chromatography and FIA systems. In chromatography, the degree of separation or resolution of two very close bands is defined as the distance between band centres divided by the average band width. If the retention and band width are measured, for two ions, in units of time, as in figure 1, the resolution Rs, is given as:

$$Rs = \frac{t_{R2} - t_{R1}}{0.5(W_1 + W_2)},$$

where W1 and W2 are band width and t_{R1} and t_{R2} are the retention times of two ions. For reasonable quantitative accuracy, resolution must be at least 1, Rs ≥ 1. These aspects might be defined relating to a detector which shows very fast response such as an ultraviolet detector (UV). Electrodes which show slower response than UV detectors, and are also applicable to chromatography or FIA systems, will exhibit tailing and irregular variations in band widths by flow rate changes. While a lower flow-rate will cause a normal large band like a UV detector, a higher flow-rate will cause an abnormally smaller band than a UV detector. In contrast, band widths have to be unchanged at each flow-rate for each detector unless the amounts of the solutes are changed. Hence, a resolution equal to 1 unit with UV detection, may not be equal to 1 unit with potentiometric detection in certain conditions. Therefore, the response time of the electrodes should be recalculated via in flow conditions with a new approach as outlined below, or the resolution defined in chromatography could be reexamined depending on the response time. On the other hand, in chromatography and FIA, eluents selected should have a low background potential for the detection of other ions injected into eluent. In fact, the detector will respond to each ion in a flowing stream of eluent, firstly from low activity to a high activity, secondly from the high activity to low activity levels. Hence, the response from high activity to low

activity is as significant as the first response as an analytical parameter in the analysis.

Consequently, to solve these problems, it is necessary to find a time, $\Delta t\alpha$, when the potential change becomes zero after a potential change occurred as shown in figure 2.

$$\text{If } Rs = \frac{t_{R2}-t_{R1}}{t\alpha+t'\alpha} \geq 1,$$

where $t\alpha+t'\alpha = \Delta t\alpha$ is the response time according to the new definition, then adequate selectivity in the column and detection by ISEs can be possible. Obviously, the resolution of detection might be better when $\Delta t\alpha$ is smaller than $t_{R2}-t_{R1}$ ($t_{R2}-t_{R1} \geq \Delta t\alpha$). The measurement of the response time by the quantity $\Delta t\alpha$ may have the following advantages when electrodes are to be used in such analysis systems; (i) it is very easy and quick to determine and calculate, (ii) it can be calculated without knowing $E\infty$ or $t\infty$ values, (iii) it is very useful to assess the electrode as a detector in chromatography or FIA, (iv) it may help in choosing the column and the eluent from the point of the separation, (v) it is possible to compare with other $t\alpha$ values which are obtained under the same conditions, (vi) moreover, it introduces a new classification of electrode parameters for use as detectors in chromatography and FIA systems.

6.5 PREPARATION OF TUBULAR FLOW THROUGH ELECTRODES AND CHEMICALS

The ISEs used were polyvinylchloride (PVC) matrix membranes which were basically tubular in form. The preparation of tubular membranes was as follows: a wire or a standard needle of 0.1 mm in outer diameter was threaded with two separate sections of PVC tubing which had 0.1 mm inner diameter. The distance between sections was 0.15 mm. The gap between tube sections was coated with PVC-tetrahydrofuran (THF) solution containing ligand and dibutylphthalate (DBP) as a plasticizer by rotation of the wire. The membrane was composed of 4% weight ligand, 68% weight DBP, and 28% weight PVC. THF was allowed to evaporate at room temperature

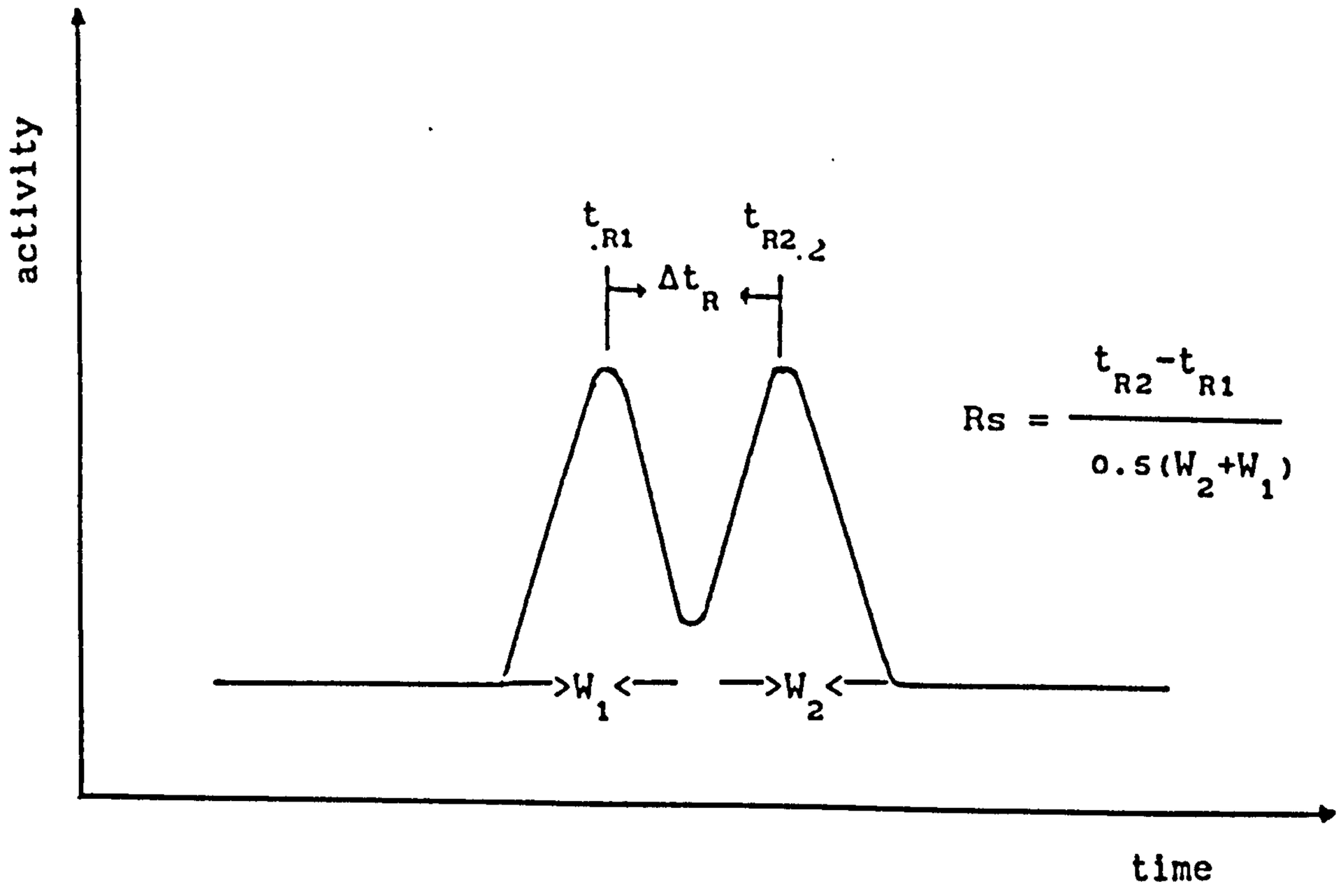


Figure 1. Definition of resolution

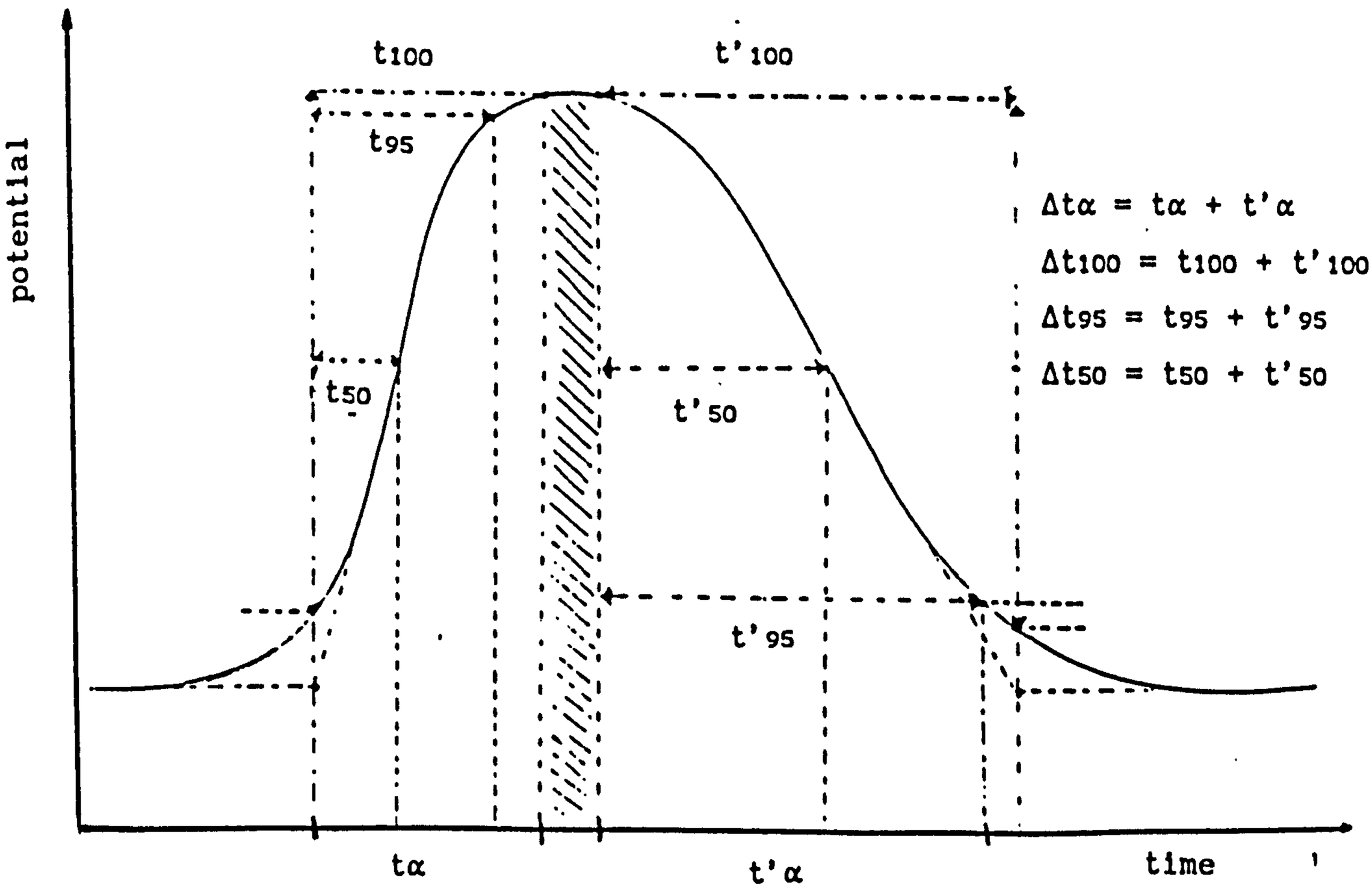


Figure 2. A new definition of response time

and open to air for four hours. Then the wire or needle connecting the two PVC tube sections was taken out. The cell had about 1.3 μl dead volume. It was conditioned for at least four hours by soaking in a 0.1 mol dm^{-3} (M) primary ion solution. Then it was fitted into the potentiometric cell. 0.1 M bromide and 0.001 M chloride mixed inner solution was used for Br^- membrane electrode. For Cl^- membrane electrode, 0.1 M chloride solution only was used. A Ag/AgCl electrode was used as inner reference. The active ligands used were tetradodecylammonium bromide (TDDA-Br), and TDDA-Cl, which was obtained from TDDA-Br by repeated exchange with analytical grade inorganic chloride in water and chloroform phases. It was then recrystallized from ethanol, and used without further purification.

The reference electrode was of the double junction calomel type (Russell, Scot.). Carrier solutions and solutions of ions tested were prepared from analytical reagent grade chemicals (BDH Chemicals, Poole England). All solutions were freshly prepared for use by direct weighing and dissolution in distilled or deionized water. All solutions were degassed by boiling before use.

6.6 PROCEDURE

The calibration curves of the electrodes were obtained by the constant volume dilution method, as previously described. The carrier stream was pumped through the injection valve and flow cell using flow-rates from 0.1 to 8 ml min^{-1}. The carrier streams were either 5.10^{-6} M solution of bromide or deionized water. Test solutions of bromide and chloride were diluted to concentrations of 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} M from stock solutions of 10^{-1} M, and were injected into carrier solution from the injection manifold with 20 μl sample loop volume by means of injection syringe. The potentiometric response of each sample of anion injected was then monitored with a millivolt meter and recorded continuously on a chart recorder.

6.7 FLOW SYSTEM

The flow system used consisted of the high pressure liquid chromatograph pump (type Perkin Elmer series 3) and the flow through cell which was connected, by PVC tubing, to the Rhodyne injection valve. The measuring system of potentiometric response consisted of a digital voltmeter (Thandar TM45), a high input impedance buffer amplifier, an off-set box, and two filters for noise reduction. Potentiometric data were recorded on a BBC model SE 120 chart recorder. A 25 µl Hamilton injector was used for sample injections.

A constant volume dilution vessel, laboratory made, was used for calibration of electrodes by constant volume dilution method. A schematic diagram of the potentiometric cell is shown in figure 3, which incorporated an indicator electrode, a reference electrode, a detector block and two short lengths of PVC tubing. Utilization of the measuring technique was accomplished after optimisation of flow-through tubular membrane cell, sample loop size, flow-cell inner tube diameter, ionic strengths and flow-rates of carrier solutions. The reference electrode was placed in a glass beaker of approximately 10 cm^3 volume filled with carrier solution. The carrier was directed towards the tubular membrane cell through to the outlet of the tubing. The distance between the tubular membrane surface and the outlet of the tubing was constant at approximately 2 cm. The distance was not critical because reproducible results were obtained at all flow-rates. To avoid further sample dispersion in the carrier solution, a length of PVC tubing 17 cm long and 0.1 cm in inner diameter was used between injection manifold and tubular membrane cell.

6.8 RESULTS AND DISCUSSION

The response curves for PVC-matrix membrane bromide and chloride selective electrodes are shown in figure 4.

The response times of the bromide selective electrode, with definitions of $t\alpha$ (α = 50, 95, 100), are shown in tables 1 and 2. The carrier solutions used were deionized water and 5.10^{-6} M bromide solution respectively. For a liquid ion exchange

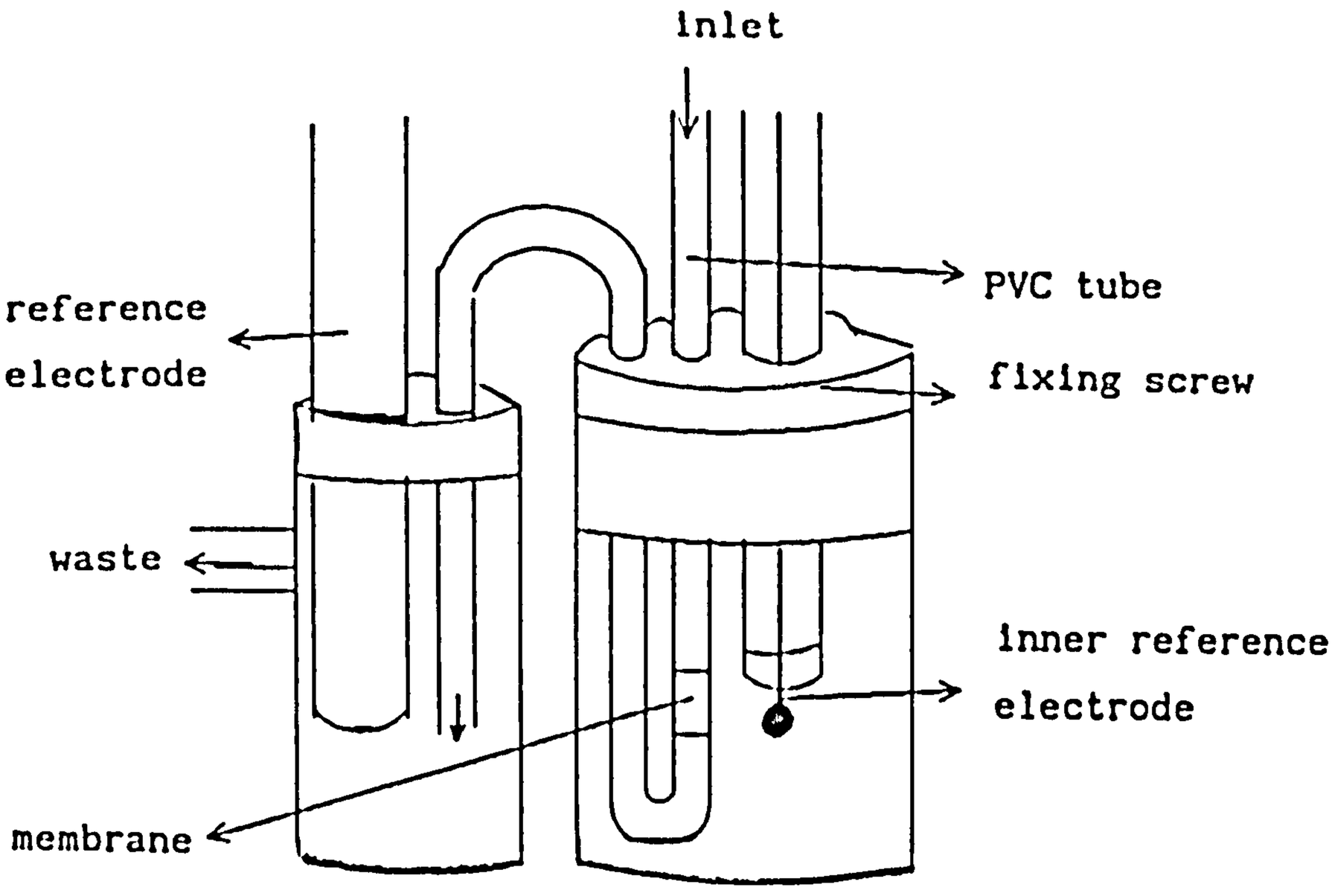


Figure 3. Schematic diagram of tubular membrane flow cell

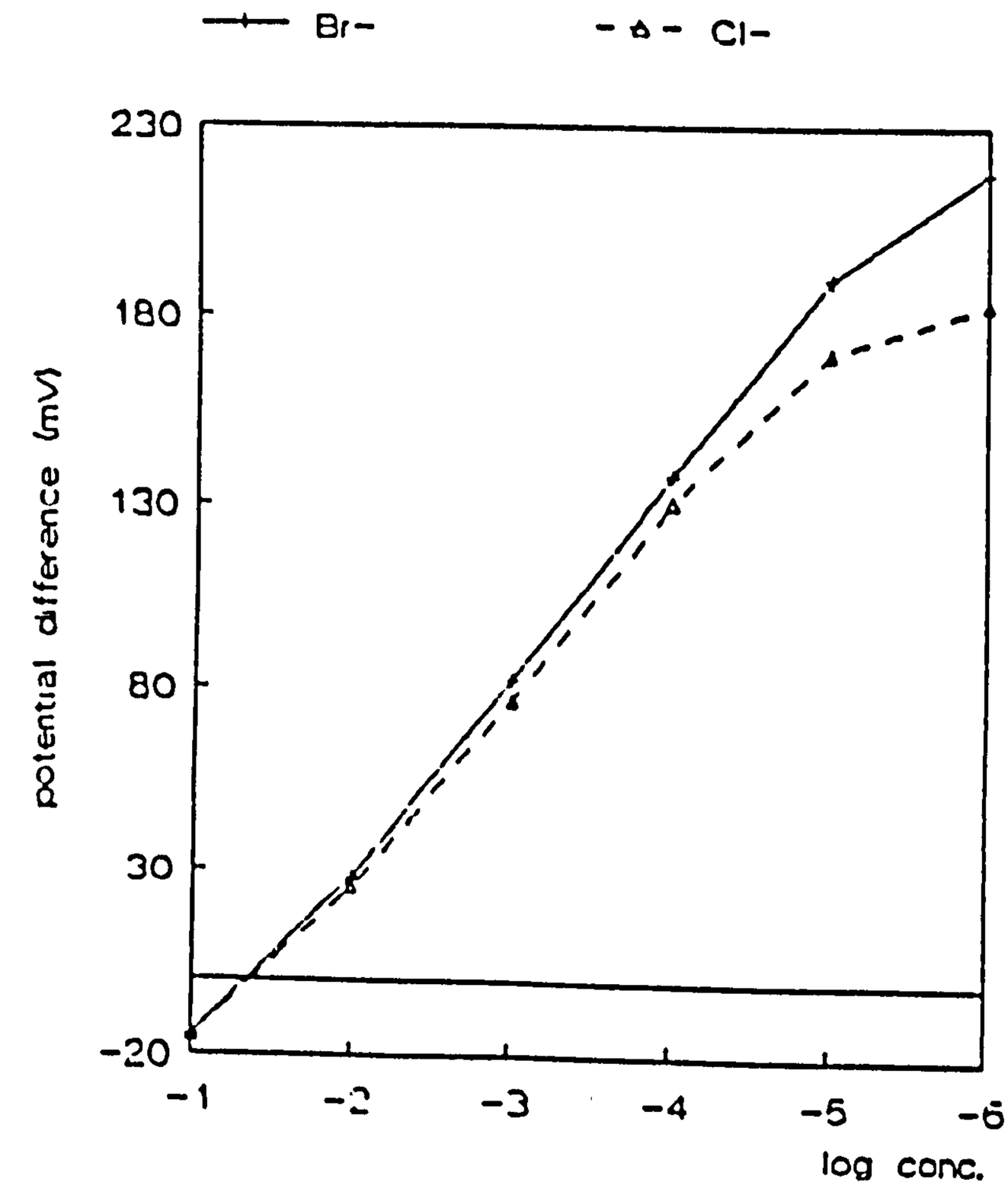


Figure 4. Response curves of the membranes

membrane, the response time values were acceptably short.
With 5.10^{-6} M bromide solution as carrier, the response times of the electrode were longer than those in deionized water as carrier solution. This might be the effect of the background potential of the carrier. The response time of bromide selective electrode and its dependence on activity change is illustrated in figure 5. The greater the activity jump, the shorter the response time, although the response time decrease was not too fast with increase of the activity, especially for t_{50} values. When flow-rate was increased, all the t_{α} values were considerably shorter. In figure 6 the t_{α} values, obtained in 5.10^{-6} M bromide solution, were found to be greater for high activities at flow-rates 1 and 2 ml min^{-1}. The effect of flow-rate on response time is shown in table 3 and figure 7. When the flow-rate increased the response time decreases.
It was noticed that a high degree of loss of the detection occurred by increasing the flow-rate of the carrier solution between 0.1 and 1 ml min^{-1}. This high degree of loss was diminished relatively with increasing flow-rates above 1 ml min^{-1}. This indicates that with flow-rate under 1 ml min^{-1} the membrane can be utilized for the detection of high amounts of solute in the sample. As the flow-rate tends to zero, the amount of the solute determined in the sample reaches the actual amount in the sample injected. Flow-rates above 1 ml min^{-1} did not significantly alter the loss. Hence, the bromide selective electrode gives the opportunity for use at high flow-rates, although, it might be suggested for use as a detector in open tubular column chromatography under slow flow-rates, because its detection volume is less than 1.5 µl at low flow-rates, and it will gain in detection of a high amount of the sample by the increasing response volume.
The results obtained with the chloride selective electrode are documented in table 4. The response times of the chloride electrode estimated are to be two times longer than the bromide electrode. The reason might be the nature of active material used

in the membrane or the thickness of the membrane. The response times of the chloride selective electrode depending on the activity changes are shown in figure 8. From the graphs, the effect of the activity change on the response times is significant, but the effect becomes negligible with increasing flow-rates, especially for t_{100} values. The significant effect of flow-rate on the response time suggests an electrode showing slow response. Such an electrode might fail to detect a solute at low activities and high flow-rate, and might be less advantageous for use in flow-through systems such as chromatography and FIA. This is caused by the volume of passing solute in contact with the membrane surface.

The response times in deionized water determined according to the new definition, ($\Delta t\alpha$, $t'\alpha$), are given in table 5a and 5b. The response times, under both definitions of $\Delta t\alpha$ and $t'\alpha$, decrease when the activity is decreased, but at 1 ml min^{-1} flow-rate, the response times were increased at low activity levels as shown in figure 9 and 10. If we compare $\Delta t\alpha$ and $t'\alpha$ values to $t\alpha$ values, it can be concluded that, more or less, all $t\alpha$ values were increased at low activity levels, while the effect on $\Delta t\alpha$ and $t'\alpha$ was contrary type, i.e. values were decreased. This indicates the second response, $t'\alpha$, of the electrode to the solute ion to be significant. Therefore, it seems that in low activity ranges, membrane bromide selective electrode can be suitable for detection of solute ions in a flowing stream. With 5.10^{-6} M bromide solution as an eluent, at low activity levels, Δt_{100} and t'_{100} values were increased but Δt_{50} and t'_{50} values were decreased as shown in figure 11 and table 6, but $\Delta t\alpha$ values were relatively higher than others, because of the second response, $t'\alpha$, of the electrode to the solute ion passing. Therefore tailing and a slow returning to baseline appeared at high activity levels.

Table 1. The response times of the bromide selective electrode for different concentrations of primary ion, obtained with delonized water as carrier at several flow-rates.

conc.	resp.%, tα	0.5 ml min^{-1}	1.0 ml min^{-1}	2.0 ml min^{1}
10^{-5}M	100	10.6	6.7	4.1
	95	9.6	6.3	3.7
	50	7.5	4.2	2.8
10^{-4}M	100	10.9	6.7	3.9
	95	9.7	5.9	3.5
	50	6.8	3.6	2.6
10^{-3}M	100	11.3	6.7	3.8
	95	10.1	5.4	3.3
	50	6.1	3.1	2.2
10^{-2}M	100	11.7	7.6	3.6
	95	10.1	5.4	3.2
	50	4.8	2.7	2.0
10^{-1}M	100	12.1	9.1	3.5
	95	10.5	5.0	3.0
	50	4.8	2.2	1.8

Table 2. The response times of the bromide selective electrode for different concentrations of primary ion, obtained with 5×10^{-6} M Br^- solution as carrier at several flow-rates.

conc.	resp.%, tα	1 ml min^{-1}	2 ml min^{-1}
10^{-5}M	100	10.4	9.4
	95	9.9	9.0
	50	6.9	6.2
10^{-4}M	100	11.9	9.5
	95	11.2	9.3
	50	7.0	6.4
10^{-3}M	100	13.9	10.5
	95	12.8	9.9
	50	7.2	6.6
10^{-2}M	100	16.6	11.5
	95	13.0	10.2
	50	7.4	6.8
10^{-1}M	100	18.1	11.8
	95	13.3	10.4
	50	7.5	6.9

Table 3. The effect of flow-rate on the response times of the bromide selective electrode for different concentrations of primary ion in deionized water as carrier.

		flow-rate (ml min^{-1})										
conc.	% tα	0.1	0.2	0.4	0.8	1.2	2.0	3.0	4.0	6.0	8.0	10.0
10^{-2}M	100	32	18	12	6.5	6.1	3.5	2.7	1.9	1.6	1.2	1.0
	95	26	15	10	5.4	5.3	3.2	2.5	1.6	1.4	1.0	0.9
	50	14	8.4	5.0	3.2	2.7	2.0	1.7	1.0	0.9	0.7	0.6
10^{-1}M	100	33	21	13	6.8	6.4	3.5	3.3	2.8	2.1	1.9	1.7
	95	27	16	11	5.6	5.3	3.0	2.8	2.4	1.8	1.6	1.5
	50	14	7.7	4.2	2.7	2.5	1.9	1.7	1.5	1.2	1.1	1.o

Table 4. The response times of the chloride selective electrode for different concentrations of primary ion, obtained with deionized water as carrier at several flow-rates.

		flow-rate (ml min^{-1})				
conc.	%tα	0.2	0.4	0.8	1.2	2.0
10^{-1}M	100	22.0	12.0	8.8	5.0	2.7
	95	18.0	9.0	7.6	4.8	2.3
	50	13.5	5.0	4.5	3.0	1.6
10^{-2}M	100	12.0	5.7	3.7	3.3	2.2
	95	9.0	4.0	3.1	2.6	1.9
	50	4.0	1.7	1.5	1.2	1.0
10^{-3}M	100	8.5	3.5	3.2	2.9	2.0
	95	7.0	3.0	2.4	2.1	1.7
	50	3.0	1.2	0.9	0.8	0.7
10^{-4}M	100	7.5	3.0	2.7	2.5	1.8
	95	6.5	2.5	2.2	2.0	1.6
	50	2.3	0.9	0.8	0.7	0.6

Table 5(a)and(b). The response times of Bromide selective electrode for different concentrations of primary ion in deionized water as carrier at several flow-rates (response times are calculated according to new definition).

(a)

conc.	%t'α	0.5 ml min^{-1}	1.0 ml min^{-1}	2.0 ml min^{-1}
10^{-5}M	100	28.0	17.7	10.8
	50	8.4	4.7	3.1
10^{-4}M	100	30.3	19.1	10.5
	50	8.2	4.5	3.1
10^{-3}M	100	33.4	21.3	10.9
	50	8.1	4.2	3.0
10^{-2}M	100	36.9	24.6	11.0
	50	8.1	4.0	3.0
10^{-1}M	100	39.6	30.9	11.5
	50	8.1	3.7	2.9

(b)

conc.	%Δtα	0.5 ml min^{-1}	1.0 ml min^{-1}	2.0 ml min^{-1}
10^{-5}M	100	38.6	24.5	14.9
	50	15.9	8.9	5.9
10^{-4}M	100	41.2	25.8	14.4
	50	15.0	8.1	5.7
10^{-3}M	100	44.7	28.0	14.7
	50	14.2	7.3	5.2
10^{-2}M	100	48.6	32.8	14.5
	50	13.6	6.7	5.0
10^{-1}M	100	51.7	40.0	15.0
	50	12.9	5.9	4.7

Table 6. The response times of the bromide selective electrode for different concentrations of primary ion in $5x10^{-6}$ M Br^- solution as carrier at several flow-rates (response times are calculated according to the new definition).

conc.	%Δt	1 ml min^{-1}	2 ml min^{-1}	%t'α	1 ml min^{-1}	2 ml min^{-1}
10^{-5}M	100	67.0	41.6	100	48.9	29.8
	50	14.4	13.0	50	7.5	6.8
10^{-4}M	100	61.4	41.5	100	44.8	30.0
	50	15.2	13.9	50	8.2	7.5
10^{-3}M	100	53.7	40.1	100	39.8	30.1
	50	16.7	15.2	50	9.5	8.6
10^{-2}M	100	48.5	39.6	100	36.6	30.1
	50	18.2	17.0	50	10.9	10.2
10^{1-}M	100	44.5	40.2	100	34.0	30.0
	50	19.9	18.1	50	12.4	11.4

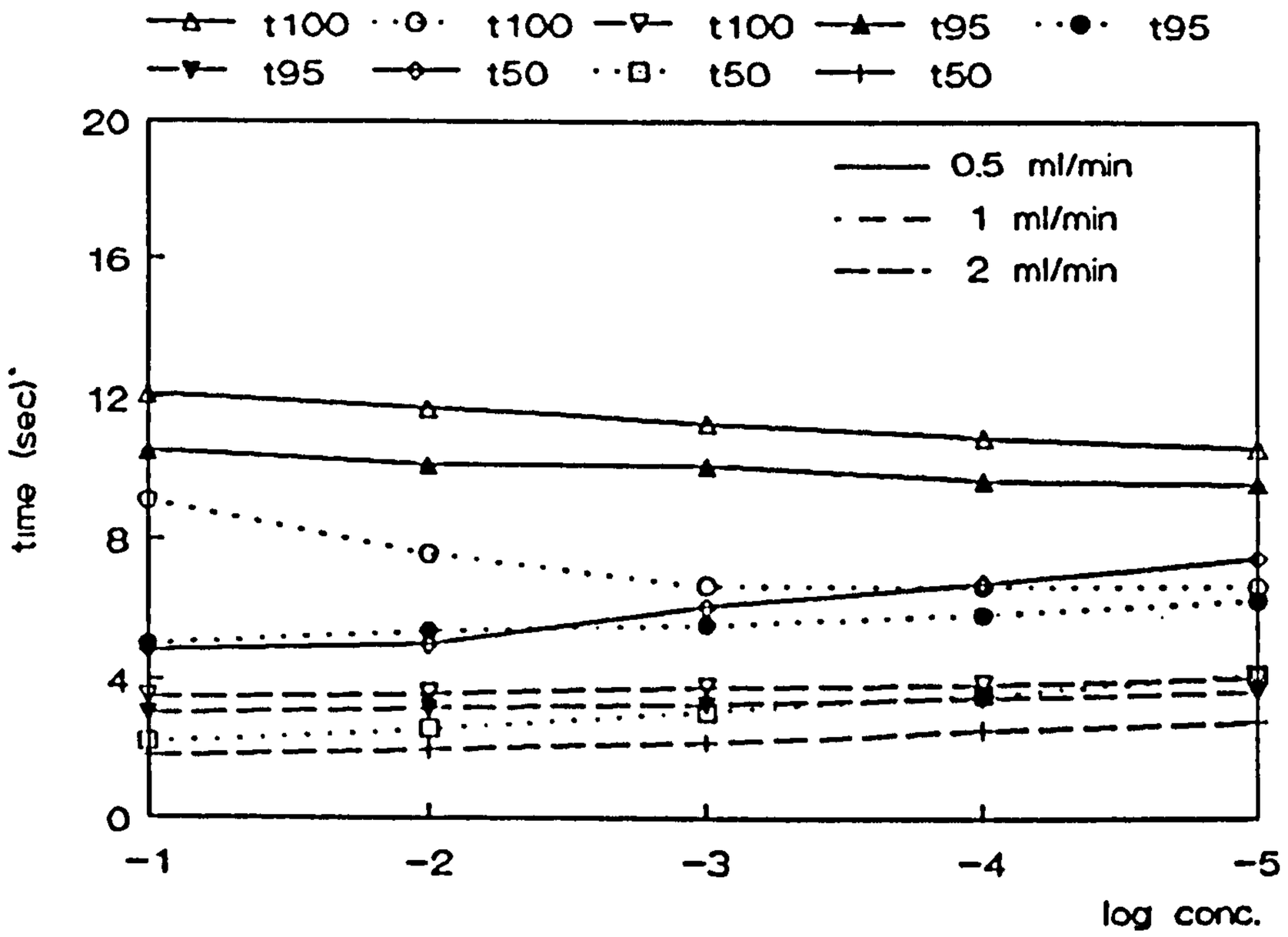


Figure 5. The effect of primary ion concentration on the response times t_{100}, t_{95} and t_{50} of the bromide selective electrode, obtained with deionized water as carrier under several flow-rates.

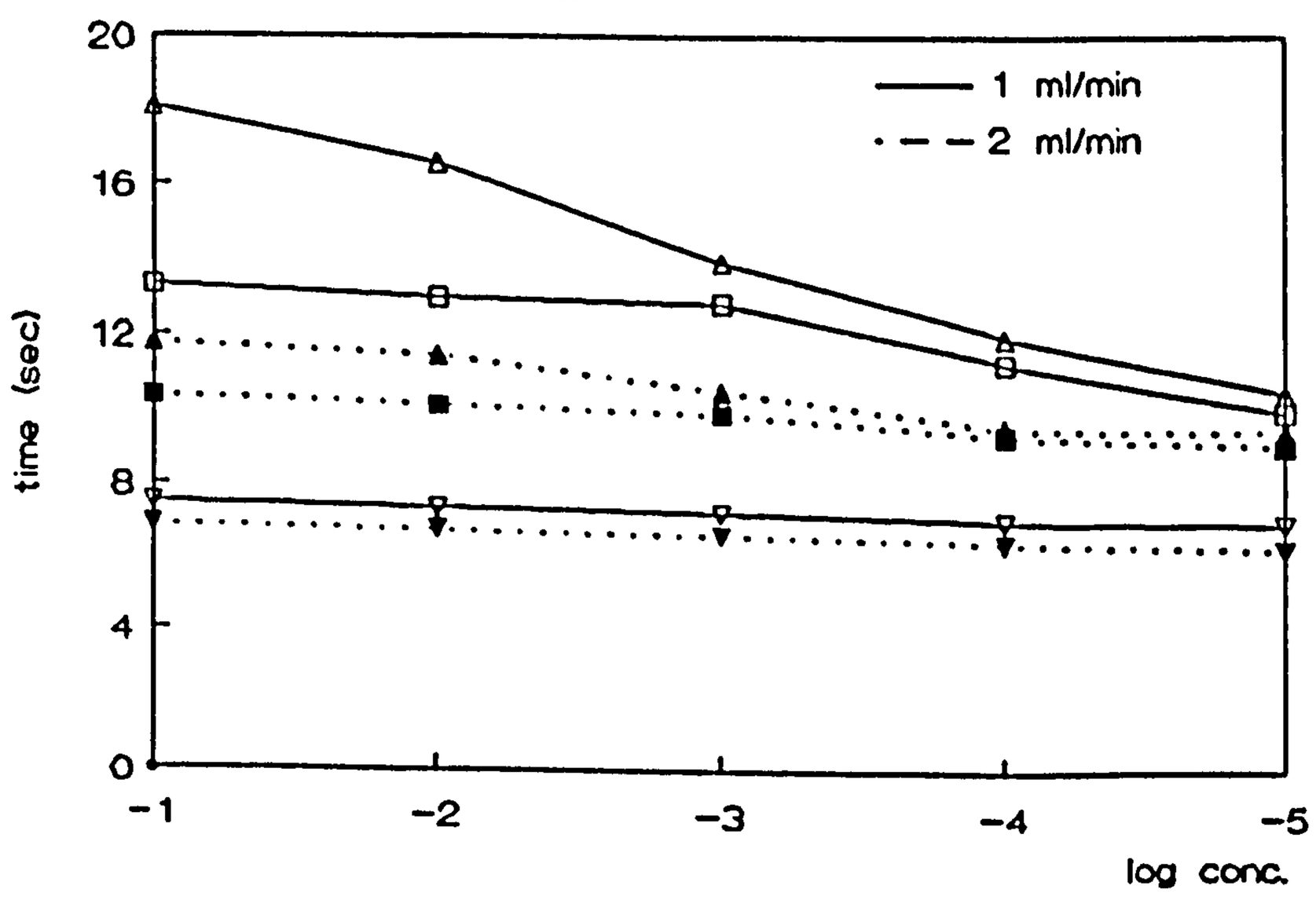
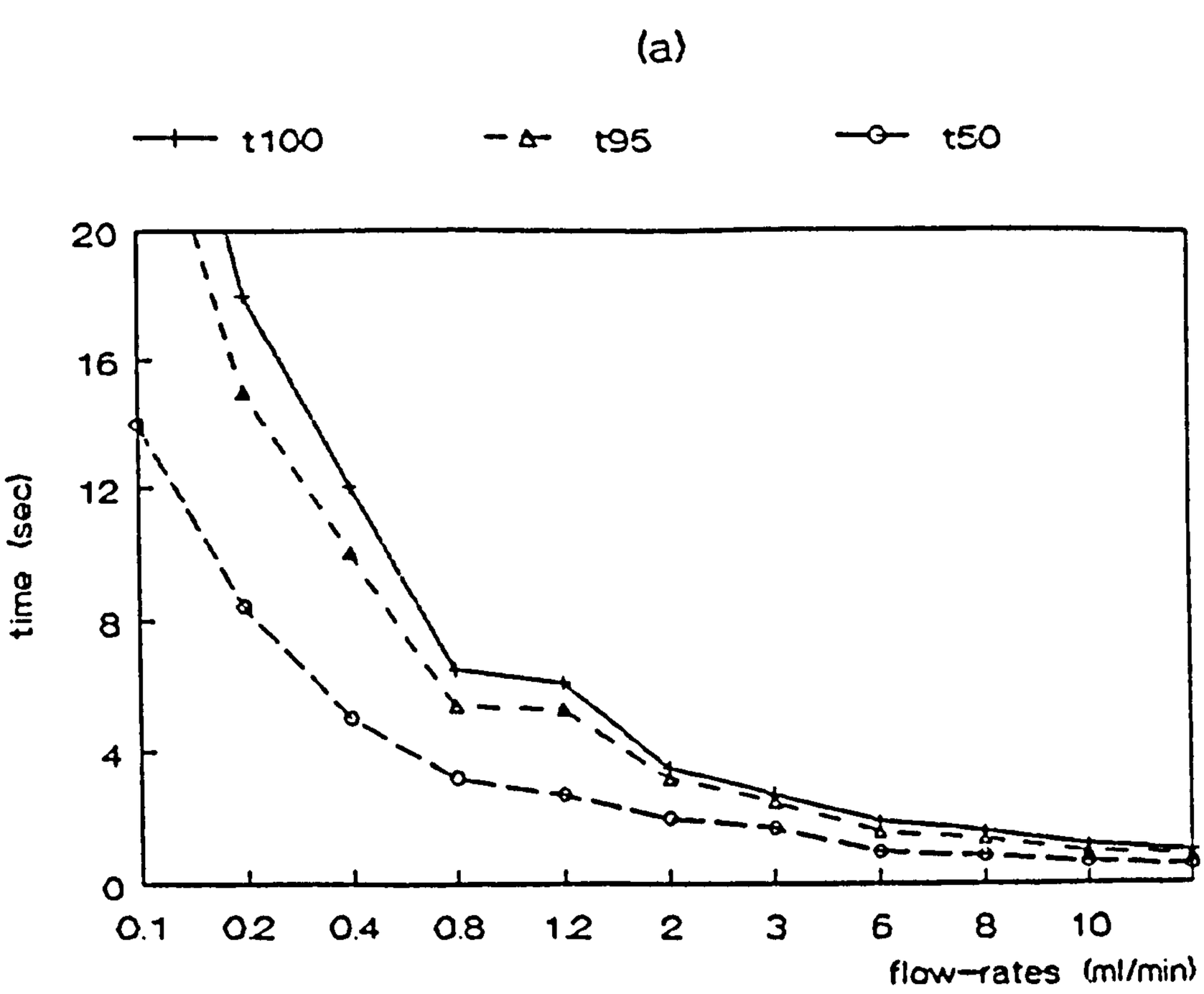


Figure 6. The effect of primary ion concentration on the response times t_{100}, t_{95} and t_{50} of the bromide selective electrode, obtained with 5×10^{-6} M Br^- solution as carrier at flow-rates 1 and 2 ml min^{-1}.



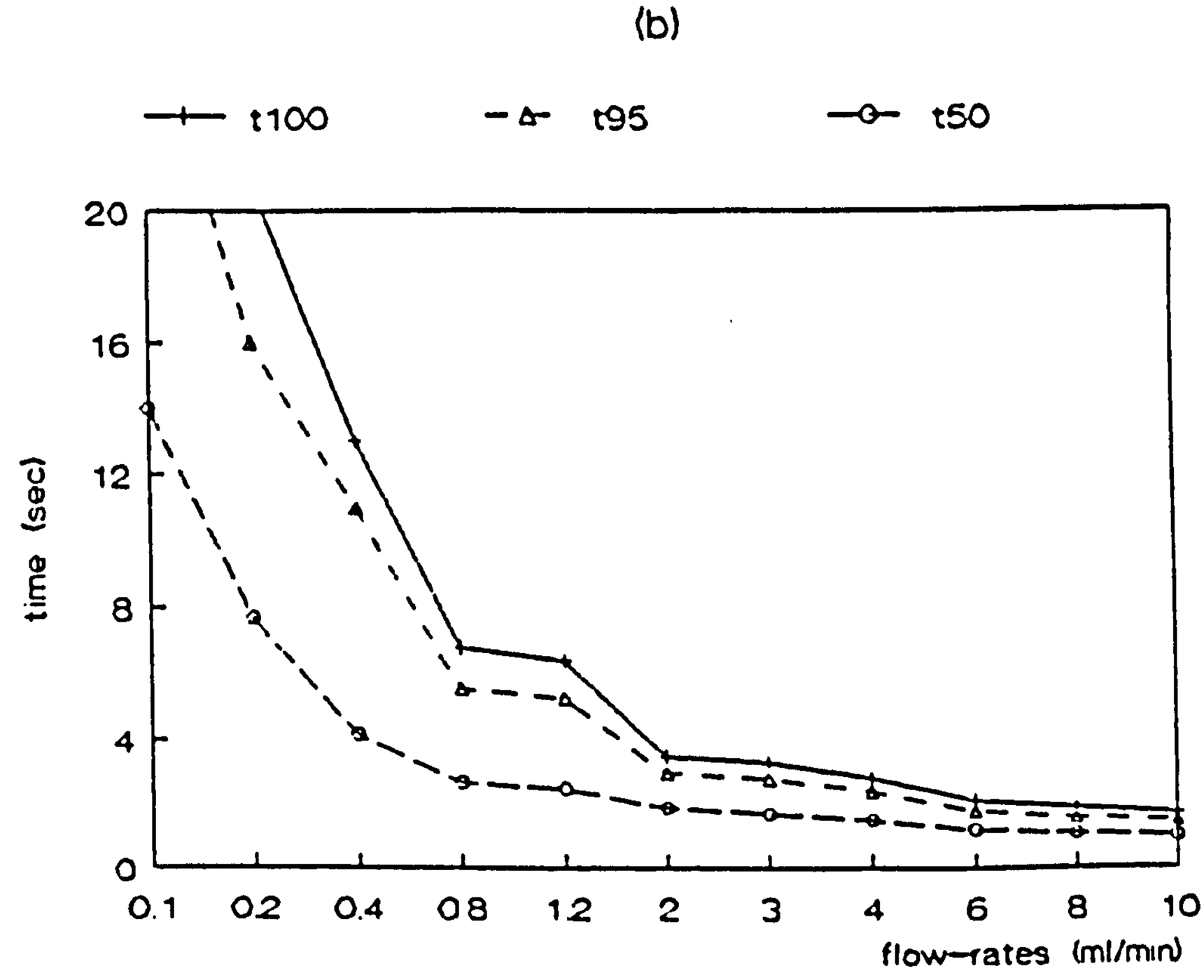
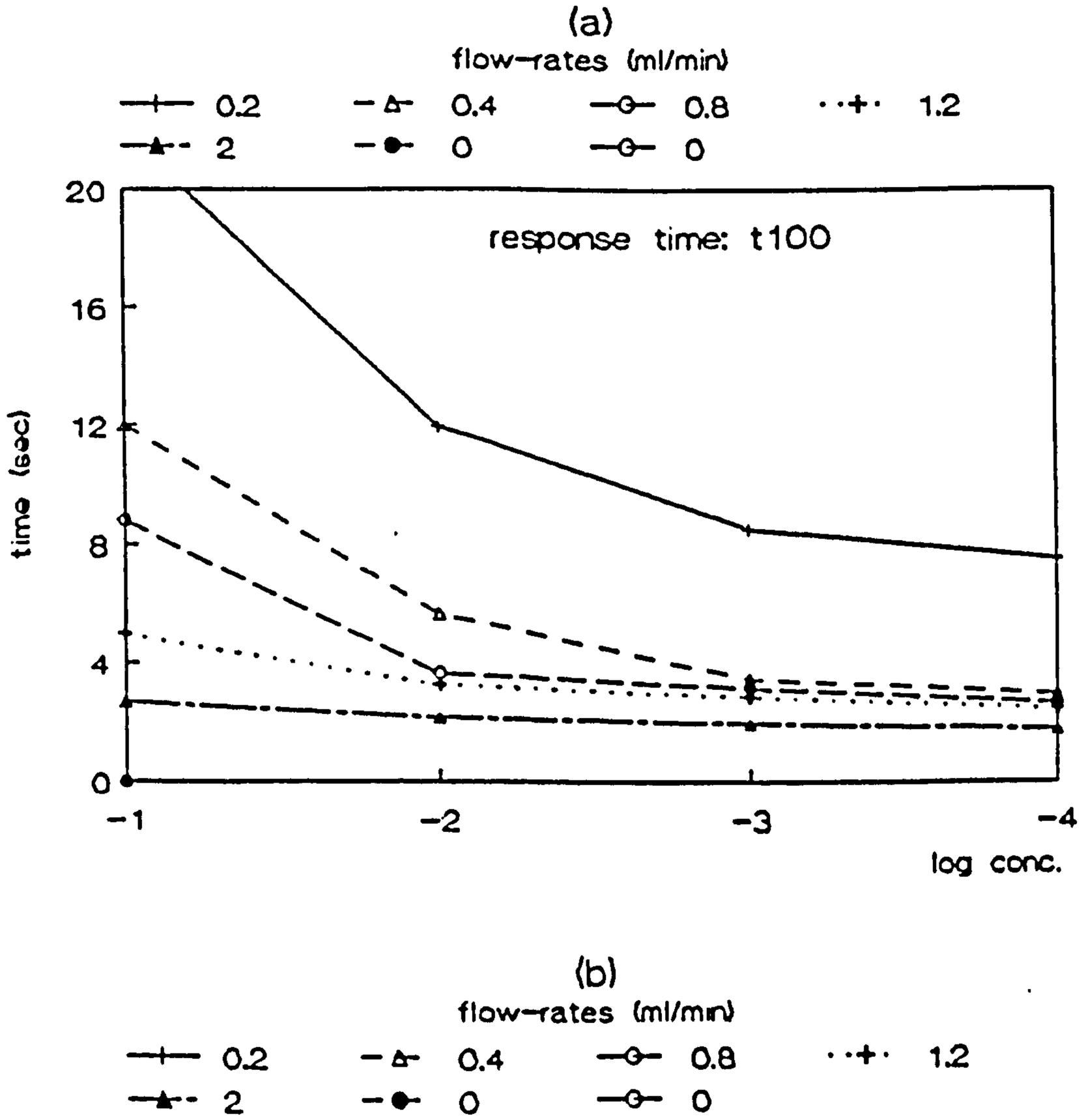
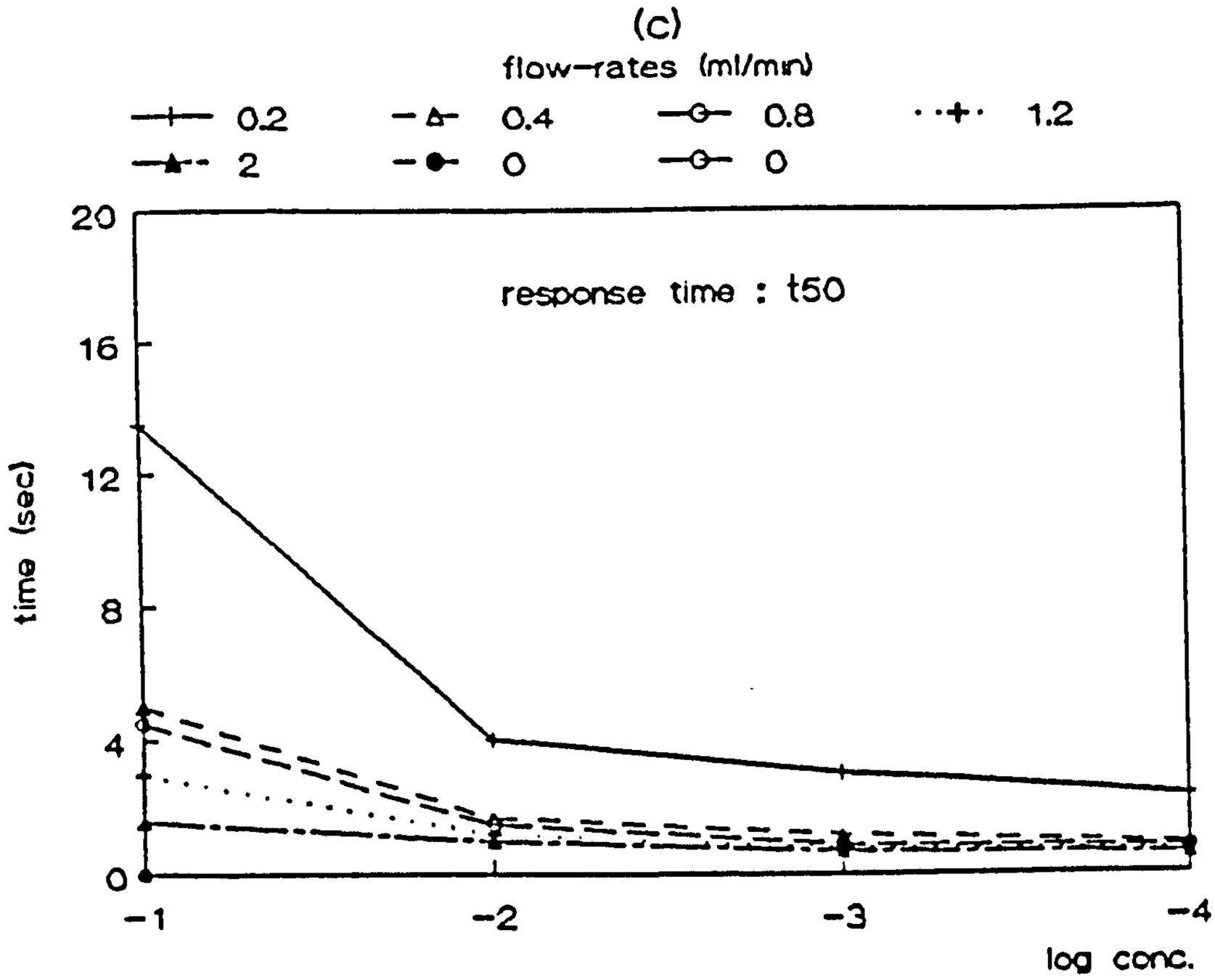


Figure 7. The effect of flow-rate on the response times Δt_{100} and Δt_{50} of the bromide selective electrode, at the concentration levels 10^{-2} M (a) and 10^{-1} M (b) of primary ion, obtained with deionized water as carrier.

Figure 8. The effect of flow-rate and primary ion concentration on the response times t_{100} (a), t_{95} (b), t_{50} (c) of the chloride selective electrode, obtained with deionized water as carrier.




(C)
flow-rates (ml/min)
0.2
0.4
0.8
1.2
2
0
0
response time : t50
time (sec)
20
16
12
8
4
0
-1
-2
-3
-4
log conc.

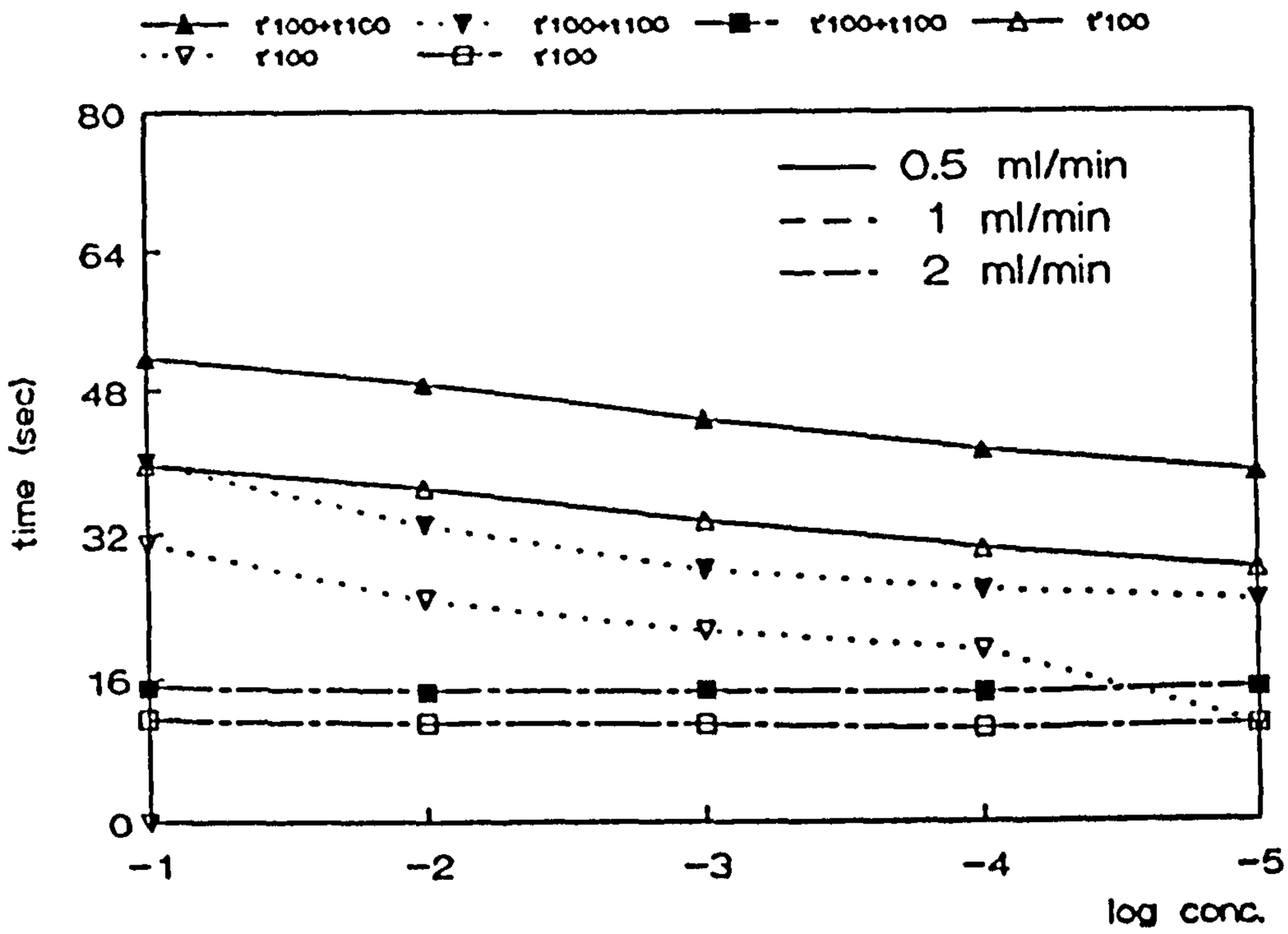


Figure 9. The effect of flow-rate and primary ion concentration on the response times Δt_{100} and t'_{100} of the bromide selective electrode, obtained with deionized water as carrier.

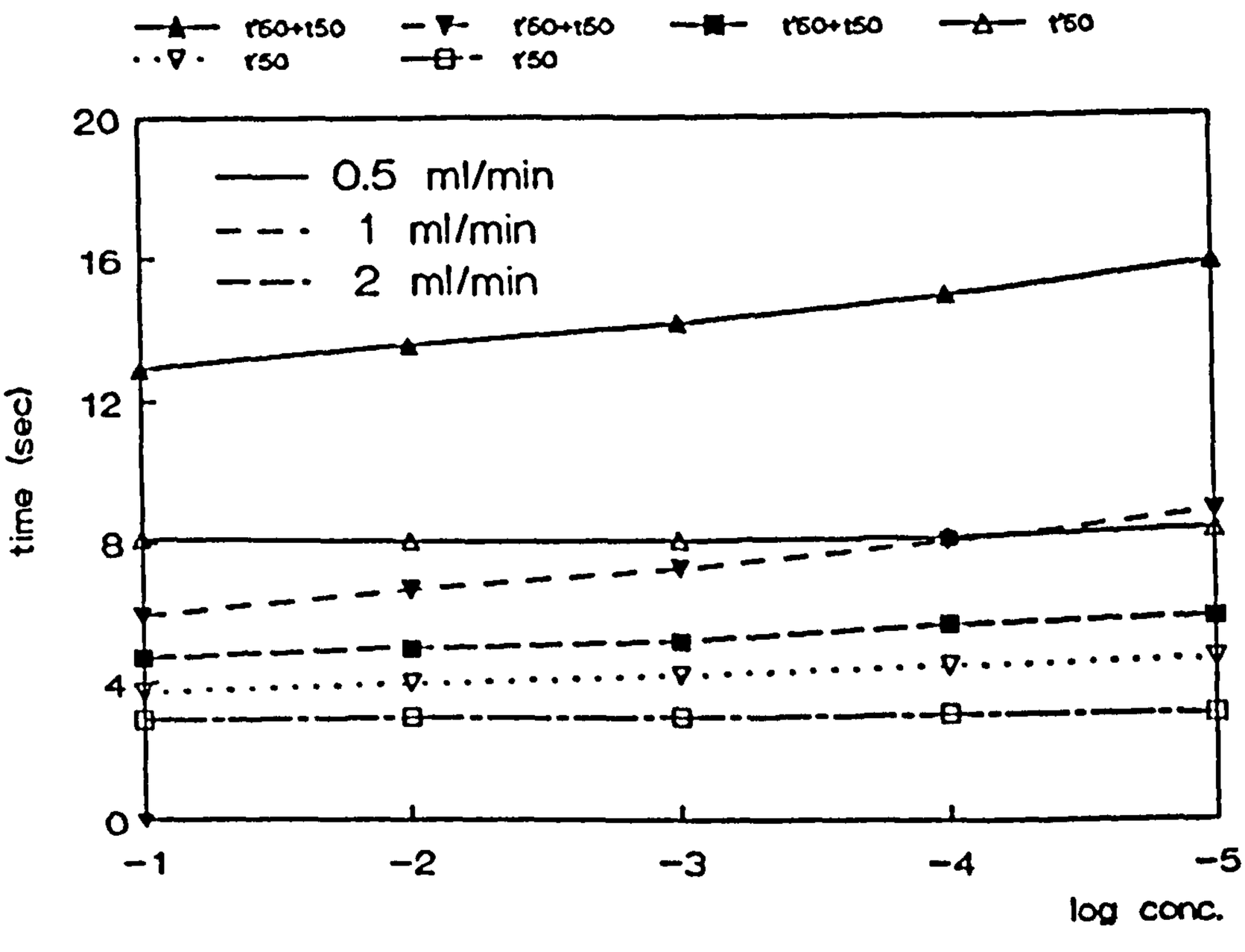


Figure 10. The effect of flow-rate and primary ion concentration on the response times Δt_{50} and t'_{50} of the bromide selective electrode, obtained with deionized water as carrier.

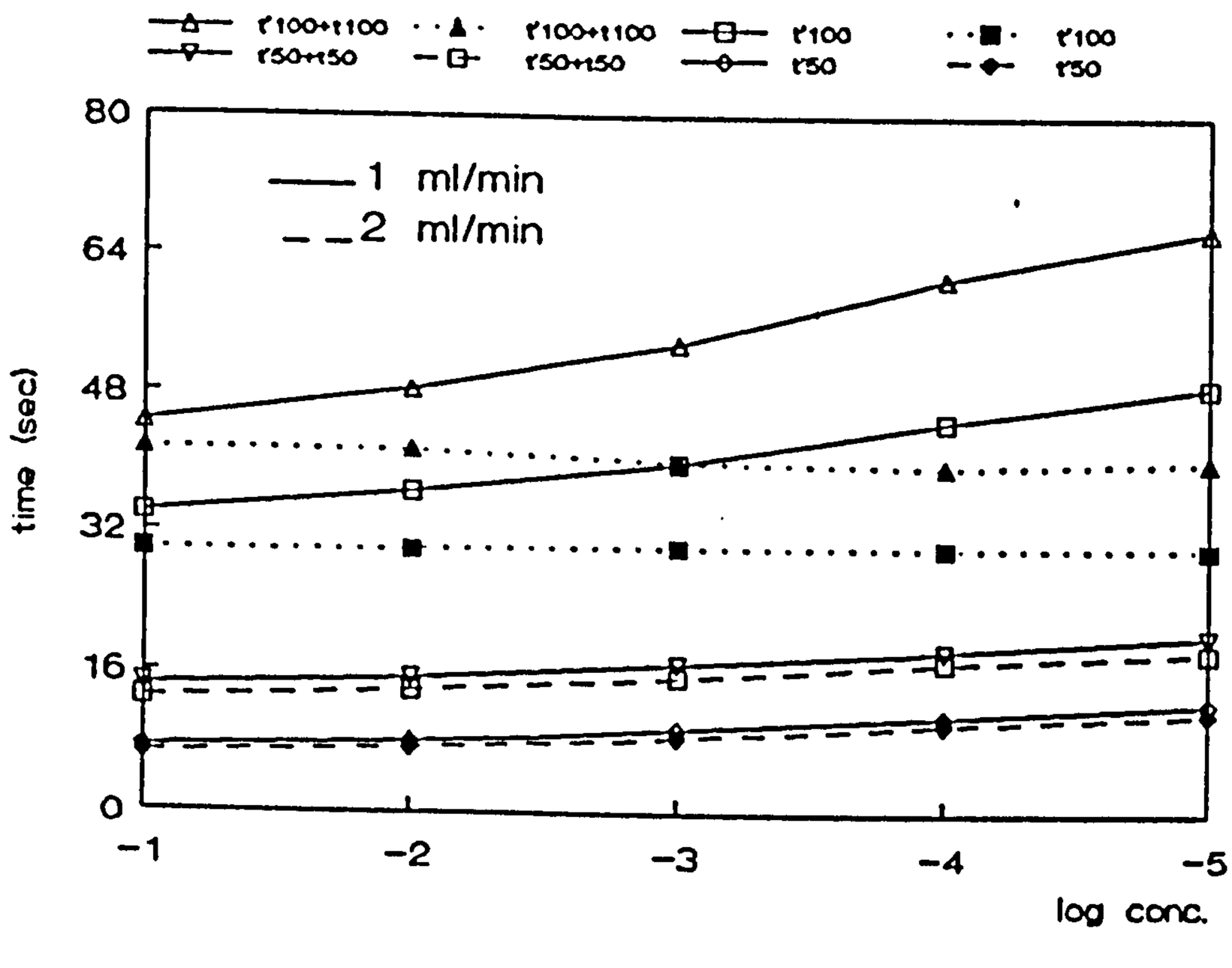


Figure 11. The effect of flow-rate and primary ion concentration on the response times Δt_{50}, Δt_{100}, t'_{50} and t'_{100} of the bromide selective electrode, obtained with 5×10^{-6} M Br^- solution as carrier.

6.9 EVALUATION OF SAMPLE DISPERSION IN FLOWING SOLUTIONS USING MEMBRANE ANION SELECTIVE ELECTRODES BASED ON PVC (INTENDED FOR USE AS DETECTORS IN ION CHROMATOGRAPHY)

6.10 INTRODUCTION

Increasing numbers of ion selective electrodes (ISEs) have been used in flowing conditions such as chromatographic[10-14] and flow injection.[15-17] There have been only a few papers related to the sample dispersion influence on the sensitivity of ion selective electrodes.[18-21] Trojanowicz and Frenzel[20] used solid-state fluoride and iodide sensitive electrodes to examine the influence of the sample dispersion in flow injection system. It was reported that, for an iodide selective electrode, the largest potential differences between experimental and theoretical data were obtained for smallest dispersion in the flow system and this was attributed to slow electrode response time. Other authors suggested adding primary ion to the carrier[22-24] or using high volume injections[25,26] in flow injection and ion chromatography studies.

To date there is no experimental study on the best conditions. Should the primary ion be added in a carrier which incorporates an affinitive ion to the electrode membrane? Therefore, water should be the basis of the experiments to examine the dispersion, response time, etc., as it is the main component of the carrier. The response times of flow through tubular membrane electrodes based on PVC were to be between 10-60 seconds depending on the factors, such as, flow-rate, direction of the activity changes and activity level. The present paper describes the evaluation of the influence and contribution of the sample dispersion on the sensitivity of tubular membrane bromide selective electrodes based on polyvinylchloride (PVC) with a new, easy and reliable approach in flowing conditions.

6.11 EXPERIMENTAL

The preparation of the tubular liquid membrane electrode was described previously in section 6.5 of this chapter. Experiments

were performed on three different flow-through tubular membrane bromide sensitive electrodes, in order to validate the data. The reference electrode was the double junction calomel type (Russell, Scot.). All components of the membrane were obtained from Fluka except DBP which was from Aldrich. Carrier and sample solutions of bromide were prepared from analytical reagent grade sodium salt in deionized water.

6.11.1 Flow System

The flow system consisted of the high pressure liquid chromatograph pump (type Perkin Elmer Series 3) and a Rhodyne injection valve, with 100 µl sample loop, and was connected to the flow through electrode cell with a PTFE tubing. The flow through tubular liquid membrane electrode cell was as described before. The membrane electrode was fitted in a flow-through cap with the inner reference solution and the Ag/AgCl electrode. The reference electrode was placed at the outlet of the tubing. The distance between the flow through tubular membrane bromide sensitive electrode and the reference electrode was 3 cm. The potential measuring system was as previously described in section 6.6 in this chapter.

6.11.2 Procedure

Carrier was pumped through the injection valve and the electrode cell using flow-rates 1, 2 and 4 ml min^{-1}. The carrier was either deionized water only or 10^{-5} mol dm^{-3} bromide solution. Samples at dispersion coefficients (dc) 1, 2 and 3 were prepared before injections by adjustment of the injection volume and concentration. 10(dc:1), 20(dc:2) and 40(dc:3) µl samples, which possess the same amount of bromide ion in deionized water, were injected into the carrier at flow-rates 1, 2 and 4 $ml.min^{-1}$ respectively. A short piece of PTFE tubing (10 cm long, 0.5 cm id.) was used between the injection manifold and the potentiometric detection system to minimize further dispersion of the sample in the carrier.

6.12 RESULTS AND DISCUSSION

From the response times of the electrode which were previously examined in flowing conditions, there was an increase in the response time with increasing activity range or decreasing flow-rate. Adding primary ion in carrier caused no significant changes in the response time of the electrodes.

The peak heights (expressed in mV) and the peak widths (expressed in cm) at different dispersion coefficients, flow-rates and activity ranges are summarized in tables 1 and 2 using deionized water and 10^{-5} mol dm^{-3} bromide solution as carriers, respectively.

With 10^{-5} mol dm^{-3} potassium bromide solution as carrier, the dispersion of sample up to dc:3 contributes to the peak height at each flow-rate as the peak height increases with decreasing flow-rate. This might be attributed to slow response of the electrode. At a flow-rate of 1 ml min^{-1}, the response volume (obtained by multiplying the time by the volume flow-rate) for a 10 µl sample at dc:1 is theoretically 10 µl, in 1 µl electrode cell used, as the sample passes through the membrane surface within 10 s. At dc:2 and 3, the response volumes are 20 and 40 µl respectively. The response time of the electrode at the same conditions was around 44 s for 10^{-5} mol dm^{3} and 67 s for 10^{-1} mol dm^{3} levels respectively (table 6). Hence, it can be suggested that the electrode is performing at less than its real response time. For a 40 µl injection (at dc:3) of 10^{-5} mol dm^{-3} of sample the loss of the response is around 9%, and around 37% for 10^{-1} mol dm^{3} levels. In the same manner for a 20 µl injection (at dc:2), the loss is around 54% and 68% at 10^{-5} and 10^{-1} mol dm^{3} levels. For a 10 µl injection (at dc:1), the loss is around 77% and 84% at 10^{-5} and 10^{-1} mol dm^{3} levels. Theoretically, each injection should yield the same electrode potential when the response time of the electrode is higher than the response volume of each sample on the electrode surface. Experimentally 40 µl injection (at dc:3) resulted in a higher potential value than for the others. The same situation was seen at each flow-rate (fig.12). The reason might be fast second response (see t'α values in table 6)

at low activity levels (high dispersion). On the other hand, the sensitivity of the electrode increased with flow-rate decrease at each dispersion level (fig. 13). There were no significant changes between peak widths due to dispersion of sample (fig 14), but, changes of flow-rate and activity range were effective. Peak tailing appeared at each flow-rate and activity range.

With deionized water as carrier, the electrode generally exhibited better sensitivity. The dispersion effect on the sensitivity of the electrode, obtained at different flow-rates for flow injection measurements, is shown in figure 15. There were no significant potential differences between flow-rates 2 and 4 ml min^{-1} as maximum sensitivity was obtained with dispersion coefficient 3 at flow-rate 1 ml min^{-1}. The sensitivity was relatively increased with the sample at dispersion coefficient 2, compared to the experiments made with the primary ion used as carrier. This may be due to decreasing the second response time of the electrode in deionized water. The response time of the electrode in the same conditions was 24.5 and 40 s for 10^{-5} and 10^{-1} mol dm^{-3} levels respectively (table 5b). For a 20 µl injection (at dc:2) of 10^{-5} mol dm^{-3} of the sample, the loss of the response is around 18% and this is 50% for 10^{-1}mol dm^{-3} level. The flow-rate effect of the carrier on the sensitivity is shown in figure 16. The highest sensitivity was obtained using flow-rate 1 ml min^{-1} at each dispersion coefficient. In figure 17, the effect of dispersion on the peak width, at different flow-rates, is shown for flow injection measurements. The peak width was slightly changed with dispersion coefficient change at each flow-rate and activity level, but sharply decreased by flow-rate increase and activity level decrease at each dispersion coefficient.

In all calibration measurements at different dispersion coefficients, the electrode exhibited non-Nernstian behaviour because of the loss of the electrode response with the increase of the response time due to increasing activity level.

Table 7. The effect of sample dispersion on peak height at different flow-rates and concentration levels using 10^{-5} mol dm^{-3} bromide solution as carrier in flowing conditions.

	dc:1			dc:2			dc:4		
conc.	flow-rate (fr): ml min-1								
mol dm^{-3}	fr:1	fr:2	fr:4	fr:1	fr:2	fr:4	fr:1	fr:2	fr:
0.00001	1.1	0.8	0.7	1.8	1.4	1.2	2.3	1.8	1.6
0.0001	1.4	1.2	1.1	2.4	2.0	1.8	3.7	3.1	2.8
0.001	3.5	3.3	3.2	4.8	4.3	4.2	5.8	5.5	5.2
0.01	7.0	6.0	5.6	9.4	6.8	6.6	11.5	8.3	7.5
0.1	10.8	9.0	8.5	13.6	10.9	10.0	15.0	12.4	12.0

Table 8. The effect of sample dispersion on the peak height (a) and width (b) at different flow-rates and concentration levels using deionized water as carrier in flowing conditions.

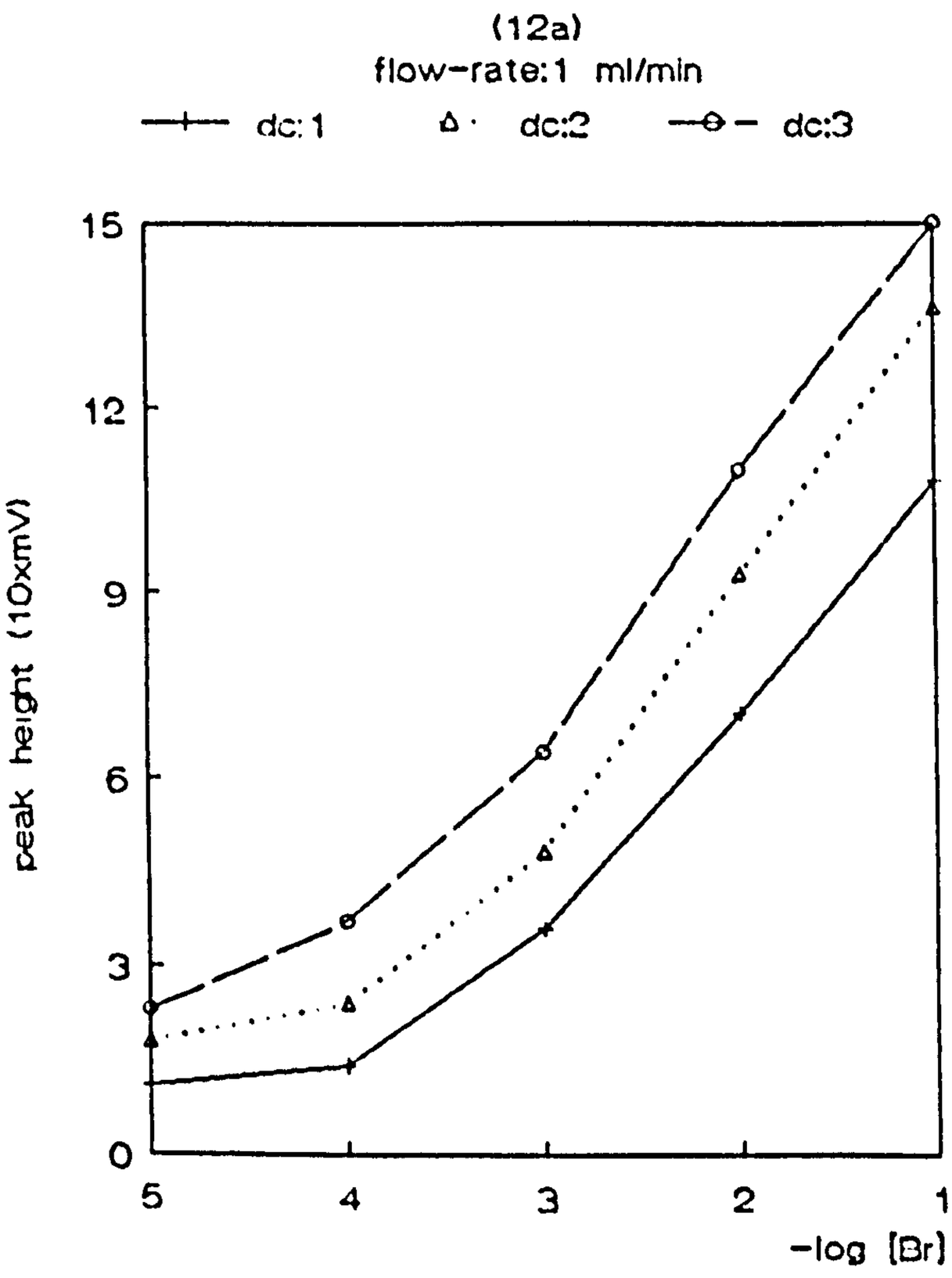
a

	dc:1			dc:2			dc:4		
conc.	flow-rate (fr): ml min^{-1}								
mol dm^{-3}	fr:1	fr:2	fr:4	fr:1	fr:2	fr:4	fr:1	fr:2	fr:4
0.00001	4.5	4.0	3.8	5.5	4.5	4.3	5.6	4.6	4.5
0.0001	5.7	5.0	4.8	6.7	5.6	5.3	6.7	5.6	5.5
0.001	9.3	8.2	7.8	10.0	8.7	8.5	10.2	9.1	9.0
0.01	13.8	11.7	11.2	14.6	12.8	12.6	15.2	13.0	12.8
0.1	17.5	14.8	13.6	18.1	15.3	15.2	18.5	15.8	15.0

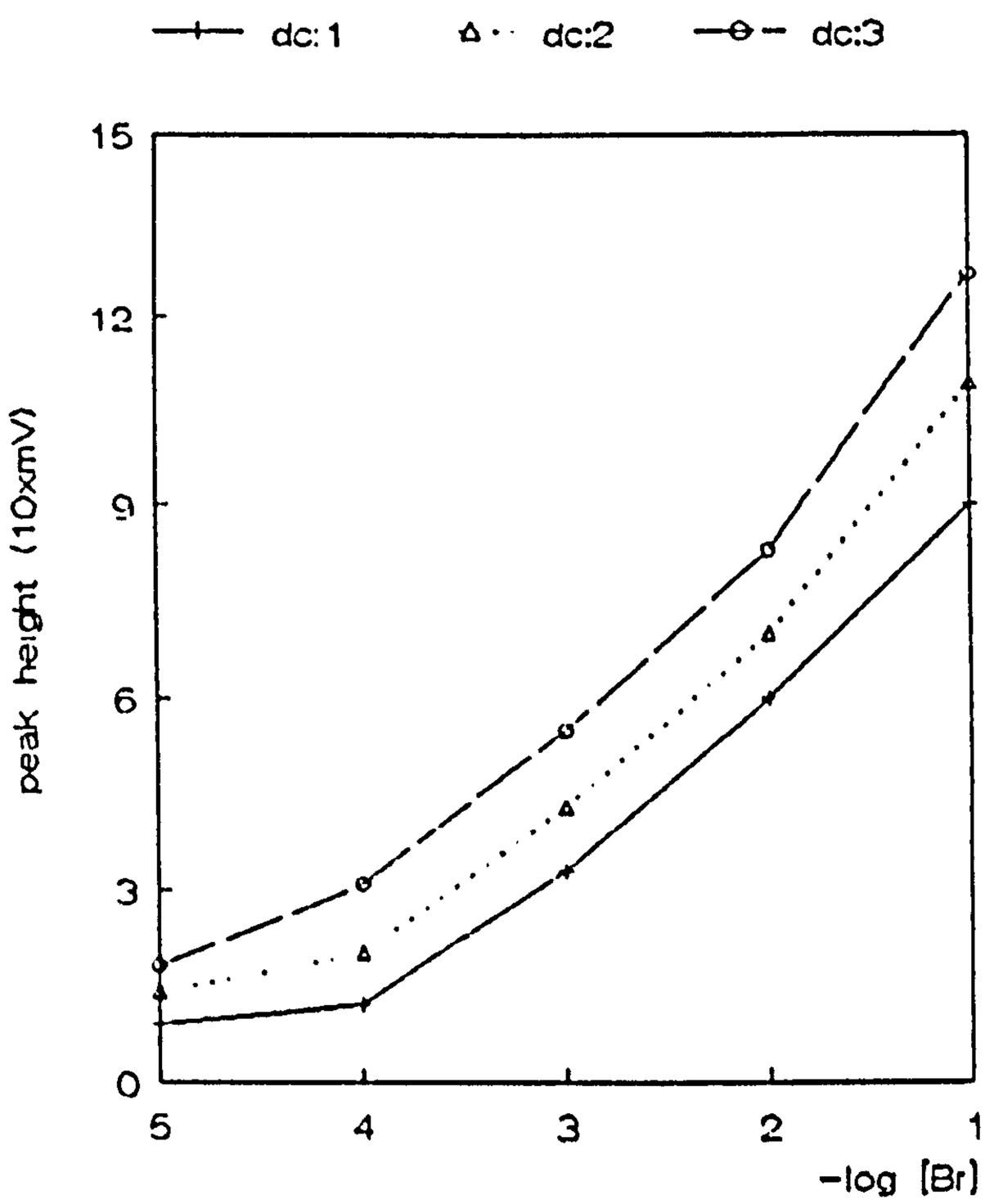
b

0.00001	0.80	0.40	0.24	0.80	0.40	0.24	0.80	0.40	0.24
0.0001	0.90	0.50	0.30	0.90	0.48	0.28	0.90	0.50	0.30
0.001	1.00	0.61	0.37	1.00	0.57	0.32	1.00	0.60	0.36
0.01	1.10	0.73	0.44	1.10	0.65	0.36	1.15	0.71	0.42
0.1	1.20	0.85	0.50	1.25	0.75	0.40	1.30	0.83	0.48

Figure 12. The effect of the dispersion on peak height at different concentration levels of bromide standard solution, using 10^{-5} mol dm^{-3} bromide solution as carrier at flow-rates 1 (a), 2 (b) and 3 (c) ml min^{-1} in the flowing conditions.



(12b)
flow-rate: 2 ml/min



(12c)
flow-rate:4 ml/min

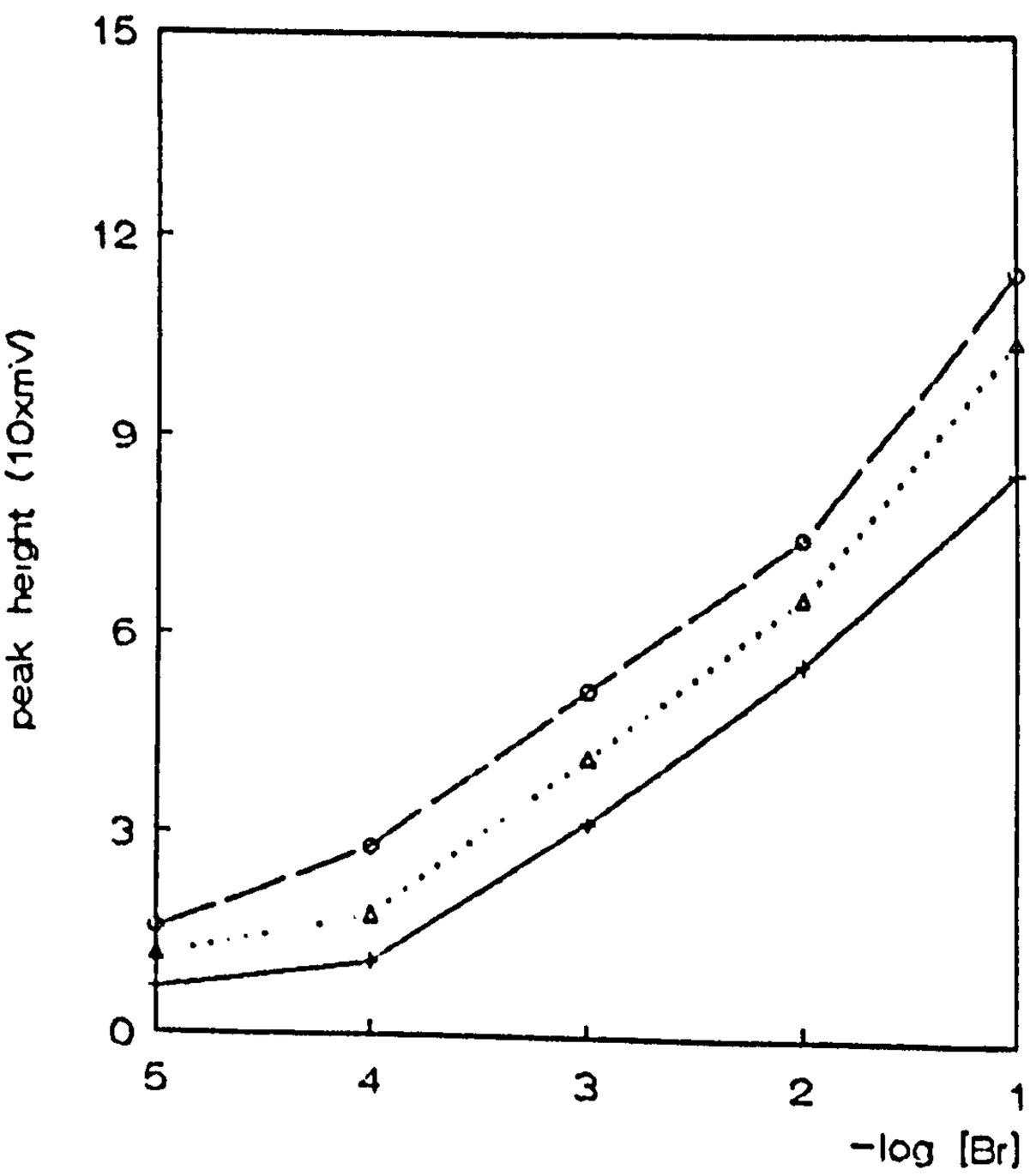
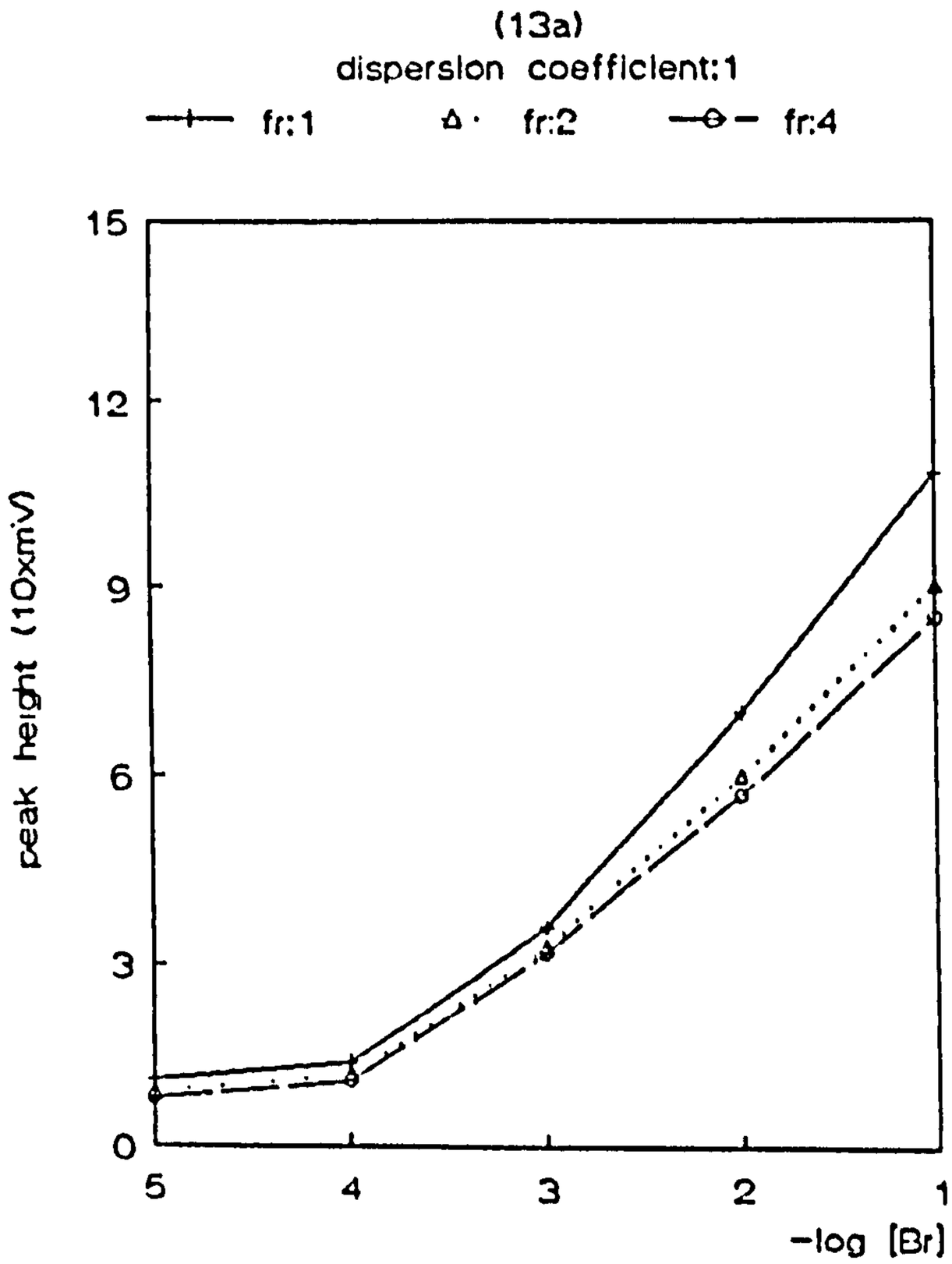
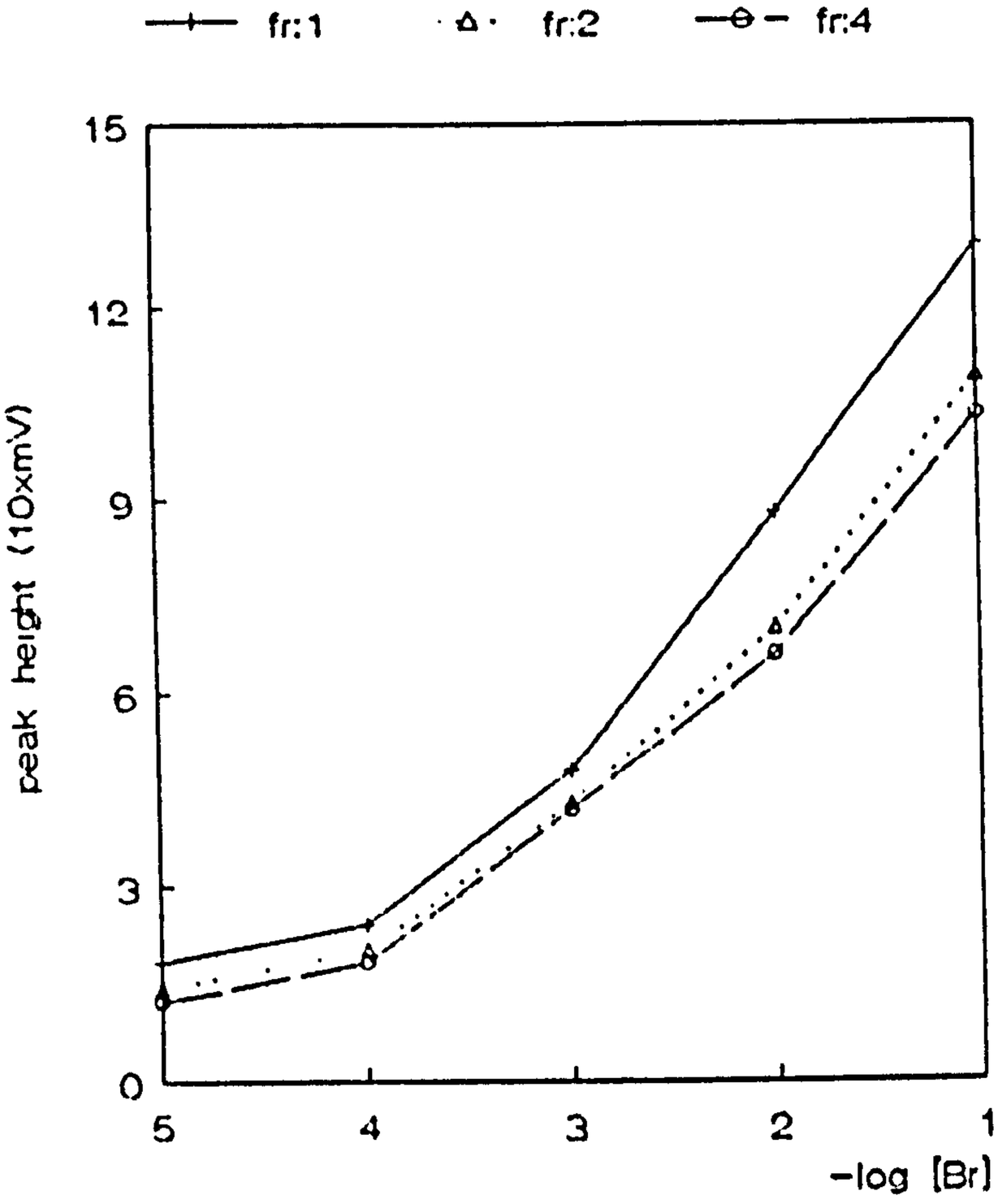


Figure 13. Measured calibration curves at the dispersion coefficients 1 (a), 2 (b) and 3 (c), obtained with 10^{-5} mol dm^{-3} bromide solution as carrier at different flow-rates in the flowing conditions.



(13b)
dispersion coefficient:2



(13c)
dispsersion coefficient:3

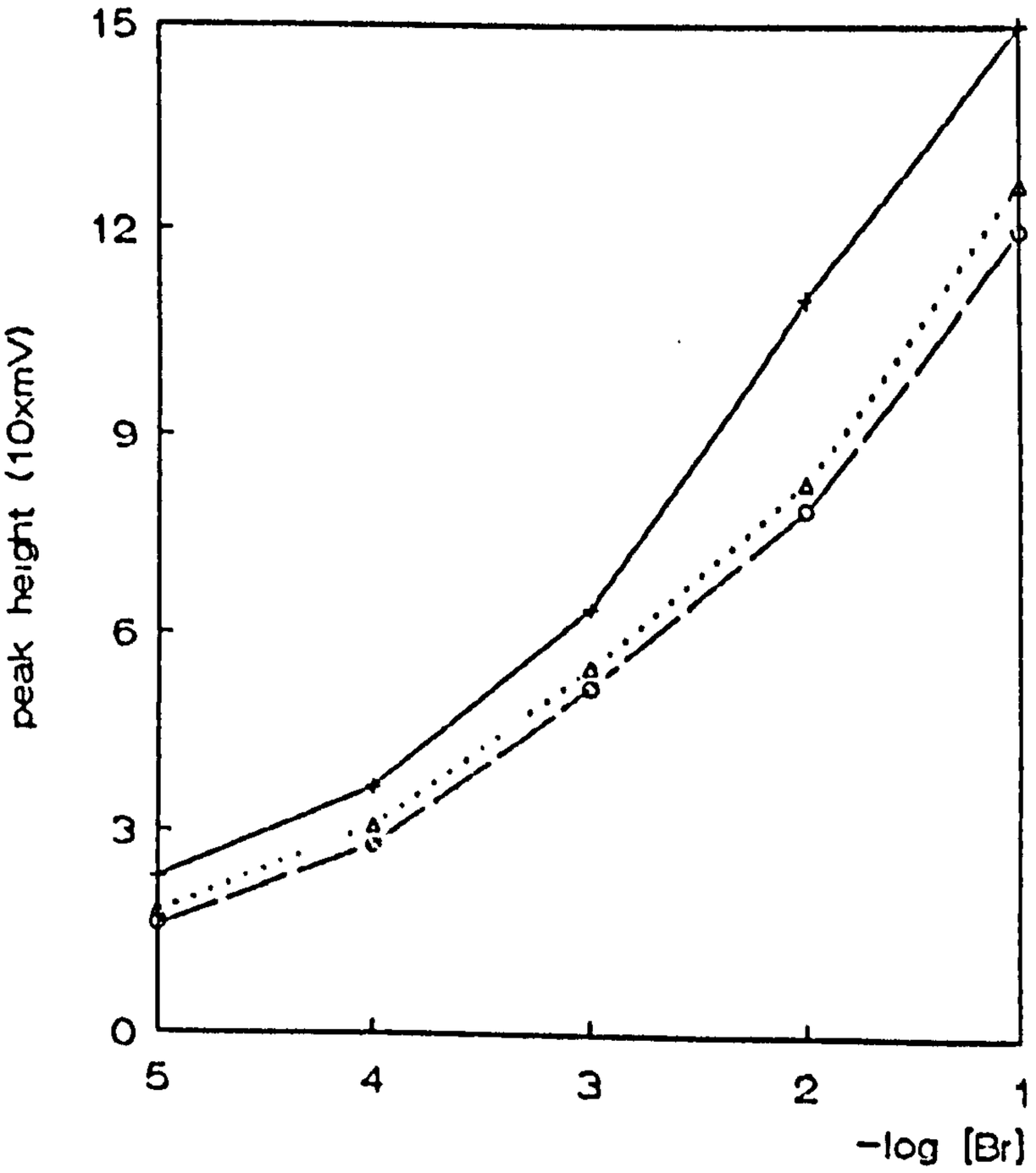
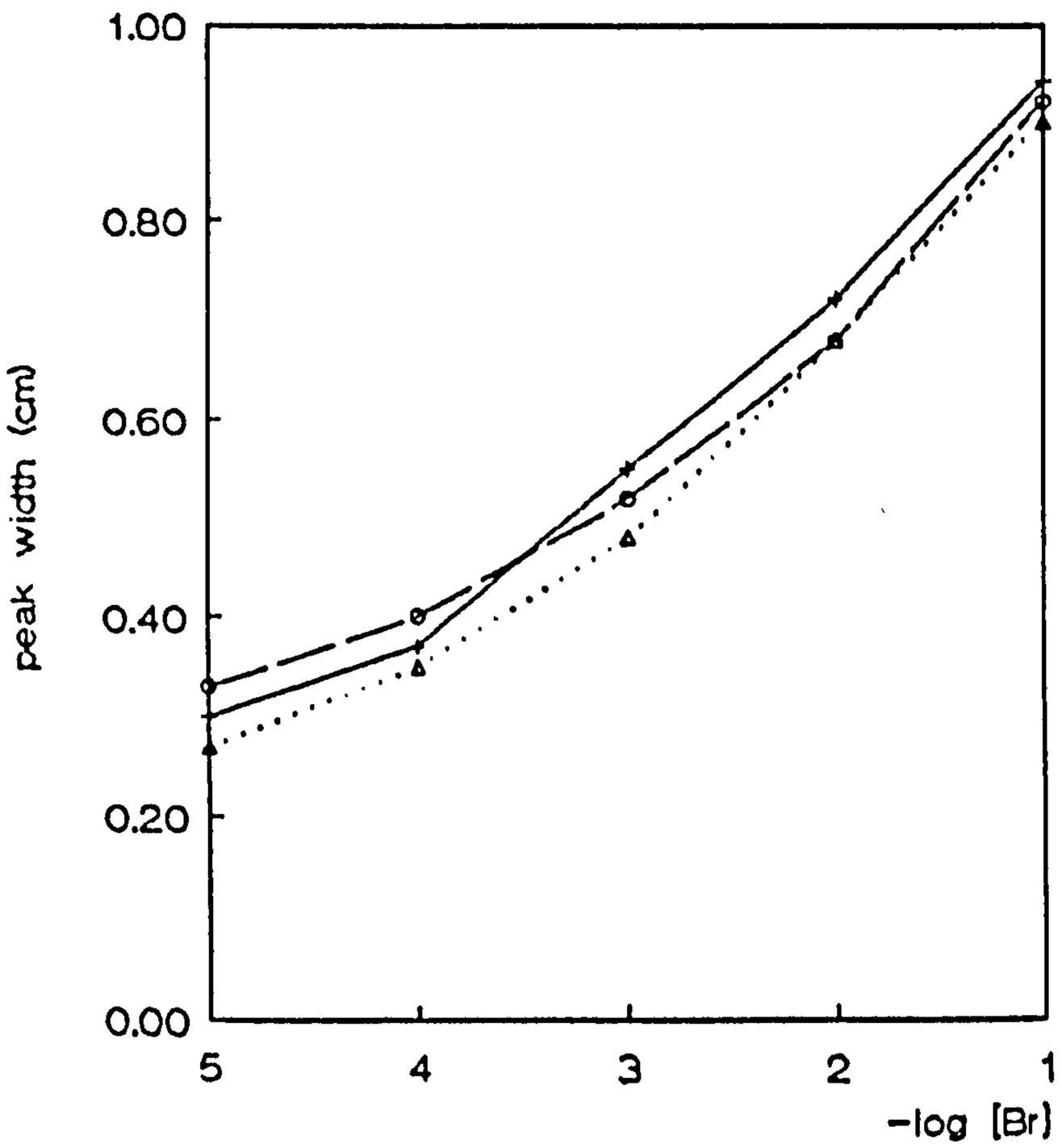
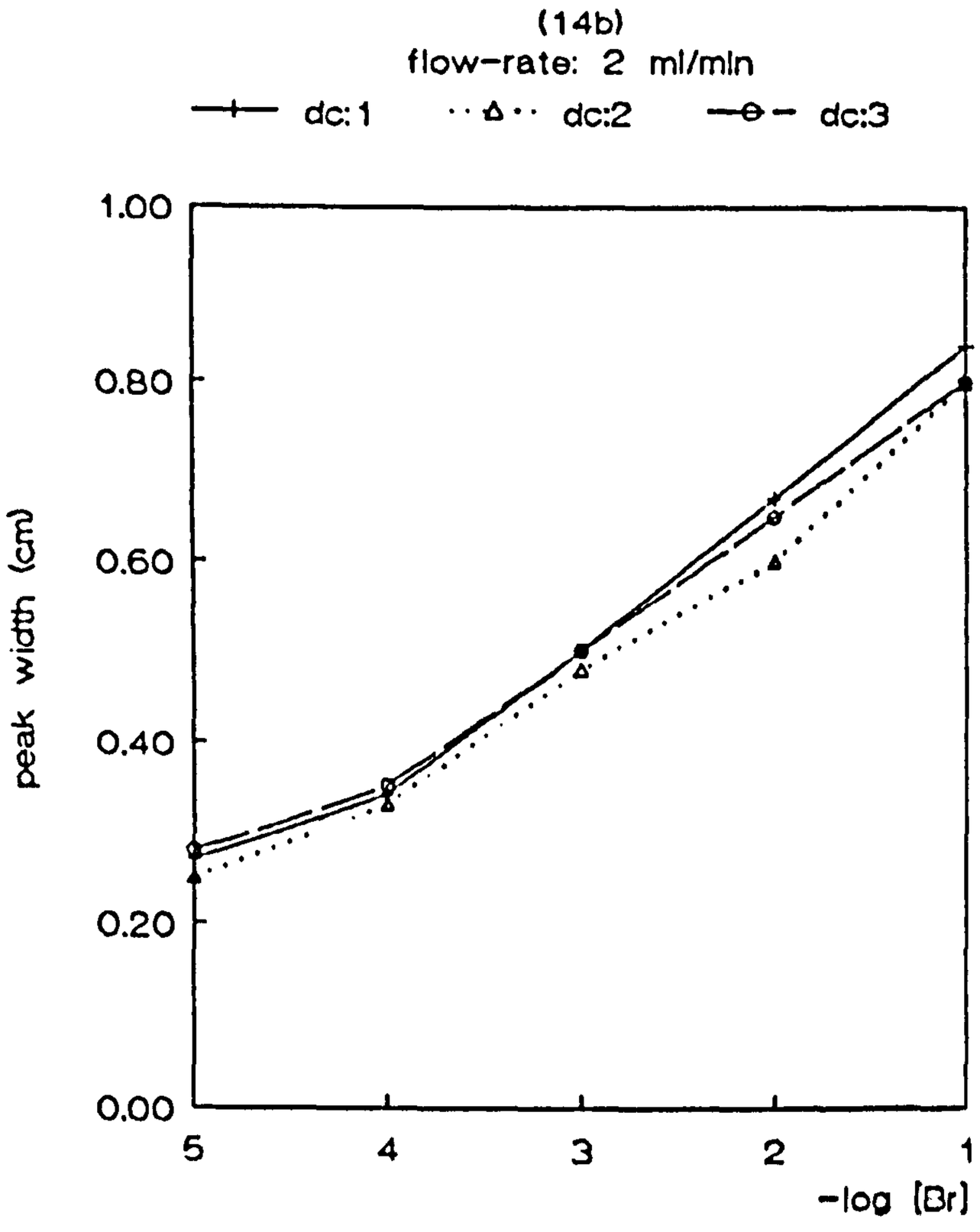


Figure 14. The effect of the dispersion on peak width at different concentration levels of bromide solution, obtained with 10^{-5} mol dm^{-3} bromide solution as carrier at flow-rates 1 (a), 2 (b) and 3 (c) ml min^{-1} in the flowing conditions.




(14b)
flow-rate: 2 ml/min
dc:1
dc:2
dc:3
1.00
0.80
0.60
0.40
0.20
0.00
peak width (cm)
5
4
3
2
1
-log [Br]

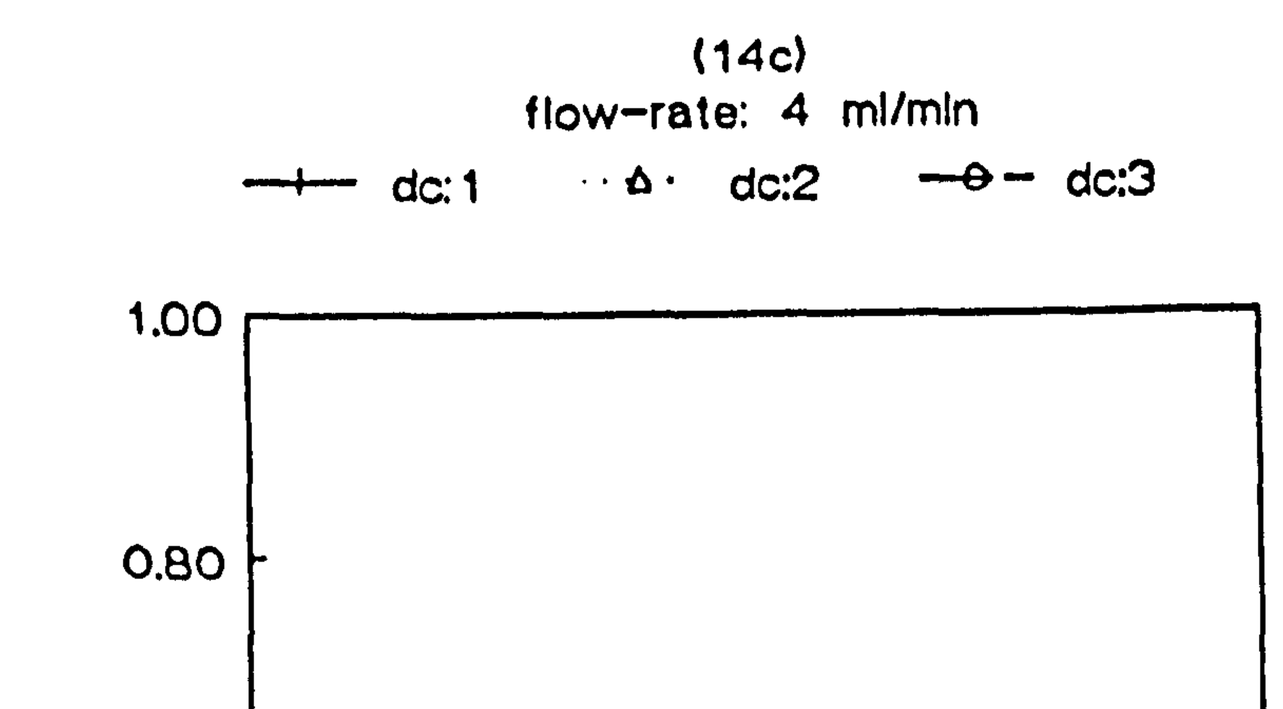
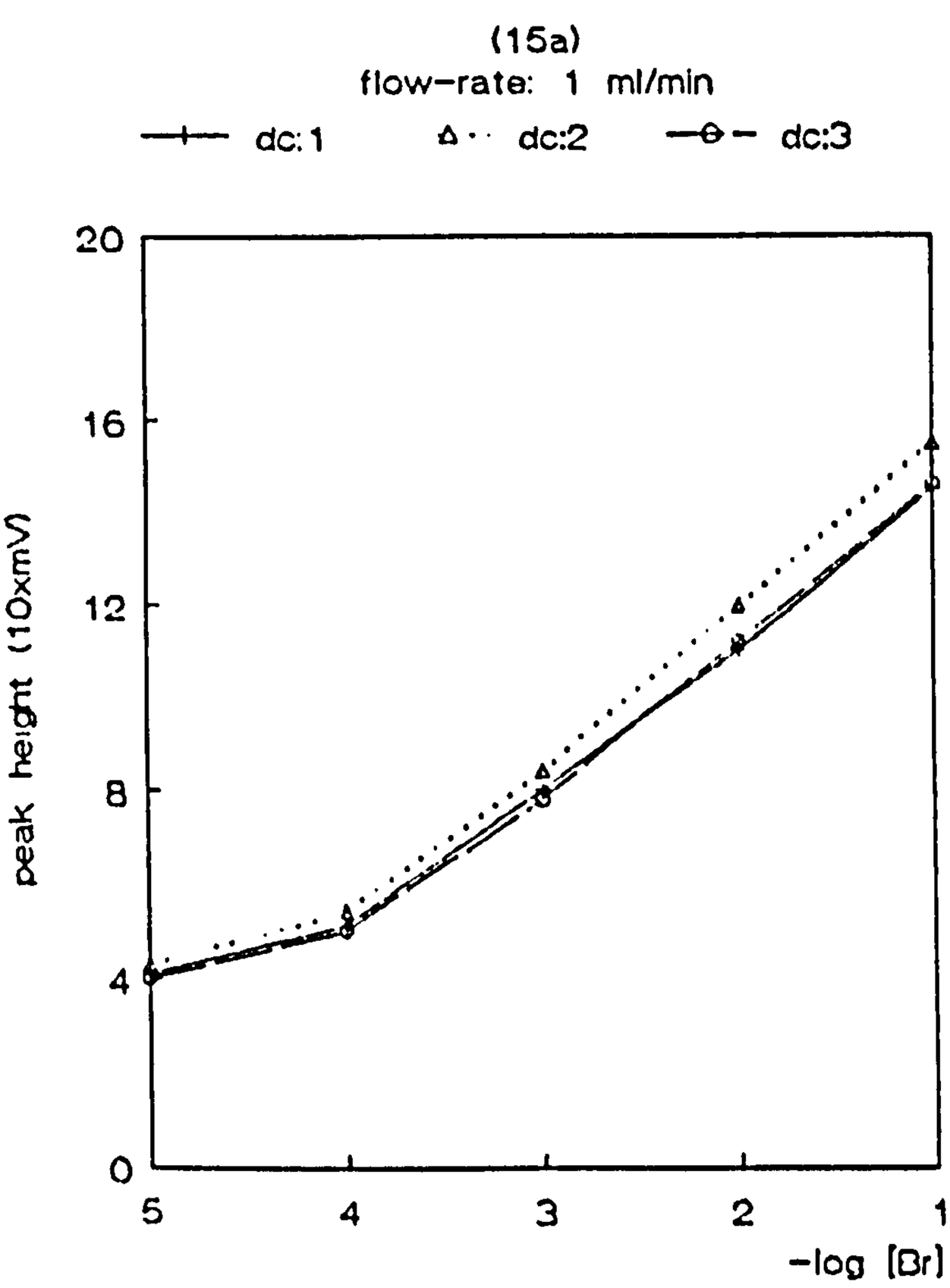
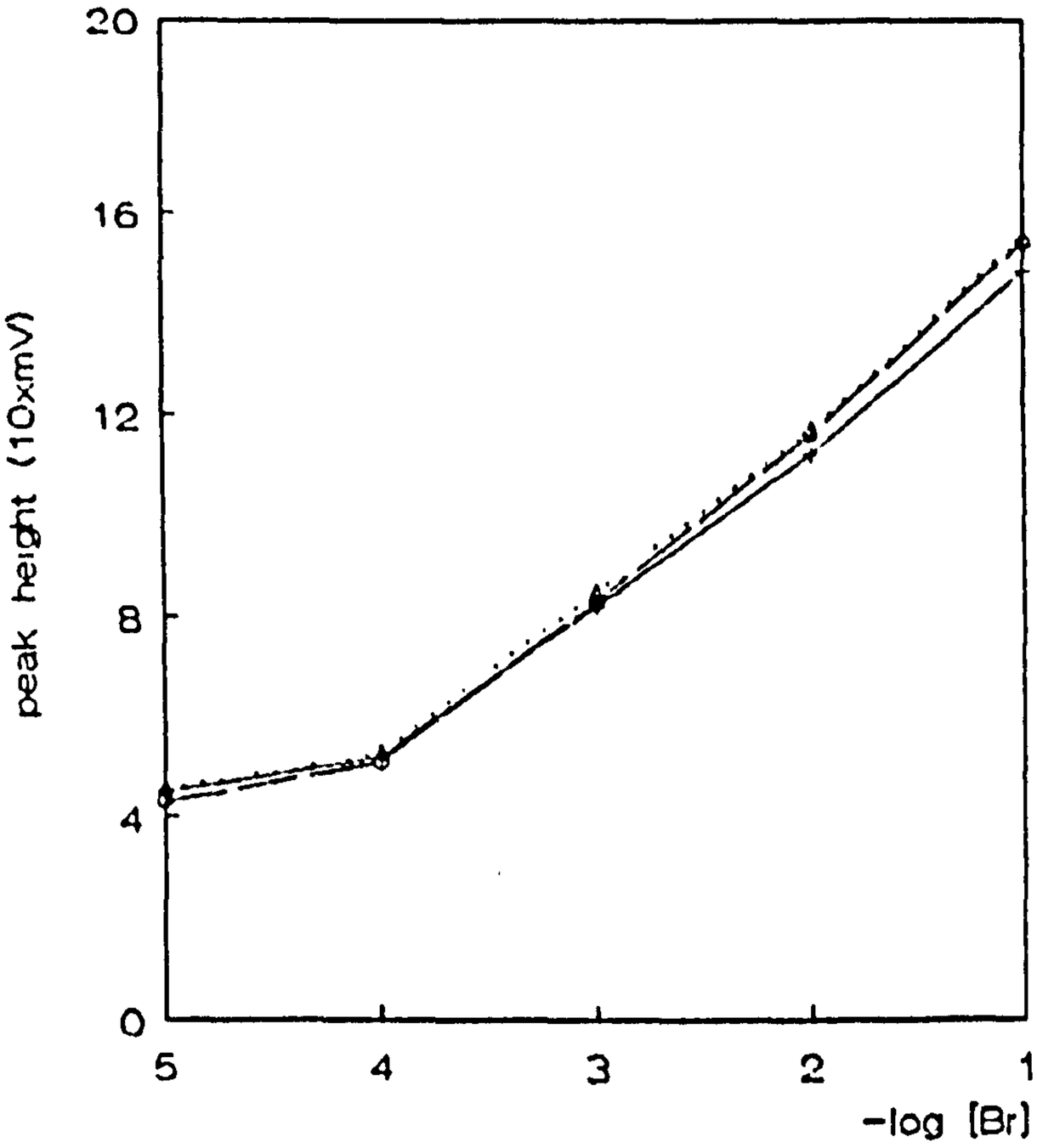

(14c)
flow-rate: 4 ml/min
dc:1
dc:2
dc:3
1.00
0.80
0.60
0.40
0.20
0.00
peak width (cm)
5
4
3
2
1
-log [Br]

Figure 15. The effect of the dispersion on peak height at different concentration levels of bromide standard solution, using deionized water as carrier at flow-rates 1 (a), 2 (b) and 3 (c) ml min^{-1} in the flowing conditions.



(15b)
flow-rate: 2 ml/min



(15c)
flow-rate: 4 ml/min

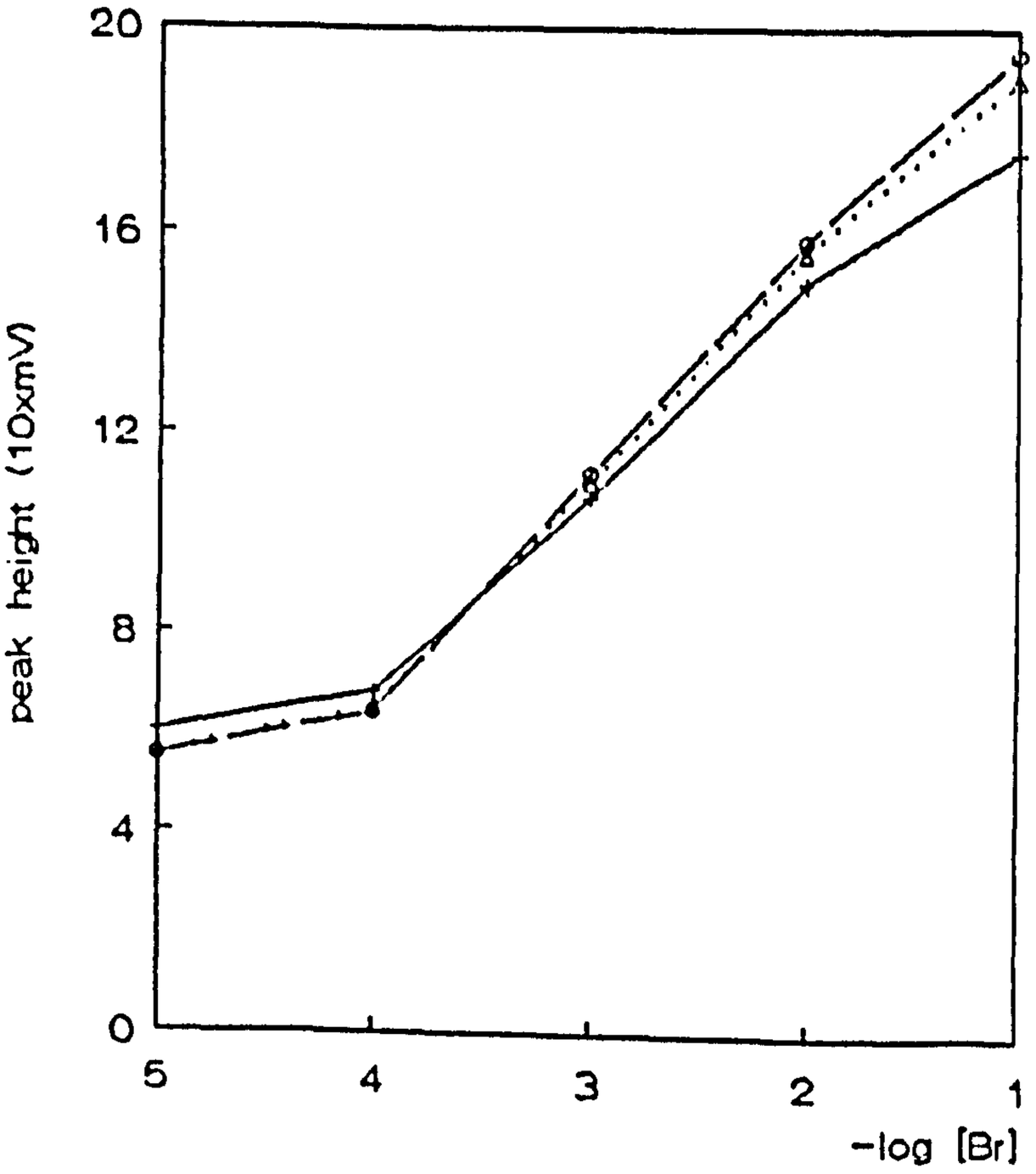
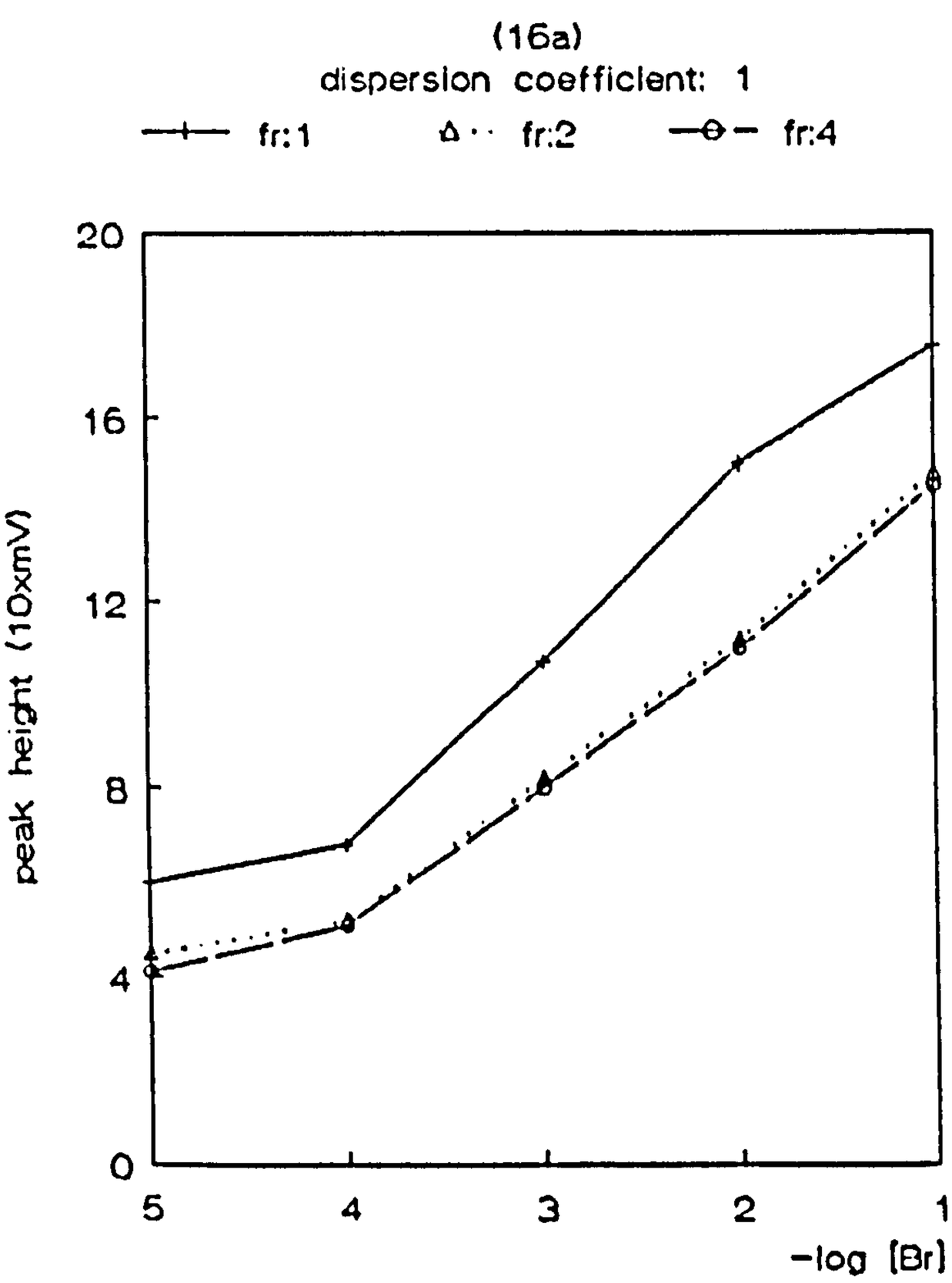
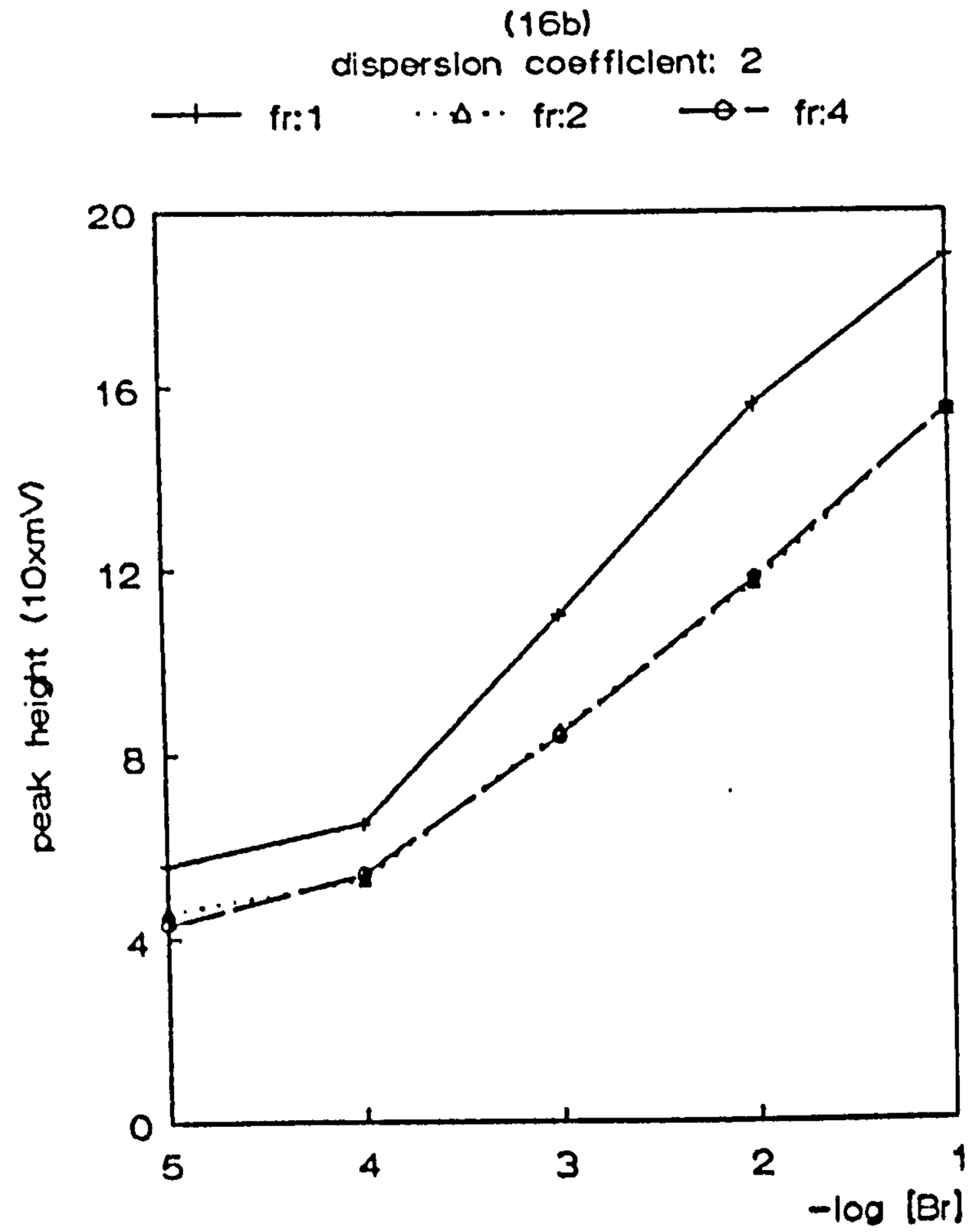


Figure 16. Measured calibration curves at the dispersion coefficients 1 (a), 2 (b) and 3 (c), obtained with deionized water as carrier at different flow-rates in the flowing conditions.



(16b)


dispersion coefficient: 2
fr:1
fr:2
fr:4
20
16
12
8
4
0
peak height (10xmV)
5
4
3
2
1
-log [Br]

(16c)

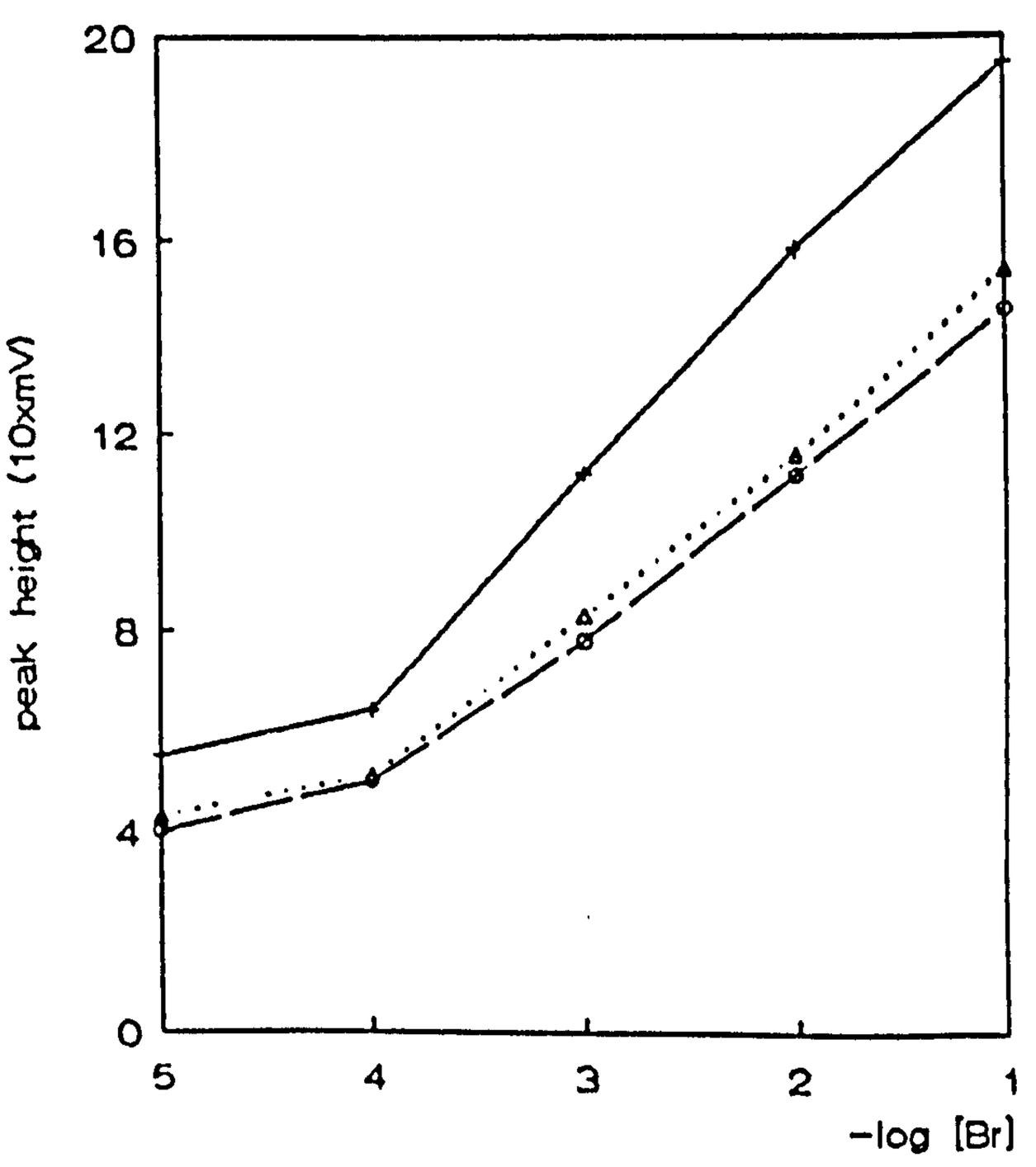
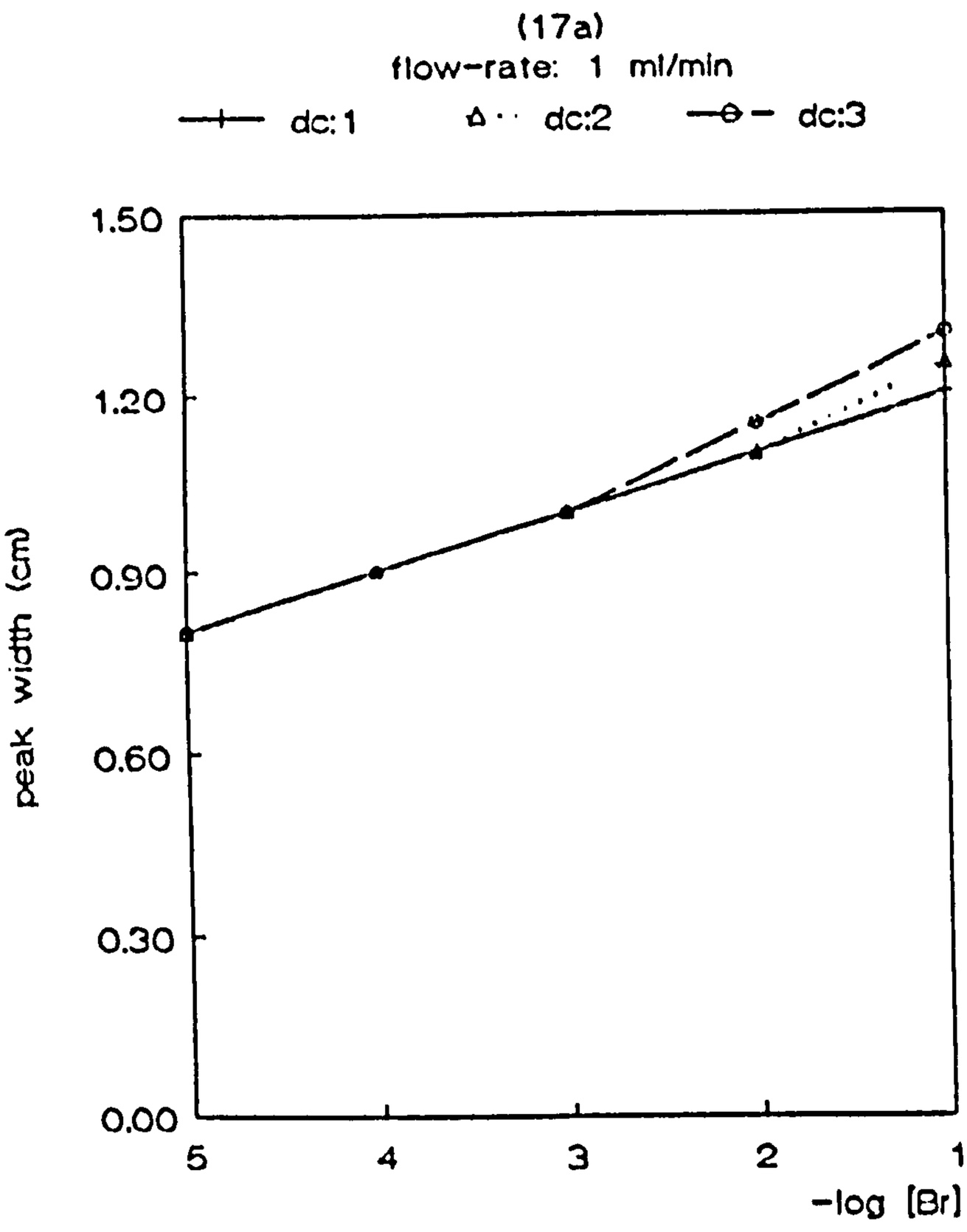
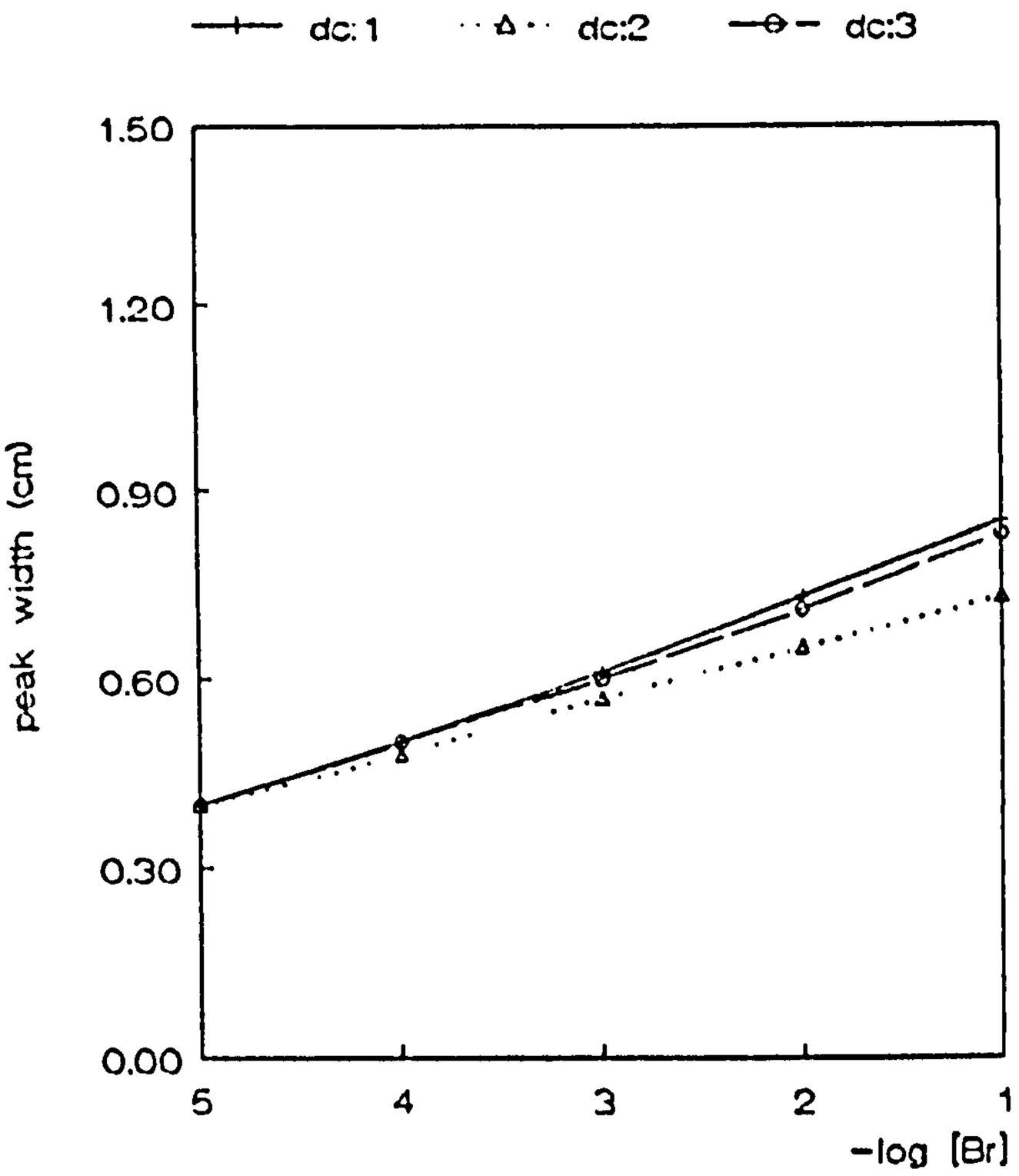

dispersion coefficient: 3
fr:1
fr:2
fr:4
20
16
12
8
4
0
peak height (10xmV)
5
4
3
2
1
-log [Br]

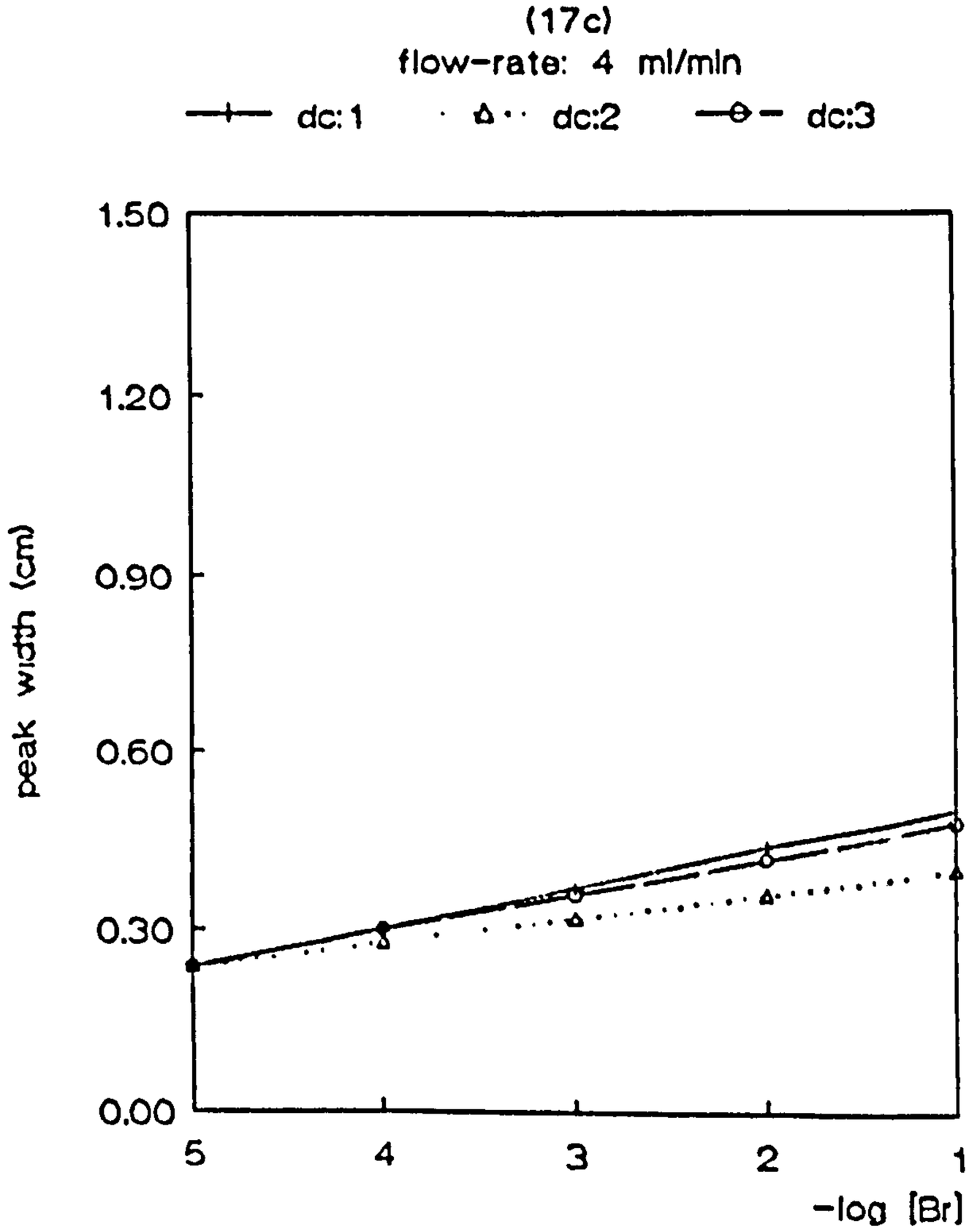
Figure 17. The effect of the dispersion on peak width at different concentration levels of bromide solution, obtained with deionized water as carrier at flow-rates 1 (a), 2 (b) and 3 (c) ml min^{-1} in the flowing conditions.



(17b)


flow-rate: 2 ml/min
dc:1
dc:2
dc:3
peak width (cm)
1.50
1.20
0.90
0.60
0.30
0.00
5
4
3
2
1
-log [Br]

(17c)


flow-rate: 4 ml/min
dc:1
dc:2
dc:3
peak width (cm)
1.50
1.20
0.90
0.60
0.30
0.00
5
4
3
2
1
-log [Br]

6.13 REFERENCES

1. Pungor E. and Umezawa Y., *Anal. Chem.*, 1983, 55, 1432.

2 Pungor E., Toth K. and Rabeczy-Pall A., *Pure Appl. Chem.*, 1979, 55, 1913.

3. I.U.P.A.C., *Anal. Chem.*, 1976, 48, 127.

4. I.U.P.A.C., *Inf. Bull.*, 1978, 1, 69.

5. Fleet B., Ryan T.H. and Brand M.J.D., *Anal. Chem.*, 1974, 46, 12.

6. Bladel W.J. and Dunwiddie D.E., *Anal. Chem.*, 1975, 47, 1070.

7. Shatkay A., *Anal. Chem.*, 1976, 48, 1039.

8. Uemasu I. and Umezawa Y., *Anal. Chem.*, 1982, 54, 1198.

9. Lindner E., Toth K.and Pungor E., *Pure Appl. Chem.*, 1986, 58, 470.

10. Suzuki K., Aruga H. and Shirai T., *Anal. Chem.*, 1983, 55, 2011.

11. Koizumi S., Imato T. and Ishibashi N., *Anal. Sci.*, 1987, 3, 319.

12. Lizhu Z., Jinglan C. and Jinyao Y., *Fenxi Huaxuse*, 1988, 16(8), 735.

13. Trojanowicz M. and Meyerhoff M.E., *Anal. Chim. Acta*, 1989, 222, 95.

14. Kolycheva N. and Muller H., *Anal. Chim. Acta*, 1991, 242, 65.

15. Petak P. and Stulik K., *Anal. Chim. Acta*, 1986, 185, 171.

16. Alonso J., Bartroli J., Lima J.L.F.C.and Machado A.A.S.C., *Anal. Chim. Acta*, 1986, 179, 503.

17. Imato T.,Yoshizuka T. and Ishibashi N., *Anal. Chim. Acta.*, 1990, 233, 139.

18. van Staden J.F., *Anal. Chim. Acta.*, 1992, 261, 381.

19. van Staden J.F., *Analyst*, 1992, 117, 51.

20. Trojanowicz M. and Frenzel W., *Z. Anal. Chem.*, 1987, 328, 653.

21. Stulik K., *Analyst*, 1989, 114, 1519.

22. Van der Linden W.E. and Oostervink R., *Anal. Chim. Acta.*, 1978, 101, 410.

23. Jyo A., Mori K. and Ishibashi N., *Bull. Chem. Soc. Jpn.*, 1983, 56, 3505.

24. Butler E.C.V. and Gershey R.M., *Anal. Chim. Acta.*, 1984, **164**, 153.

25. Fucsko J., Toth K., Kunovits J. and Puxsbaum H., *Anal. Chim. Acta.*, 1987, **194**, 163.

26. Dovey D.E., Mulcaky D.E. and O'Connell G.R., *Anal. Lett.*, 1986, **19**, 1387.

CHAPTER 7

7.1 POTENTIOMETRIC DETECTION AT PPB-RANGES OF CHLORIDE, BROMIDE, NITRITE, NITRATE, IODIDE, THIOCYANATE AND BENZOATE ANIONS SEPARATED IN ION-EXCHANGE CHROMATOGRAPHY IN A SINGLE RUN

7.2 INTRODUCTION

Classical ion chromatography can be conveniently carried out with conventional high performance liquid chromatographic equipment and classical ion-exchangers using a single column and a UV absorbing eluent ion. The preparation of new ion-exchange resins featured the development of improvements in suppressed ion chromatography[1,2] and in high efficiencies despite very low ion-exchange capacities. It is now the leading analytical method for determining of anions in aqueous samples. Gradient elution has rarely been used in ion chromatography with conductometric detection,[3-7] mainly because many important ions can be separated and eluted isocratically, for example, the halides and some common anions. However, the time to elute strongly retained anions, which have high affinity for ion-exchangers, such as I^-, SCN^-, ClO_4^-, in one run is unacceptably long. It is necessary to vary the composition of the eluent or very strong eluents should be chosen.[7-11] Contaminants in the eluents altering during the run causes severe baseline shifts and makes gradient elution in ion chromatography difficult. To avoid the eluent suppression step, several alternative methods have been developed. These methods use either direct and indirect conductometric detection on the effluent from the separation column, or other detection methods such as UV absorbance, refractive index or electrochemical measurements. In some cases, potentiometric detection of eluted ions has been used, which was reviewed earlier. As suggested in chapter 5, ISEs based on PVC are less selective and could therefore be used for the chromatographic detection of a wide range of analyte ions. An all solid-state tubular type PVC matrix membrane electrode has been used throughout this work. The practical advantages of PVC-matrix bromide selective membrane electrode as detector in chromatography are demonstrated for

non-suppressed elution. Also, the possibility of separation of such strongly retained anions in an acceptable time in a single ion-exchange chromatography run, and increase their detection limits via potentiometric detection is examined. Using a solution of 3 mM phosphate as eluent causes these anions to elute in 13 minutes from an anion-exchange column in a single run, and a PVC-matrix membrane bromide selective electrode as detector permits the detection of most anions at sub-ppb levels.

7.3 EXPERIMENTAL

7.3.1 Preparation of sensors

The construction of a flow-through tubular PVC matrix membrane electrode without an inner reference solution was carried out as described by Alegret et al.[12] This consisted of two perspex holders into which 3 mm diameter channels were drilled and one perspex cylinder body in which a conductive support with 1.5 mm diameter channel was drilled. The membrane was applied into the hole of the conductive support which was epoxy resin loaded with graphite. The detector cell consisted of a flow-through tubular PVC matrix membrane electrode and a reference electrode as is shown in figure 1. When the sensing membrane solution had been coated on dropwise, the inner diameter of the channel was reduced to ca.1.2 mm. When not in use the tubular electrode was kept dry after washing with deionized water. It was reconditioned with primary ion solution before use. The calibration curve of the detector was obtained by constant volume dilution method as previously described,[13] and is shown in figure 2. The sensing membrane solution, incorporating TDDA-Br, DBP, PVC, THF and KTPB, was prepared as described in chapter 6. The epoxy resin mixture used to bind the graphite in preparing the internal conducting support of the electrode was made from Araldite 2005a and hardener 2005b (both from Ciba-Geigy) in a ratio of 1:0.4. The powdered graphite was mixed with epoxy resin in a ratio of 1:1.

All standard solutions of anions and eluent were prepared from their analytical reagent grade sodium or potassium salts in deionized water, then diluted to the desired concentrations. The identification of species was performed by comparing retention times of peaks with those of peaks in standards.

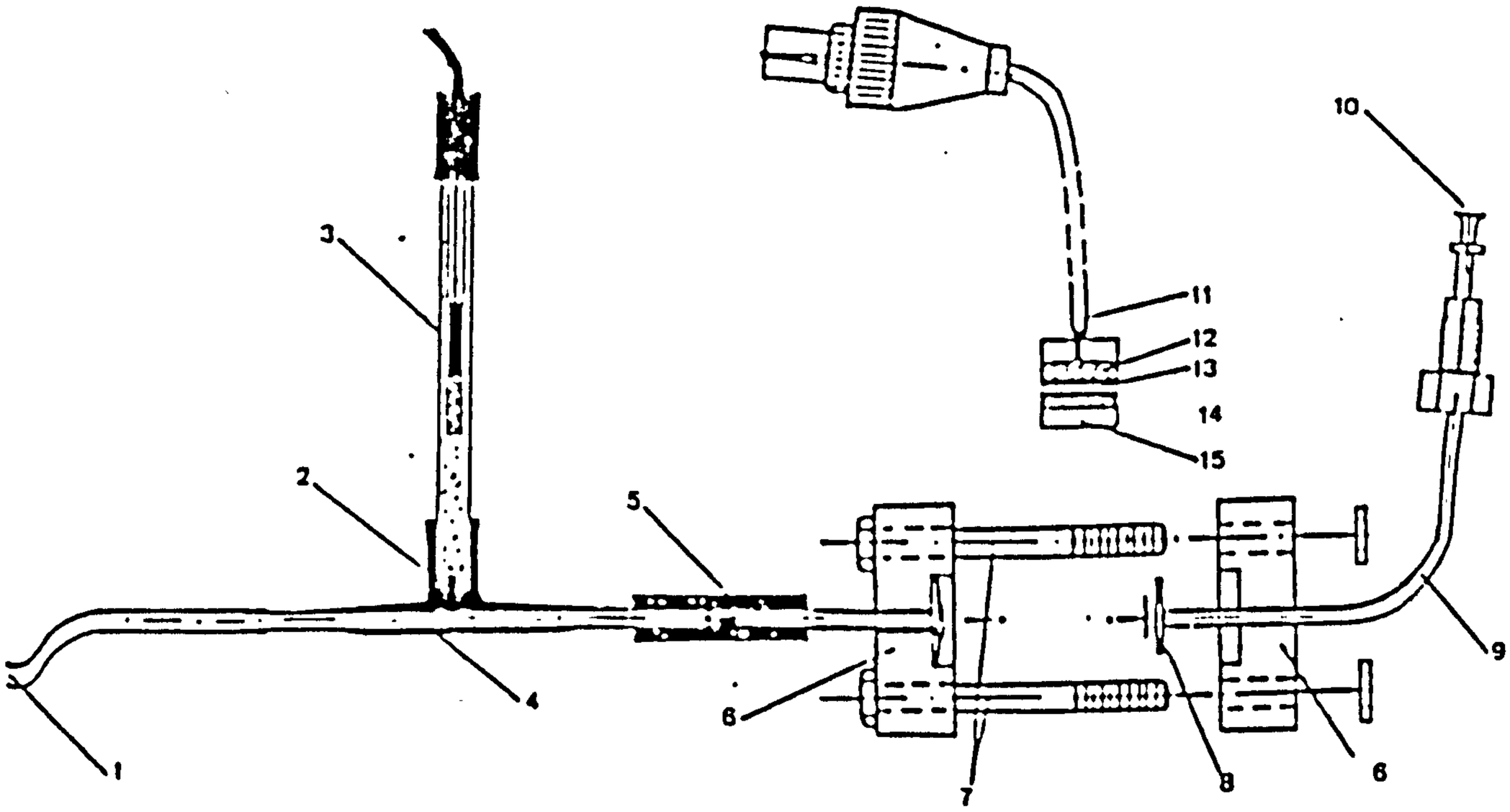
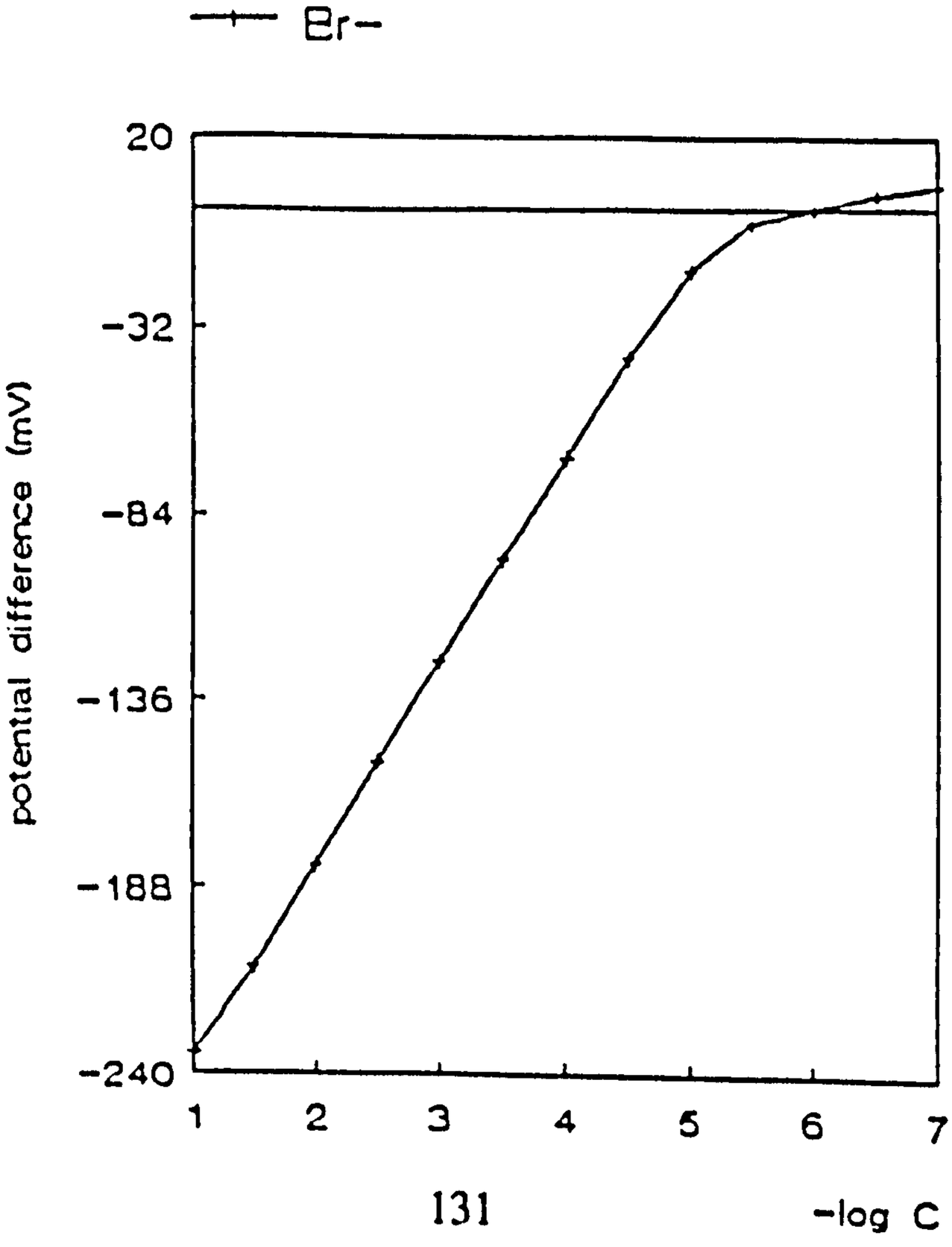


Figure 1. Detector cell: 1. outlet; 2. inlet frit; 3. reference electrode; 4. glass holder; 5. adapter; 6. perspex holders; 7. screws; 8. O-rings; 9. PTFE-tubings; 10. inlet; 11. electric cable; 12. conducting epoxy cylinder; 13. channel; 14. PVC membrane; 15. perspex cylinder body.

Figure 2. Response curve of the electrode to primary ion solution



7.3.2 Apparatus

Experiments were carried out with the HPLC apparatus with 20 μl sample loop as described in chapter 6. The separations of standard solutions were achieved with either IonPac-AS4A and IonPac-AG4A columns, or a single column. The detector cell was placed immediately after the end of the separation column. Other components of the system used for the recording of the chromatogram and for the reading of the potential were as described in chapter 6.

7.4 RESULTS AND DISCUSSION

The main limitation of the conductometric detector is its non-specificity, and the concentration of the eluent should therefore be close to that of the injected ions, since the background conductivity is too high without suppression. Strongly retained anions such as I^- and SCN^- produce broad peaks or may not be eluted with known eluents used for anion exchange columns from Dionex. Such anions, uneluted in an acceptable time, leave the column later and interfere with determinations and reproducibility or cause background conductivity of the eluent to increase. In order to improve the peaks of the strongly retained anions, the concentration of the eluent must be increased, but this is in contradiction to the requirements for conductometric detection with or without suppression (because of suppressor capacity). On the other hand, whilst the potentiometric detector shows high sensitivity, the choice of phosphate solution as eluent results in relatively short retention times and good resolution for strongly retained anions. The phosphate eluent was stronger than carbonate eluent by as much as eight times in concentration when used with the anion exchange column.

Previous reports[14,15] indicated that a background level of an electroactive species should be included in the eluent to give a stable electrode potential. It was found that with a liquid membrane ion selective electrode at any flow-rate, some electroactive species which have a slight affinity towards the membrane serve as baseline supporting electrolytes and no addition of primary ion to the eluent is required. It was also concluded

in chapter 6 on response time measurements of liquid membrane electrodes that adding the primary ion to the flowing stream (which was deionized water) caused a slight increase in response time. A typical chromatogram for a mixture of eight anions is shown in figure 3 The resolution and the sensitivity indicate the shortness of the response time of the membrane and non-selectivity to each anion.

Iodide and thiocyanate are very strongly retained anions on anion-exchange columns, primarily due to adsorption and dilution rather than ion-exchange. In the past, very strong eluents, such as 8 mM CO_3^{-2} in suppressed chromatography, are used to elute I^- only from the anion guard column.[16] Under these conditions, the peak shape was often unsymmetrical, making quantitation difficult. In addition, high strength eluent requiring frequent regeneration is necessary. The separation of six anions including iodide and thiocyanate was achieved on a Dionex guard column using only 0.35 mM phosphate as eluent, and is shown in figure 4.

The analysis of trace iodide has been a significant analytical problem for a number of years. For many applications the levels of iodide are in the ppb range and adequate sensitivity cannot be achieved with conductivity detection. Potentiometric detection by membrane ion selective electrodes offers increased sensitivity and selectivity over other methods and at the same time eliminates the need for a suppressor column. Using 1.5 mM phosphate as eluent, separation of seven anions including iodide and thiocyanate was achieved on Dionex analytical and guard columns, as shown in figure 5. In order to resolve iodide and thiocyanate in a reasonable time, the change of the eluent ionic strength was apparently necessary to shorten the time between nitrate, iodide and thiocyanate. A chromatogram obtained, using 3 mM phosphate as eluent, is shown in figure 6. As can be seen in the figure, seven common anions can be effectively separated on the Dionex analytical and guard anion exchange columns with this eluent within 16 minutes. Retention times for I^- and SCN^- are shorter than those obtained with other eluents used with such chromatographic columns.

In spite of large differences in affinity, good resolution was observed within reasonable time. A chromatogram of the anions

obtained with only one analytical column is shown in figure 7. The high sensitivity of the detector allows determination of each anion in most analytical samples without any sample preconcentration process. The detection limits estimated were sub-ppb levels for each anion with 20 μl injection volume. The reproducibility of peak heights for repeated injections of all anions at all concentrations was generally less than 2%. The lower reproducibility might occur when the distance between the working electrode and reference electrode is too large. Studies on response time measurements indicated that 2 cm distance between working electrode and reference electrode had caused no interference.

Ion chromatography with potentiometric detection is an excellent method of determining ppm and ppb levels of common anions plus iodide and thiocyanate in various matrices. The eluent was very effective for the separation of common and long retained anions. Applications of the electrode to various sample matrices will be attractive.

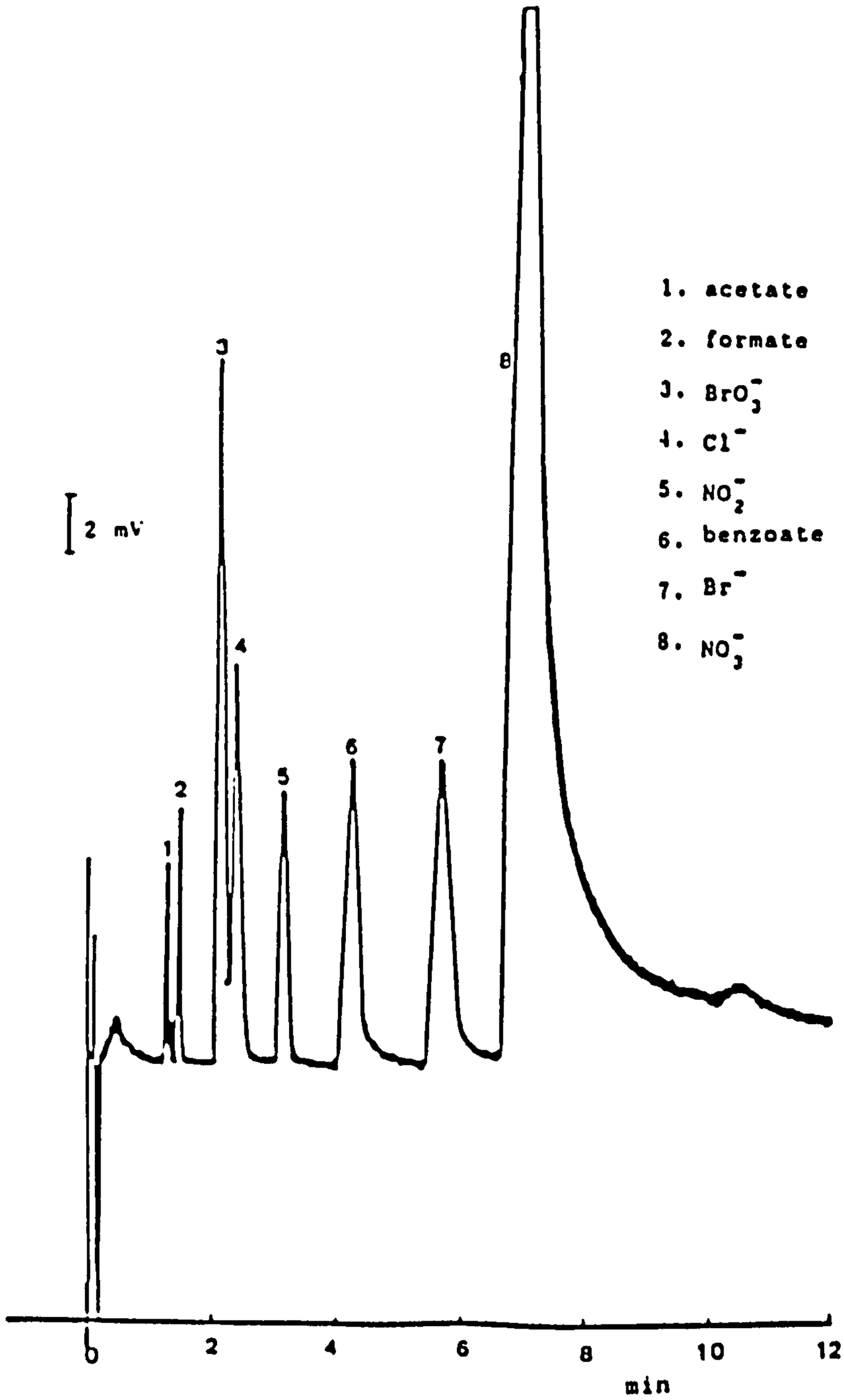


Figure 3. Separation of inorganic and organic anions on Dionex HPIC-AS4A and AG4A columns using 0.3 mM phosphate as eluent, and potentiometric detection, flow-rate: 2 ml min^{-1}, injection: 20 µl of 10^{-5} molar standard solution of each anion except benzoate, NO_2^-, Br^- and NO_3^- were $5x10^{-6}$ molar.

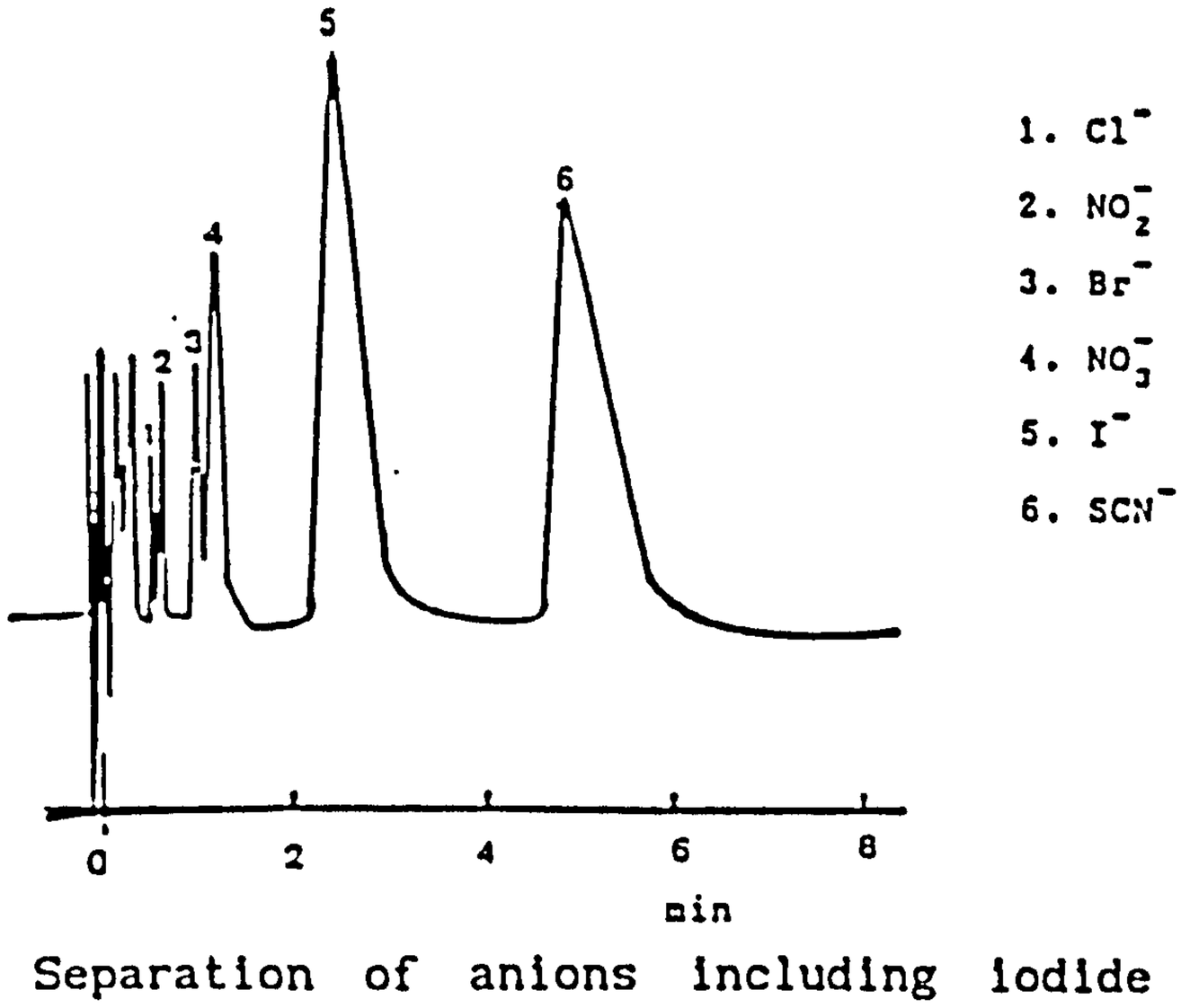


Figure 4. Separation of anions including iodide and thiocyanate on Dionex HPIC-AG4A column using 0.35 mM hydrogen phosphate as eluent, and potentiometric detection, flow-rate: 2 ml min^{-1}, injection: 20 µl of 10^{-5} molar standard solution of each anion except I^- and SCN^- were $5x10^{-6}$ molar.

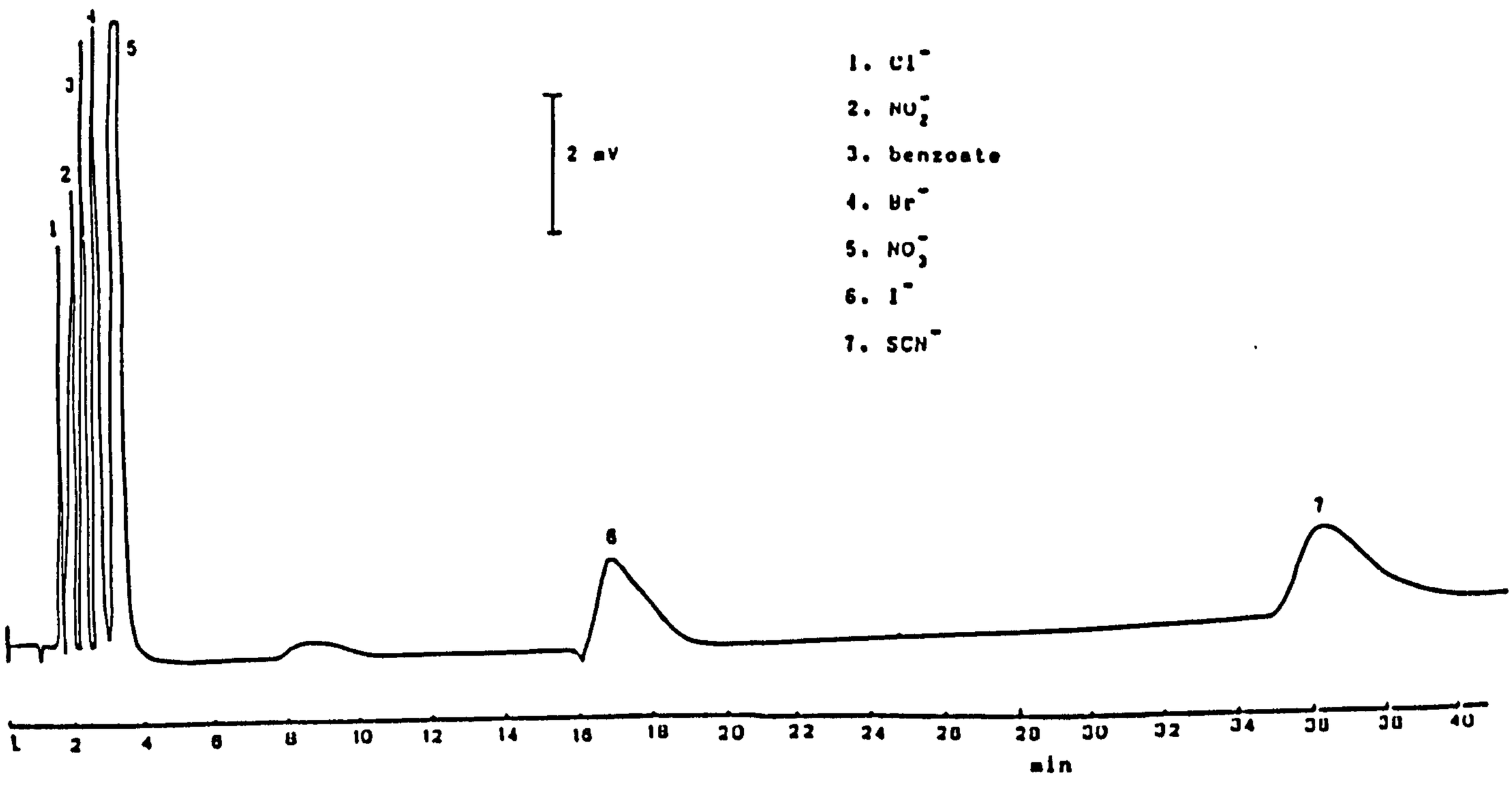


Figure 5. Separation of anions including iodide and thiocyanate on Dionex HPIC-AS4A and AG4A columns using 1.5 mM phosphate as eluent, and potentiometric detection, flow-rate: 2 ml min^{-1}, injection: 20 µl of 10^{-5} molar standard solution of each anion except I^- and SCN^- were $5x10^{-6}$ molar.

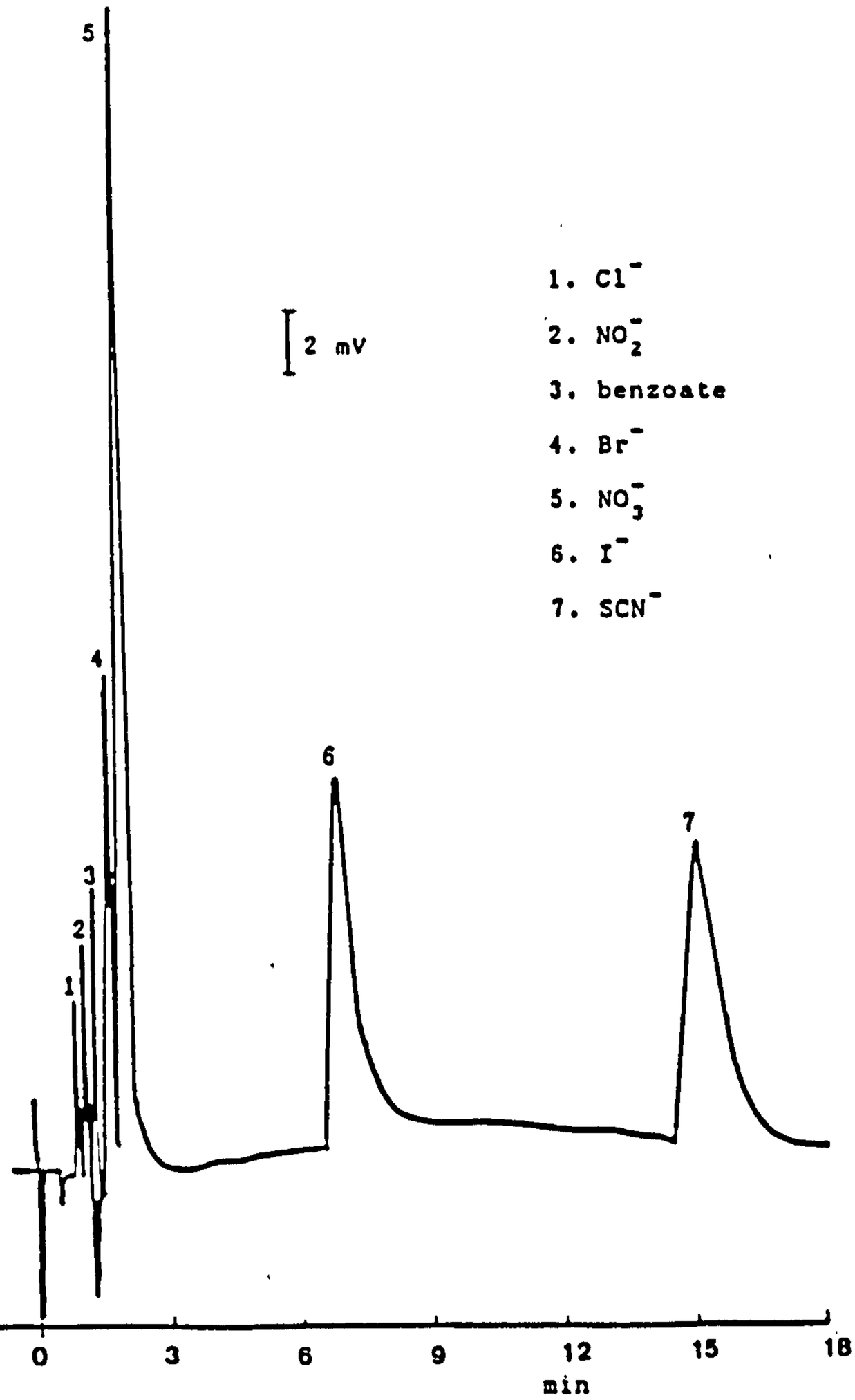


Figure 6. Separation of anions including iodide and thiocyanate on Dionex HPIC-AS4A and AG4A columns, using 3 mM phosphate as eluent, and potentiometric detection, the other conditions were as in figure 5.

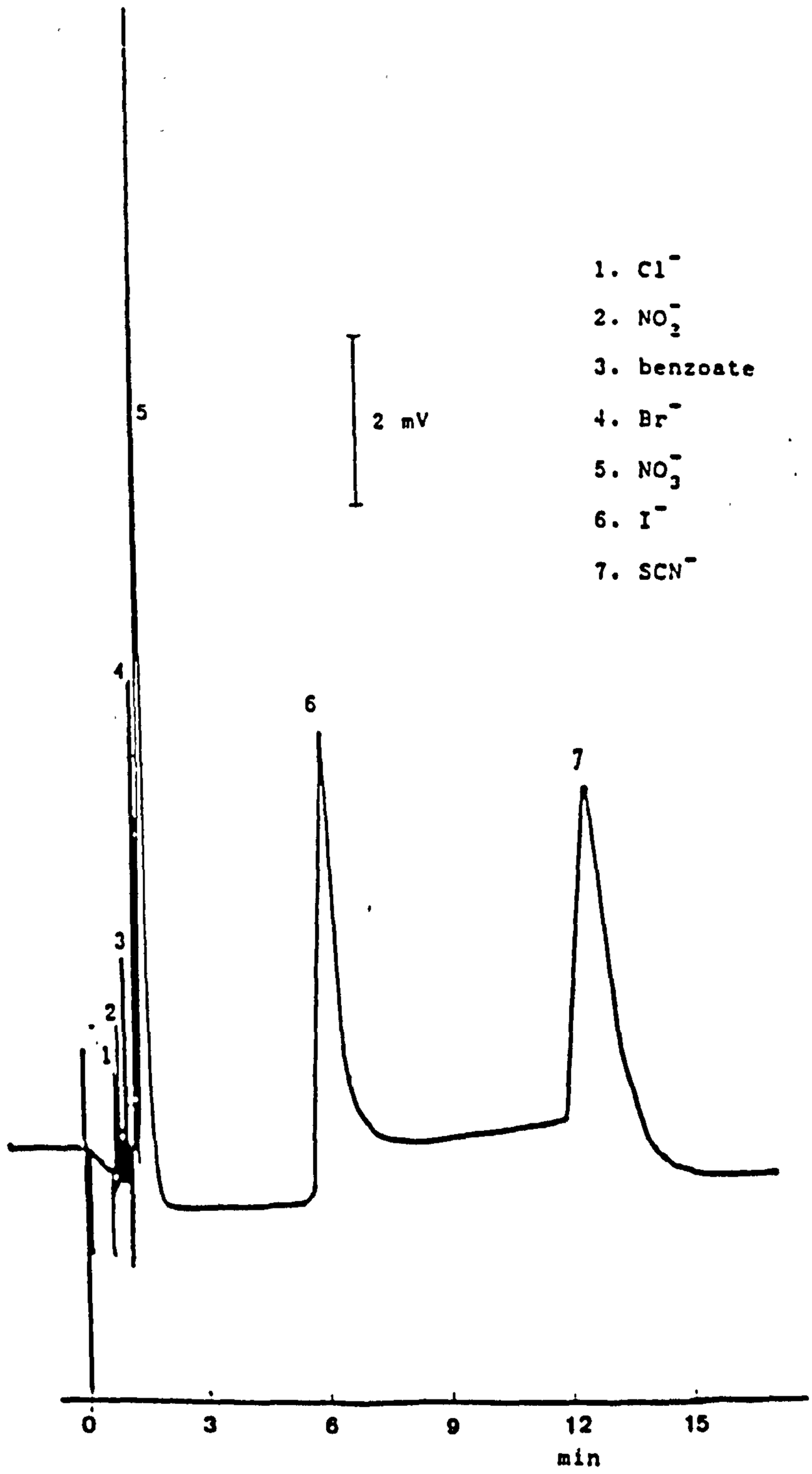


Figure 7. Separation of anions including iodide and thiocyanate on Dionex HPIC-AS4A column using 3 mM phosphate as eluent, the other conditions were as in figure 5.

7.5 POTENTIOMETRIC DETECTION OF ELEVEN ANIONS SEPARATED IN AN ANION-EXCHANGE COLUMN IN A SINGLE RUN AND APPLICATION FOR RIVER, SEA AND DRINKING WATER

7.6 INTRODUCTION

Most desired separations can be achieved with the correct combination of eluent and stationary phase. Whilst adequate resolution of sample components can generally be achieved with isocratic elution, the distribution of peaks throughout the chromatogram may not be optimal. For example, resolution of a group of early eluted species may be possible by using a weak eluent, but only with a long analysis time. Resolution of some later eluted species may not be possible in view of the weakness of the eluent. Chromatography aims for simultaneous separation of a large number of ions in short times and detection at lower concentrations. This chapter describes a method in which isocratic elution is used to determine organic and inorganic anions from a single sample injection. Separation of eleven inorganic and organic anions, on an ion-exchange column, was achieved in an acceptably short time and good resolution. These included, acetate, formate, chloroacetate, bromate, chloride, nitrite, cyanate, benzoate, bromide, chlorite and nitrate. Potentiometric detection has been succesfully employed, and in most cases a direct detection of ionic species on the column was carried out. When compared with other detection methods use of a membrane anion selective electrode as a detector offers the best simultaneous sensitivity toward all anions. The detection limit was sub-ppb for most of anions using 20 μl injection. The detector cell volume was 2 μl.

7.7 EXPERIMENTAL

All standard anion stock solutions were prepared from their analytical reagent grade sodium or potassium salts, and then diluted to desired concentrations. All other conditions and components of the systems were as described in section 7.3. Samples of river and drinking water taken from local areas of Newcastle Upon Tyne and were diluted before use. Rain water standard sample was prepared in accordance with NIST SRM 2694-I.[17]

The identification of species was performed by comparing retention times of peaks of interest with those of peaks in a standard.

7.8 RESULTS AND DISCUSSION

When ISEs are used in flow conditions, the streaming potential strongly interferes with precise potential measurement.[18] It was suggested[14,15,19] that a background level of an electroactive species should be included in the eluent to suppress streaming potentials. Although there were some advantages to this approach, the baseline supporting electrolyte can cause negative peaks when the potential of an elute in the sample is less than the electrolyte background potential, or give no peaks when the analyte potential is equal to the background potential.[20] The potential depends on the selectivity of the electrode rather than the nature of the analytes. The streaming potential is mainly generated by pulsing,[21] which may influence the density and viscosity of the eluent, the diffusion rate of the analyte molecules, the eluent flow-rate and temperature of the system, and the interactions between eluent, column packing and analytes. Schultz and Mathis[22] described the use of a membrane nitrate ion selective electrode as detector in chromatography but made no mention of streaming potentials. This was probably due to the affinity of the eluent for the membrane. Ishibashi et al.[23] described a membrane nitrate ion selective electrode as a detector for HPLC. The electrode showed baseline drift and selectivity. To reduce streaming potentials, 10^{-4} M nitrate solution with eluent was used as baseline supporting electrolyte. Because of the selectivity of the electrode to the phosphate solution down to 10^{-4} molar as eluent in the experiments, the eluent functions as a baseline supporting electrolyte as well. Therefore in most cases direct detection of anionic species, especially for monovalent anions, in the column effluent was possible. If a pump is used which supplies a constant pressure of eluent, there should effectively be no streaming potential. An eluent with a moderate affinity on the membrane of the electrode leaves the electrostatic effect itself as the only source of the noise. The detector response to common anions is shown, using a Dionex HPIC-AG4A guard column with hydrogen phosphate and phosphate as eluents, in

figures 8, 9, 10 and 11. Use of ion selective electrode as detector in ion chromatography has the advantage that some anions were not detected, as shown in figures 12 and 19, and did not interfere in the determination of the detected anions. Determination of anions at low concentrations in several matrices may lead to more efficient identification of contamination. Applications to river, drinking and rain water samples are shown in figures 13, 14 and 15. The peak heights were directly proportional to the concentrations of the analytes in the samples. Without gradient elution, an eluent strong enough to elute nitrate would generally cause fluoride and the most weakly retained organic acid anions to co-elute in the void volume. But using isocratic elution with hydrogen phosphate eluent, all of these anions have been separated and determined at ppb levels with potentiometric detection, as shown in figure 16. An explanation of the separation is that the eluent was very strong, even at low concentrations, to elute, and the column's capacity was fully functional at low eluent concentration ranges, because there is more interaction between analytes and column packing when the total concentration is decreased within the column. The electroselectivity effect of the column packing material is increased in diluted eluent. The eluent ion charge varies with pH and the elution power can be adjusted within a wide range. A chromatogram using 0.3 mM phosphate as eluent is shown in figure 17. Calibration plots varied from one anion to another when peak height was used. Figure 18 shows the curves obtained for each peak height plotted against log concentration. The combined influence of concentration and ion-exchange capacity determine the peak potentials. The calibrations of the electrode potential versus concentrations of analytes show either a linear or logarithmic relationship, depending on the concentration range studied. When the total concentrations of analytes at the electrode surface is very low, a linear relationship was not observed. Under optimum conditions, the detection limits for most anions, defined as the amount for a signal to noise ratio of 2, are of the order of few, or tens, of ppb for an injected volume of 20 µl. The detection limit essentially depends on the ability to prepare low level standard samples and handle samples without

contamination. The sensitivity of most constructed detectors remained almost constant for at least 2.5 months. The reproducibility of peak heights for repeated injections of all anions at all concentrations was generally better than 2%. Some of the reasons for good reproducibility of potential response might be the continuous washing of membrane surface of the electrode with fresh eluent solution, the nature of the eluent and membrane, lack of sample contamination and the design of the detector cell. The selectivity of the electrode toward monovalent anions results in some other anions being undetected. Figure 19 shows the chromatographic separation with potentiometric detection of eleven anions. The sample also included some divalent anions which were not detected. This can be called *monovalent anion selective detection*; no interference occurs unless the concentration is approximately 10^{-3} M in each species.

Figures 20 and 21 show the application of the method to the river and drinking water samples. Ion chromatography provides a powerful method for the identification and quantitation of ionic contaminants in samples. Use of a membrane ion selective electrode in ion chromatography has also the another advantage that some anions are undetected, because of absence of an affinity of an anion to the membrane, and hence such anions can be employed as eluents to separate anions and leads to a high sensitivity. An eluent with a very low affinity for the membrane minimizes baseline drift and creates effective eluent concentration gradients so that sensitive detection of large electroactive species might be possible. Consequently, because of selective detection, the separation of the analytes of the sample in the analytical column can only be achieved for monovalent anions, not for others. It may also be possible to have universal detection with an array of the liquid membrane electrodes designed so that there may be a very sensitive detection for all anions. Further it can be suggested that post-column arrangement, which can be employed with phosphate eluent, allows another possibility of detection with UV spectrophotometry. Moreover, because of low concentration of the eluent to achieve separation, the eluent can be employed in non-suppressed or suppressed ion chromatography with conductivity detection.

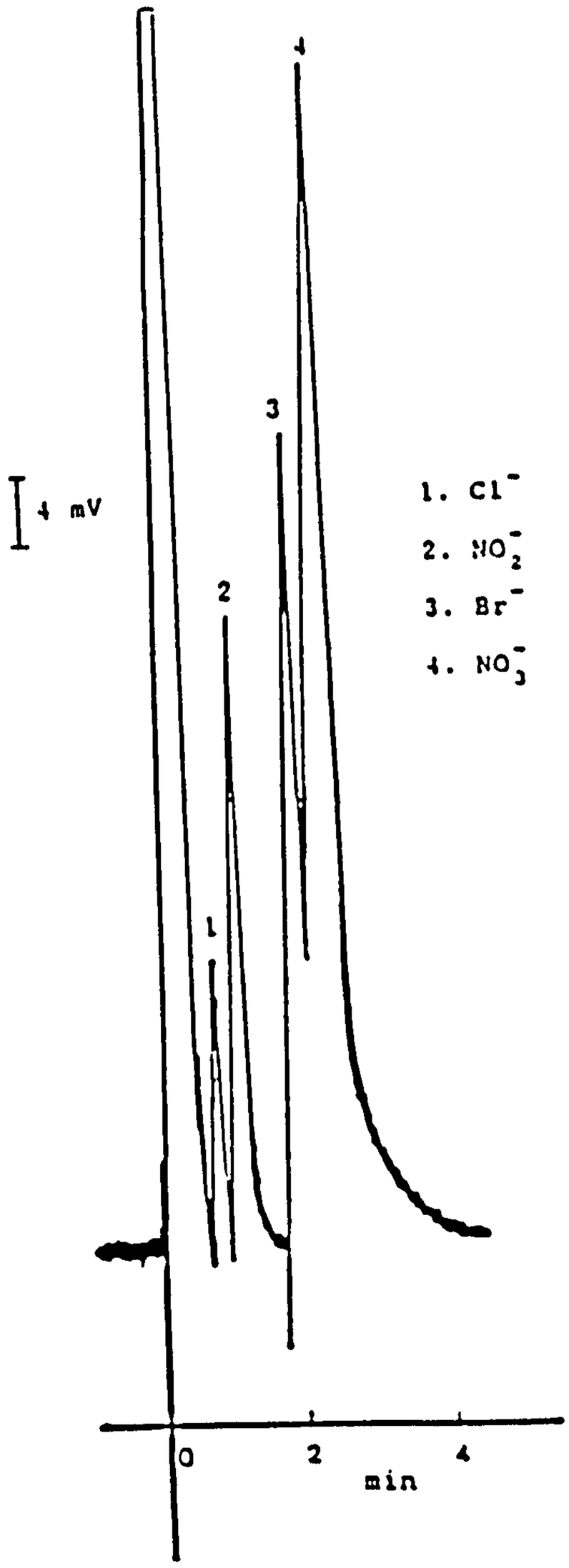


Figure 8. Potentiometric detection of anions using a 0.1 mM phosphate as eluent, column: Dionex HPIC-AG4A guard, flow-rate: 1.5 ml min^{-1}, injection: 20 µl of 10^{-4} molar standard solution of each anion.

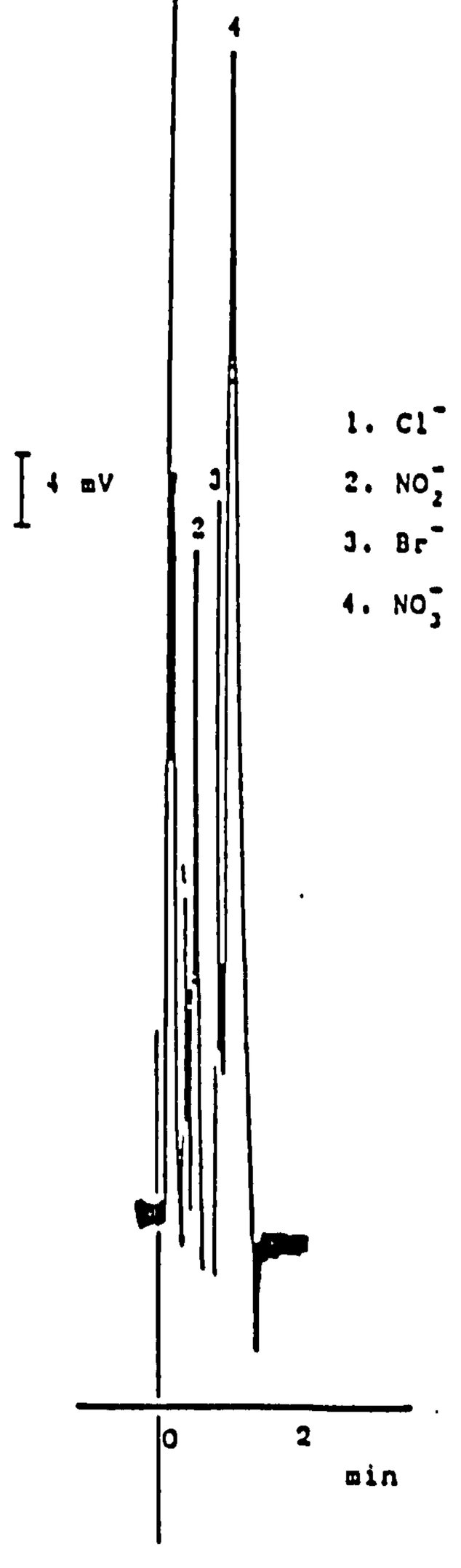


Figure 9. Potentiometric detection of anions using a 0.15 mM phosphate as eluent, the other conditions were as in fig. 8.

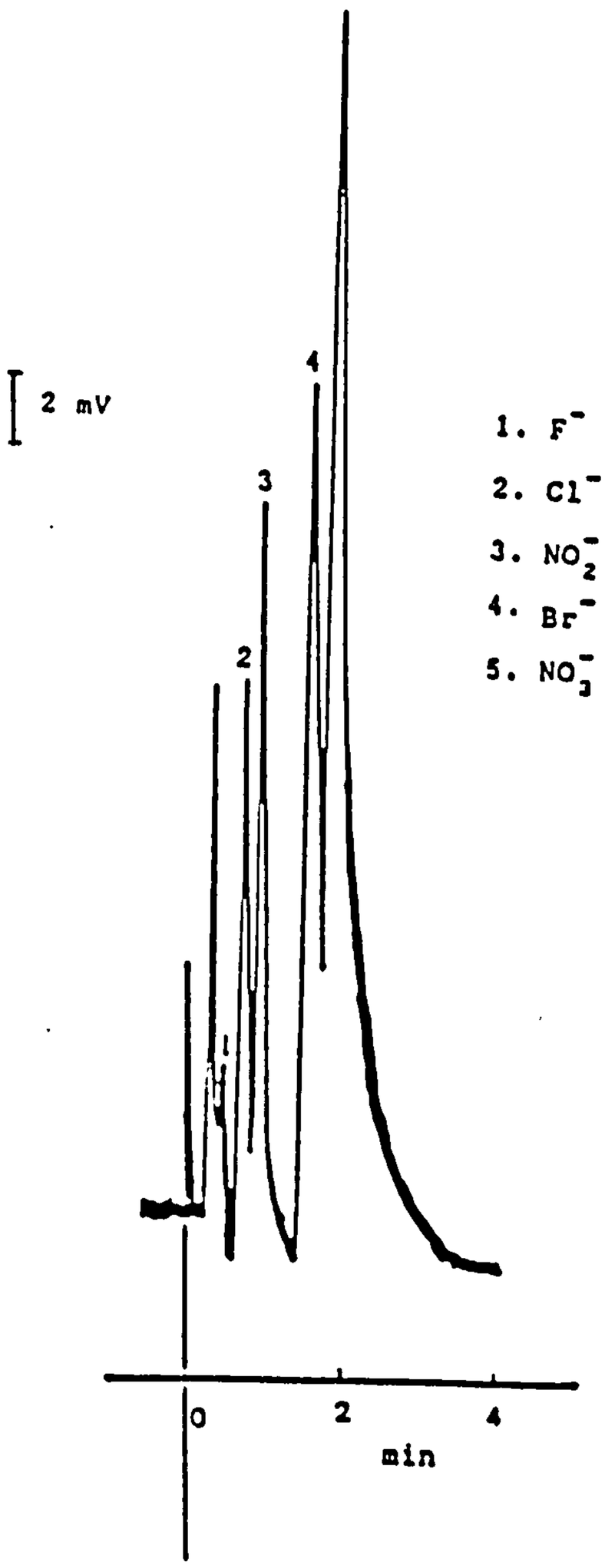


Figure 10. Potentiometric detection of anions using a 0.35 mM hydrogen phosphate as eluent, column: Dionex HPIC-AG4A guard, flow-rate: 1.5 ml min^{-1}, injection: 20 µl of $5x10^{-5}$ molar standard solution of each anion except fluoride was $5x10^{-4}$ molar.

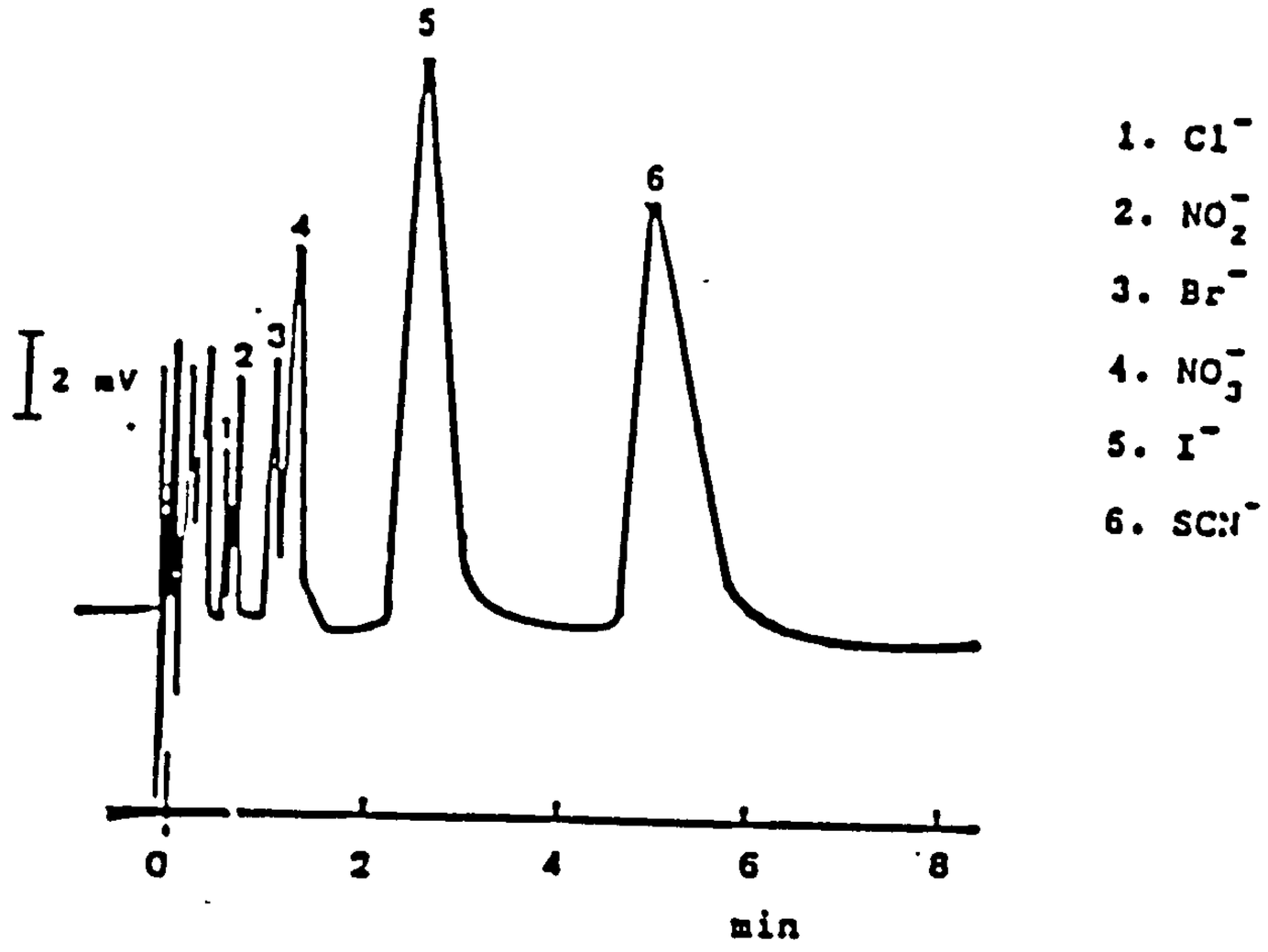


Figure 11. Potentiometric detection of anions including iodide and thiocyanate using 0.35 mM hydrogen phosphate as eluent, column: Dionex HPIC-AG4A, flow-rate: 2 ml min^{-1}, injection: 20 µl of 10^{-5} molar standard solution of each anion except I^- and SCN^- were $5x10^{-6}$ molar.

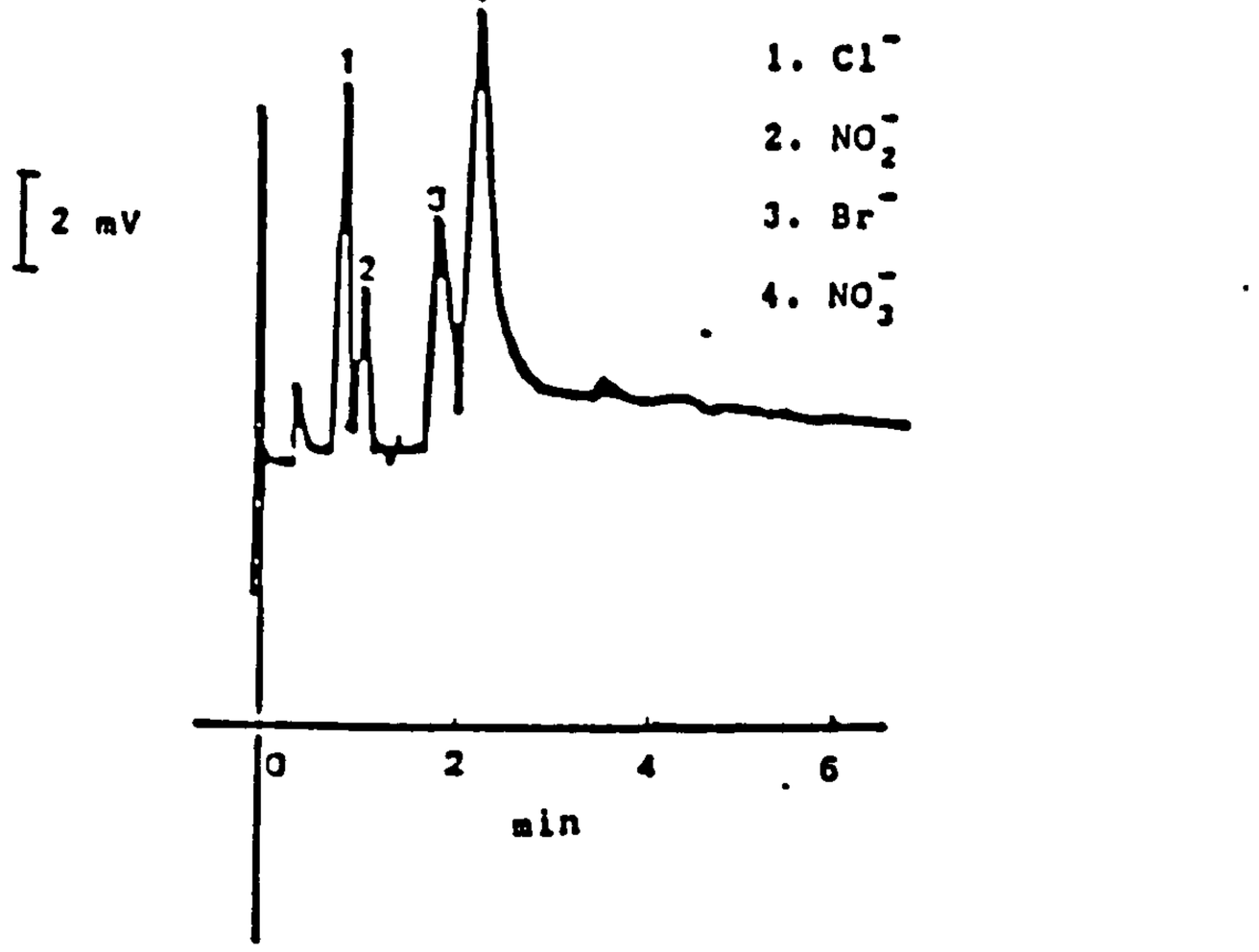


Figure 12. Potentiometric detection of anions, injection: 20 µl of $6x10^{-6}$ molar standard solution of each anion except PO_4^{-3}, CO_3^{-2} and SO_4^{-2} were 10^{-3} molar (which were undetected), the other conditions were as in figure 10.

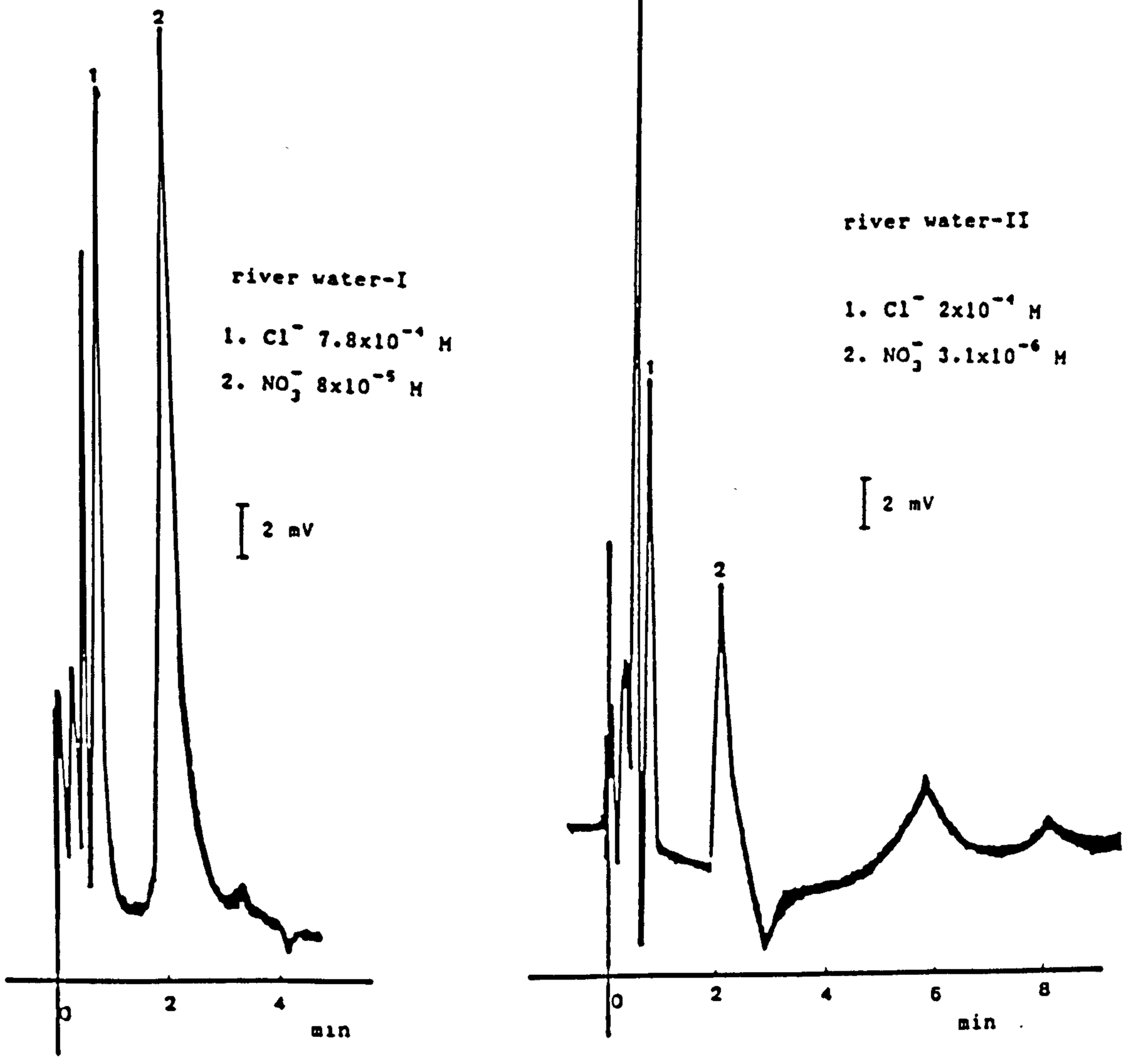


Figure 13. Potentiometric detection of anions in river water-I and II (diluted two fold) samples, the other conditions were as in fig. 10.

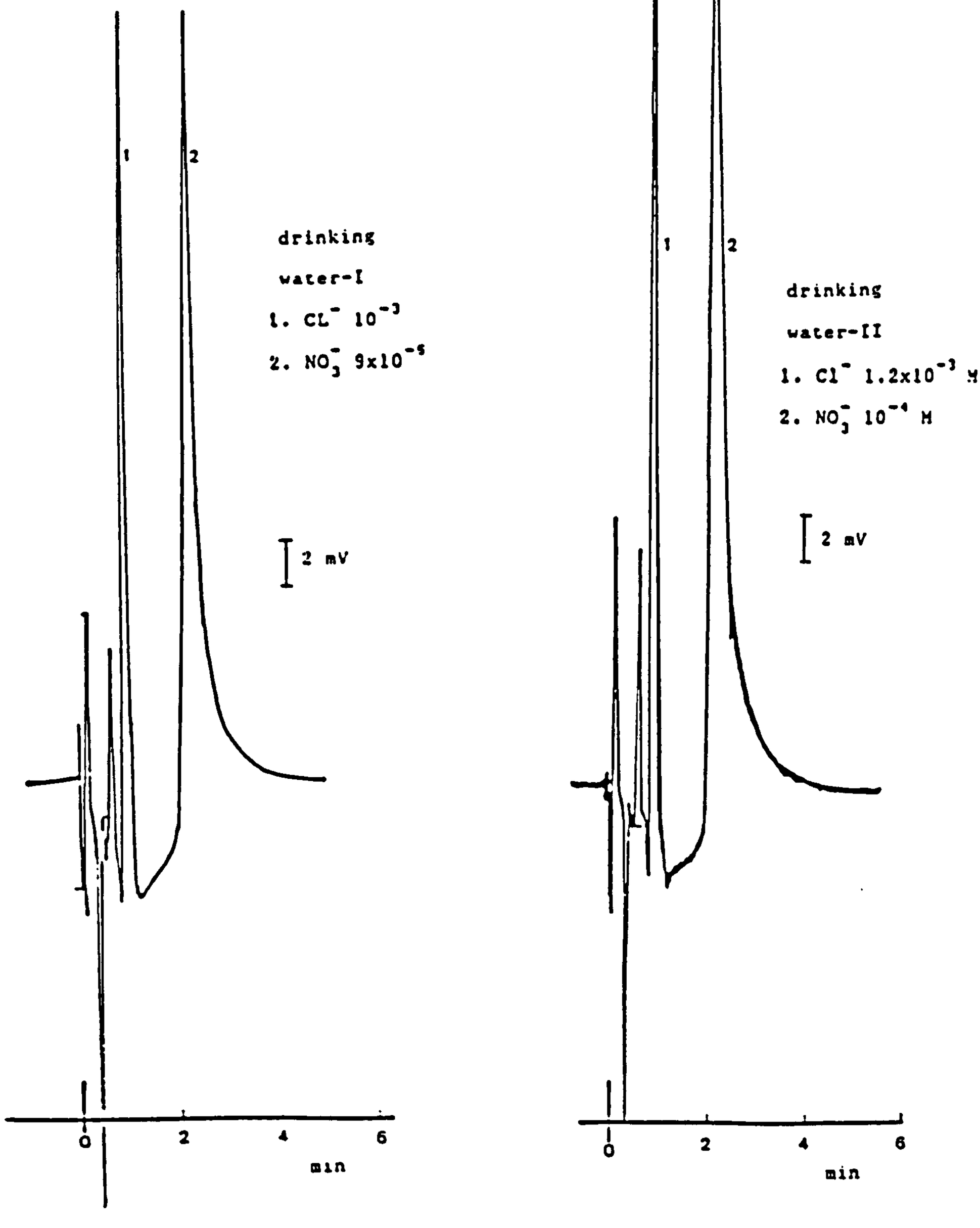


Figure 14. Potentiometric detection of anions in drinking water-I and II samples, the other conditions were as in fig. 10.

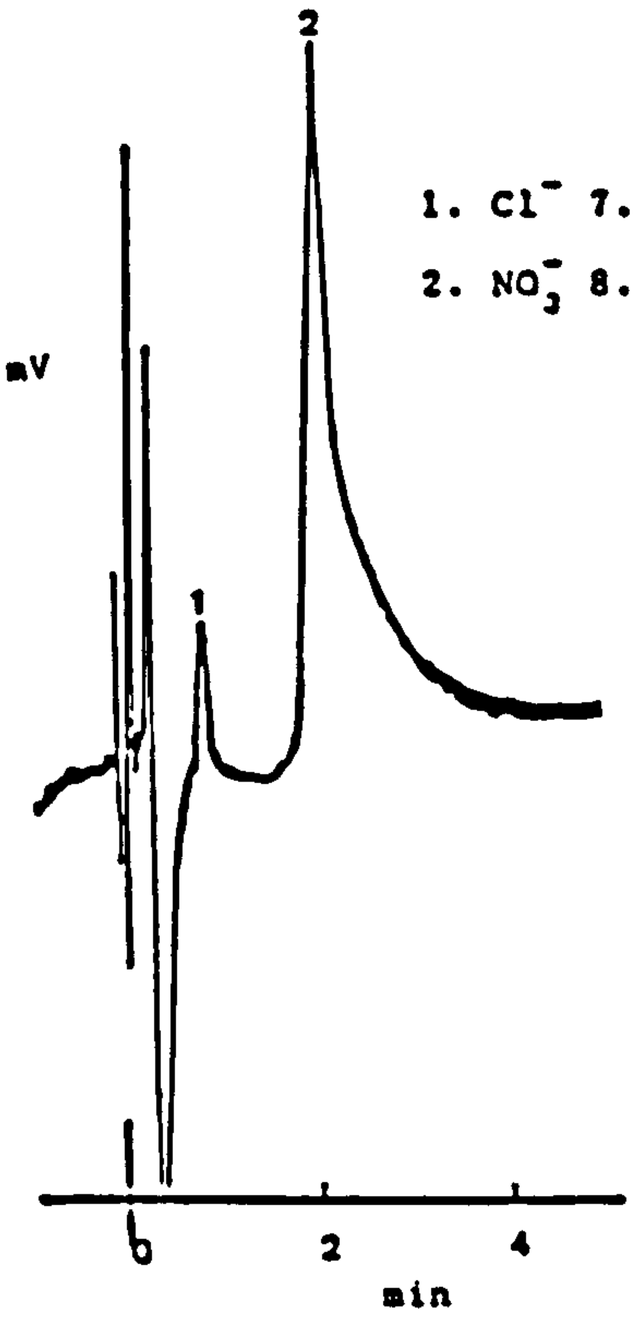


Figure 15. Potentiometric detection of anions in rain water standard sample (SRM 2694-1), the other conditions were as in fig. 10.

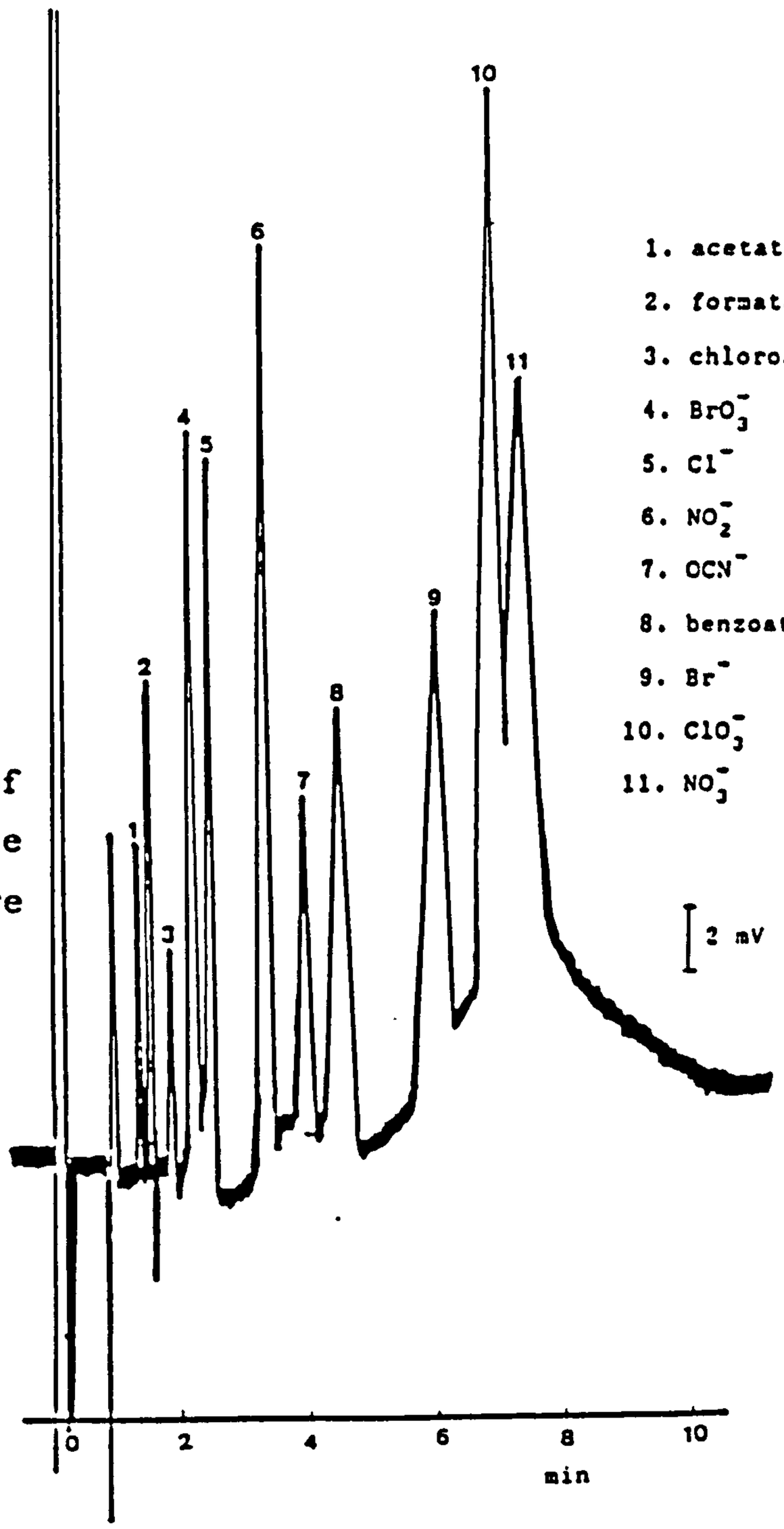


Figure 16. Potentiometric detection of anions, using 0.7 mM hydrogen phosphate as eluent on Dionex HPIC-AS4A and AG4A columns, flow-rate: 2 ml min^{-1}, injection: 20 µl of 5x10^{-6} molar standard solution of each anion except Br^-, ClO_3^- and NO_3^- were 10^{-6} molar.

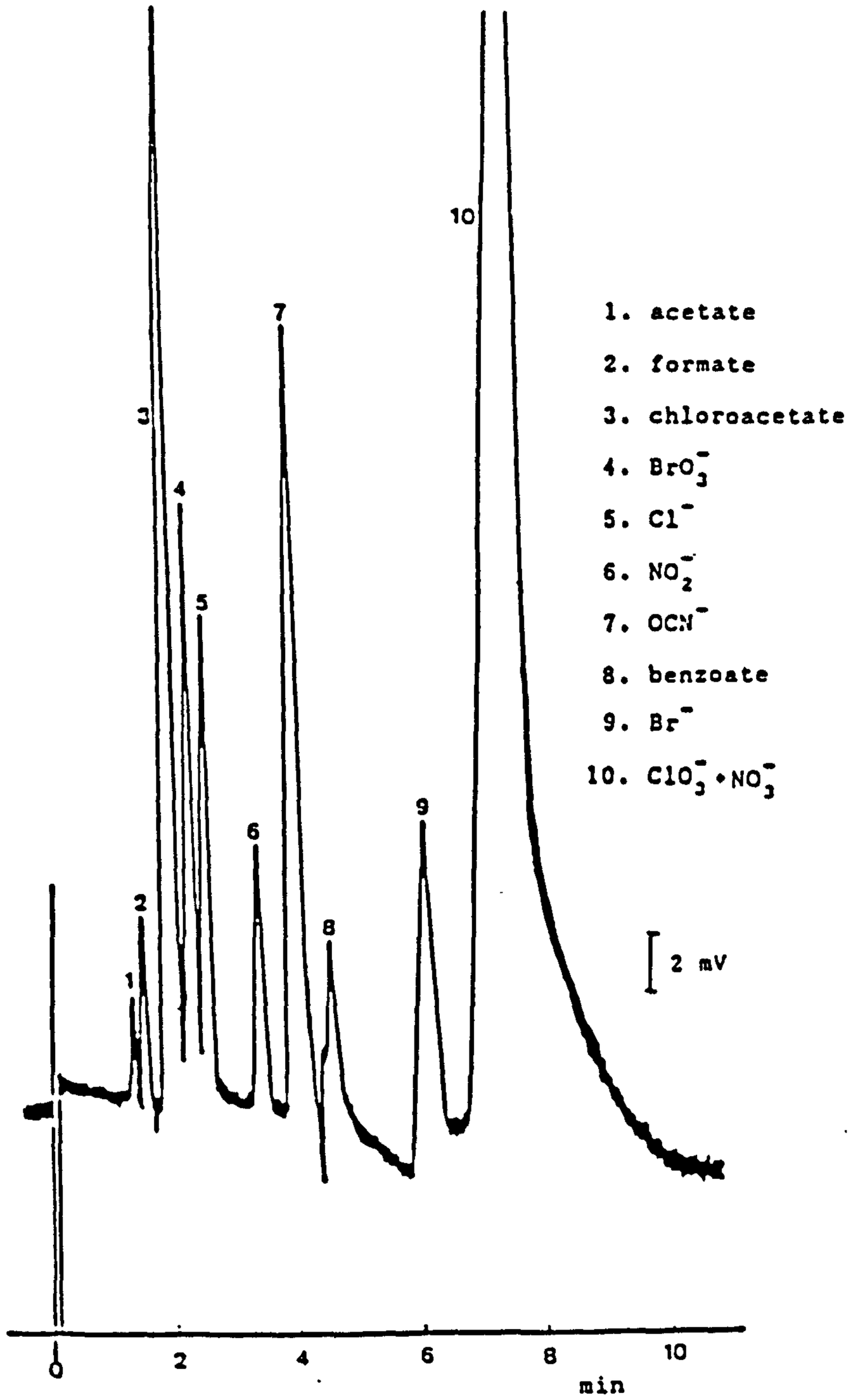
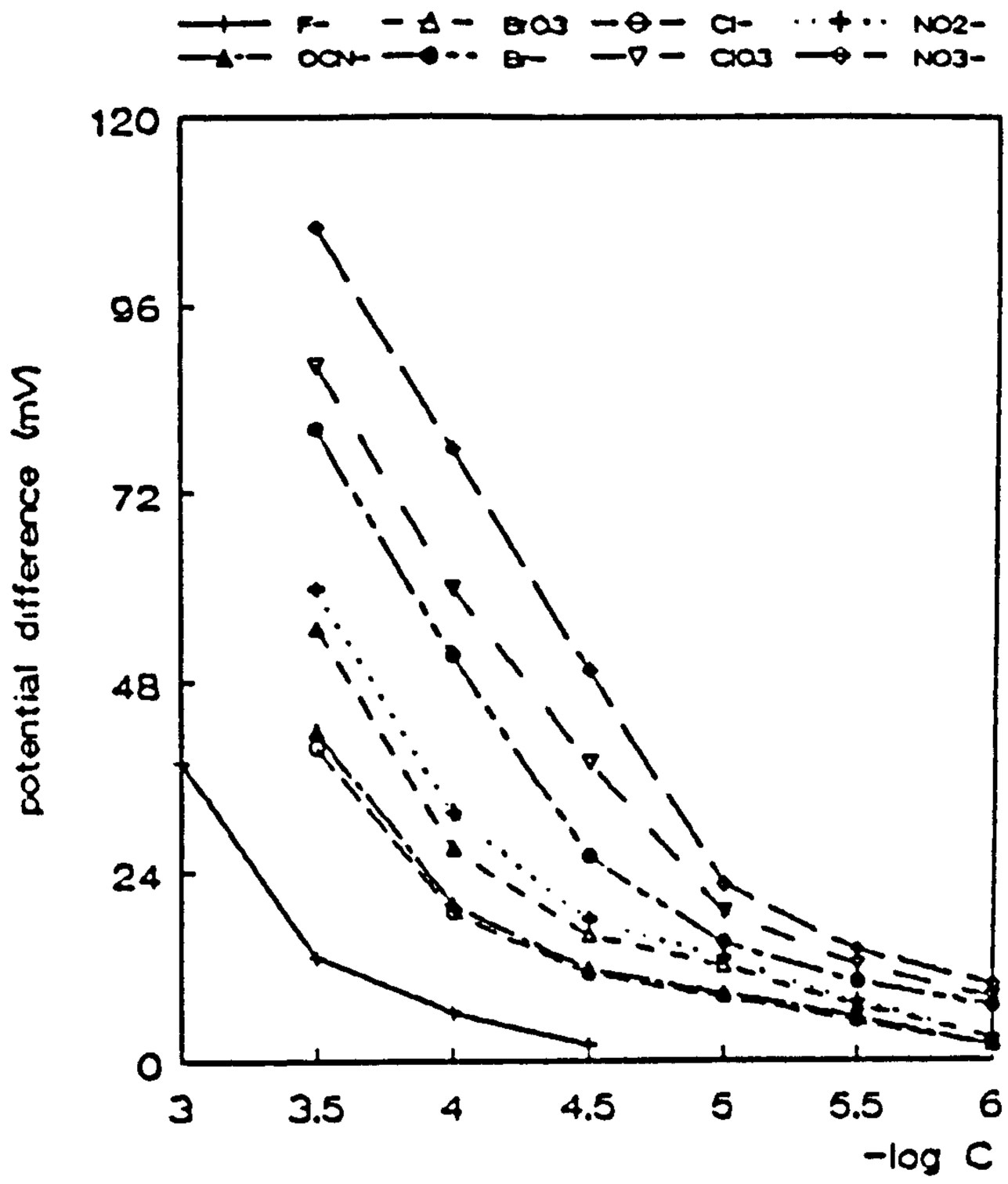


Figure 17. Potentiometric detection of anions using 0.3 mM phosphate as eluent, injection: 20 µl of 10^{-5} molar standard solution of each anion, the other conditions were as in fig. 16.

(a)



(b)

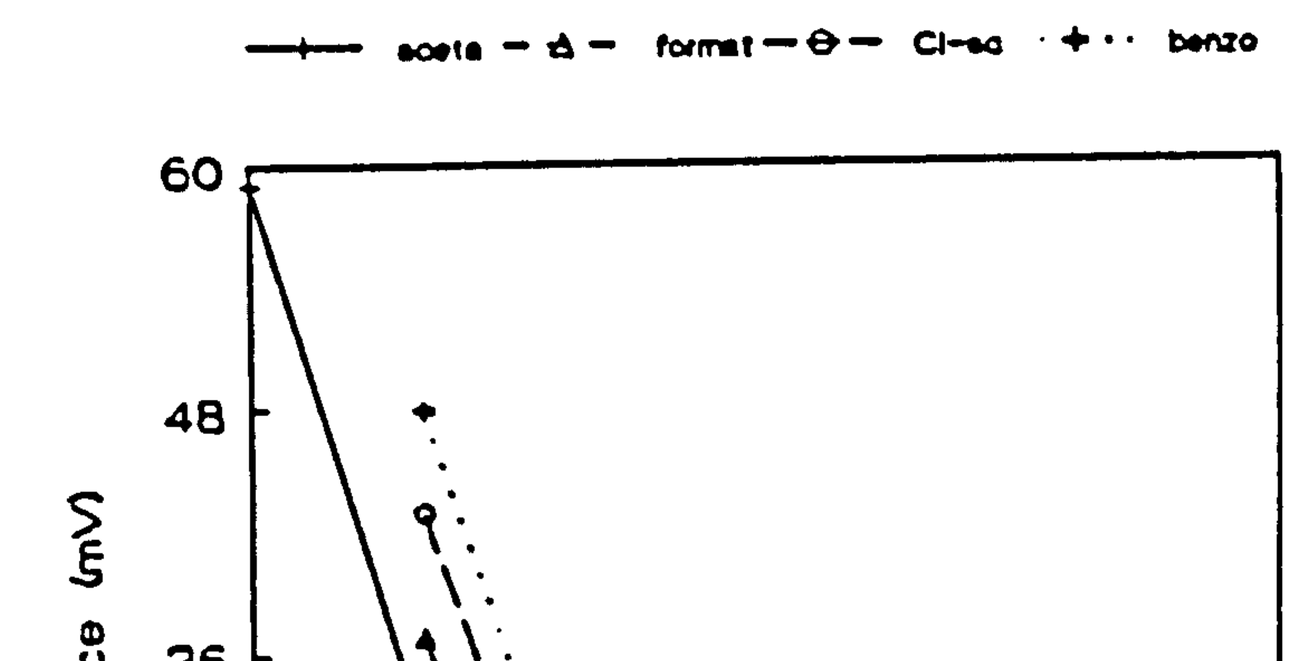


Figure 18. Calibration of the electrode potential of analytes (peak heights plotted versus log concentrations of analytes).

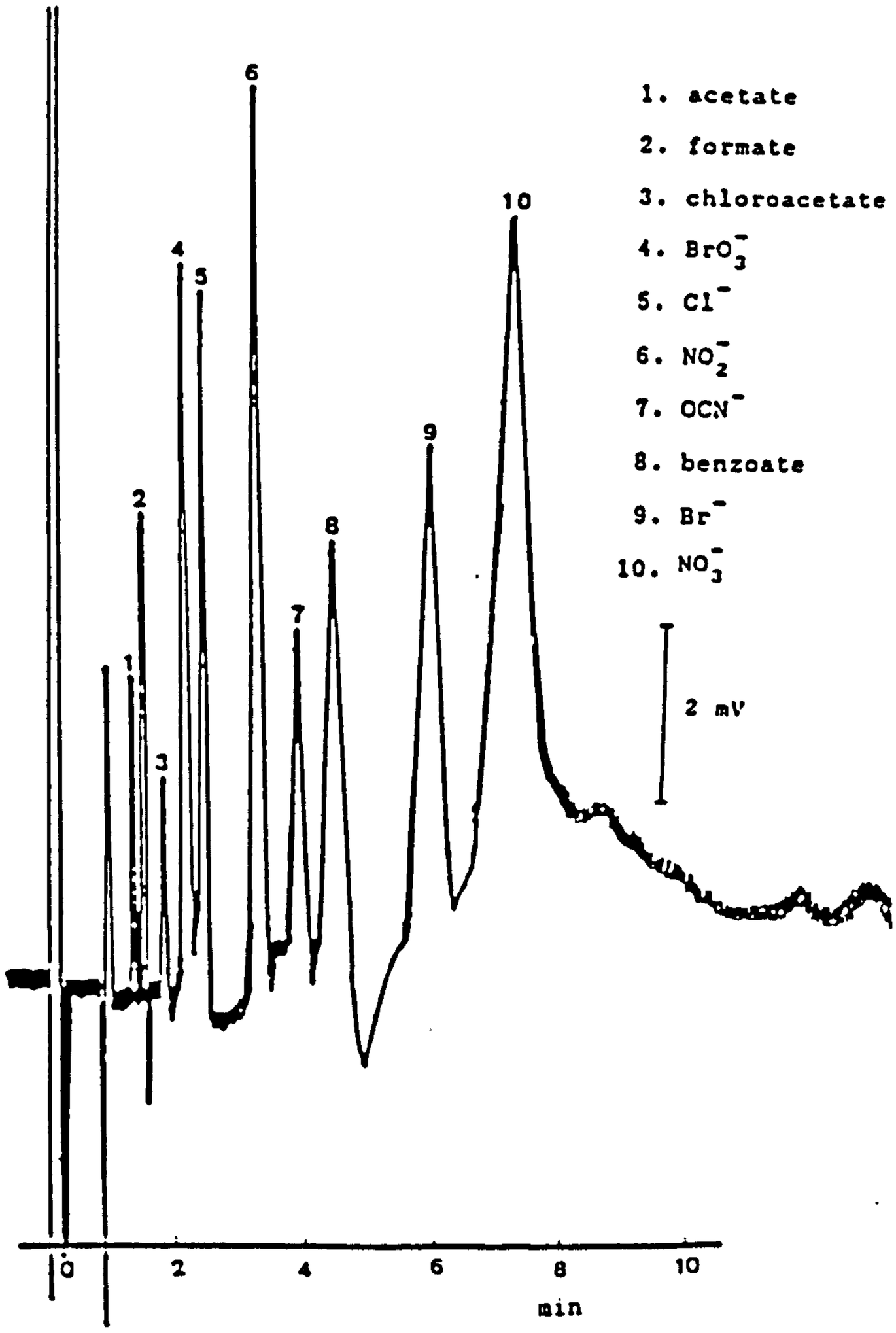


Figure 19. Potentiometric detection of anions using 0.7 mM hydrogen phosphate as eluent on Dionex HPIC-AS4A and AG4A columns, flow-rate: 2 ml min^{-1}, injection: 20 µl of 10^{-5} molar standard solution except Br^-, NO_2^- and NO_3^- were $5x10^{-6}$ molar and phosphate, sulphate, arsenate, stannate, gluconate, succinate, perborate, carbonate, tartarate, Na-citrate and hydrogen phosphate were 10^{-3} molar.

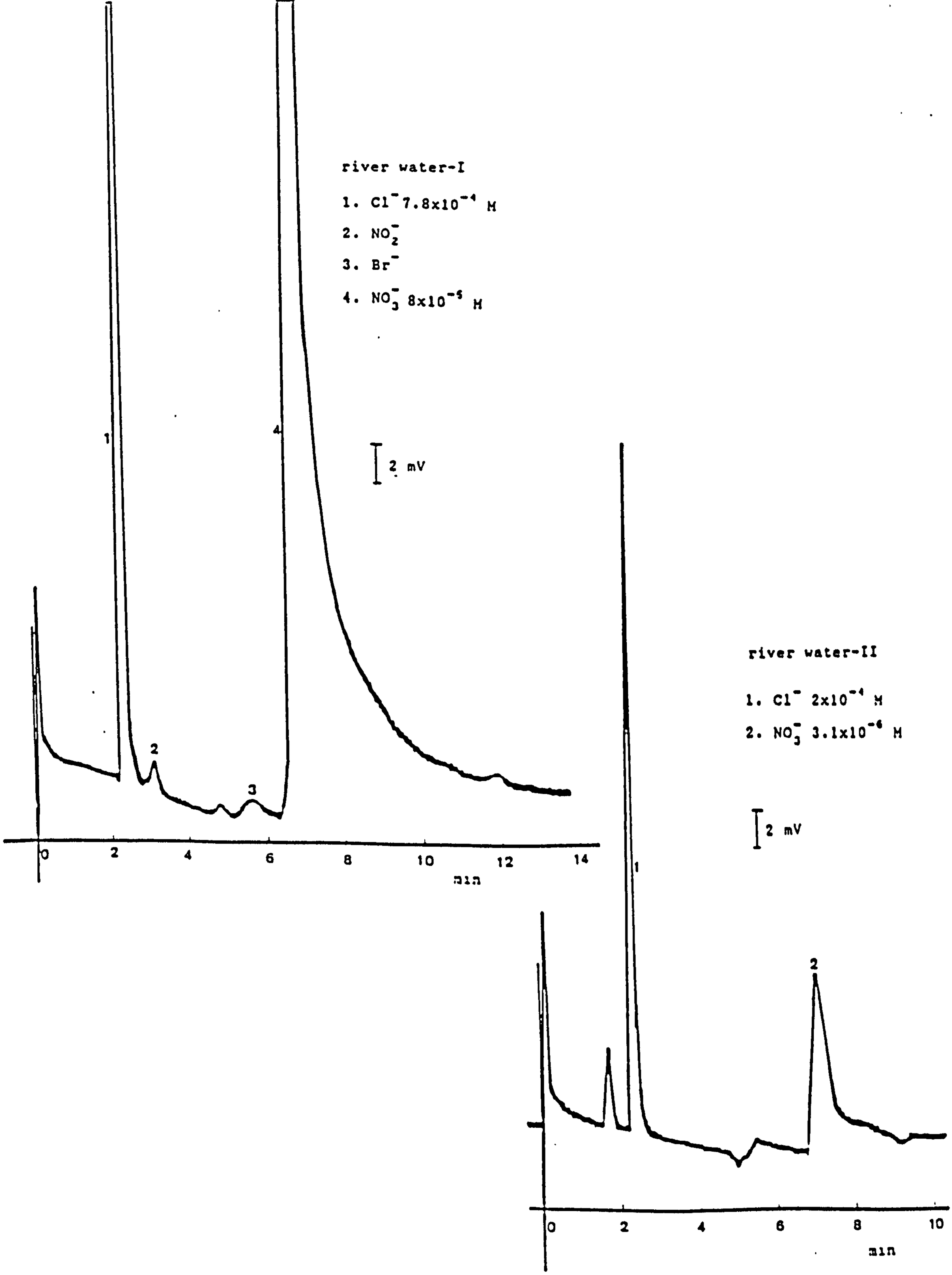


Figure 20. Determination of anions in river water-I and II' (diluted two fold) samples, the other conditions were as in fig. 16.

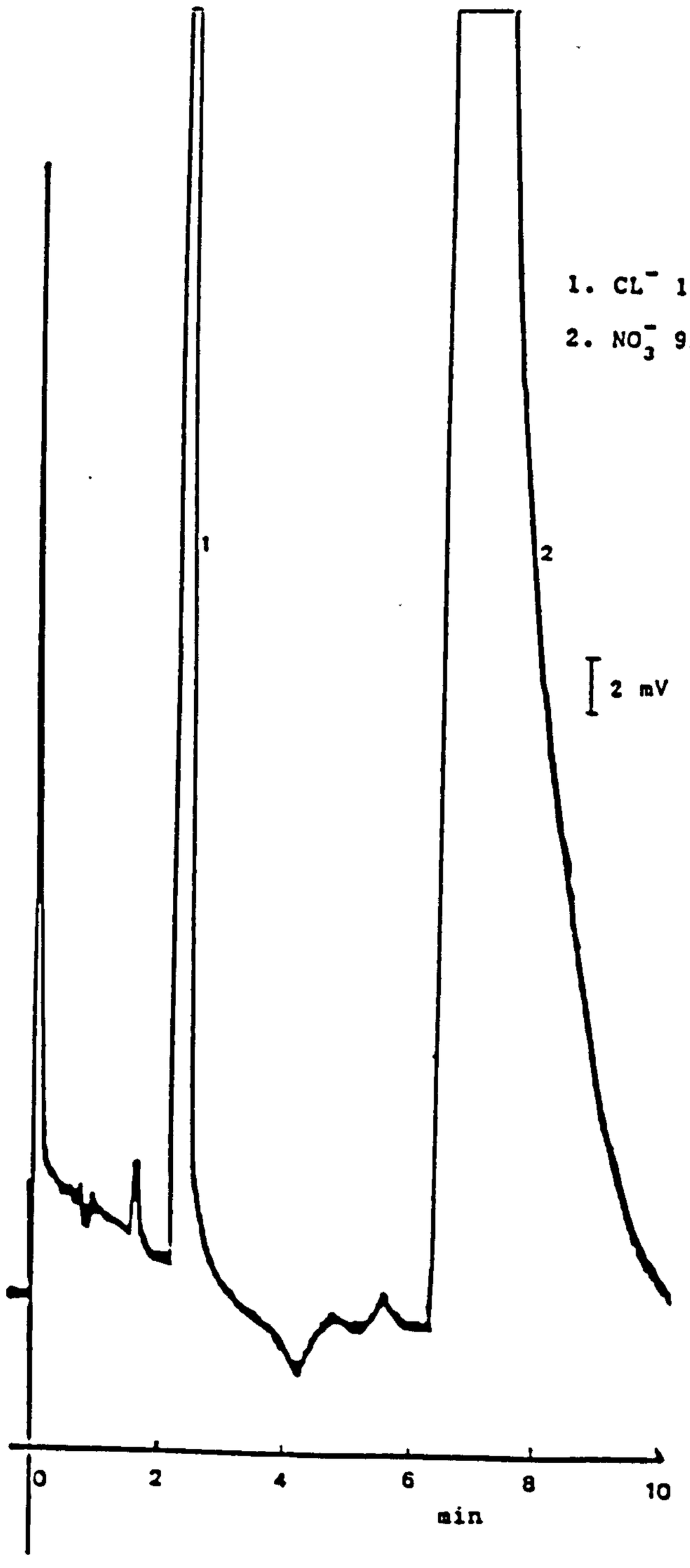


Figure 21. Determination of anions in drinking water-I sample, the other conditions were as in fig. 16.

7.9 REFERENCES

1. Stevens T S., Denis J C. and Small H., *Anal. Chem.*, 1981, 53, 1488.

2. Shintani H. and Dasgupta P K., *Anal. Chem.*, 1987, 59, 802.

3. Dasgupta P K., *Anal. Chem.*, 1987, 59, 769.

4. Rocklin R D., Christopher A P. and Schibler J A., *J. Chromatography*, 1987, 411, 107.

5. Sunden T., Lindgren M. and Gedergen A., *Anal. Chem.*, 1983, 55, 24.

6. Lockridge J E., Fortier N E., Schmuckler G. and Fritz J S., *J. Chromatography*, 1987, 192, 41.

7. Jones W R., Jandik P. and Heckenberg A L., *Anal. Chem.*, 1988, 6, 1979.

8. Muller H., *4th Symposium On Ion Selective Electrode*, Matrafured, 1984, 553, Ed. E. Pungor.

9. Schmuckler G., Jakoe A L., Girard J E. and Buell P E., *J. Chromatography*, 1986, 356, 413.

10. Butler E C V. and Gershey R M., *Anal. Chim. Acta*, 1984, 164, 153.

11. Buchberger W., *J. Chromatography*, 1988, 439, 129.

12. Alegret S., Alonso J., Batroli J. and Martinez-Fabregas E., *Analyst*, 1989, 114, 1443.

13. Covington A K. and Whalley P D., *J. Chem. Soc.*, Faraday Trans 1, 1986, 82, 1209.

14. Jyo A., Mori K. and Ishibashi N., *Bull. Chem. Soc. Jpn.*, 1983, 56, 3507.

15. Butler E C V. and Gershey R M., *Anal. Chim. Acta*, 1984, 164, 153.

16. Dionex *Application Note 37*, 1982.

17. Koch W F., Marinenko G. and Paule R C., *J. Research of the National Bureau of Standards*, 1986, 91, 33.

18. Van der Winkel P., Mertens J. and Massart D L., *Anal. Chem.*, 1974, 46, 1765.

19. Van der Linden W E. and Ostervink O., *Anal. Chim. Acta*, 1978, 101, 410.

20. Haddad P R. and Jackson P E., "*Ion Chromatography*", Elsevier, Amsterdam, 1990, p.335.

21. Berek D. and Macko T., *Pure and Appl. Chem.*, 1989, **61**, 2041.
22. Schultz F E. and Mathis D E., *Anal. Chem.*, 1974, **46**, 2253.
23. Ishibashi N., Jyo A. and Imato T., *Anal. Chem. Symp. Ser.*, 1983, 17 (Chem. Sens.), 570.

CHAPTER 8

8. SEPARATION OF MANY IONS WITH VARIOUS ELUENTS IN SUPPRESSED ION CHROMATOGRAPHY WITHOUT GRADIENT ELUTION AND CONDUCTIVITY DETECTION AND APPLICATION FOR RIVER, SEA AND DRINKING WATER SAMPLES

8.1 INTRODUCTION

To eliminate the interferences which merge into one peak on the chromatogram, gradient elution,[1-4] or selective detection,[5] have been usually applied whenever possible. The use of eluent concentration gradients in ion chromatography has been quite difficult when conductivity and spectrophotometric detection were used. Most published works on ion chromatography have used isocratic elution. However, in any liquid chromatographic technique, the eluent composition provides greatest flexibility for manipulating the retention of solutes in order to achieve a desired separation. Developments in ion chromatography are necessary to promote rapid and effective methods for the determination of ionic concentrations in aqueous solutions. Utilizations of a composition of HCO_3^-/CO_3^{-2} buffer solution or a phosphate solution as eluents are demonstrated for the separation of many common inorganic and organic anions. In six minutes, twelve anions can be separated with good resolution.

8.2 EXPERIMENTAL

Chromatography was performed on a Dionex-100 ion chromatograph, which consisted of a gradient pump and a conductivity detector. All components of the chromatographic system in contact with samples or eluent are made of non-metallic materials to avoid metallic contamination. A Dionex anion trap column was installed before the ion-exchange valve and used to minimize interferences from the contaminant anions. Separations were performed on a Dionex HPIC-AS4A separator and HPIC-AG4A guard column, with the eluent suppressed with a Dionex micromembrane suppressor and 12.5 mM sulphuric acid regenerant. Suitable compositions of the eluents were adjusted by dilution of the HCO_3^-/CO_3^{-2} buffer solution from Dionex or dilution of a phosphate stock solution which was prepared

from analytical reagent grade sodium salt in deionized water. All standard sample solutions were prepared from their analytical reagent grade chemicals in deionized water. Sample matrices of river, sea and drinking water were taken from local areas of Newcastle Upon Tyne, and were diluted before injections. During the process, 20 µl of samples and standard solutions were injected. The samples were filtered through Millipore filters (pore size 0.45 µm). The identification of species is performed by comparing retention times of peaks of interest with those of peak in a standard.

8.3 RESULTS AND DISCUSSION

The determination of common anions in drinking, river and sea water is an important environmental problem. To eliminate interferences in the samples, a selective detection or a complete separation is usually necessary. Separation of samples, containing many early eluting anions which have less affinity on the ion-exchange resin, can be achieved using low concentrations of carbonate and bicarbonate buffers. The chromatogram of such anions obtained using 0.52 mM CO_3^{-2} and 0.50 mM HCO_3^- buffer solution as eluent, is shown in figure 1. The peak shapes and resolutions for ten species are reasonably good. When eluent concentration was increased one and a half or two times, the separation of anions were still possible as is shown in figures 2 and 3. The effect of the concentration of the eluent on retention times of the anions was fair because of the higher selectivity of the ion-exchange resin toward the anions at low eluent concentrations.

The sensitivity of the detection system toward each anion might be increased, since the background conductivity of the suppressed eluent was reduced when eluent concentration decreased. The method for the determinations of anions was applied to drinking, river and sea water samples respectively. Chromatograms of the samples are shown in figure, 4, 5, and 6. The peak heights were directly proportional to the quantity of the elutes in the sample.

Conductometric detection can be employed with phosphate as eluent for the determination of the anions since its lower concentration enables the separation of the anions in an equivalent time. The conductometric detection in suppressed ion chromatography with

phosphate eluents was examined and a chromatogram is shown in figure 7.

The nature of the eluent can be changed to a form of phosphoric acid by the suppressor which produces hydrogen ions. Phosphoric acid exists in an unassociated form in the solution since it is a stronger acid. Hence the background conductivity was enormously increased by hydrogen ions emerging with the eluent. Due to low dissociation of some species (e.g. organic anions and nitrite) there was a decrease of background conductivity of the eluent. Therefore, as expected, these anions emerged as negative peaks in the chromatogram while others were positive. When the flow-rate of the regenerant solution was reduced, which caused a decrease of hydrogen ion concentration in the eluent, the heights of the negative peaks were found to be smaller while the height of the positive peaks increased. The situation is called direct and indirect detection in suppressed chromatography since both mechanisms exist in one run. The effect of the regenerant flow-rate on chromatograms is shown in figure 8. To obtain direct detection, the regenerant flow-rate should be reduced to attain a pH which permits the dissociation of weak acid anions, or eluent concentration should be increased. The use of hydrogen phosphate as a more concentrated eluent than phosphate produces positive peaks, which means direct detection. The chromatogram is shown in figure 9.

Analyses of drinking, river and sea water samples were examined using hydrogen phosphate as eluent, the chromatograms are shown in figures 10, 11 and 12. Almost the same sensitivities were obtained with the hydrogen phosphate eluent, when compared to carbonate/bicarbonate eluent. The comparison was made by injection of the same samples for both eluents. A very low concentration of phosphate as eluent is an advantage, and functions very well for separation of ten anions. Further studies with phosphate eluent are required to optimize the results, and possibly to increase the sensitivity. The eluent may be suitable for use with post column reaction detection too.

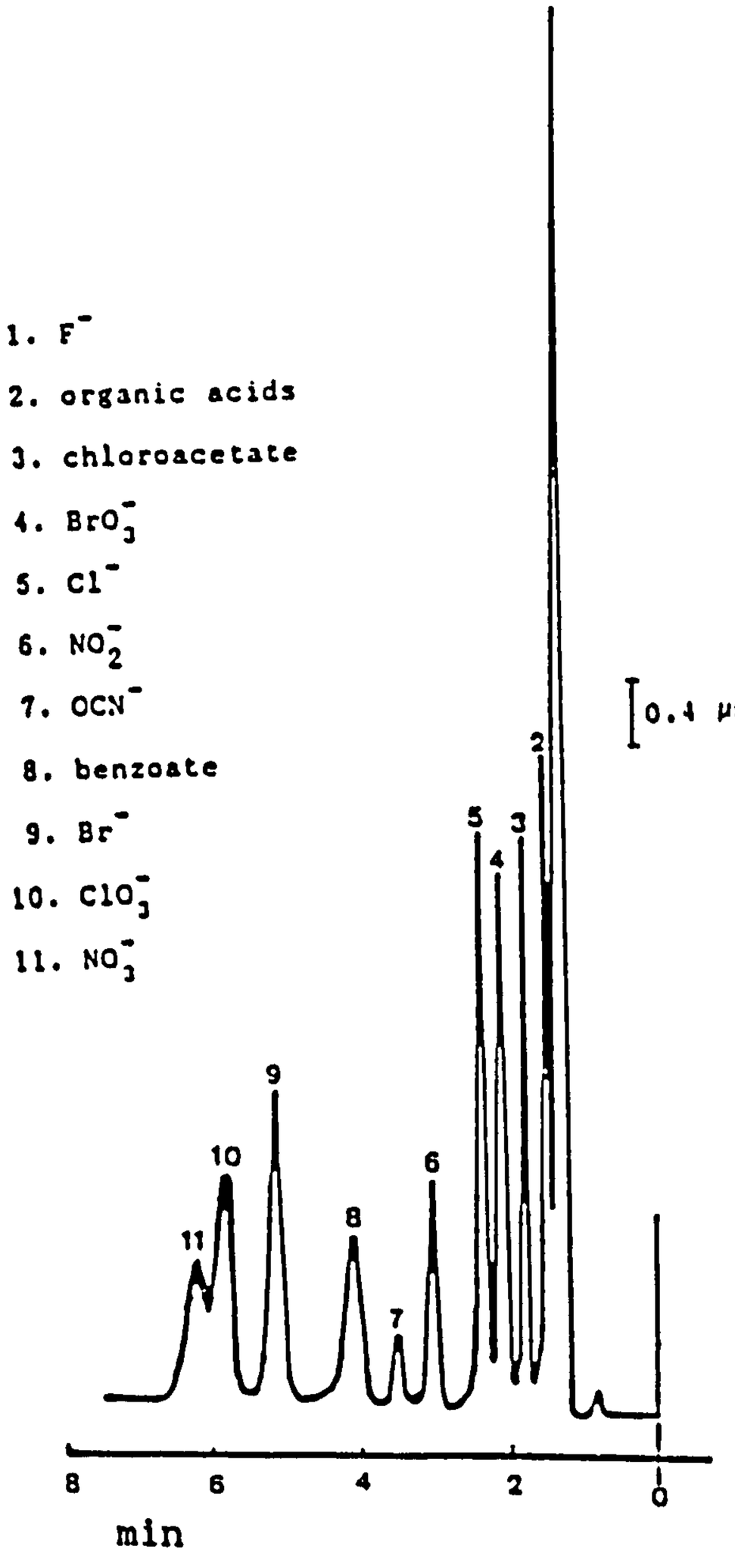


Figure 1. Separation of anions on Dionex HPIC-AS4A and AG4A columns using 0.52 mM carbonate and 0.50 mM hydrogen carbonate buffer as eluent, eluent flow-rate: 1.8 $ml.min^{-1}$, regenerant flow-rate: 1 ml min^{-1}, injection: 20 µl of $6x10^{-5}$ molar standard solution of each anion.

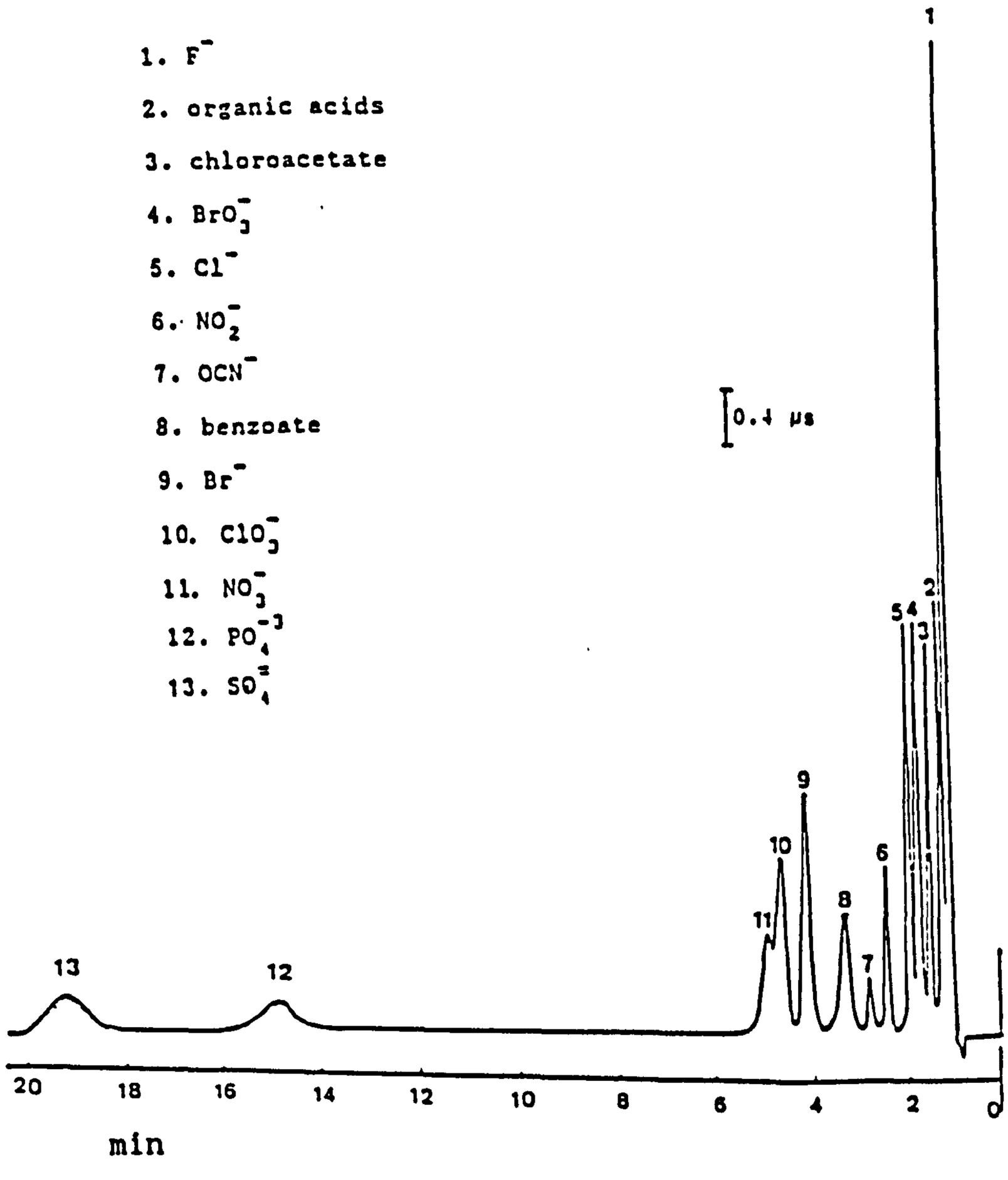


Figure 2. Separation of anions using a 0.72 mM carbonate and 0.68 mM hydrogencarbonate buffer as eluent, the other conditions were as in fig. 1.

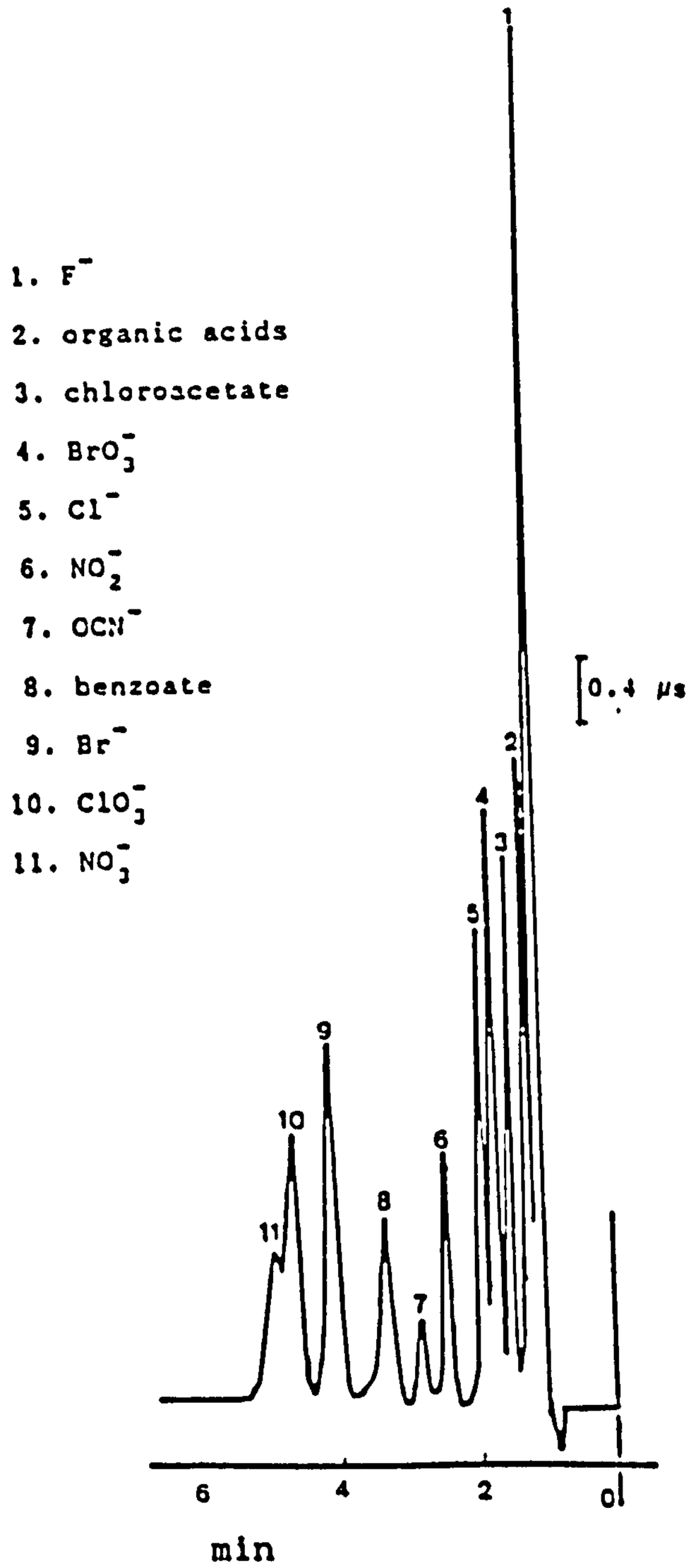
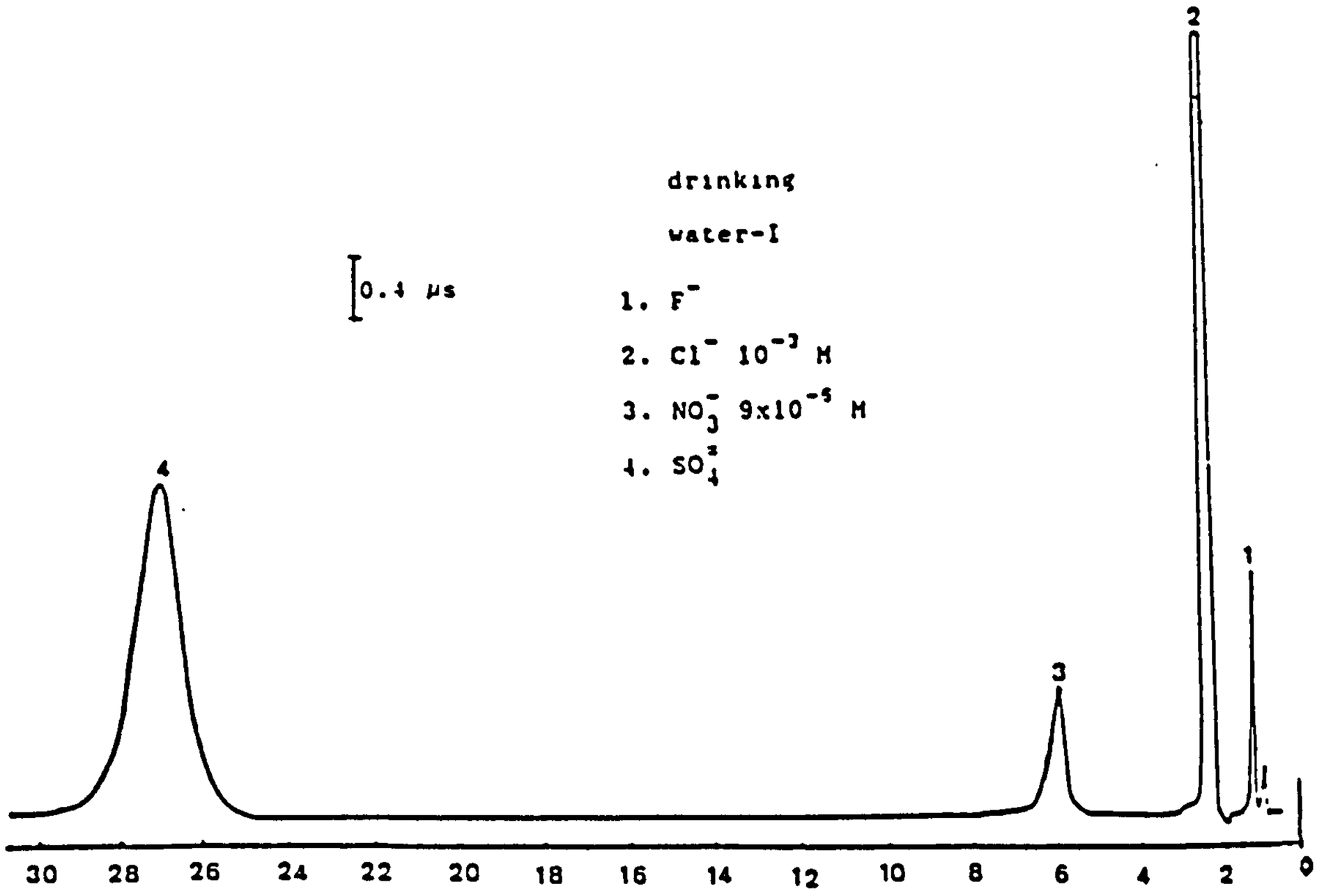


Figure 3. Separation of anions using 1 mM carbonate and 0.94 mM hydrogencarbonate buffer as eluent, the other conditions were as in fig. 1.



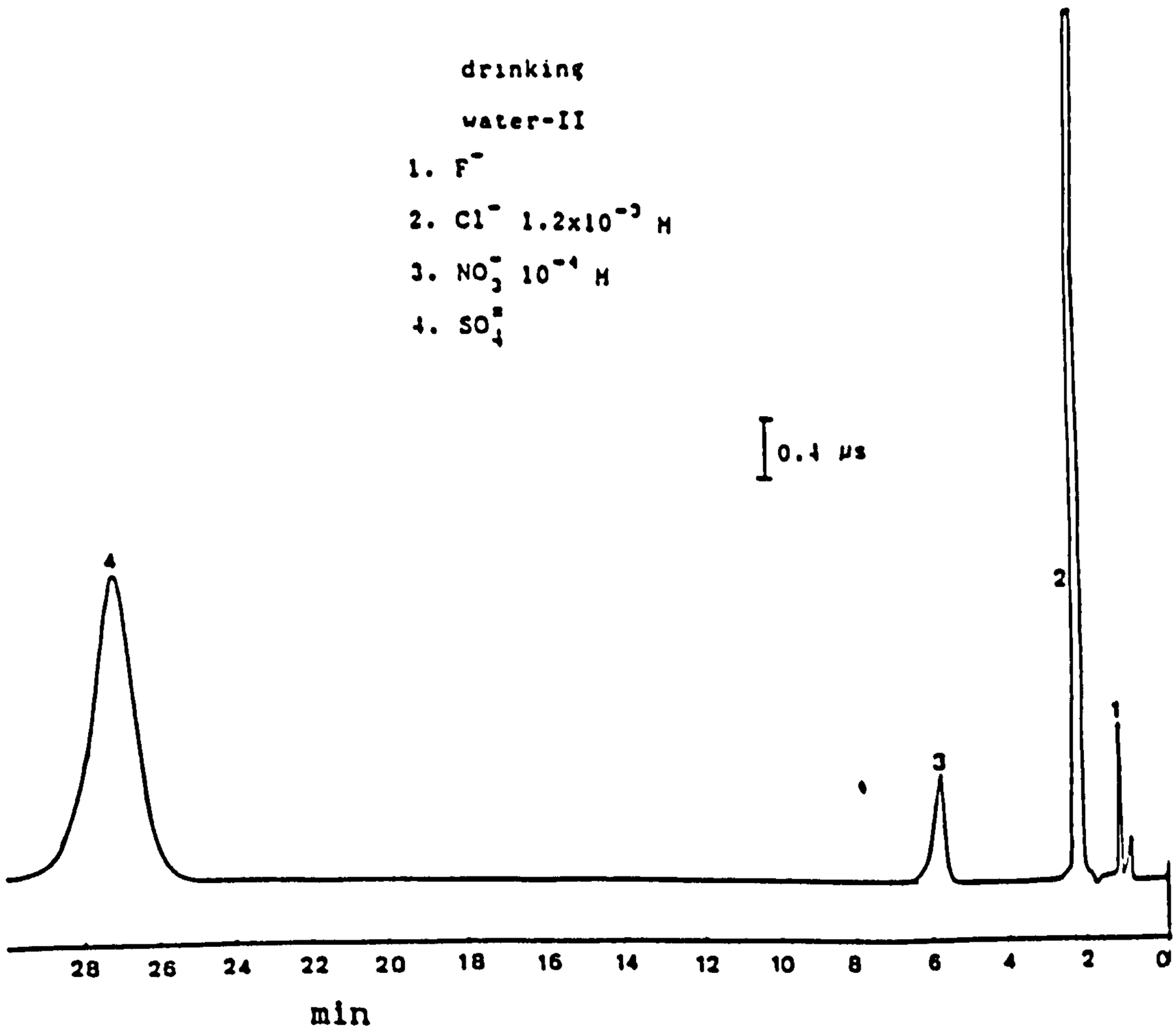


Figure 4. Anions in drinking water-I and II samples, the other conditions were as in fig. 1.

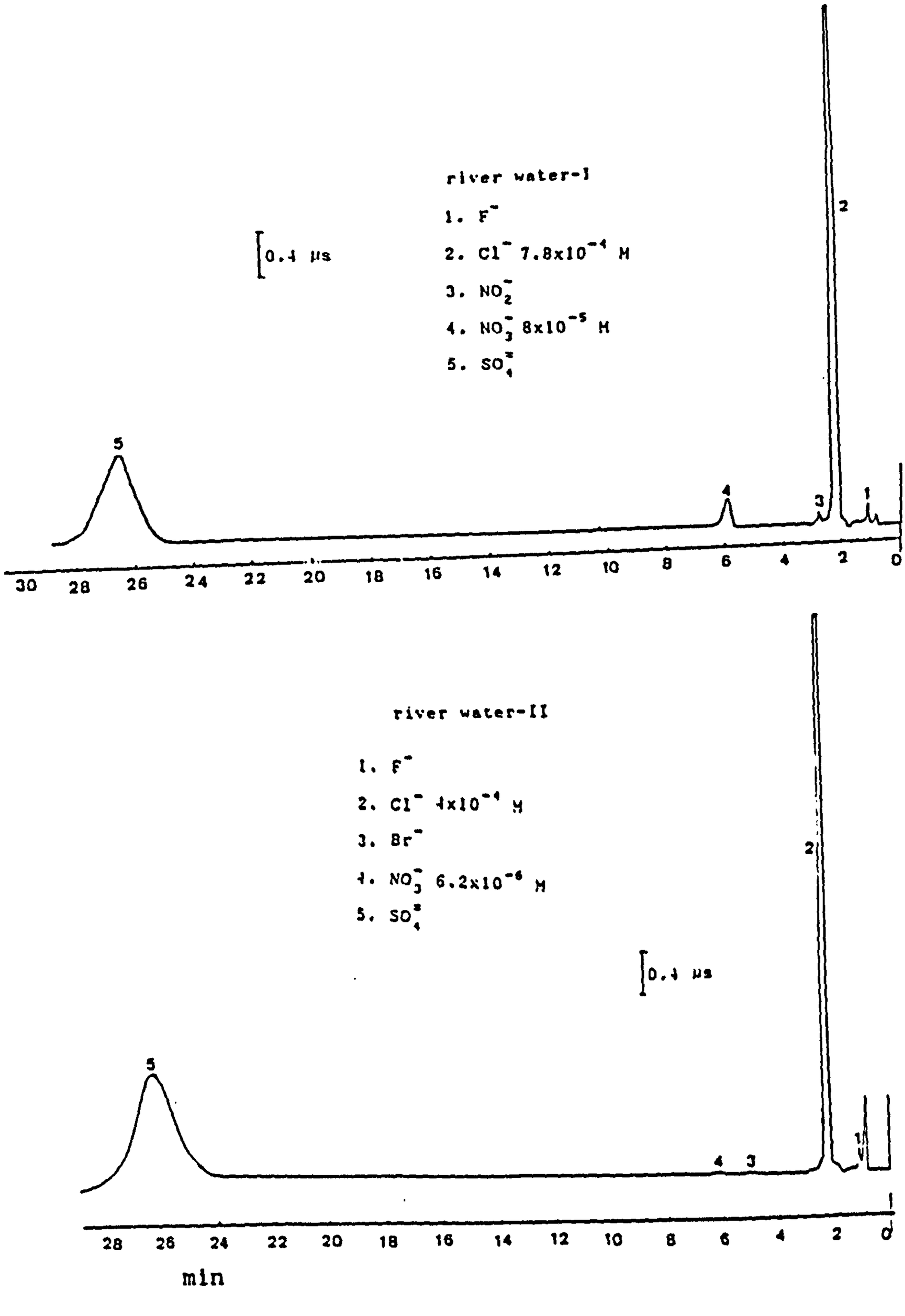


Figure 5. Anions in river water-I and II samples, the other conditions were as in fig. 1.

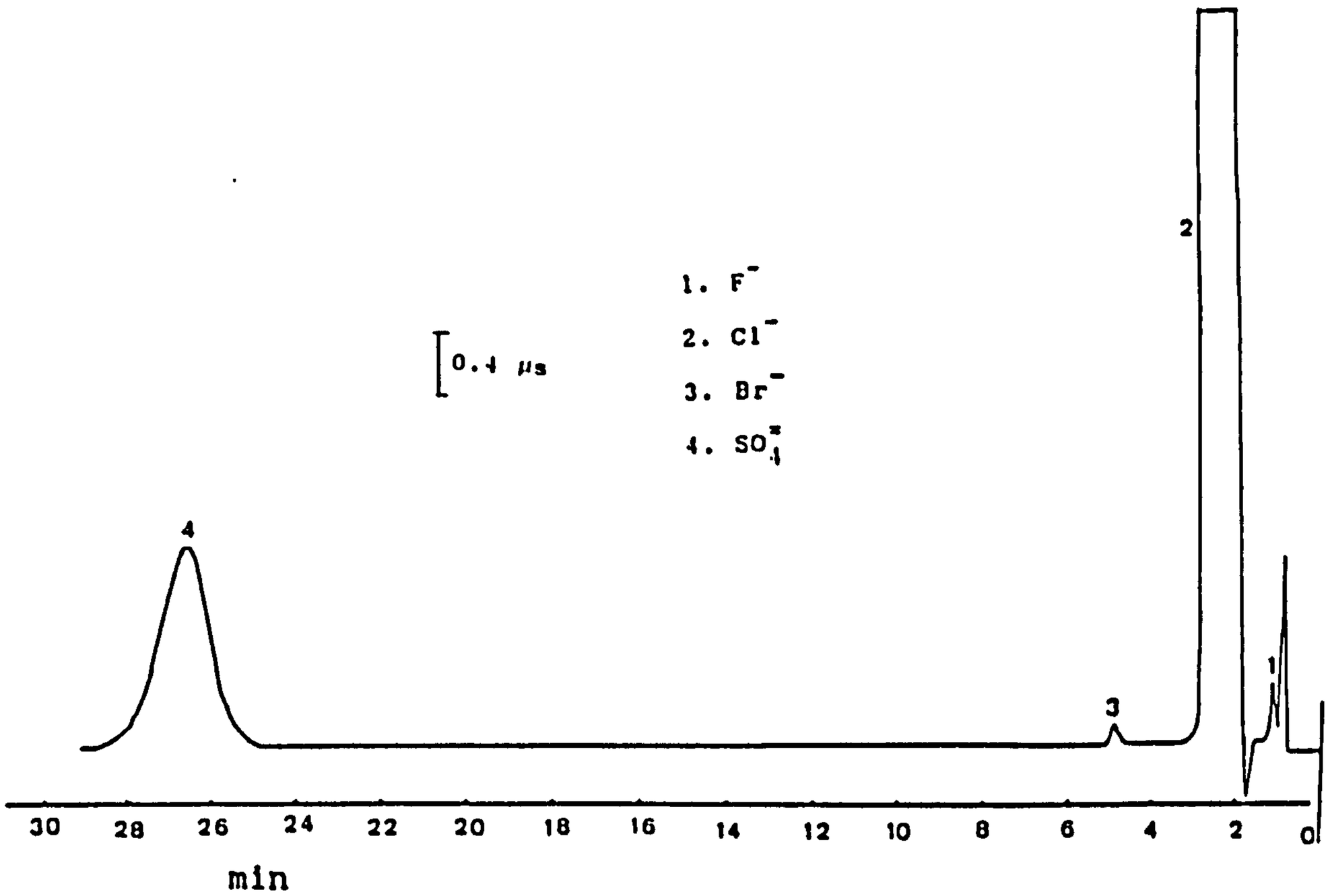


Figure 6. Anions in sea water-sample diluted hundred fold, the other conditions were as in fig. 1.

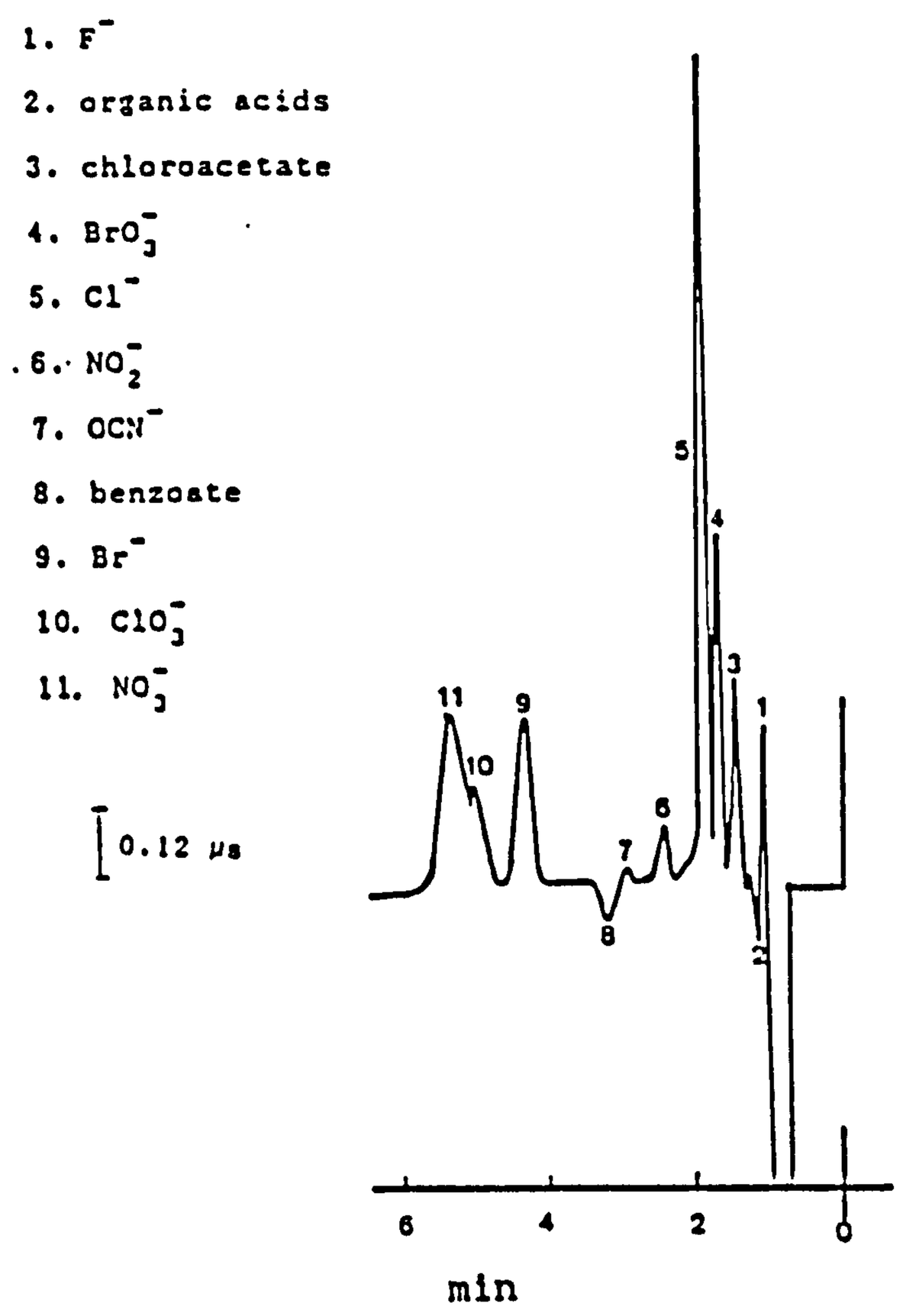


Figure 7. Separation of anions on Dionex HPIC-AS4A and AG4A columns using 0.35 mM phosphate as eluent and suppressed conductivity detection, eluent flow-rate: 1.8 ml min^{-1}, injection: 20 μl of 10^{-5} molar standard solution of each anion, regenerant flow-rate: 0.3 ml min^{-1}.

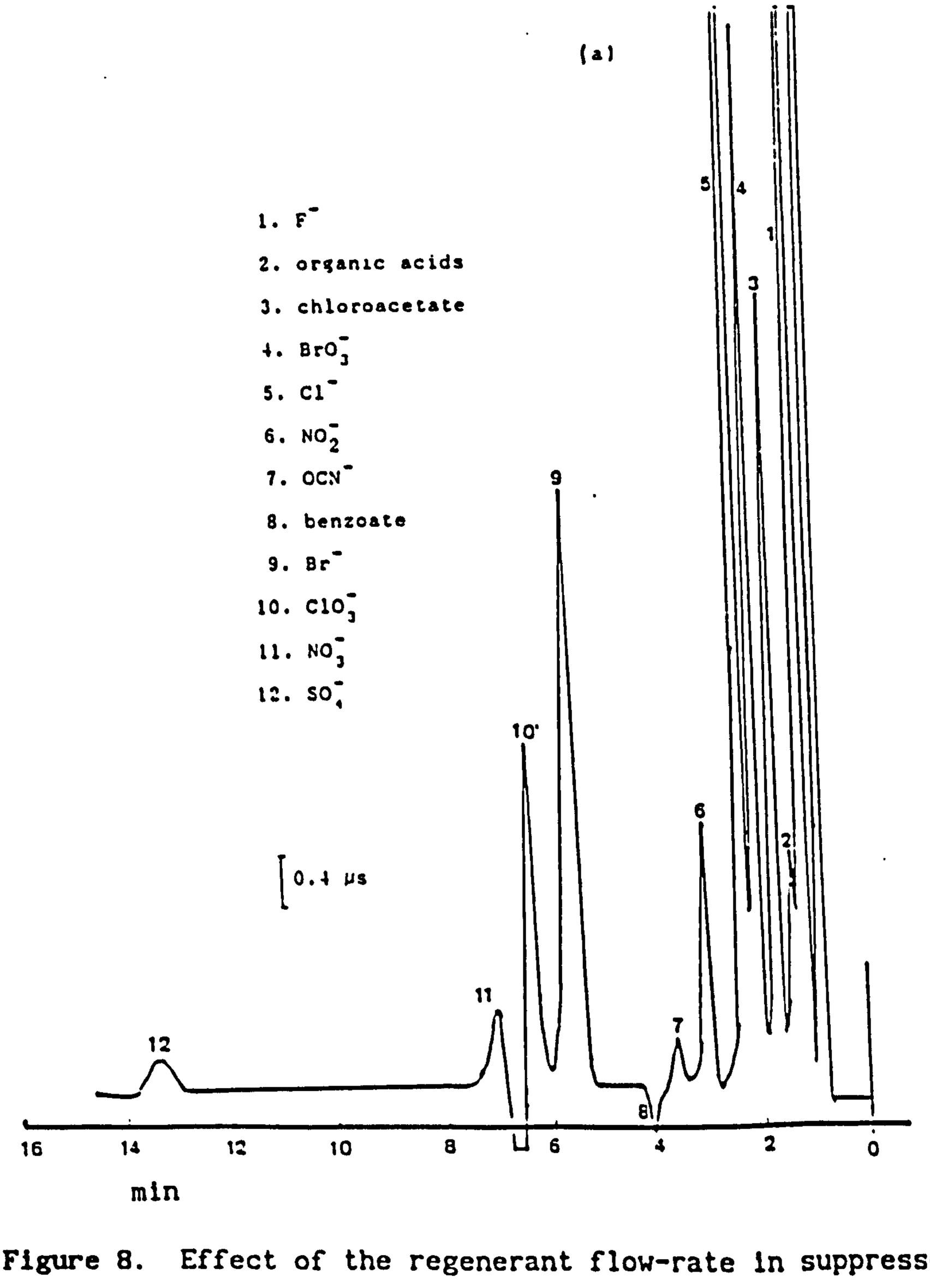
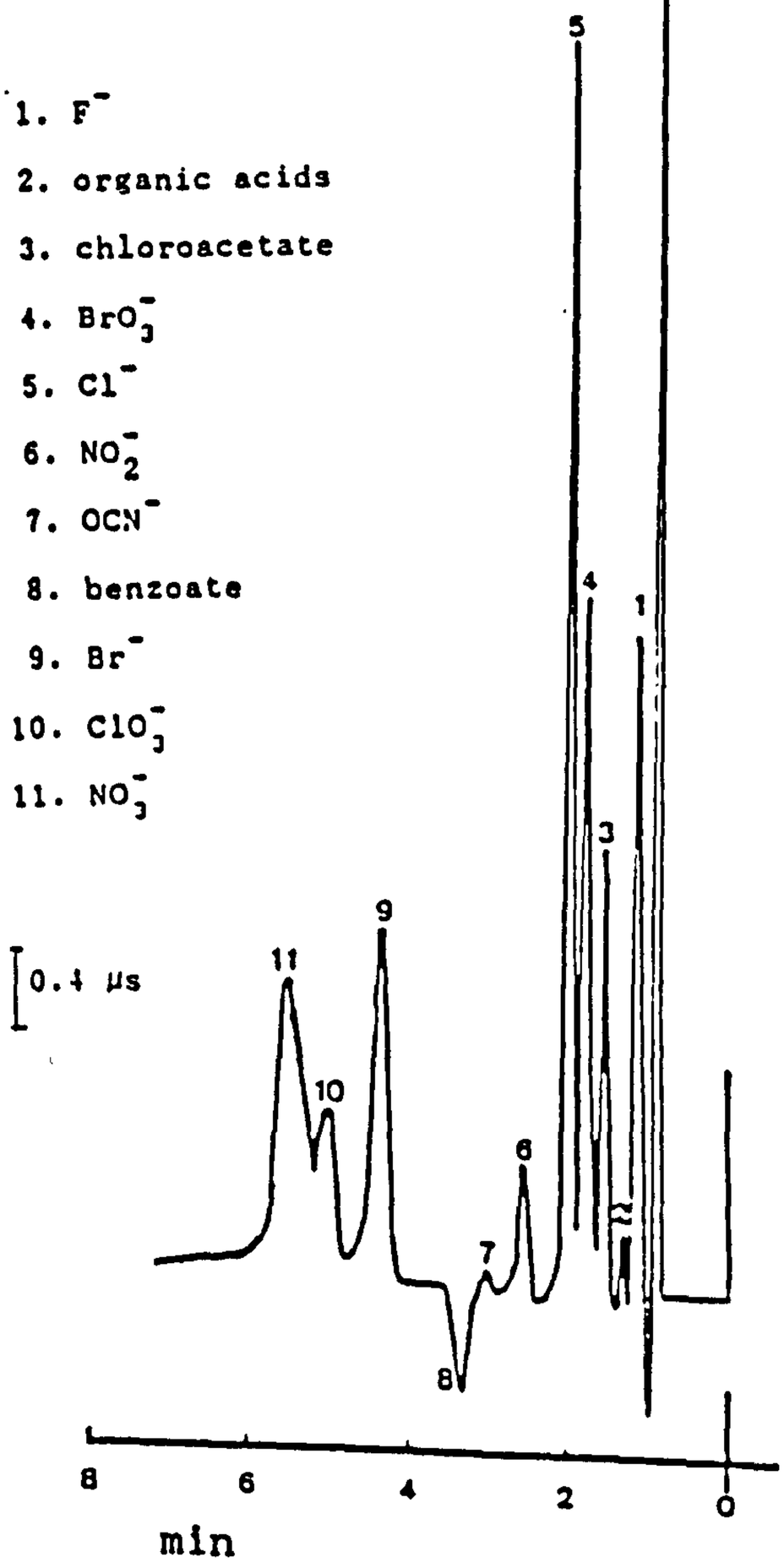


Figure 8. Effect of the regenerant flow-rate in suppressed conductivity detection, column: Dionex HPIC-AS4A and AG4A, eluent: 0.3 mM phosphate, injections: 20 µl of 10^{-4} molar standard solution of each anion, regenerant flow-rate: 0.3 ml min^{-1} for (a), 0.5 ml min^{-1} for (b), and 0.7 ml min^{-1} for (c).

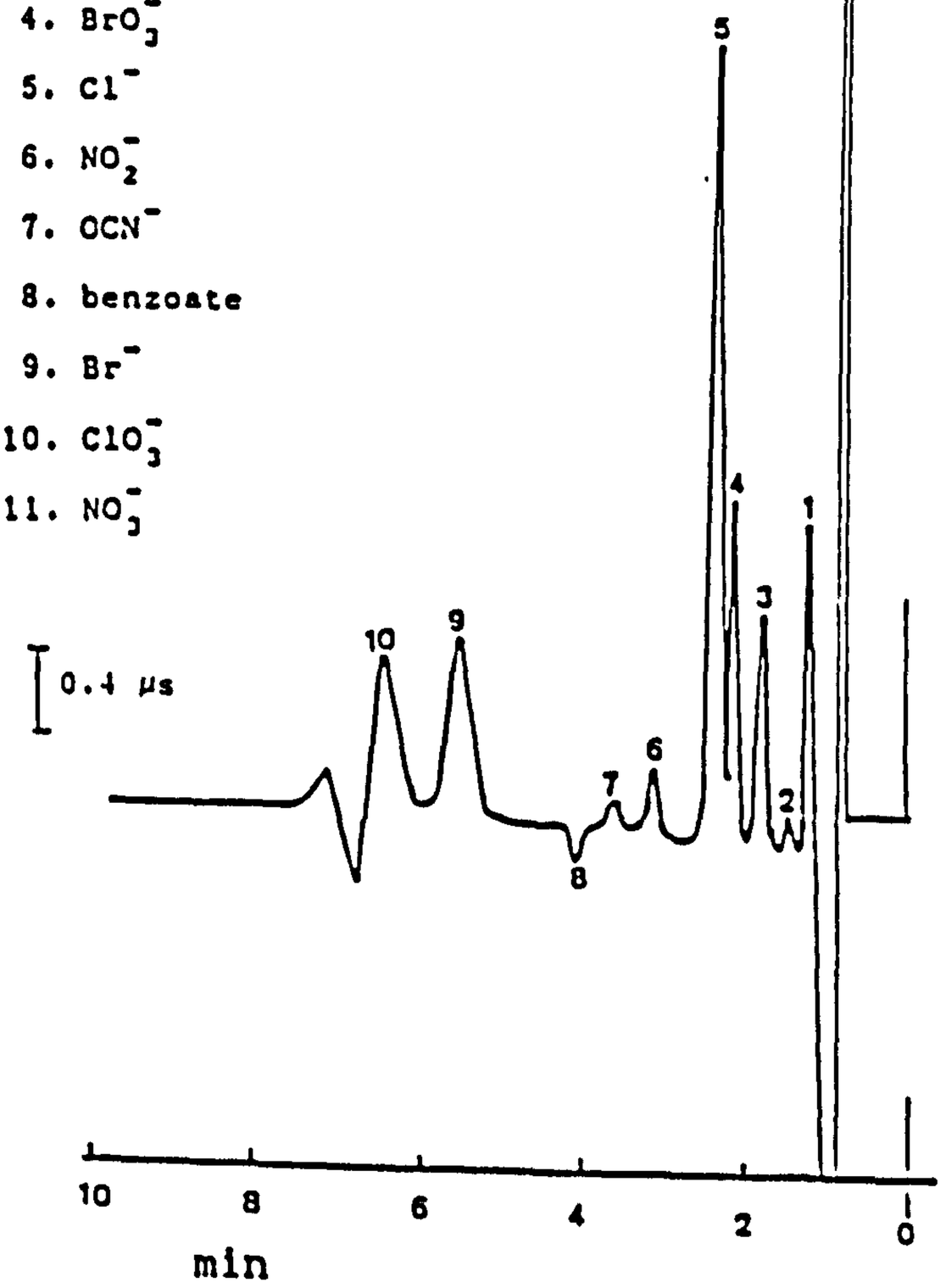
(b)

1. F^-
2. organic acids
3. chloroacetate
4. BrO_3^-
5. Cl^-
6. NO_2^-
7. OCN^-
8. benzoate
9. Br^-
10. ClO_3^-
11. NO_3^-



(c)

1. F^-
2. organic acids
3. chloroacetate
4. BrO_3^-
5. Cl^-
6. NO_2^-
7. OCN^-
8. benzoate
9. Br^-
10. ClO_3^-
11. NO_3^-



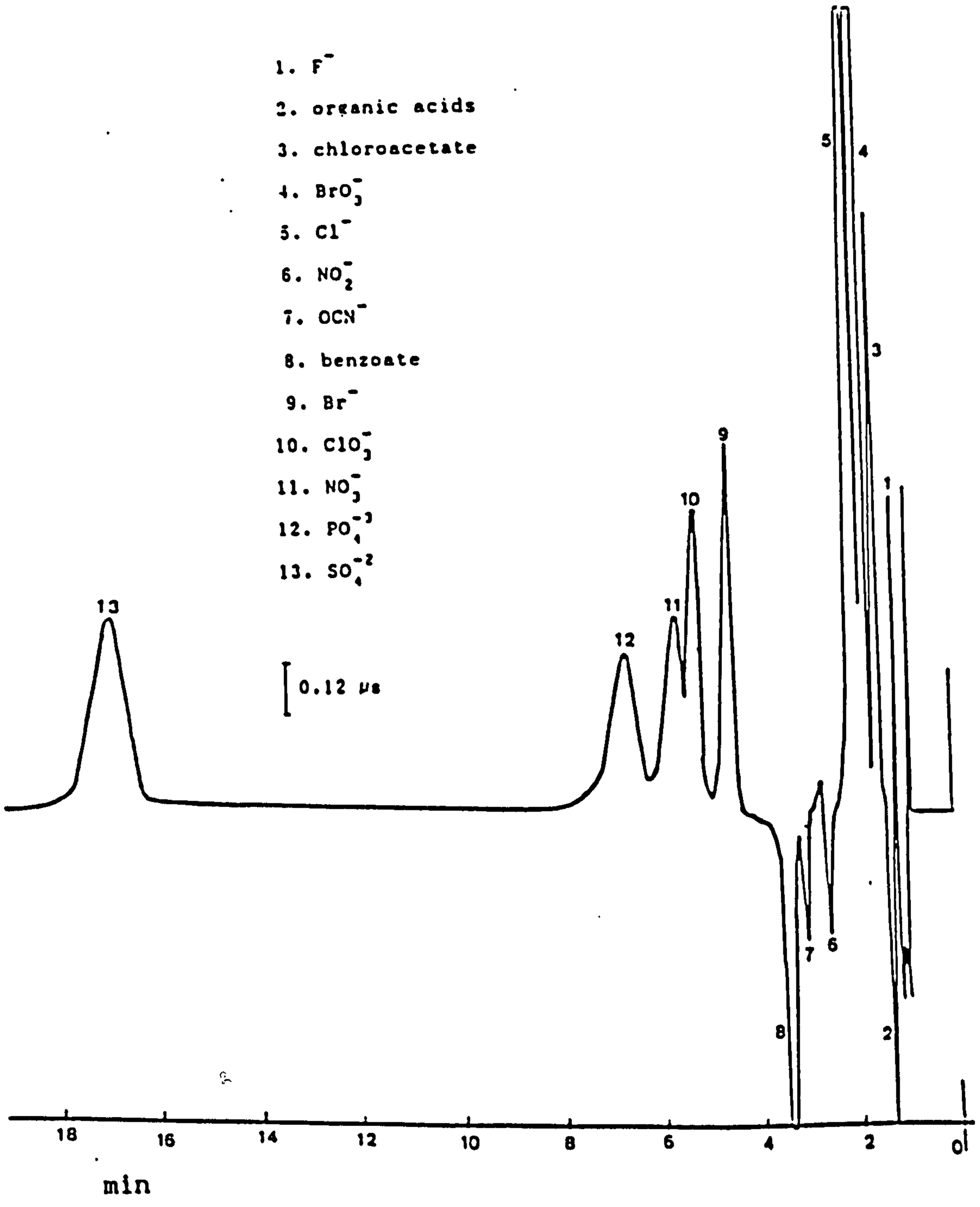


Figure 9. Separation of anions on Dionex HPIC-AS4A and AG4A columns using 0.7 mM hydrogen phosphate as eluent and suppressed conductivity detection, eluent flow-rate: 1.8 ml min^{-1}, injection: 20 µl of 0.1 mM standard solution of each anion, regenerant flow-rate: 1 ml min^{-1}.

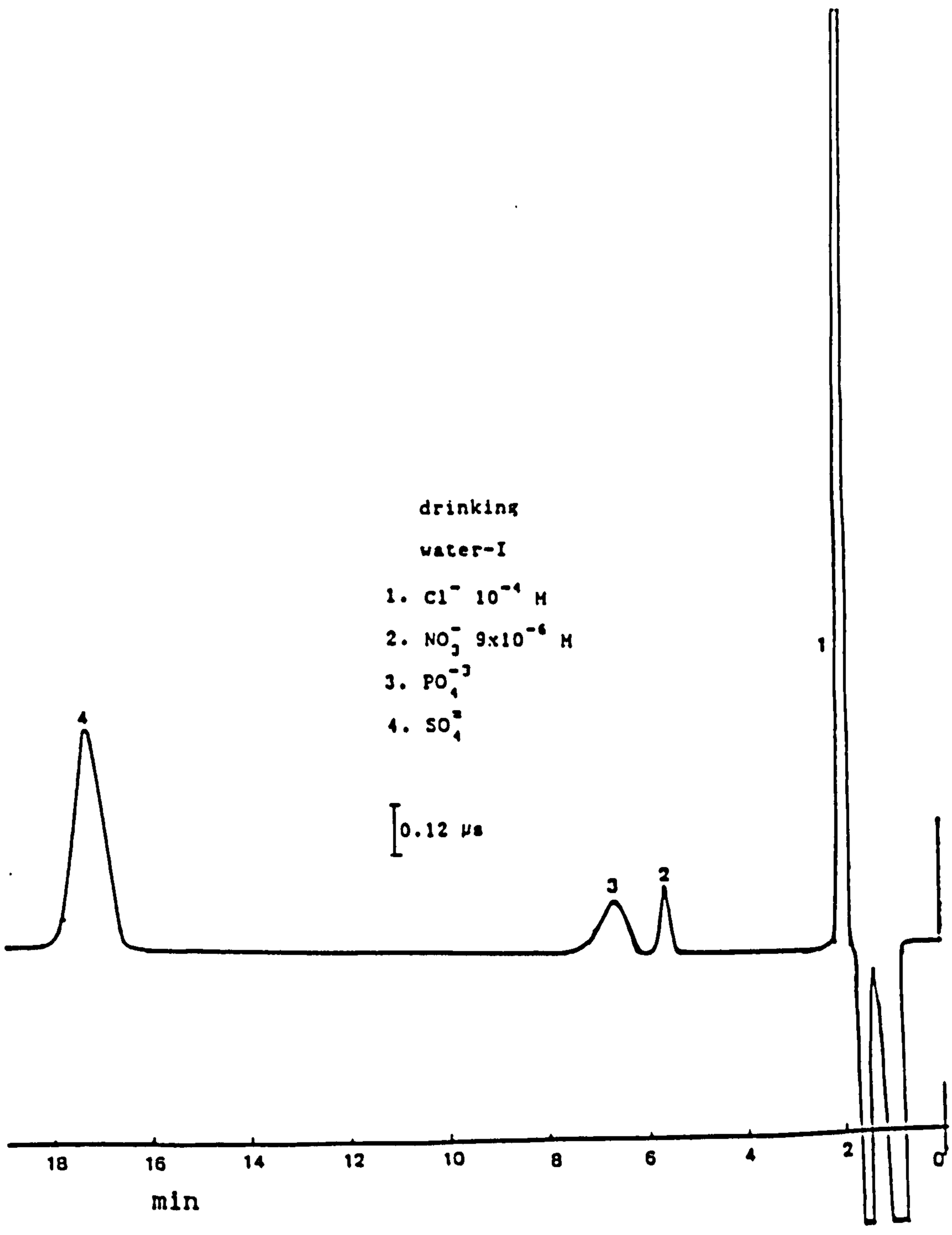
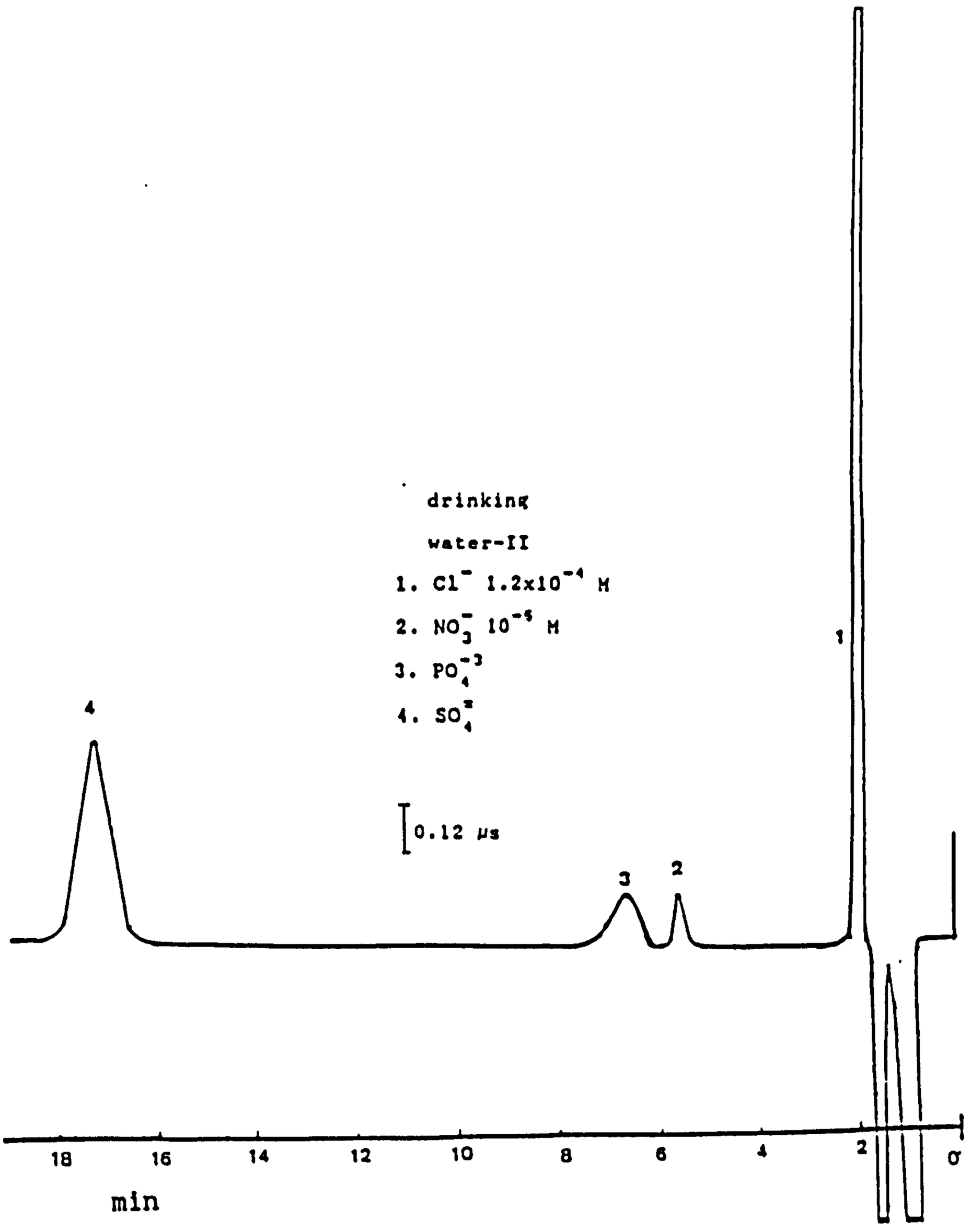


Figure 10. Anions in drinking water-I and II samples diluted ten fold, the other conditions were as in fig. 9.


drinking
water-II
1. Cl^- $1.2x10^{-4}$ M
2. NO_3^- 10^{-5} M
3. PO_4^{-3}
4. $SO_4^=$
0.12 μs
3
2
1
18
16
14
12
10
8
6
4
2
0
min

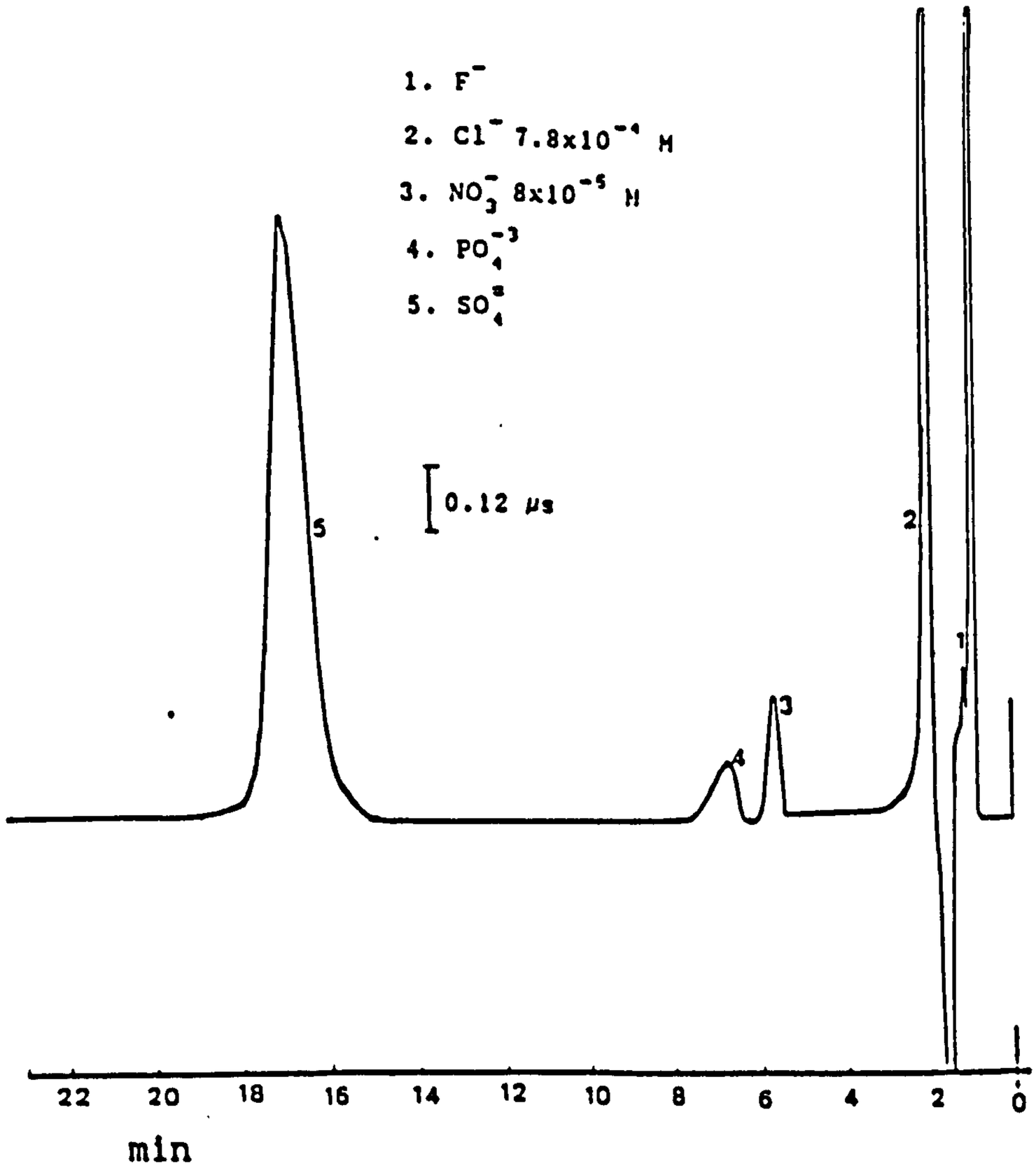


Figure 11. Anions in river water-I sample, the other conditions were as in fig. 9.

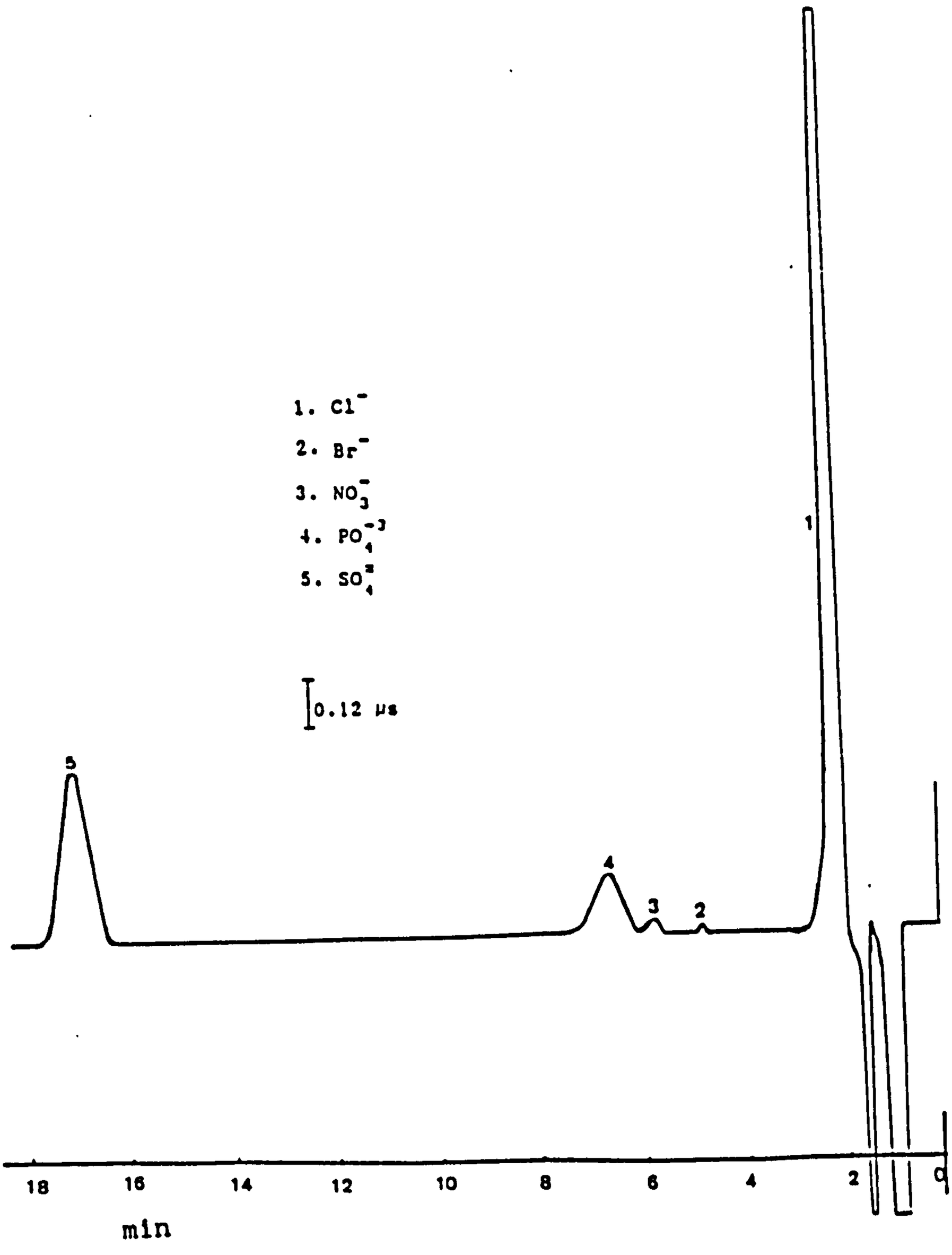


Figure 12. Anions in sea water-sample diluted hundred fold, the other conditions were as in fig.9.

8.4 REFERENCES

1. Shintani H. and Dasgupta P K., *Anal. Chem.*, 1987, 59, 802.

2. Lockridge J E., Fortier N E., Schmuckler G. and Fritz J S., *J. Chromatography*, 1987, 192, 41.

3. Jones W R., Jandik P. and Heckenberg A L., *Anal. Chem.*, 1988, 6, 1979.

4. Rocklin R D., Christopher A P. and Schibler J A., *J. Chromatography*, 1987, 411, 107.

5. Rocklin R D. and Johnson E L., *Anal. Chem.*, 1983, 55, 4.

CHAPTER 9

9.1 POTENTIOMETRIC DETECTION AND ION CHROMATOGRAPHIC SEPARATION OF MONOVALENT CATIONS USING AN ALL SOLID-STATE CONTACT MEMBRANE CATION SELECTIVE ELECTRODE BASED ON PVC

9.2 INTRODUCTION

With phosphate and sulphate anions as eluents, simple, selective and sensitive methods in ion chromatography with potentiometric detection of monovalent anions were described in chapter 7. The methods were based on the use of an anion-exchange resin column with the adaptation of quite dilute eluents with very low background potential for the detector.

It seemed feasible to develop a simple and selective method for separation of inorganic and organic monovalent cations that is analogous to the method developed for anion chromatography. Thus, monovalent cation separations would be achievable with quite dilute eluent with a very low background potential for the detector, and separated cations would be detected by an all solid-state contact monovalent cation selective electrode as a detector placed immediately after the separation column.

It has been suggested in chapter 2 that neutral carriers based on crown compounds generally exhibit high selectivity for several metal cations of similar size or charge but much lower sensitivity for another group of ions. This property can be a useful tool, for sensitive detection of a wide range of monovalent cations, if such compounds are used in the membranes of electrodes as detectors in ion chromatography. Another advantage of this system is that a large variety of chemicals could be tried as eluents for more efficient separation and sensitive detection. However, the use of copper and magnesium salts as eluents with an all solid-state contact tubular liquid membrane potassium selective electrode incorporating dibenzo-18-crown-6 compound as active material, gives a highly selective and sensitive potentiometric detection of Na^+, NH^+, K^+, Rb^+, Cs^+, Tl^+ and TMA^+ (tetramethyl

ammonium) cations, and and exhibits an efficient separation in ion chromatography.

The applications of the method for drinking, river, spring and sea water samples, and orange juice, urine and saliva samples, are illustrated. As the detector is highly selective and sensitive to only monovalent cations, with no interference from other cations, it can be easily applied to many sample types. Examples, such as the determination of Na^+ and K^+, adsorbed on the surface of glassware during the fabrication stage and in many inorganic and organic chemicals, are given.

9.3 EXPERIMENTAL

9.3.1 Preparation of Sensors

The construction of flow-through tubular PVC matrix membrane electrodes without an inner reference solution was as previously described in chapter 7.

The potentiometric cell consisted of two perspex cylinder body in which a conductive support with 1.5 mm diameter channel was drilled. The PVC-tetrahydrofuran (THF) solution containing the active ligand, potassium tetraphenylborate (KTPB) to reducer the membrane resistivity, and plasticizer was applied into the hole of conductive support which was epoxy resin loaded with graphite. The sensing membrane consisted of 28 weight % PVC, 4 weight % dibenzo 18-crown-6 as active ligand, 2 weight % KTPB and 66% dioctyl sebacate (DOS) as plasticizer after evaporation of THF at room temperature open to air for four hours. Once the sensing membrane solution had been coated dropwise, the inner diameter of the channel was reduced to ca. 1.2 mm. When not in use for a long time, the tubular electrode was stored dry after washing with deionized water. It was reconditioned for one hour with primary ion solution before use.

9.3.2 Instrumentation and Chemicals

Chromatography was performed on a Perkin Elmer (series 3) high performance liquid chromatograph (HPLC), which consists of a dual channel pump and injection valve with 20 µl sample loop.

Separations were performed on Dionex IonPac-CS3 analytical and guard columns. An all solid-state contact flow-through tubular PVC matrix membrane electrode as indicator and a double junction calomel electrode as reference were used for potentiometric detection of monovalent cations. A high input impedance buffer amplifier and digital voltmeter were connected to the electrodes for potential recording during the experiments. A SE 120 BBC chart recorder was used for obtaining the chromatograms of monovalent cations.

Chemicals for preparation of sensing membrane were from Fluka except the DBP which was from Aldrich. All standard sample solutions were prepared from their analytical reagent grade chemicals in deionized water. Sample matrices of river, sea and drinking water were taken from local areas of Newcastle upon Tyne and were diluted before injections when required. Spring water, orange juice, milk, urine and saliva samples were supplied from different sources indiscriminately. Inorganic and organic chemicals, in which constituents were determined, were either analytical or laboratory reagent grade. 20 µl of samples and standard solutions were always injected. Samples were filtered through Millipore filters (pore size: 0.45 µm). Suitable compositions of eluents were prepared freshly before use. The identification of species was performed by comparing retention times of peaks with those of standards.

9.4 RESULTS AND DISCUSSION

Figure 1 shows the chromatograms of a mixture of sodium, ammonium, potassium, rubidium, cesium and thallium at different concentration levels and the high selectivity and sensitivity of the detector towards monovalent cations at lower concentration levels.

Separation was affected by cation-exchange chromatography incorporating copper sulphate as eluent. The detector response is determined by the selectivity of the electrode membrane towards cations injected. The retention time for solutes can be controlled to some extent by controlling the eluent concentration, which

affects the degree of cation-exchange of solutes. Since the concentration of the eluent was not a significant parameter for sensitivity and selectivity of the detector, it could be easily altered. To demonstrate the flexibility of the method, separations and sensitive potentiometric detection of inorganic and organic monovalent cations were obtained using different concentration levels of copper sulphate as eluent and cation-exchange columns. Results are shown in figures 2-4.

Also, separation and highly sensitive and selective detection of monovalent inorganic and organic cations is possible using dilute magnesium sulphate solution as eluent, which is shown in figure 5. The determination of monovalent cations in drinking, mineral, sea, river water samples, and orange juice, urine, milk and saliva samples was carried out using the method developed and are shown in figures 6-16.

The lower detection limit for cations, especially for potassium and sodium, is at sub-ppb levels, which allows the method to be applied to the determination of sodium and potassium, adsorbed on the surface of glassware at the fabrication stage. The washing of the glassware surface with deionized water diminishes the sodium and potassium levels as illustrated in figure 17.

Inorganic and organic impurities in organic salts can be determined simultaneously with the method is shown in figure 18.

Ion chromatography with potentiometric detection can also be applied to many other sample types as the method is highly selective and sensitive, with no interference from higher charged cations or anions. A wide variety of inorganic and organic chemicals are used in every laboratory in industry, and the exact concentration levels of impurities is required for quality control. Sodium and potassium levels are especially significant as they are the most common contaminants.

Potentiometric detection and ion chromatographic separation of sodium in a variety of potassium salts is shown in figures 19 and 20. Figures 21, 22 and 23 are of potassium determination in a variety of sodium salts. Figures 24-27 show the determination of sodium and potassium in a variety of inorganic and organic

chemicals. The solubility products of sparingly soluble organo-potassium salt can also be calculated by chromatography as shown in figure 28. The method is very simple and requires only dissolution, dilution and injection of samples without further processing.

The method could be applied to a variety of sample types for monovalent cation determination where high sensitivity and selectivity are of prime importance.

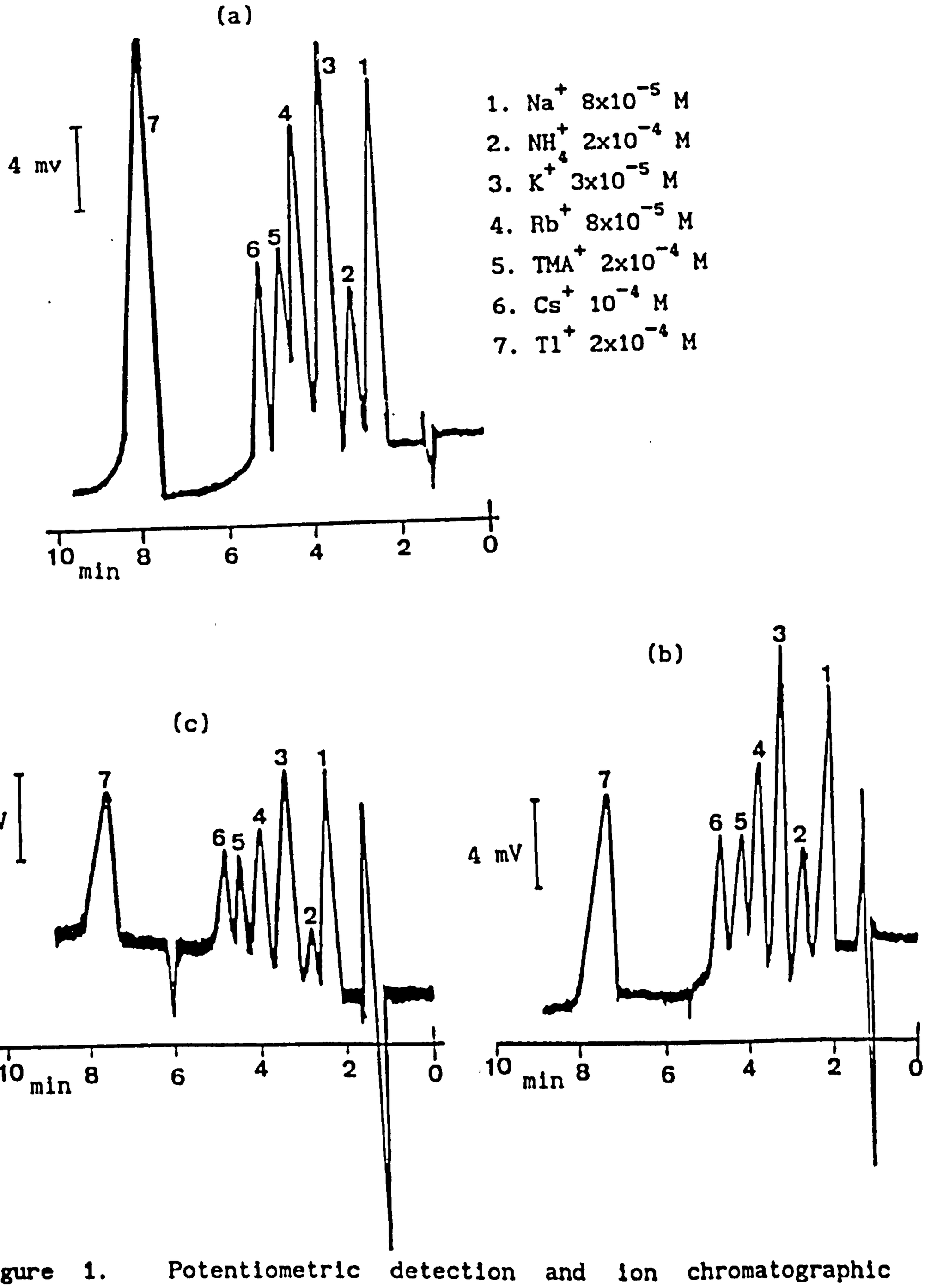
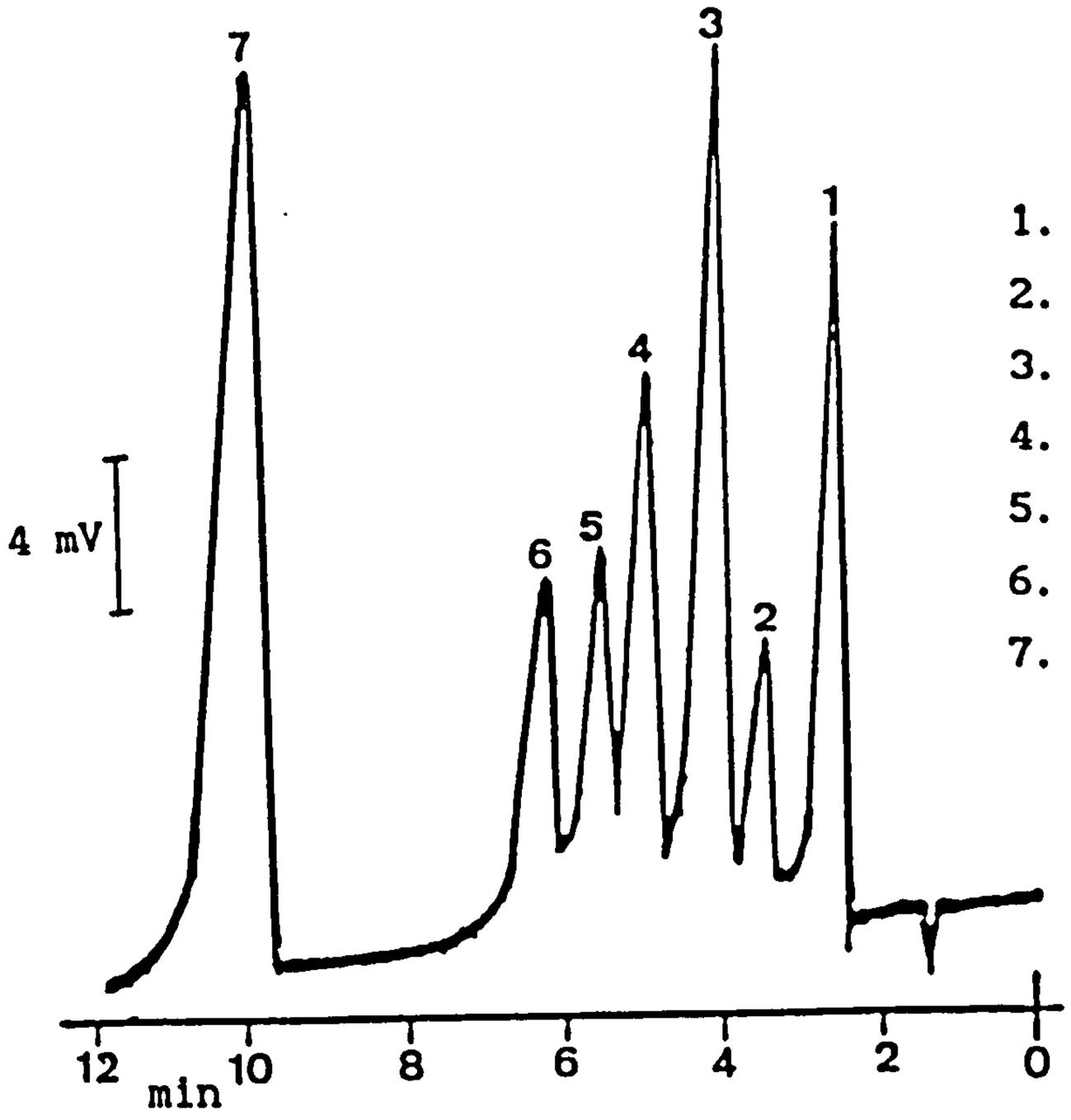
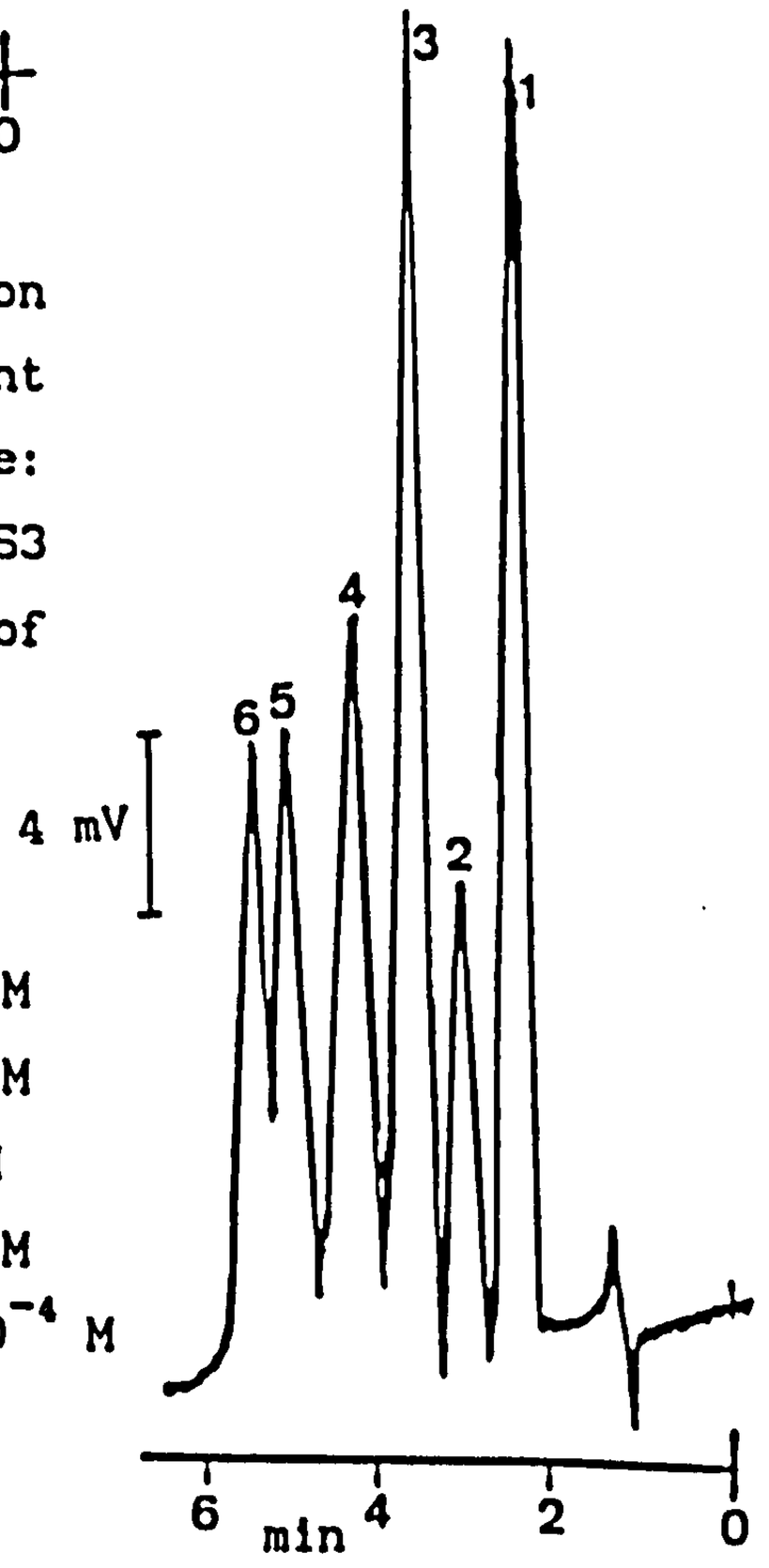


Figure 1. Potentiometric detection and ion chromatographic separations of monovalent cations at different concentration levels, eluent: 1 mM $CuSO_4$, flow-rate: 1.5 ml min^{-1}, column: Dionex HPIC-CS3 analytical and guard columns, injection: 20 µl of the standard solution of cations (a), 5 times diluted (b) and 10 times diluted (c).



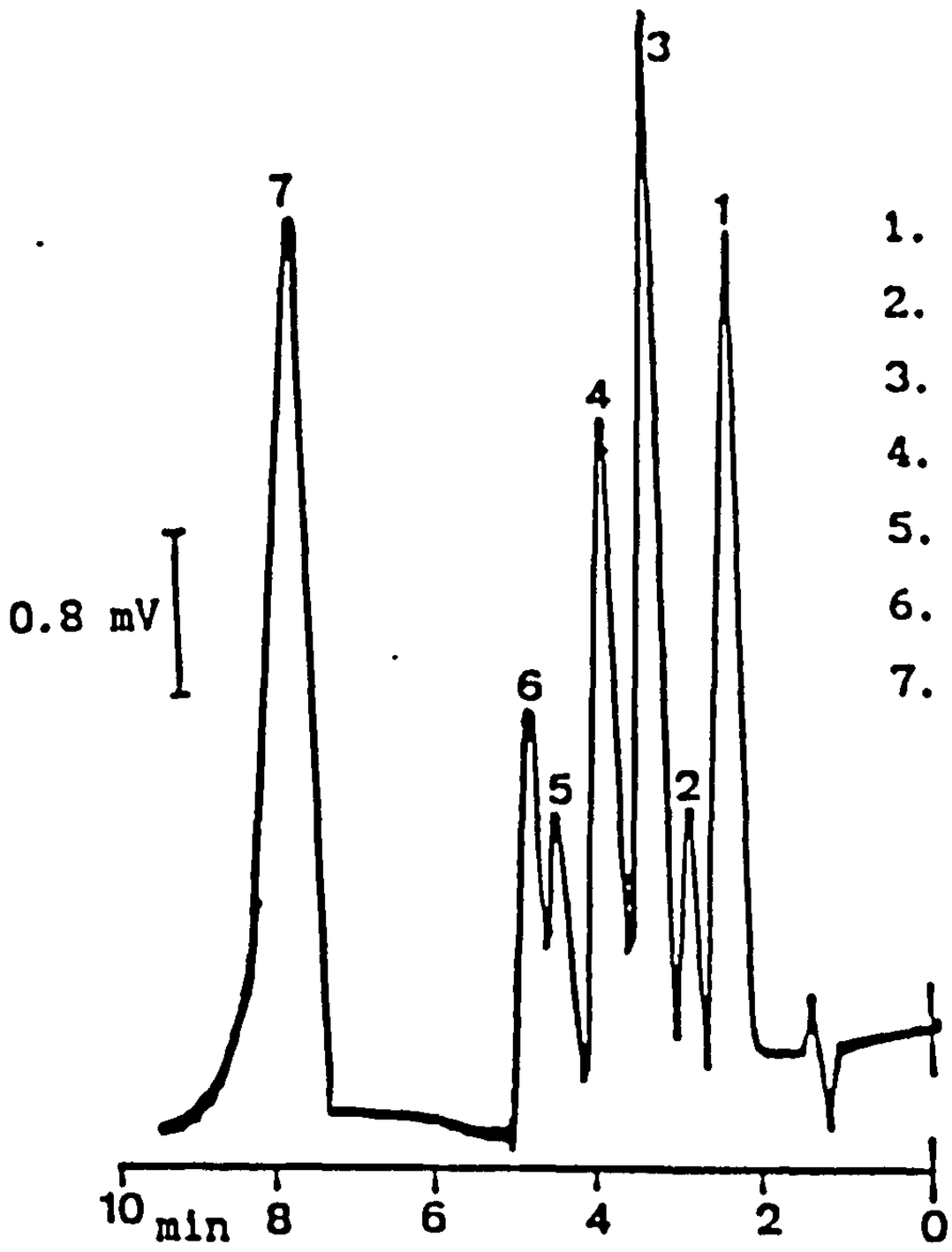
1. Na^+ $8x10^{-5}$ M
2. NH_4^+ $2x10^{-4}$ M
3. K^+ $3x10^{-5}$ M
4. Rb^+ $8x10^{-5}$ M
5. TMA^+ $2x10^{-4}$ M
6. Cs^+ 10^{-4} M
7. Tl^+ $2x10^{-4}$ M

Figure 2. Ion chromatographic separation and potentiometric detection of monovalent cations, eluent: 0.5 mM $CuSO_4$, flow-rate: 1.5 ml min^{-1}, column: Dionex HPIC-CS3 analytical and guard, injection: 20 µl of the standard solution of cations.



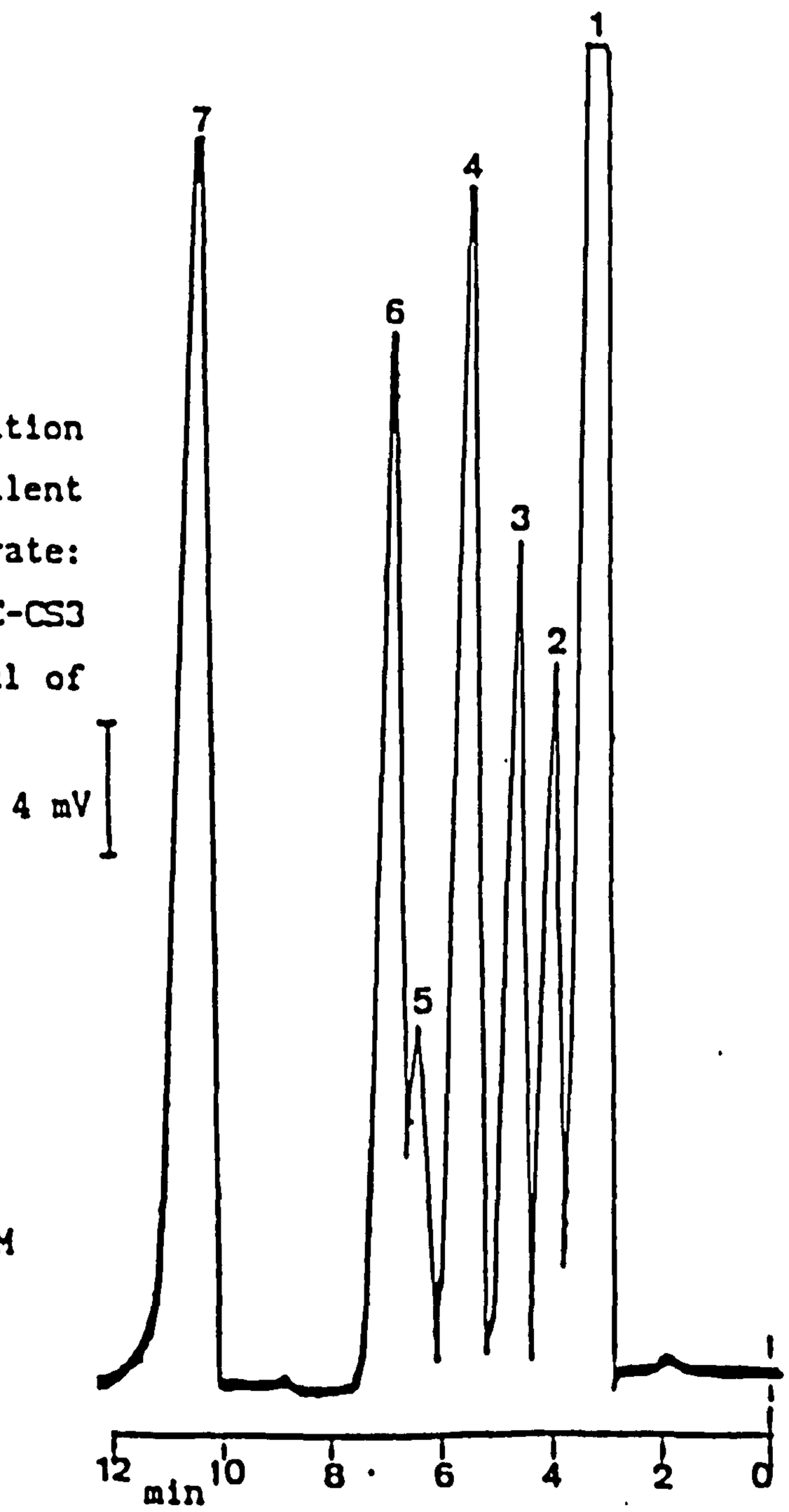
1. Na^+ $8x10^{-5}$ M
2. NH_4^+ $2x10^{-4}$ M
3. K^+ $3x10^{-5}$ M
4. Rb^+ $2x10^{-4}$ M
5. $TriMA^+$ $2x10^{-4}$ M
6. Cs^+ 10^{-4} M

Figure 3. Ion chromatographic separation and potentiometric detection of monovalent cations including $triMA^+$, eluent: 1 mM $CuSO_4$, flow-rate: 1.3 ml min^{-1}, column: Dionex HPIC-CS3 analytical and guard, injection: 20 µl of of the standard solution of cations.



1. Na^+ $8x10^{-5}$ M
2. NH_4^+ $2x10^{-4}$ M
3. K^+ $3x10^{-5}$ M
4. Rb^+ $8x10^{-5}$ M
5. TMA^+ $1x10^{-4}$ M
6. Cs^+ 10^{-4} M
7. Tl^+ $2x10^{-4}$ M

Figure 4. Ion chromatographic separation and potentiometric detection of monovalent cations, eluent: 2 mM $CuSO_4$, flow-rate: 1.3 ml min^{-1}, column: Dionex HPIC-CS3 analytical and guard, injection: 20 µl of the standard solution of cations.



1. Na^+ $2x10^{-4}$ M
2. NH_4^+ $2x10^{-4}$ M
3. K^+ $5x10^{-5}$ M
4. Rb^+ $2x10^{-4}$ M
5. TMA^+ $2x10^{-4}$ M
6. Cs^+ $2x10^{-4}$ M
7. Tl^+ $2x10^{-4}$ M

Figure 5. Ion chromatographic separation and potentiometric detection of monovalent cations, eluent: 1.2 mM $MgSO_4$, flow-rate: 1.2 ml min^{-1}, column: Dionex: HPIC-CS3 analytical and guard, injection: 20 µl of the standard solution of cations

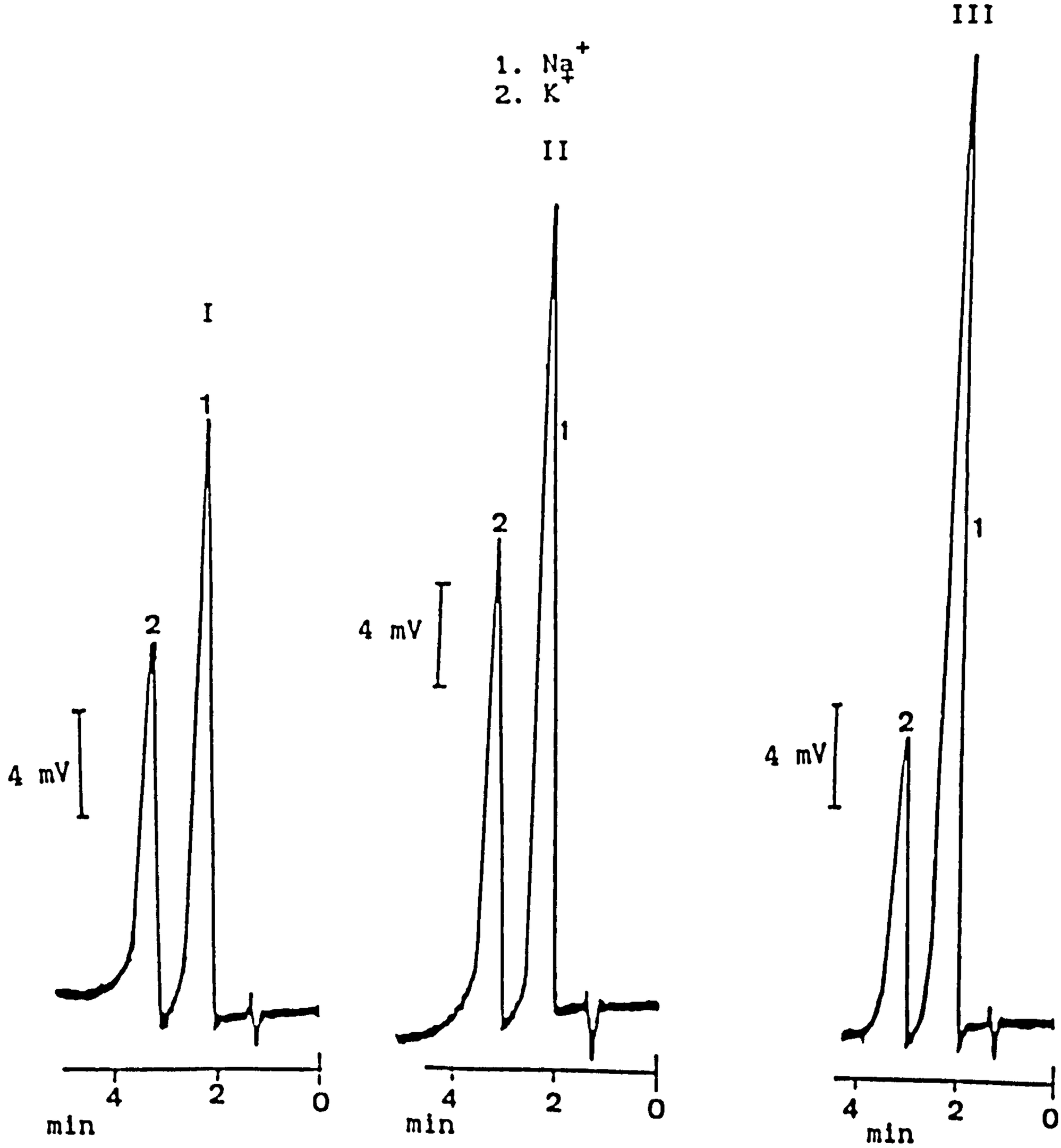


Figure 6. Potentiometric detection of monovalent cations in three different river water I, II and III samples, the other conditions were as in figure 1.

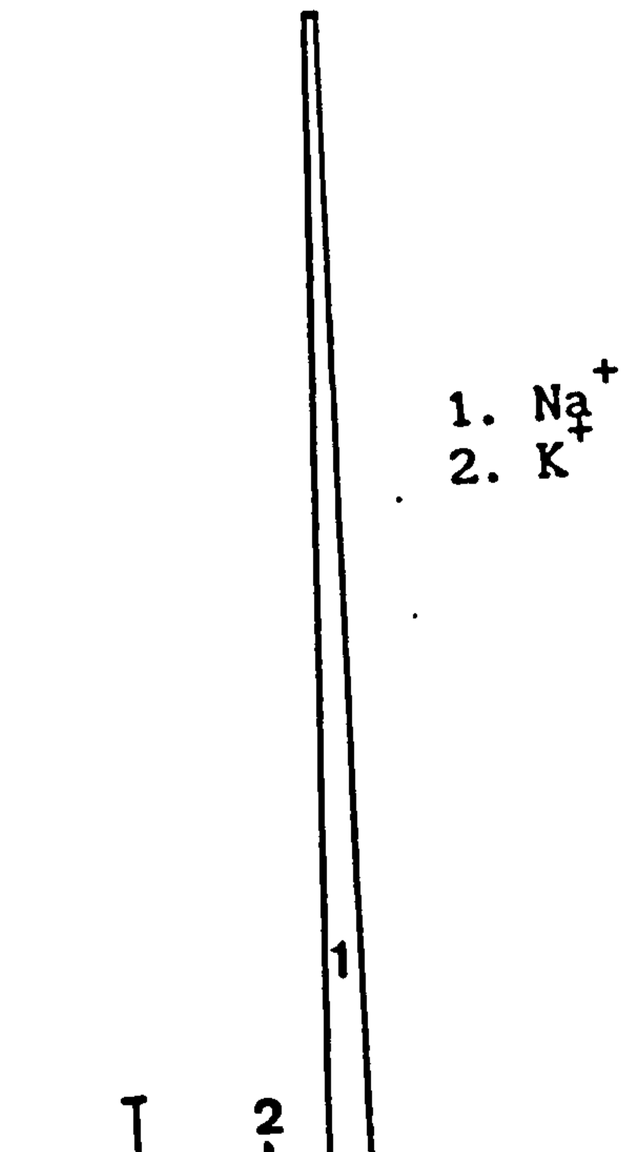


Figure 7. Potentiometric detection of monovalent cations in sea water 200 times diluted, the other conditions were as in figure 1.

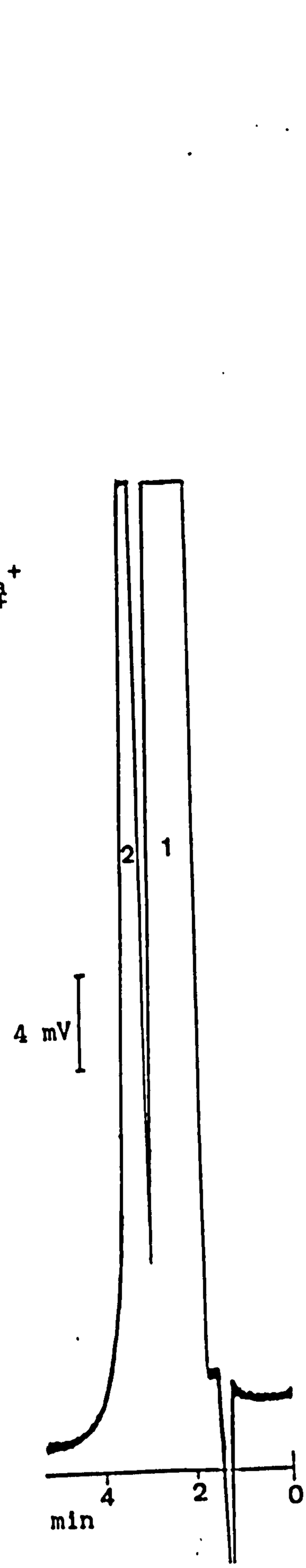


Figure 8. Potentiometric detection of monovalent cations in saliva 25 times diluted, the other conditions were as in figure 1.

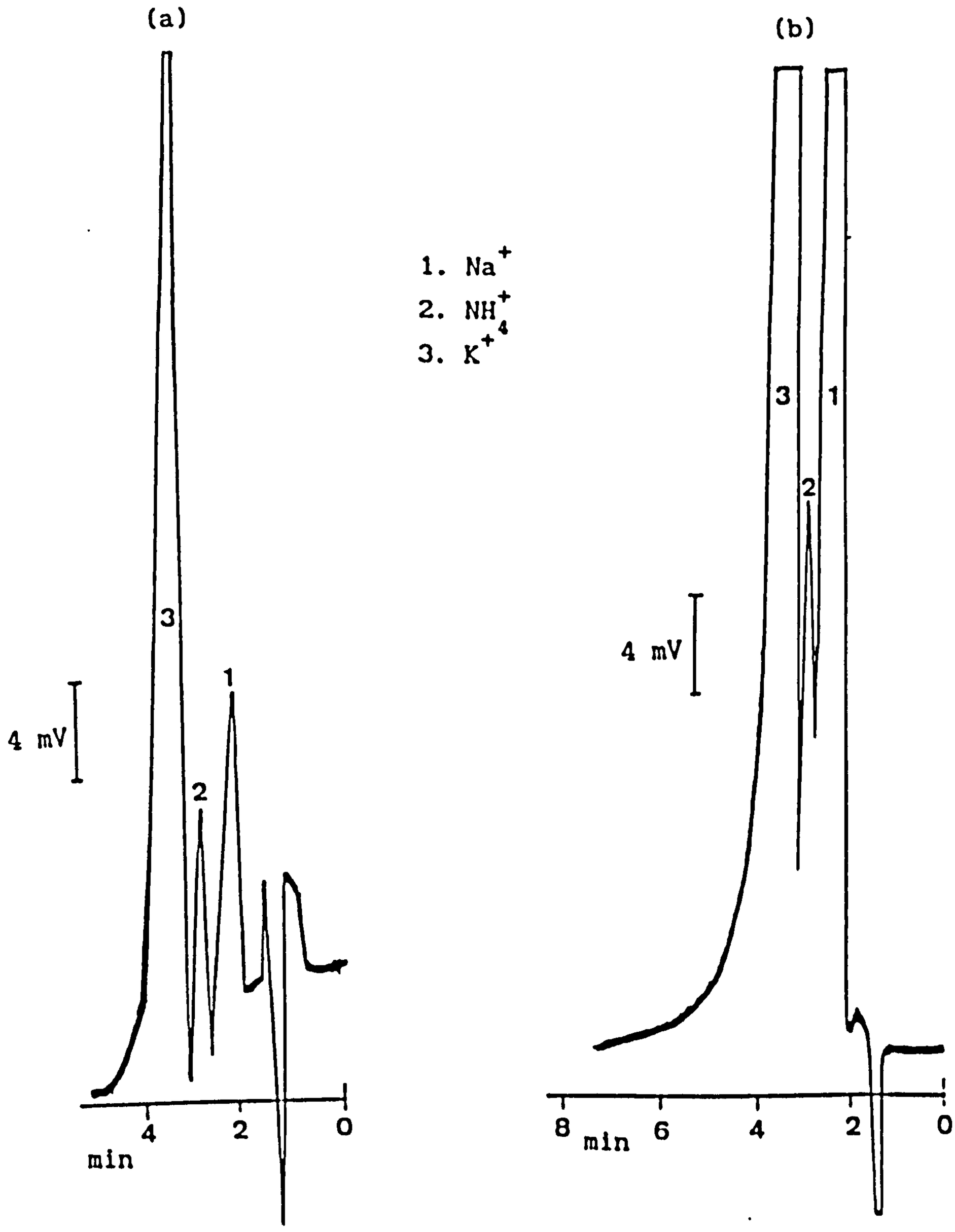


Figure 9. Potentiometric detection of monovalent cations in urine 25 times diluted (a), not diluted (b), the other conditions were as in figure 1.

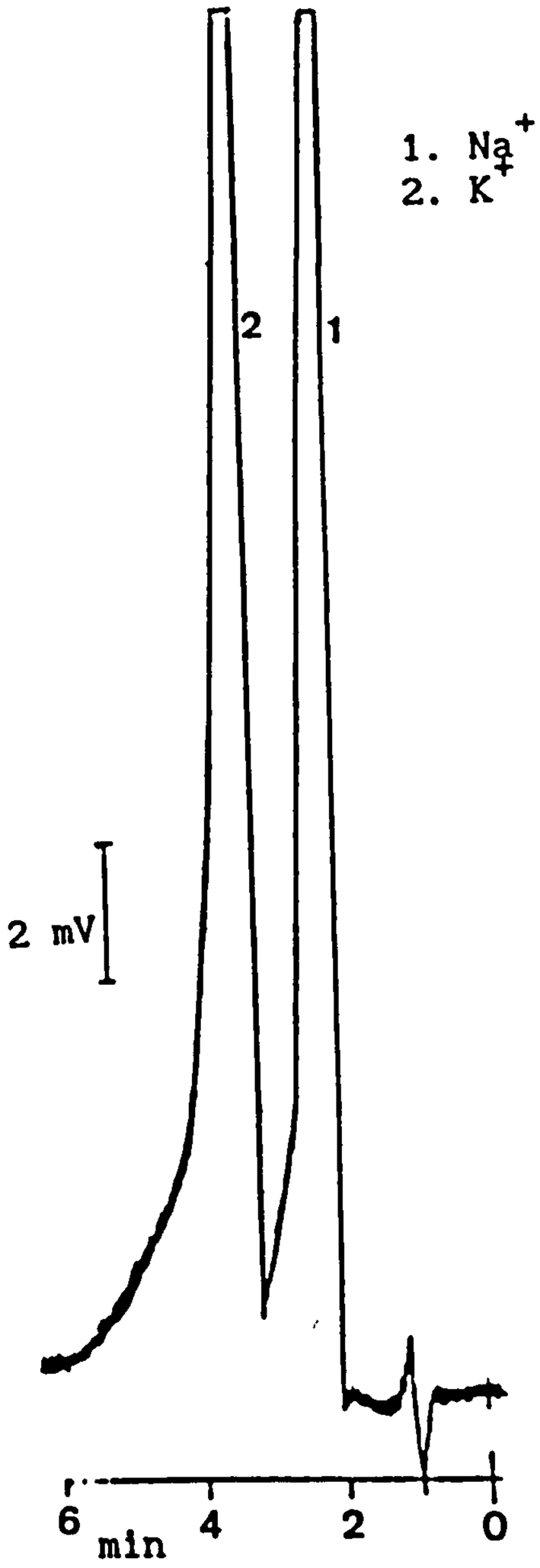


Figure 10. Potentiometric detection of monovalent cations in semi skimmed milk 100 times diluted, the other conditions were as in figure 1.

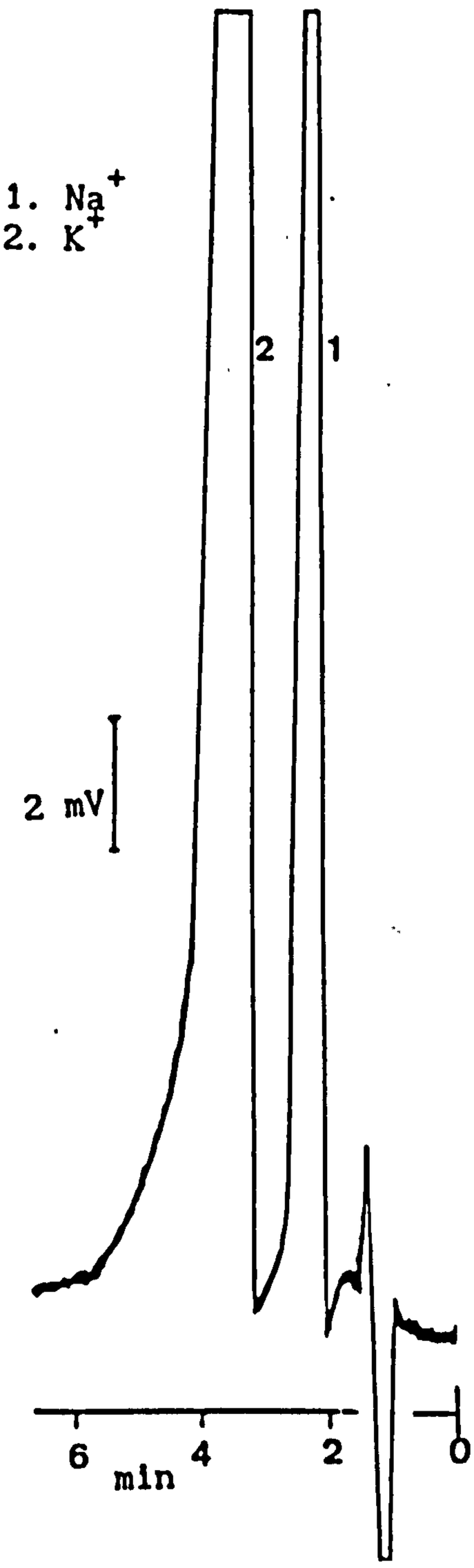


Figure 11. Potentiometric detection of monovalent cations in orange juice 50 times diluted, the other conditions were as in figure 1.

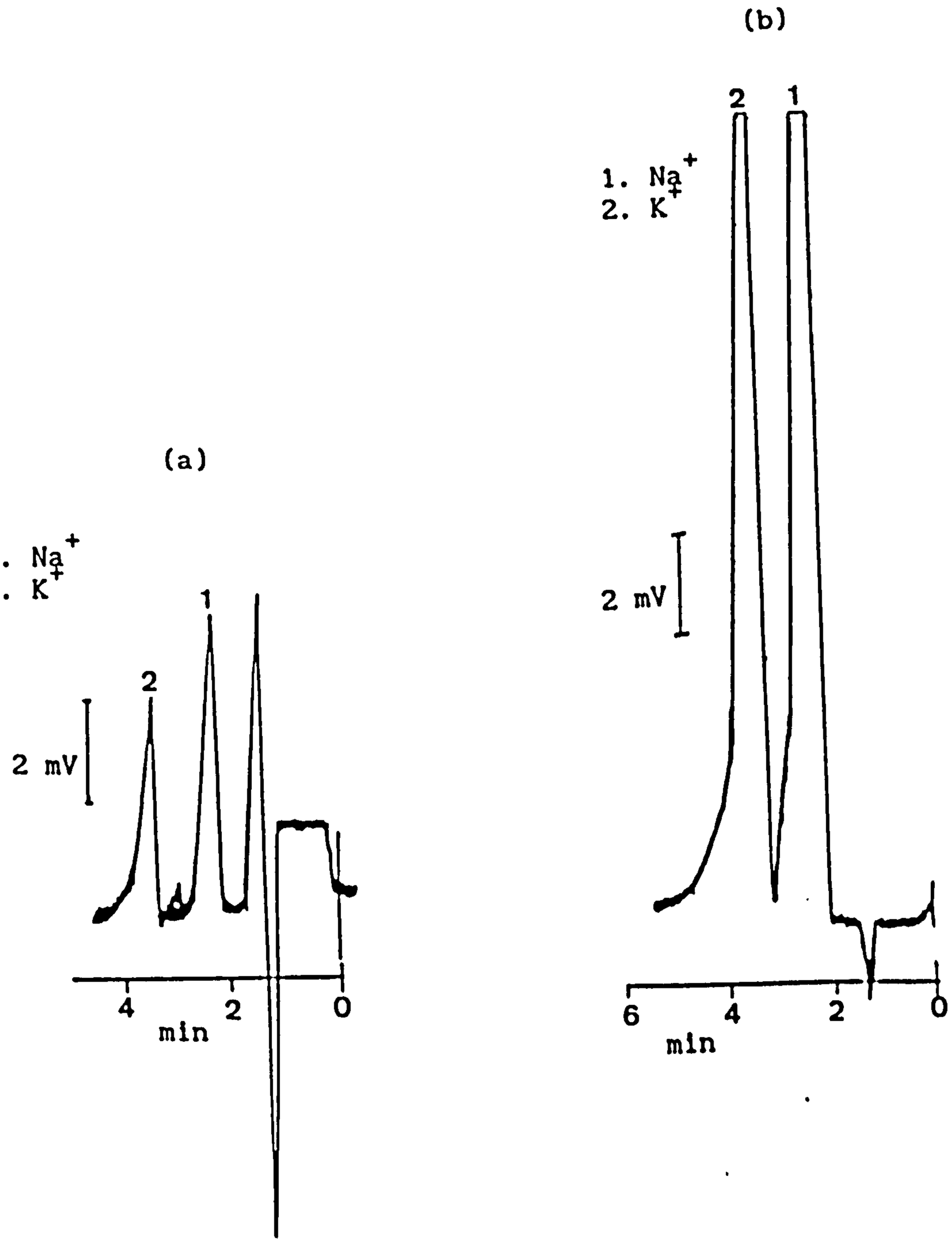


Figure 12. Potentiometric detection of monovalent cations in spring water (Volvic) 10 times diluted (a), not diluted (b), the other conditions were as in figure 1.

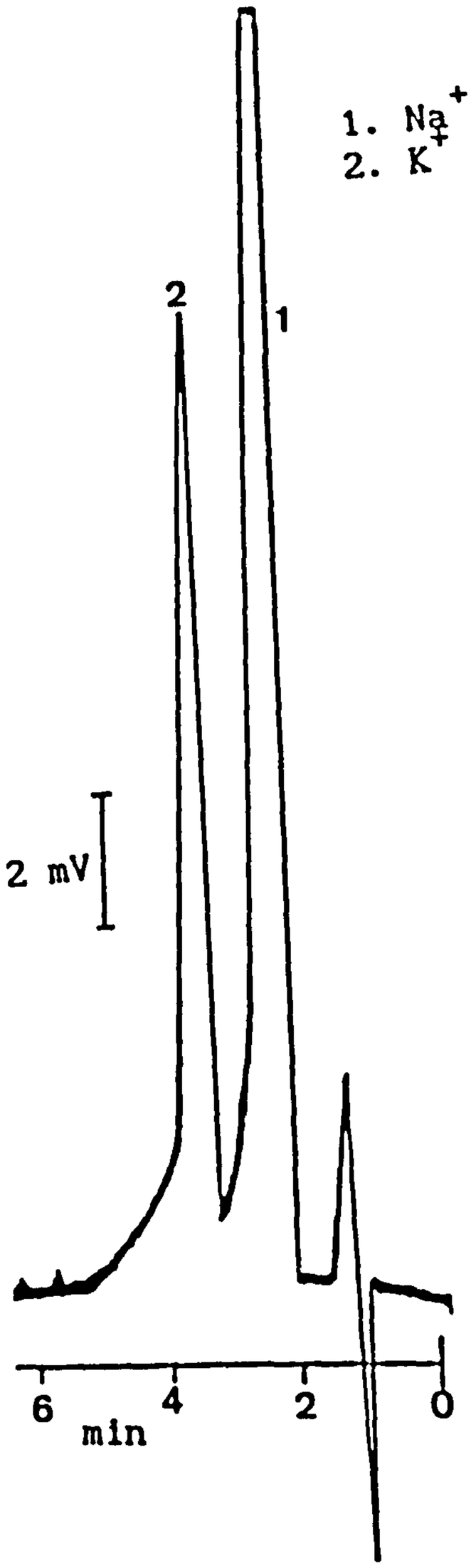


Figure 13. Potentiometric detection of monovalent cations in spring water (St.Michel) not diluted, the other conditions were as in figure 1.

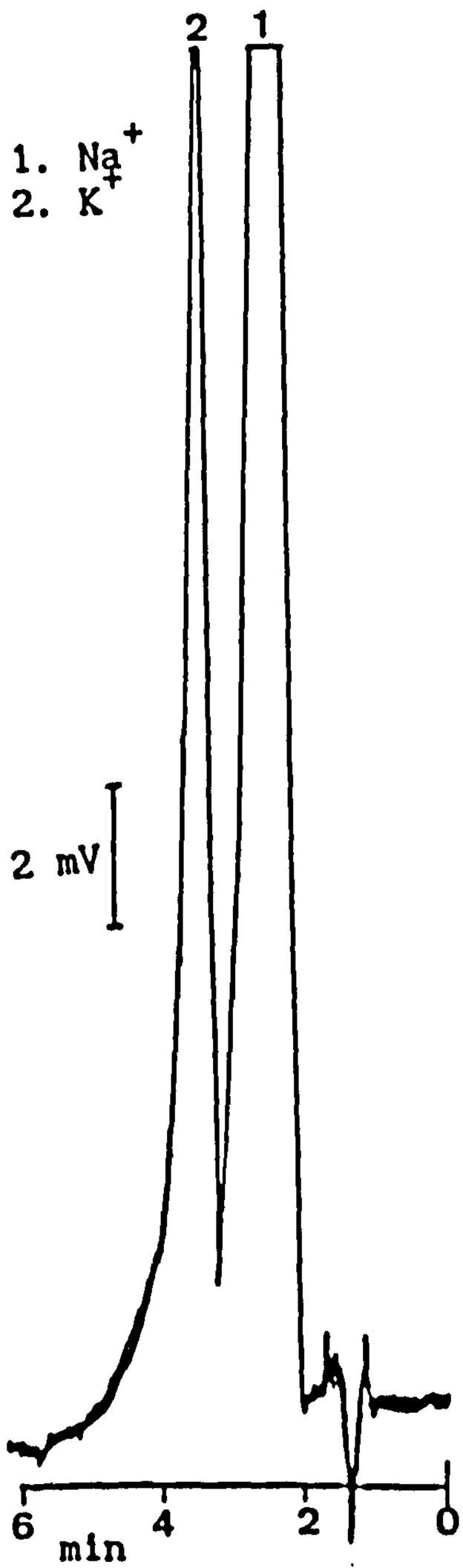


Figure 14. Potentiometric detection of monovalent cations in spring water (Strathmore) not diluted, the other conditions were as in figure 1.

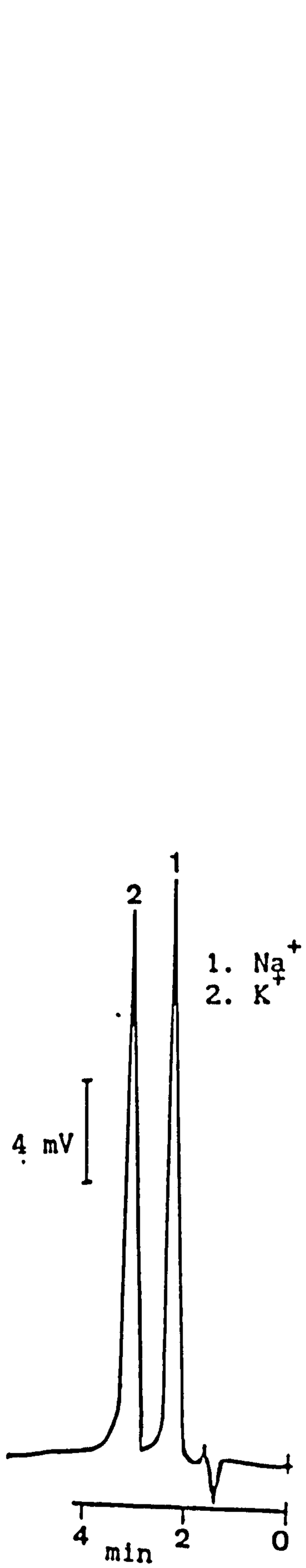


Figure 15. Potentiometric detection of monovalent cations in drinking water not diluted, the other conditions were as in figure 3.

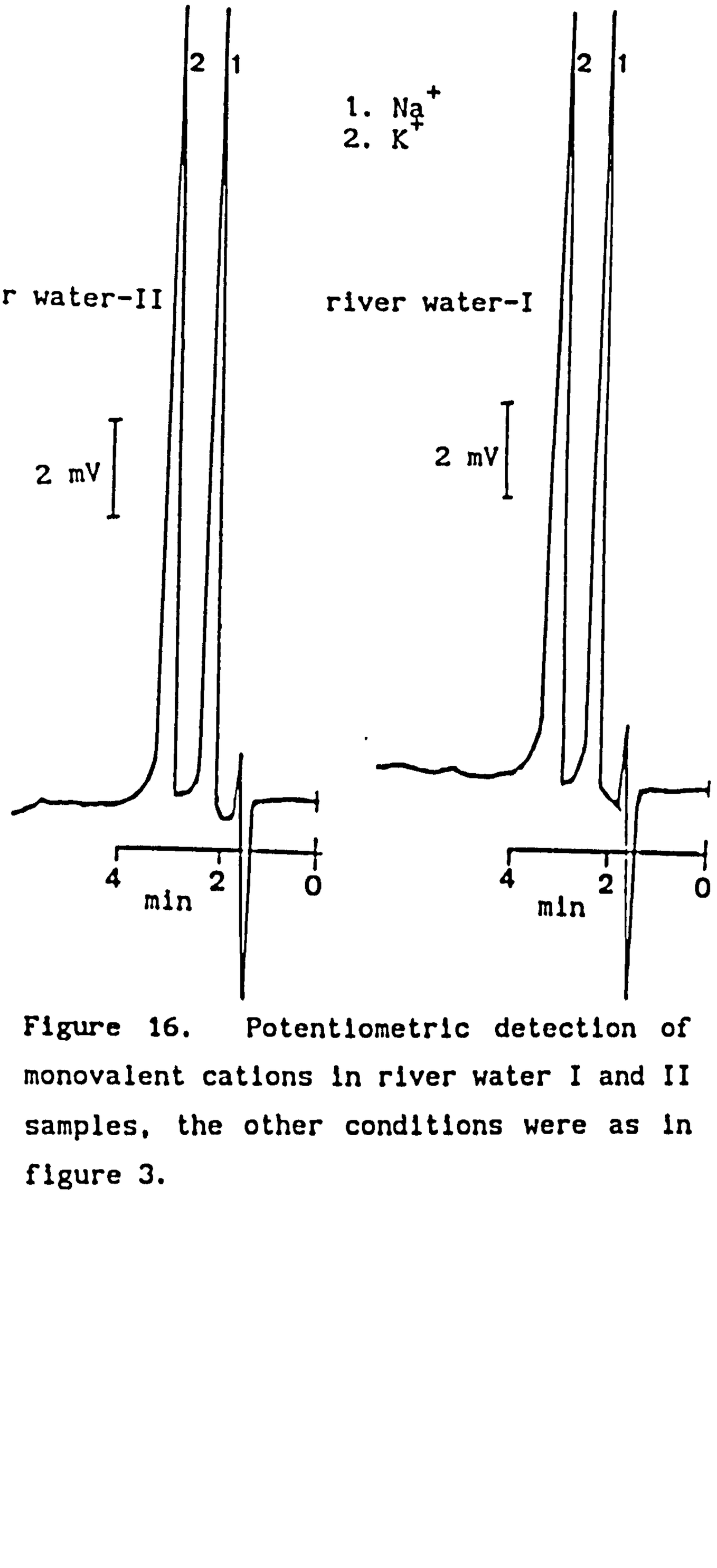


Figure 16. Potentiometric detection of monovalent cations in river water I and II samples, the other conditions were as in figure 3.

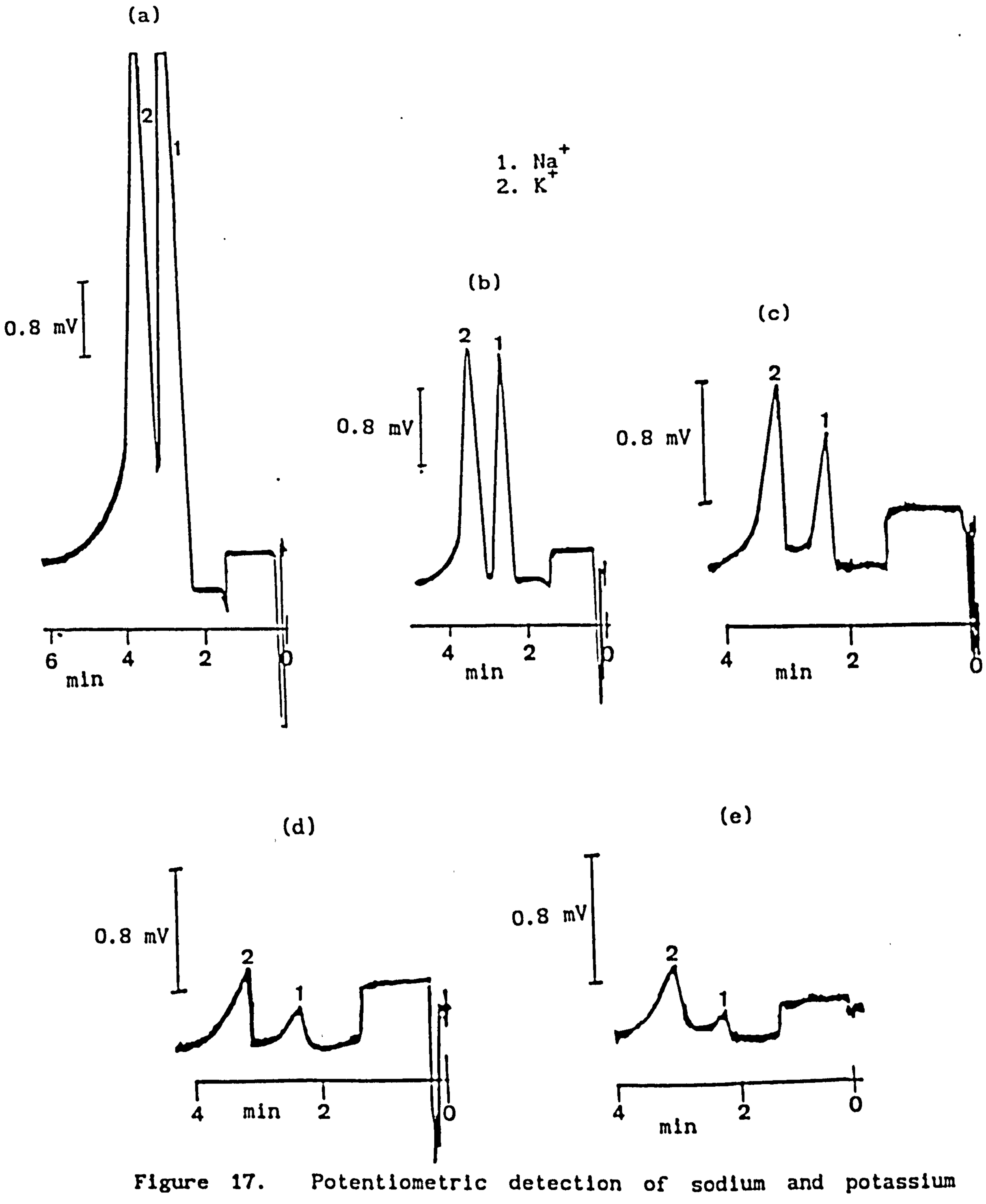


Figure 17. Potentiometric detection of sodium and potassium adsorbed on the surface of glassware in fabrication stage, eluent: 1.2 mM $CuSO_4$, flow-rate: 1.1 ml min^{-1}, column: Dionex HPIC-CS3 analytical and guard. injections: 20 µl of first washing sample with deionized water (a), of second washing sample (b), of third washing sample (c), of fourth washing sample (d), and of seventh washing sample (e),

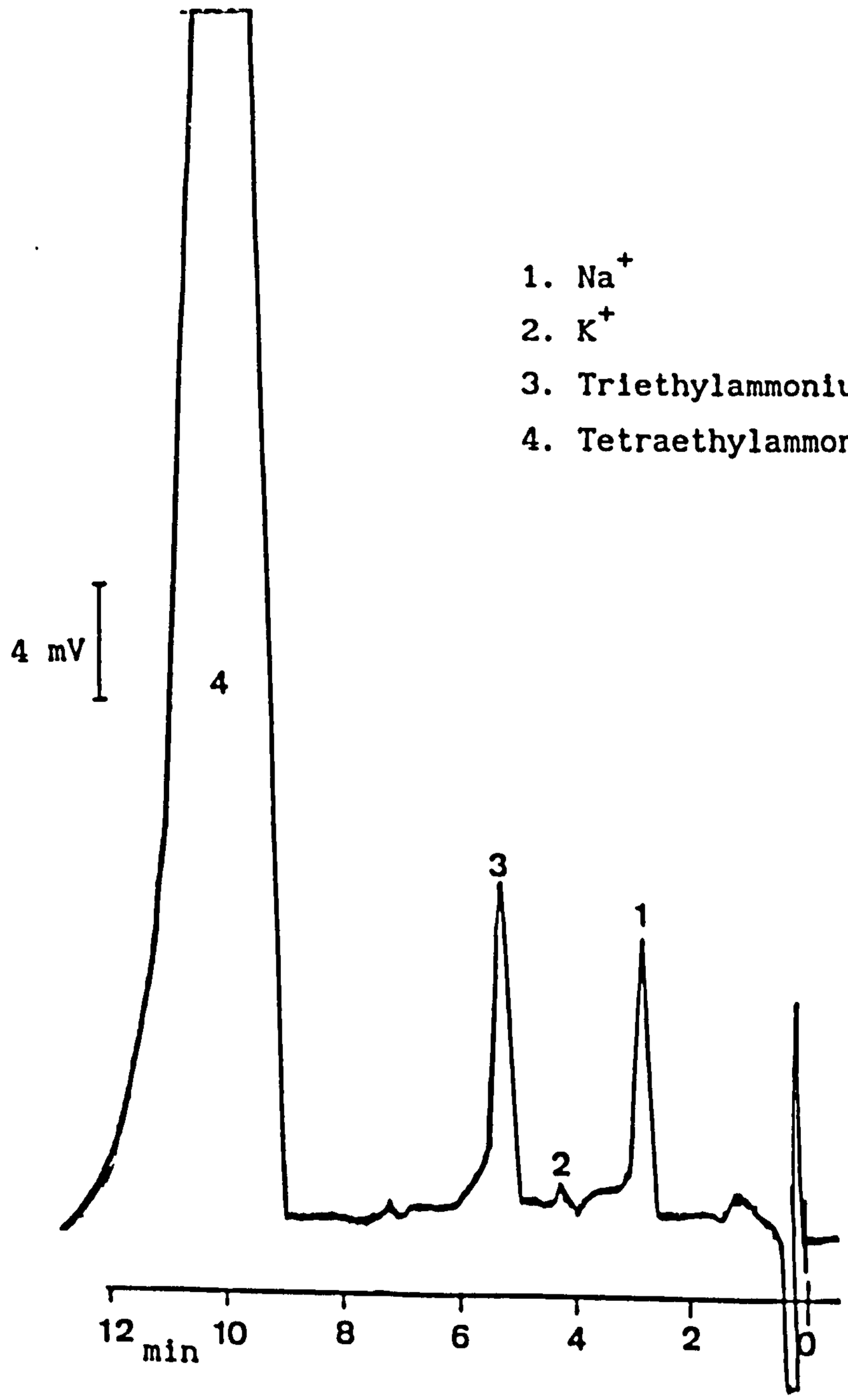


Figure 18. Potentiometric detection of TEA^+ and impurities, injection: 20 µl of 0.05% weight TEA^+ solution, the other conditions were as in figure 2.

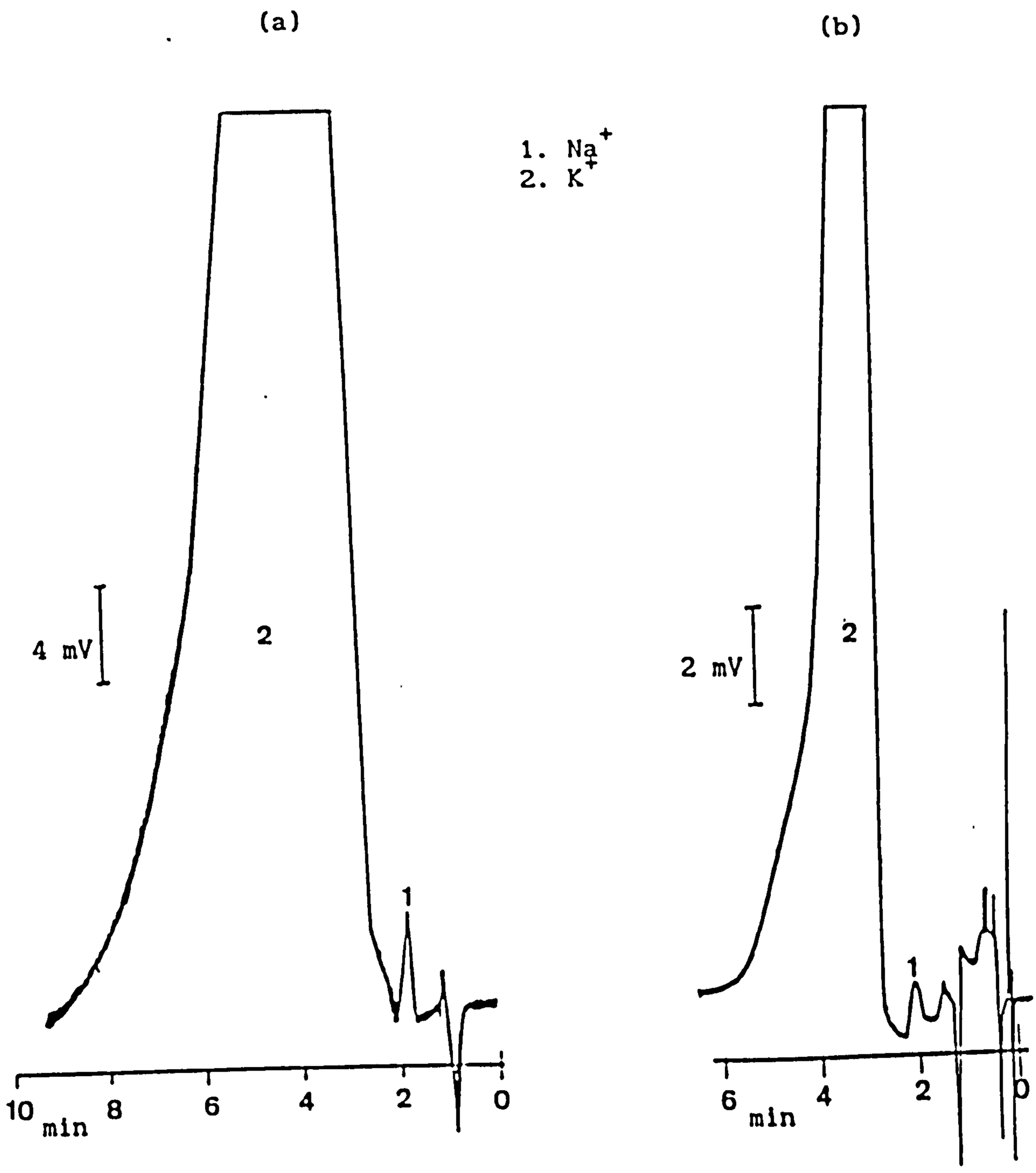


Figure 19. Potentiometric detection of sodium in 20 µl of 0.03 (a) and 0.005 (b) molar solution of KH_2PO_4 (AnalaR), eluent:1.5 mM $CuSO_4$, flow-rate: 1.3 ml min^{-1}, column: Dionex HPIC-CS3 analytical and guard.

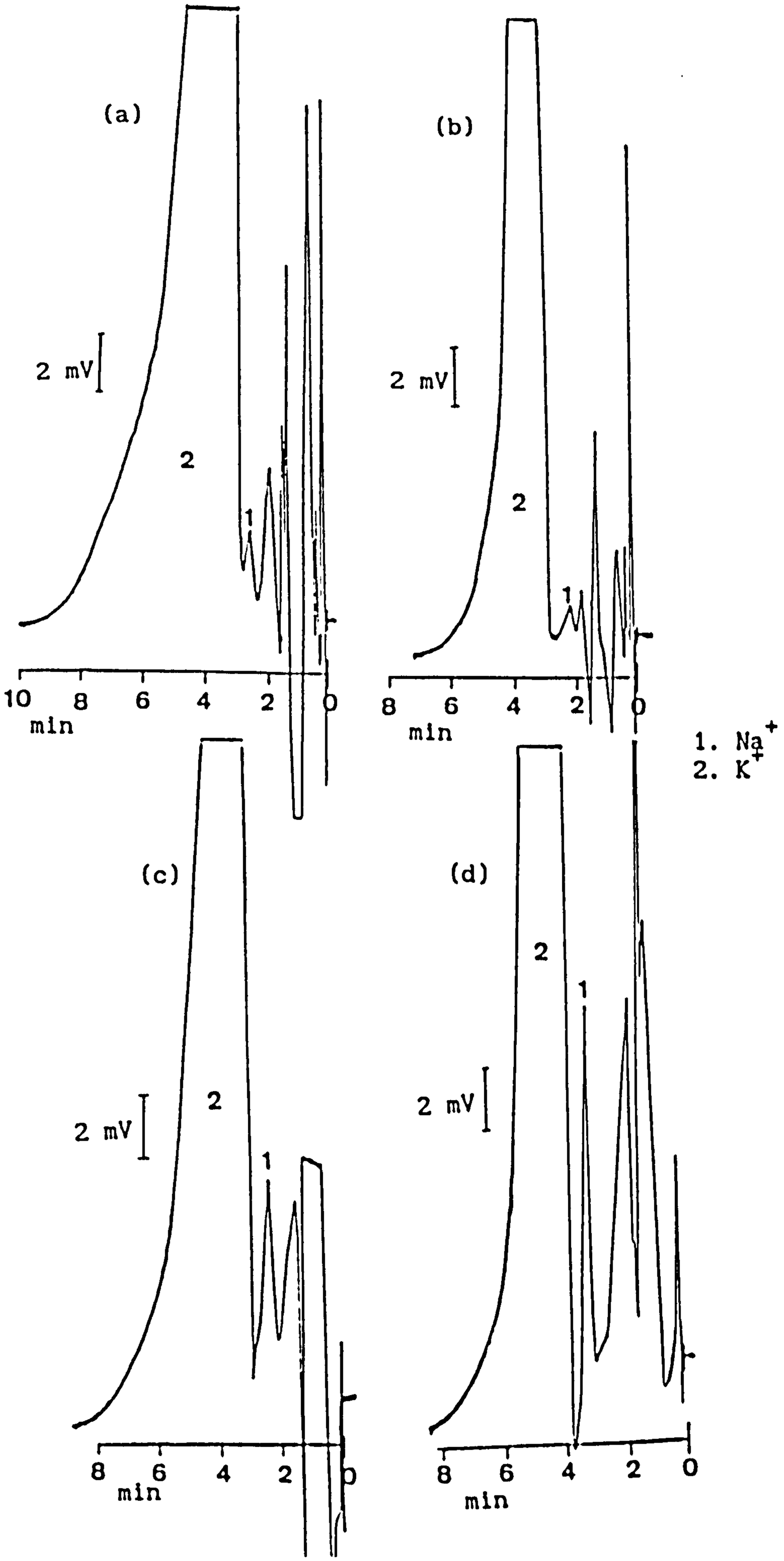


Figure 20. Potentiometric detection of sodium in 20 µl of, 0.005 M solution of KCl (AnalaR) (a), 0.008 M solution of K_2SO_4 (AnalaR) (b), 0.02 M solution of KF (c), and 0.5 M solution of K-tetraoxalate (d), the other conditions were as in figure 19.

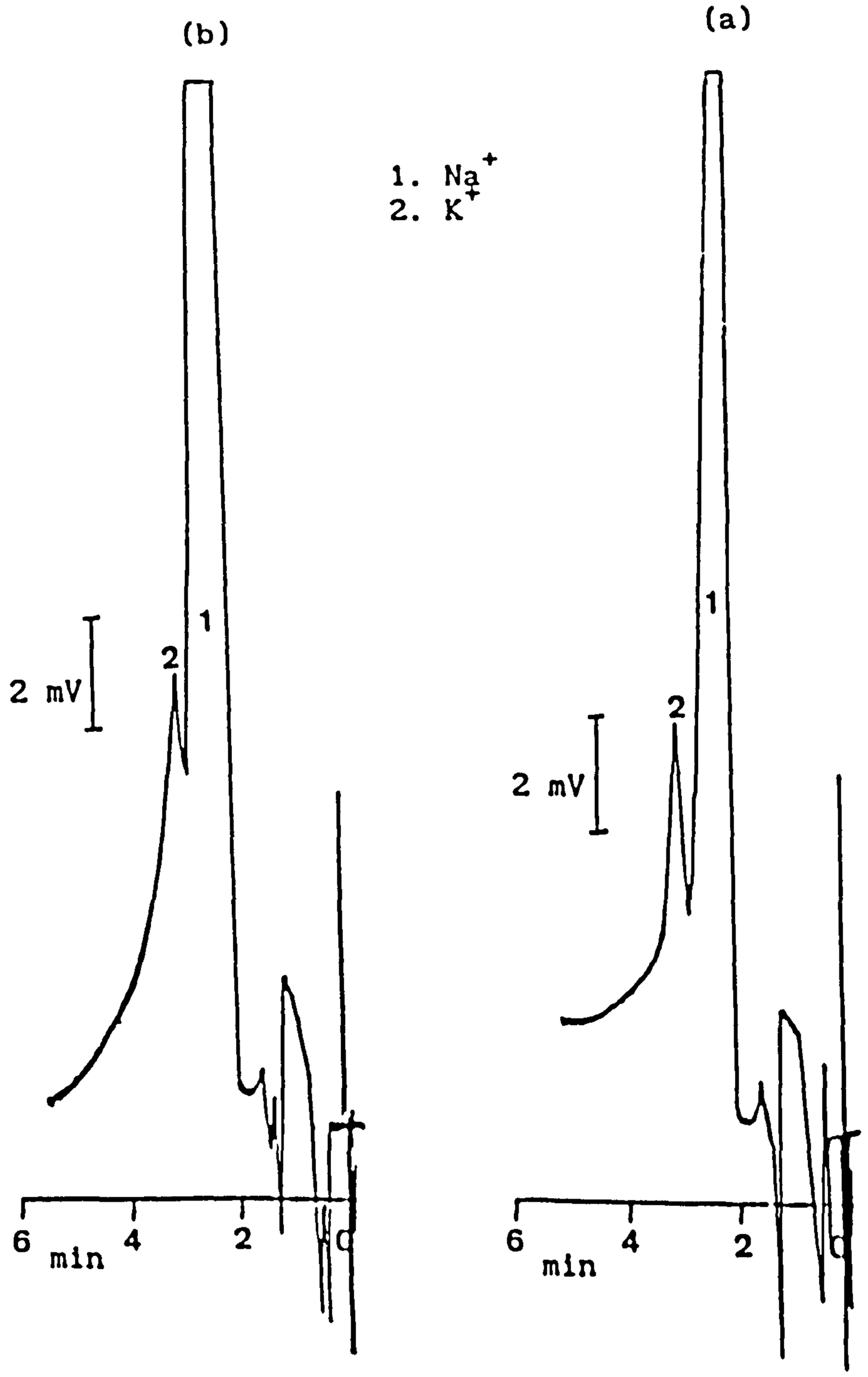


Figure 21. Potentiometric detection of potassium in 20 µl of, 0.005 M (a) and 0.01 M (b) solution of NaF, the other conditions were as in figure 19.

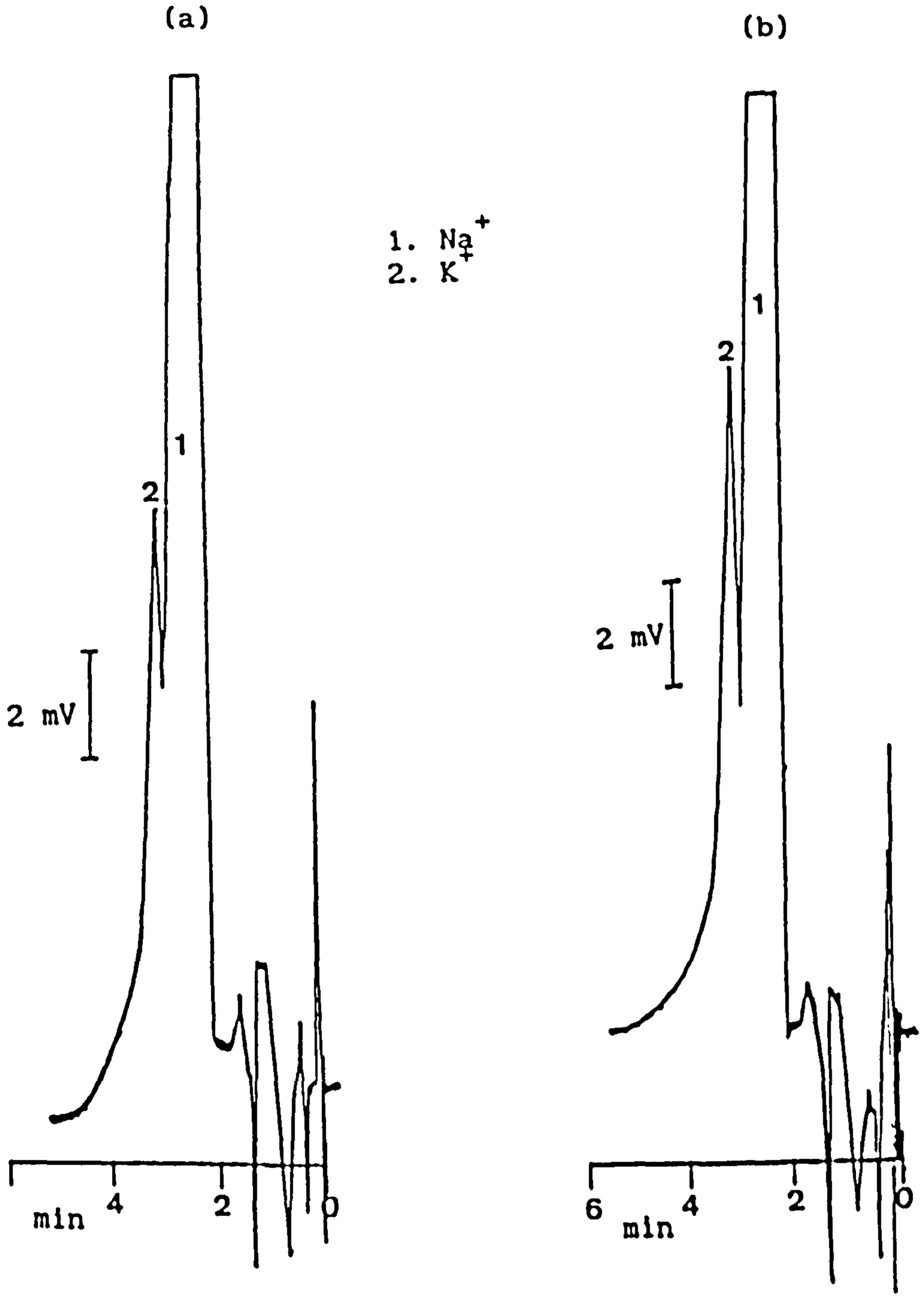


Figure 22. Potentiometric detection of potassium in 20 µl of, 0.003 M (a) and 0.005 M (b) solution of NaH_2PO_4, the other conditions were as in figure 19.

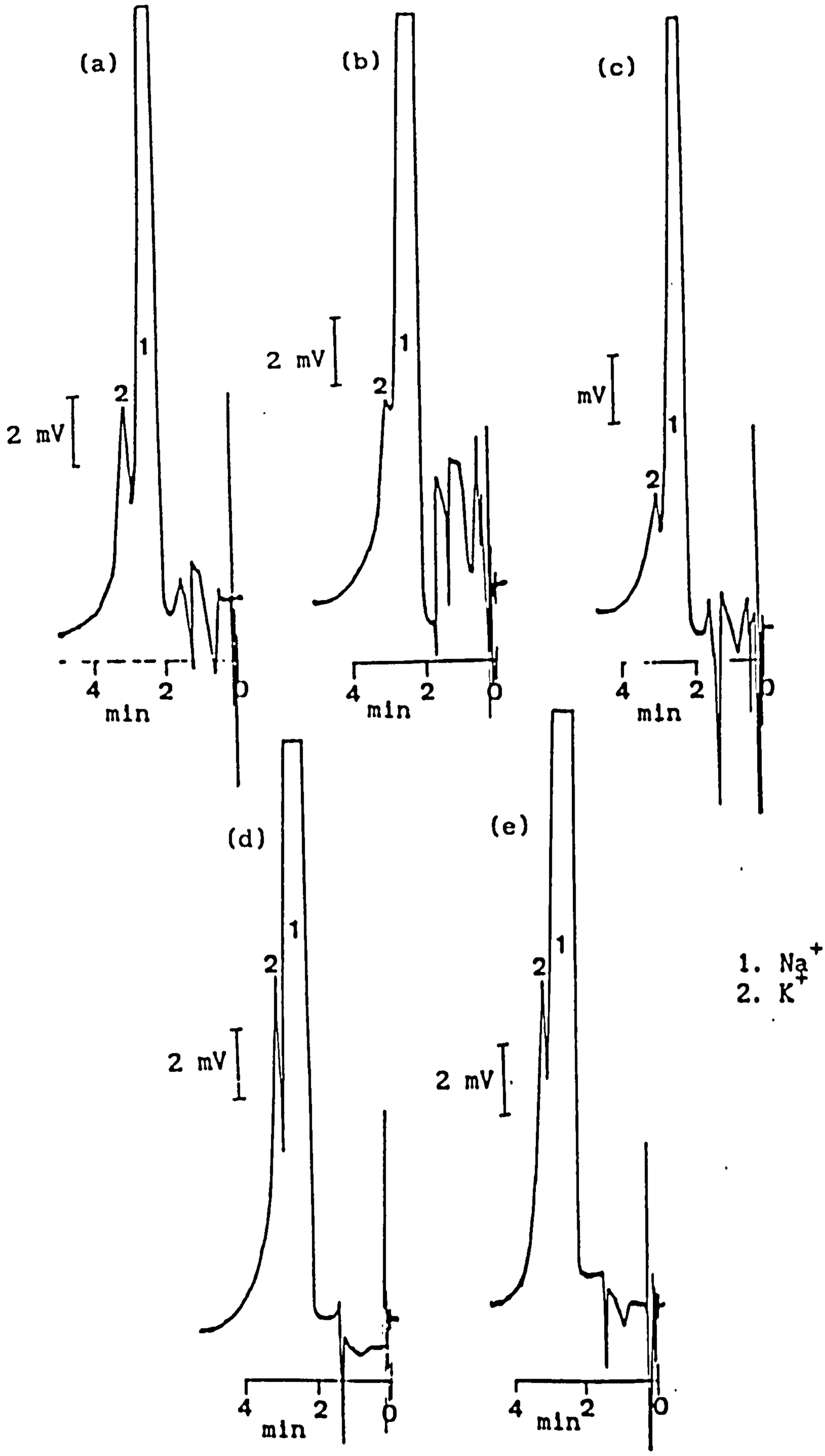


Figure 23. Potentiometric detection of potassium in 20 µl of, 0.0025 M solution of Na_2SO_4 (a), 0.0015 M solution of Na_2CO_3 (AnalaR) (b), 0.0015 M solution of $NaHCO_3$ (AnalaR) (c), 0.0015 M solution of Na-acetate (AnalaR) (d), and 0.0015 M solution of Na-citrate (e), the other conditions were as in figure 19.

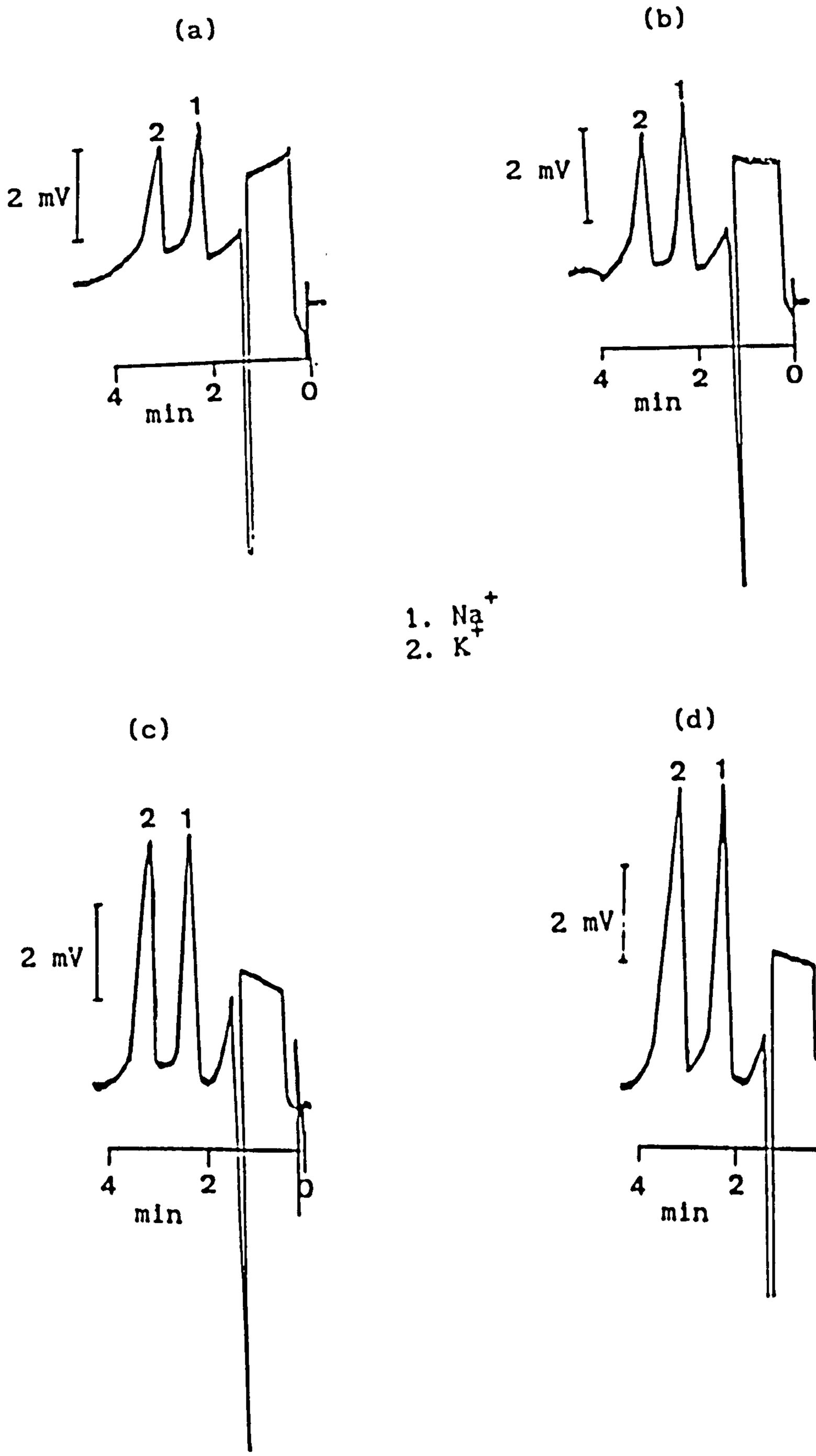


Figure 24. Potentiometric detection of sodium and potassium in 20 µl of, 0.20 g (a), 0.40 g (b), 0.60 g (c), and 1 g (d) solution of $CaCO_3$ treated in 4 ml deionized water, the other conditions were as in figure 19.

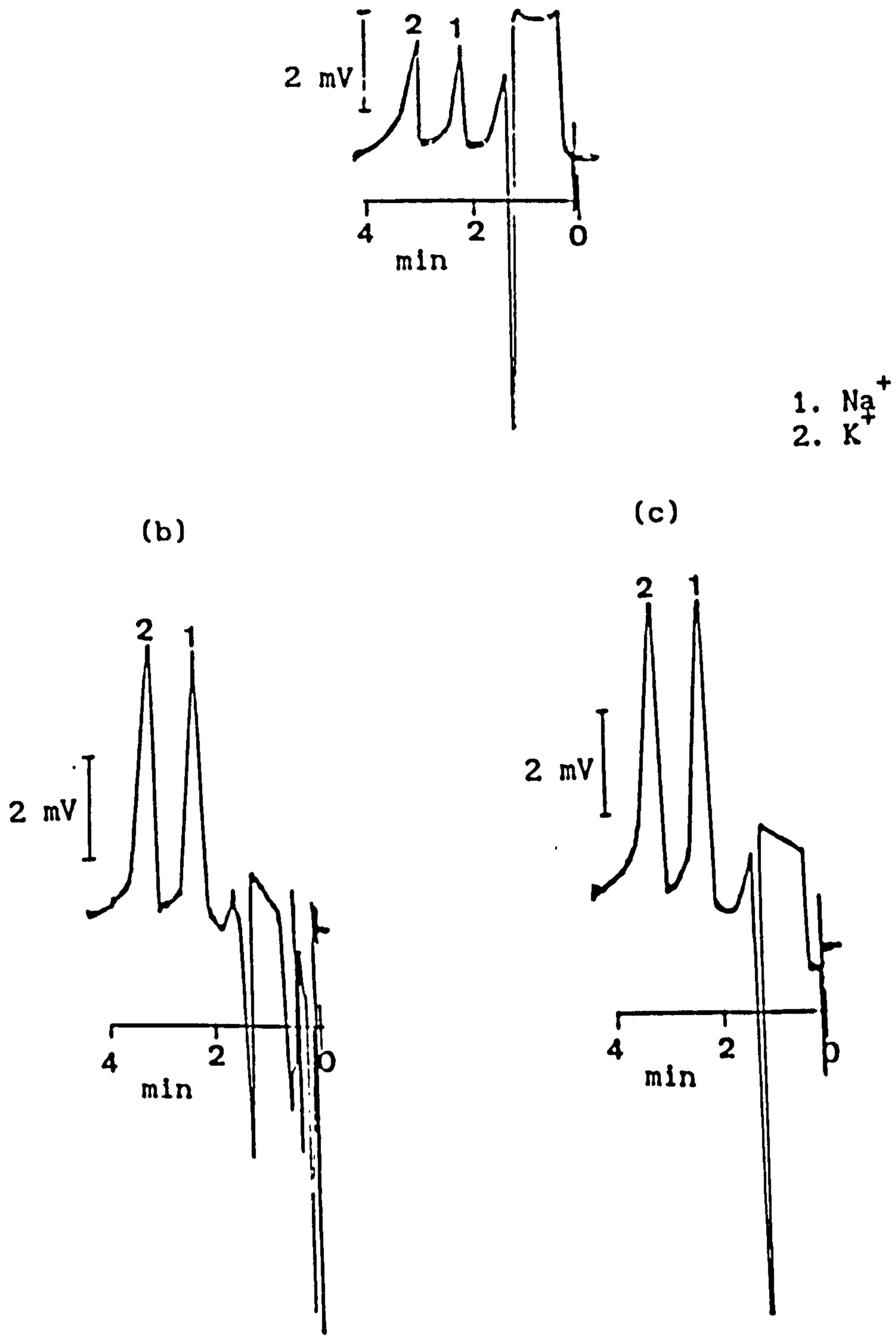


Figure 25. Potentiometric detection of sodium and potassium in 20 µl of, 0.30 g $BaCO_3$ (a), 0.20 g $MgCO_3$ (b) and 1.00 g (c) solution of $MgCO_3$ treated in 4 ml deionized water, the other conditions were as in figure 19.

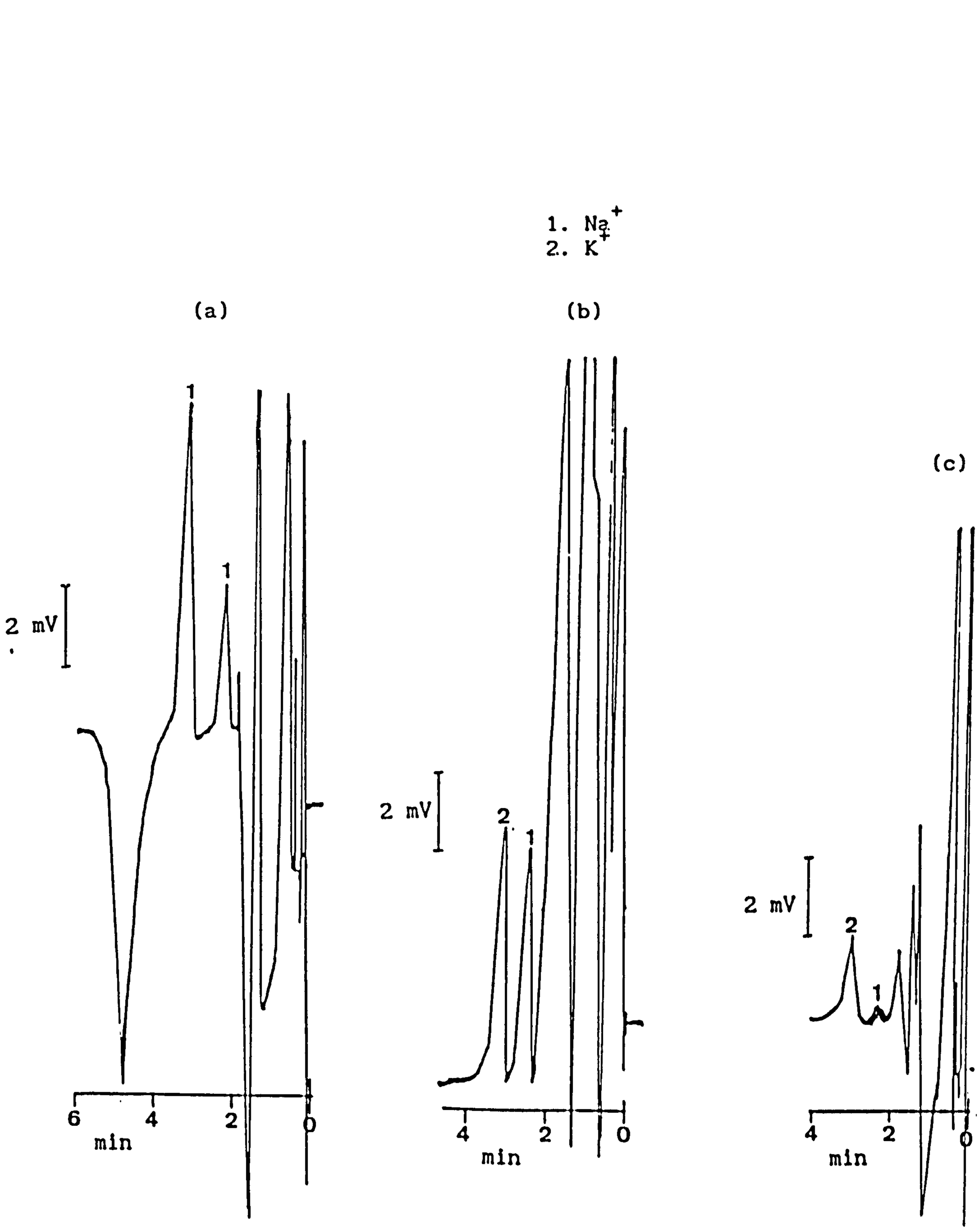


Figure 26. Potentiometric detection of sodium and potassium in 20 µl of, 0.015 M solution of $MgSO_4$ (a), 0.01 M solution of LiBr (Koch) (b), and 0.01 M solution of HCl (AnalaR) (c), the other conditions were as in figure 19.

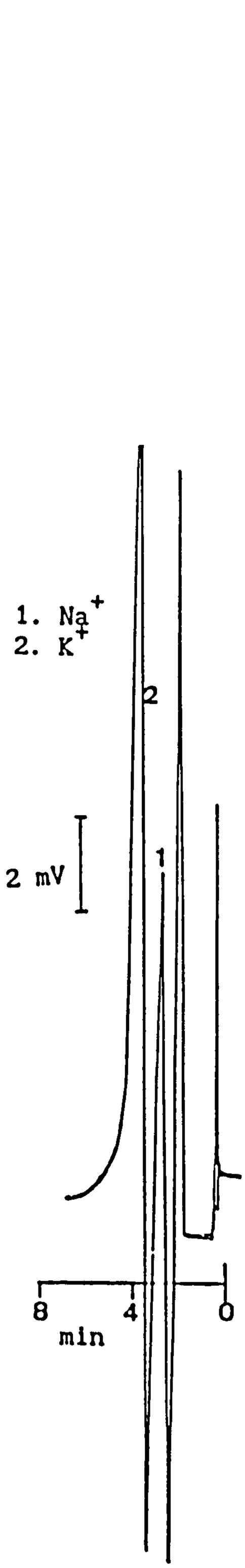


Figure 27. Potentiometric detection of sodium and potassium in 20 µl of 0.05 M solution of $CaCl_2$ (AnalaR), eluent: 1.2 mM $CUSO_4$, flow-rate: 1.2 ml min^{-1}.

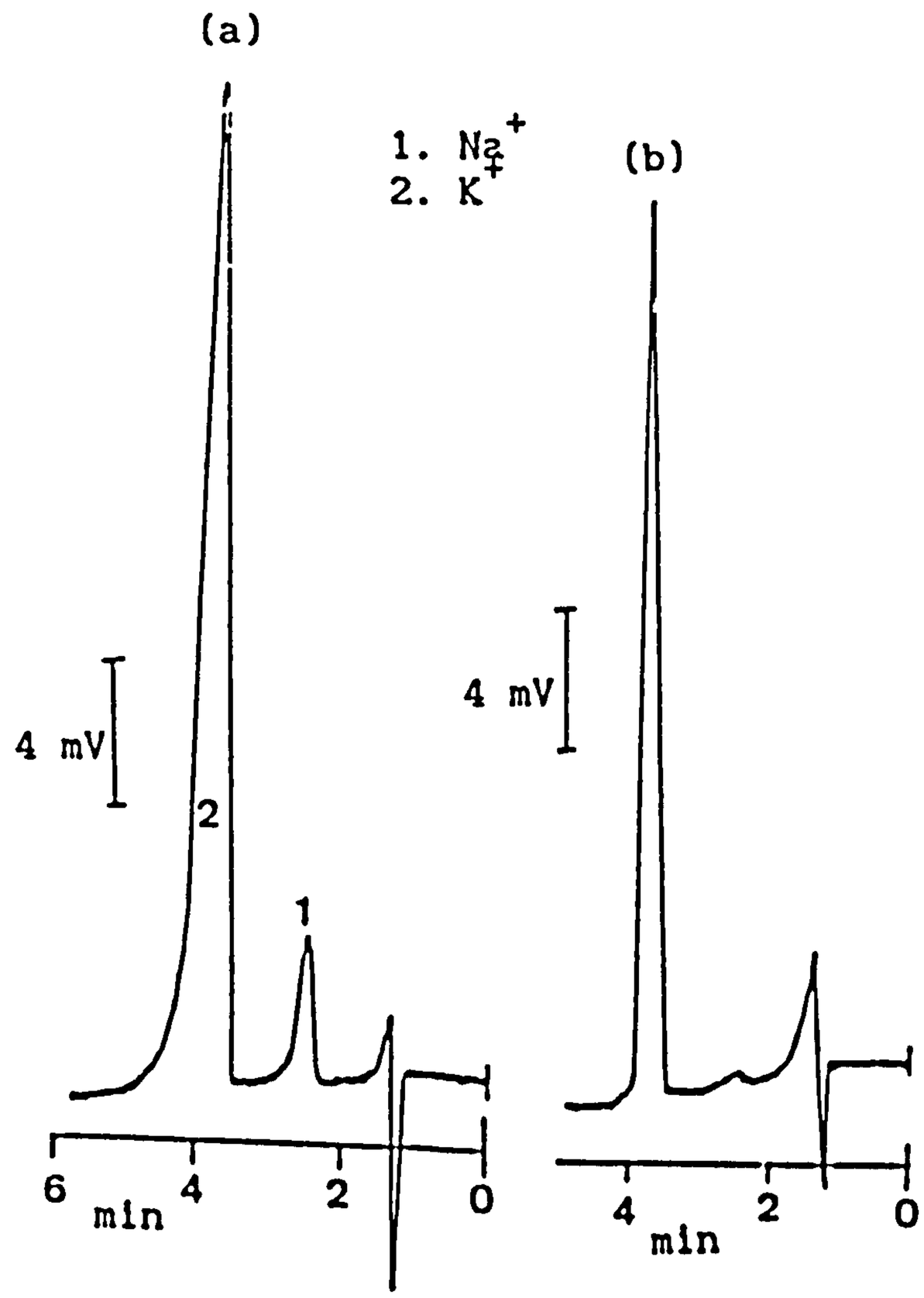


Figure 28. Potentiometric detection of sodium and potassium in 20 µl of 0.50 g KTPB (Aldrich) salt treated in 10 ml of deionized water (a), and 20 µl injection of 10^{-4} M standard solution of K^+ (b), the other conditions were as in figure 27.

CHAPTER 10

10. POTENTIOMETRIC DETECTION OF FOURTEEN INORGANIC AND ORGANIC MONOVALENT ANIONS AND CATIONS USING DIONEX ANION AND CATION COLUMNS IN TANDEM.

10.1 INTRODUCTION

Apart from activation analysis, ion chromatography is most capable technique for performing both cation and anion determinations simultaneously.[1] The routine separation and detection of inorganic common anions and cations at low levels in a simultaneous system is the goal for achieving the over-all efficiency of ion chromatographic procedures. The main drawback to simultaneous detection of a large variety of anions and cations is non-specificity of detection methods used in simultaneous determination. Therefore several approaches have been envisaged for simultaneous separation and detection of anions and cations.[2-6] Potentially the most straightforward approach to simultaneous determination is a single channel system in which an anion-exchange and a cation-exchange column are connected in series. The ion-exchange capacities of the two columns can be manipulated to give appropriate retention times for both species, and a suitable detection mode can be used to perform simultaneous analysis, conductometric,[7-9] indirect photometric detection [3,10] and potentiometric detection[11] modes. This chapter describes a full independent separation and simultaneous detection method in which isocratic elution is used to determine a group of fourteen inorganic and organic monovalent anions and cations simultaneously. The method is highly selective since no other species can interfere with analysis. No over-lap problems arise when the retention time of a cation is equal to that of an anion. The high sensitivity towards most ions may facilitate the application and preparation of a wide variety of samples such as industrial, environmental and clinical etc. The chromatographic

run time in for good resolution is about eight minutes. Several examples of independent separation and detection modes for both anions and cations using a cation and an anion-exchange column connected in series, a single injection and two potentiometric detectors one for anions another for cations are examined.

10.2 DETECTION LIMITS AND RETENTION TIMES

The detection limits for anions and cations vary very slightly between different modes of the system. Under all operating conditions the detection limits for Na^+, K^+, Rb^+, Cs^+, Cl^-, NO_2^-, CNO^-, Br^- and NO_3^-, defined as the amount for a signal noise ratio of 2, are of the order of tens of ppb for an injected volume of 20 µl. For other ions values are of the order of hundreds of ppb.
The relative retention times of anions, to the first eluted anion, with different modes of the system are essentially the same. For the cations, the situation changes. The selectivity of the anion-exchange column to the cations influences their retention times. Hence, the retention times of cations change with different modes of the system. However, the retention times do not exceed ten minutes for both anions and cations.

10.3 IDENTIFICATION

The identification of anions and cations was performed by comparing the retention time of each peak of interest with that of a standard. The separations of anions and cations were done independently, as there is no interference for anions from cations, or for cations from anions. Therefore, chloride salts of the cations and sodium salts of the anions were used for the identification and the output potential from each detector was recorded independently. The separation sequence did not change, except for organic anions, with different modes of the system. Inorganic cations were relatively more affinitive than organic cations on the anion-exchange column. So, for example, tetraethylammonium (TEA^+) cation overlaps with the thallium (Tl^+)

cation when only the cation-exchange column was used. But the anion exchange column selects Tl^+ rather than TEA^+ and causes elution of Tl^+ later than TEA^+.

10.4 REPRODUCIBILITY

Over three years, the sensitivity of most detectors constructed remained almost constant for at least 2.5 months. The reproducibility of peak heights for repeated injections of all anions and cations at all concentrations was generally better than 2%. The lower reproducibility might occur if the distance between working electrode and reference electrode is too large. The study on response time measurements (chapter 6) indicated that a 3 cm distance between working electrode and reference electrode had caused no interference.

10.5 CALIBRATION

As the detection limits for the cations change with different modes of the system, calibrations of anions and cations should be made with each mode. The calibration of electrode potential versus concentrations of analytes showed either a linear or logarithmic relationship, depending on concentration range studied. When the total concentration of analytes at the electrode surface was very low a linear relationship was not observed.

10.6 EXPERIMENTAL

The construction of flow-through tubular PVC matrix membrane electrodes without an inner reference solution was as previously described in chapters 7 and 9.

Chromatography was performed on a Perkin Elmer (series 3) high performance liquid chromatograph (HPLC), which consist of the dual channel pump and injection valve with 20 µl sample loop. Separations were performed on Dionex IonPac-AS4A and -CS3 analytical and guard columns. Suitable compositions of eluents were prepared freshly before use. Two all solid-state

flow-through tubular PVC matrix membrane working electrodes as detectors, with a double junction calomel electrode as reference, were used for detection of anions and cations. Two high input impedance buffer amplifiers and digital voltmeters were connected to the electrodes for potential recording during the simultaneous determinations. A SE 120 BBC chart recorder was used for obtaining chromatograms of both anions and cations.

Chemicals for preparation of sensing membrane were from Fluka except DBP and DBC-KBr which were from Aldrich. All standard sample solutions were prepared from their analytical reagent grade chemicals in deionized water. Sample matrices of river, sea and drinking water were taken from local areas of Newcastle Upon Tyne and were diluted before injections when required. During the experiments, 20 µl of samples and standard solutions were injected. Samples were filtered through Millipore filters (pore size 0.45 µm). The identification of species was performed by comparing retention times of peaks of interest with those of peak in a standard.

10.7 RESULTS AND DISCUSSION

Figures 1 and 2 show single detection of anions and cations obtained using the anion and cation-exchange column separately. The time required for each determination was less than ten minutes. In figure 3, simultaneous detection was obtained using cation selective electrode at the end of the first column which was cation-exchange, and anion selective electrode at the end of two columns in series. Working with this mode, the Tl^+ cation can be efficiently separated and detected. When comparing the retention times of anions with cations, this mode exhibits an optimized configuration. Figure 4 shows the simultaneous detection of the anions and cations using the anion selective electrode after anion-exchange column which was the first, and the cation selective electrode at the end of two columns in series. It can be seen from the chromatograms that the retention time of the anions has not significantly changed. Also the cations and

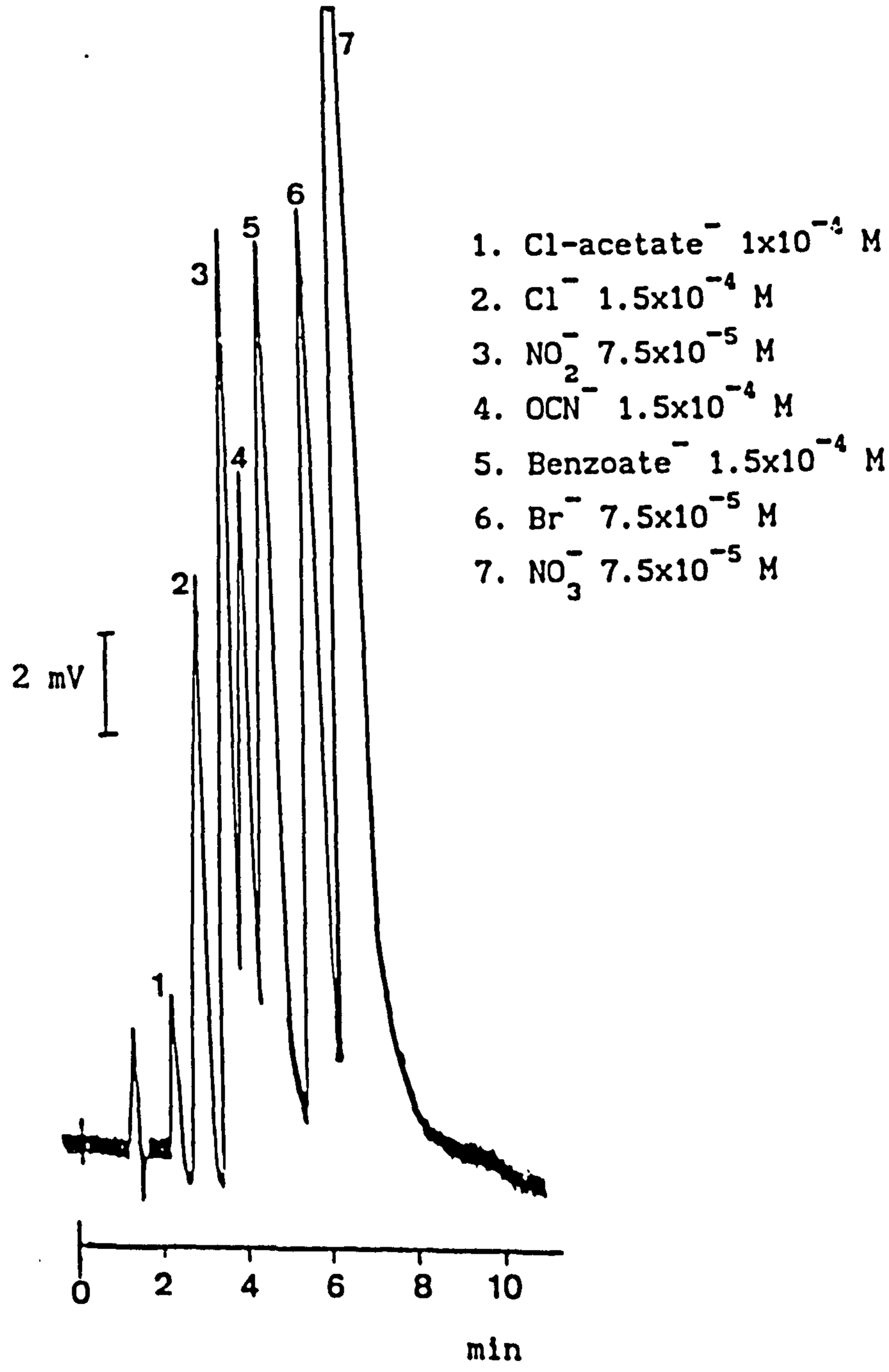
the anions are independently separated and simultaneously detected using both anion and cation selective electrodes at the end of two column in tandem as shown in figure 5. In the last two figures 4 and 5, the detection of the Tl^+ was excluded because the cation selective electrode was used after the anion-exchange column, the retention times of the cations, except the Tl^+, were slightly changed. In the case of the Tl^+ the situation was different, the higher affinity of the Tl^+ to the anion-exchange resin influences its retention and results in broadening the peak shape. This effect is illustrated in figure 6 using both electrodes at the end of the two columns in tandem, and a more concentrated eluent. It is worthy of mention here that the chromatograms demonstrate the reproducibility and the completely resolved peaks. When samples included species such as amino acids or organic ions with long chains, the reproducibility becomes lower. Amino acids and organic ions with long chains may cover the surface of the electrode membrane and influence its sensitivity.

The selectivity characteristics of an anion-exchange column for cations, or of a cation-exchange column for anions, can be explored easily using ion selective electrodes as detectors as the anions did not interfere with the detection of cations or vice versa. Consequently, independent detection was employed with the anion-exchange and the cation-exchange column as shown in figure 7 and 8. For the anion-exchange column, a cation and an anion standard solution were injected. As can be seen from the chromatogram, the anion-exchange column exhibits high selectivity for cations. Only anion standard solution was used with cation-exchange column which exhibits no selectivity to the anions. Hence, the use the cation selective electrode as detector before the anion-exchange column is an advantage.

The three different modes of independent separation and detection system can also be applied with three columns in series. An extra guard anion-exchange column was connected to the analytical column. Figures 9, 10 and 11 illustrate simultaneous determinations with three columns using more concentrated eluent.

These examples illustrate the flexibility and the capability of the method developed here.

The capability of the simultaneous detection of independently separated anions and cations is further illustrated in figures 12, 13, 14 and 15 for drinking, river and sea water samples. The high sensitivity of the ion selective electrodes as detectors allows the determination of each anion and cation in most analytical samples without any sample preconcentration. In figure 15, the determination of the anions and cations was performed on IonPac-AS4A column only as it illustrates its capability for separation of cations.



1. Cl-acetate^{-} 1x10^{-4} M
2. Cl^{-} 1.5x10^{-4} M
3. NO_2^- 7.5x10^{-5} M
4. OCN^{-} 1.5x10^{-4} M
5. Benzoate^{-} 1.5x10^{-4} M
6. Br^{-} 7.5x10^{-5} M
7. NO_3^- 7.5x10^{-5} M

Figure 1. Potentiometric detection of anions using anion selective electrode as detector, eluent: 1.1 mM $MgSO_4$, flow-rate: 1.1 ml min^{-1}, column: Dionex HPIC-AS4A.

1. Na^+ $2x10^{-4}$ M
2. NH_4^+ $2x10^{-4}$ M
3. K^+ $5x10^{-5}$ M
4. Rb^+ $2x10^{-4}$ M
5. TMA^+ $2x10^{-4}$ M
6. Cs^+ $2x10^{-4}$ M
7. Tl^+ $2x10^{-4}$ M

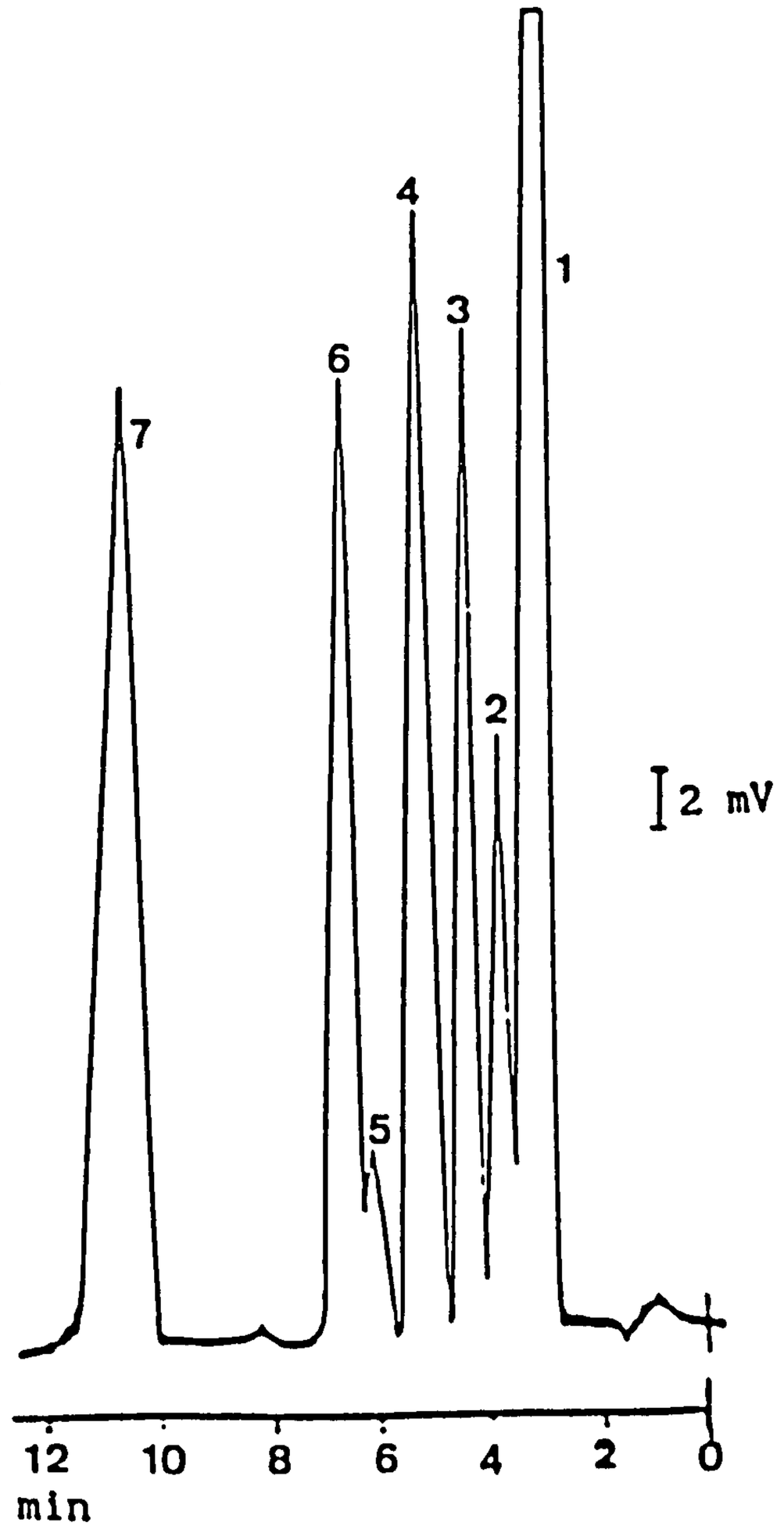


Figure 2. Potentiometric detection of cations using cation seletive electrode as detector, eluent: 1.1 mM $MgSO_4$, flow-rate: 1.1 ml min^{-1}, column: Dionex HPIC-CS3.

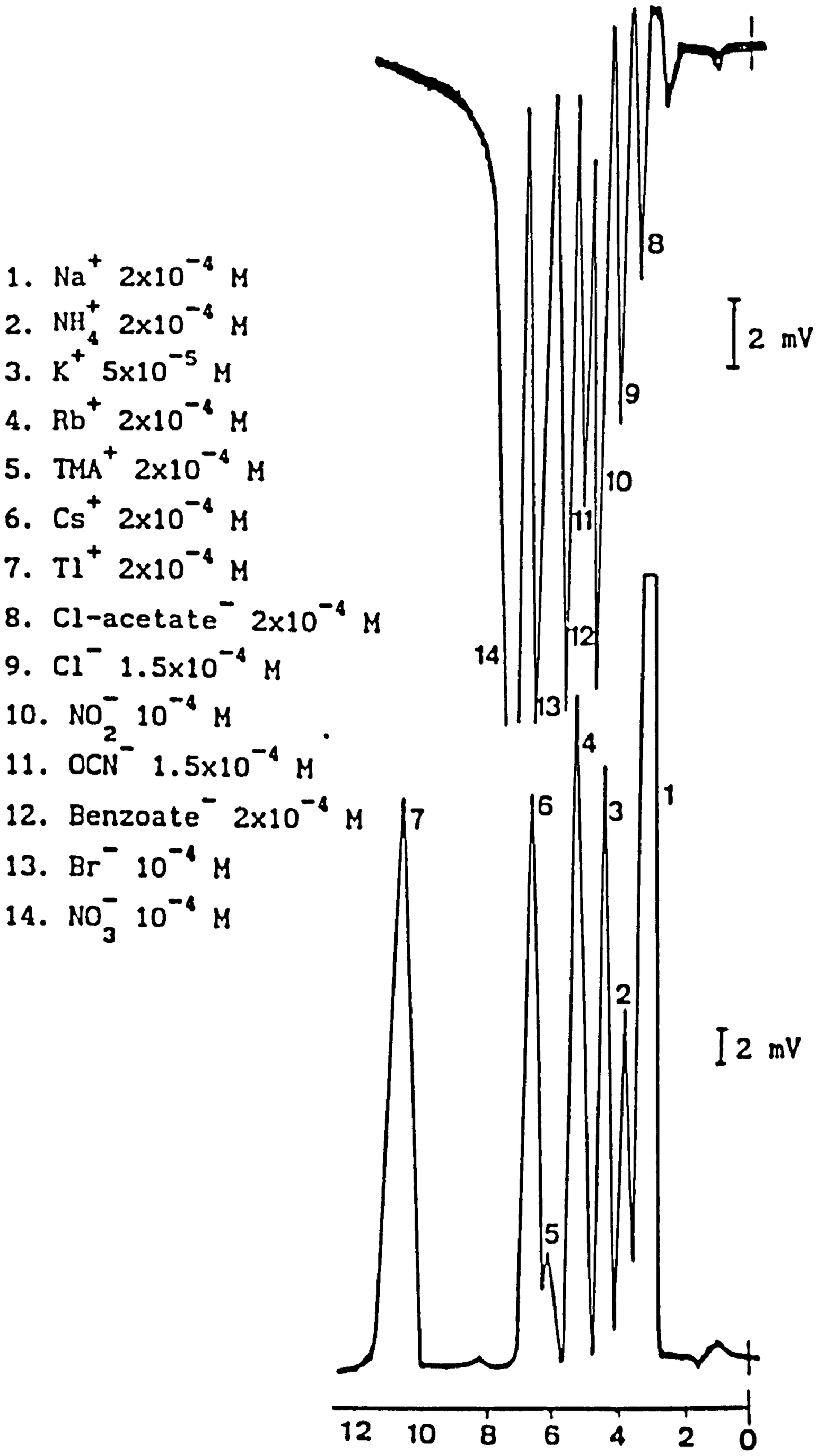
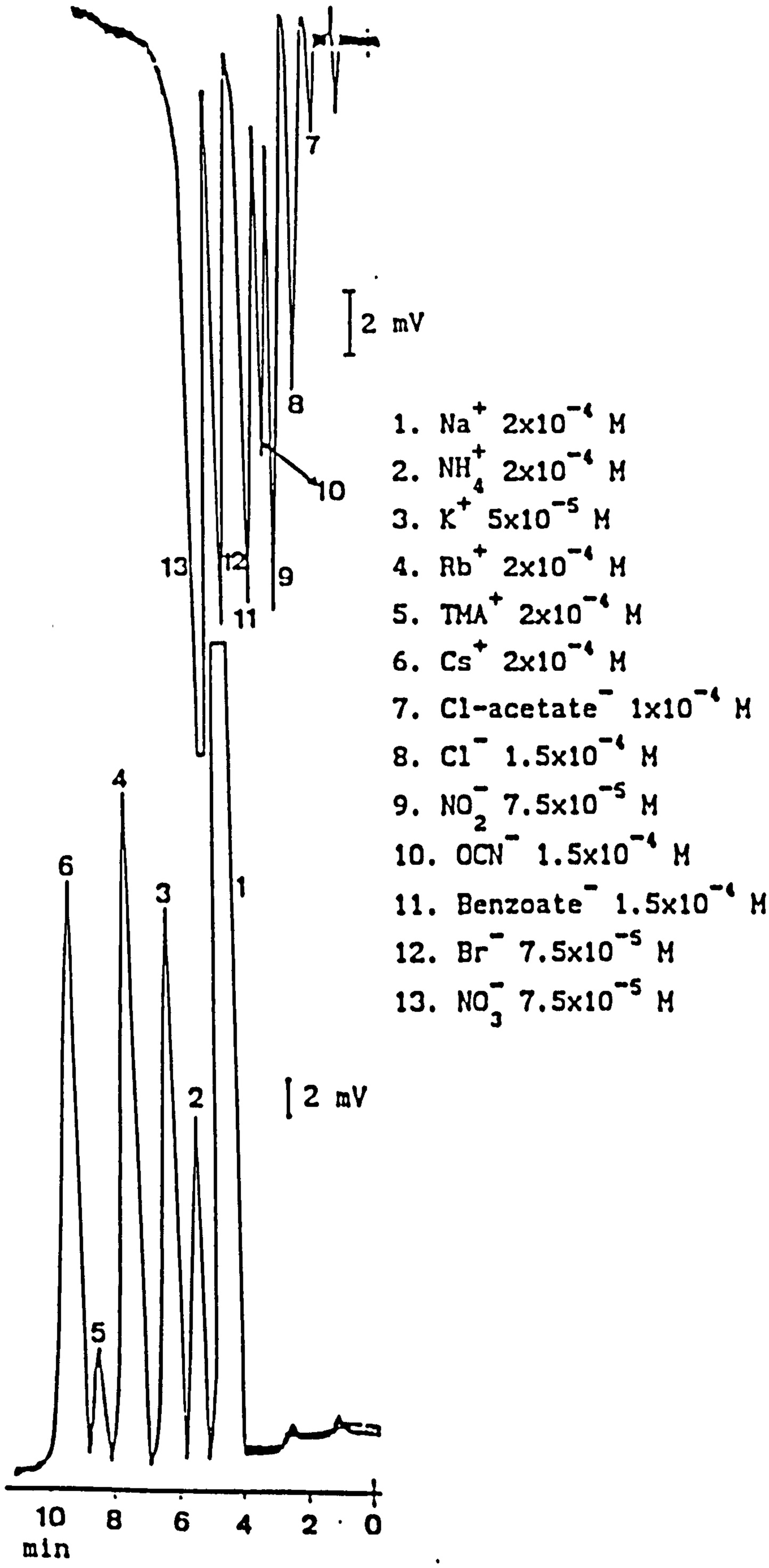


Figure 3. Independent potentiometric detection of anions and cations using two ion selective electrodes as detectors. The cation selective electrode was fixed at the end of first column which was cation-exchange and the anion selective electrode was fixed at the end of two column in tandem, columns: HPIC-CS3 and AS4A, the other conditions were as in fig. 1.



1. Na^+ $2x10^{-4}$ M
2. NH_4^+ $2x10^{-4}$ M
3. K^+ $5x10^{-5}$ M
4. Rb^+ $2x10^{-4}$ M
5. TMA^+ $2x10^{-4}$ M
6. Cs^+ $2x10^{-4}$ M
7. Cl-acetate$^-$ $1x10^{-4}$ M
8. Cl^- $1.5x10^{-4}$ M
9. NO_2^- $7.5x10^{-5}$ M
10. OCN^- $1.5x10^{-4}$ M
11. Benzoate$^-$ $1.5x10^{-4}$ M
12. Br^- $7.5x10^{-5}$ M
13. NO_3^- $7.5x10^{-5}$ M

Figure 4. Independent potentiometric detection of anions and cations using the anion selective electrode at the end of first column which was anion-exchange and the cation selective electrode at the end of two column in tandem, columns: Dionex HPIC-AS4A and CS3, the other conditions as in fig. 1.

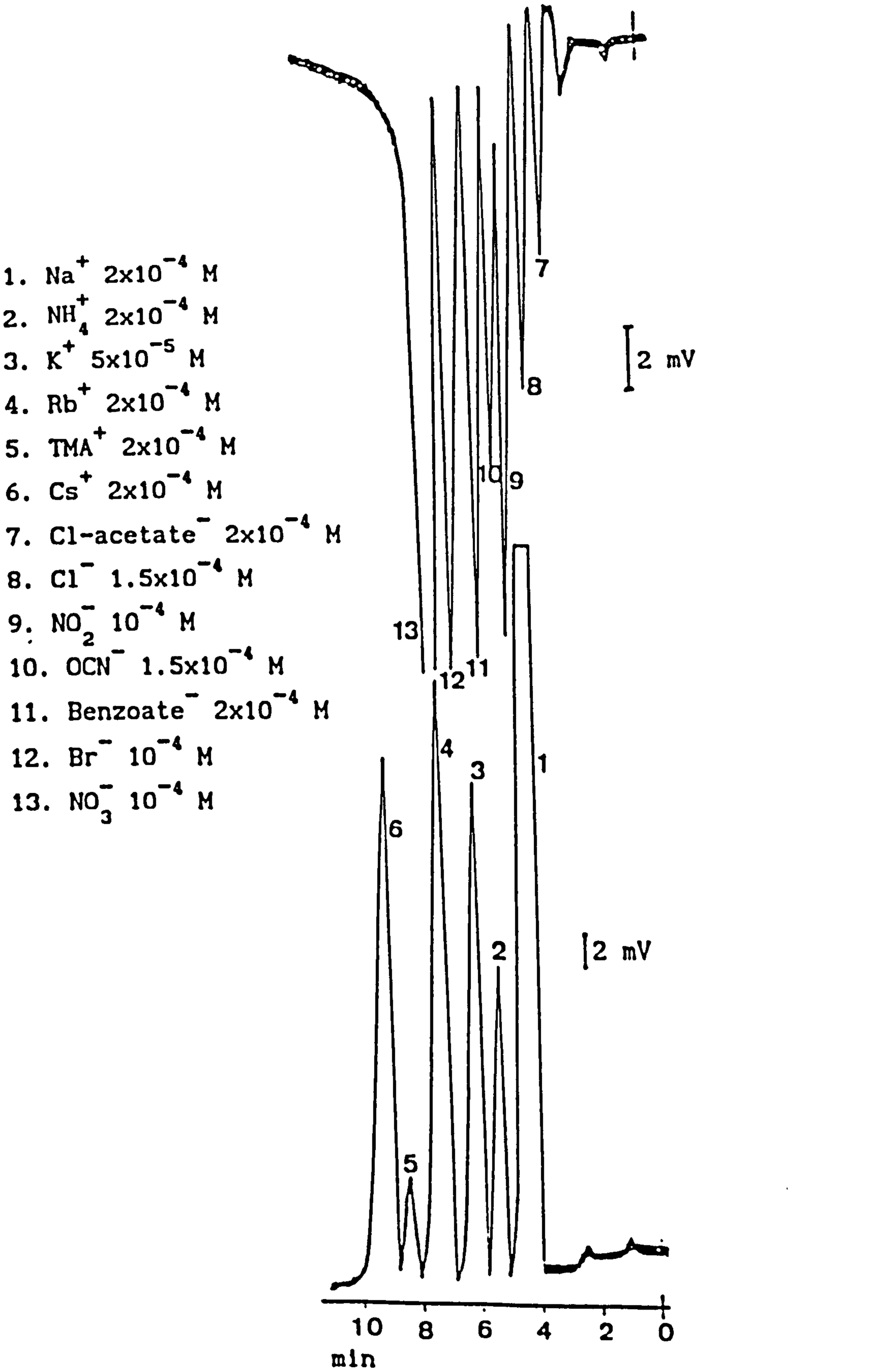


Figure 5. Independent potentiometric detection of anions and cations using two ion selective electrodes at the end of two column in tandem, columns: Dionex HPIC-AS4A and CS3, the other conditions were as in fig. 1.

1. Na^+ 10^{-4} M
2. NH_4^+ $2x10^{-4}$ M
3. K^+ $5x10^{-5}$ M
4. Rb^+ $1x10^{-4}$ M
5. TMA^+ $2x10^{-4}$ M
6. Cs^+ 10^{-4} M
7. Tl^+ $2x10^{-4}$
8. Cl^- $2x10^{-4}$ M
9. NO_2^- 10^{-4} M
10. OCN^- $1.5x10^{-4}$ M
11. $Benzoate^-$ $2x10^{-4}$ M
12. Br^- 10^{-4} M
13. NO_3^- 10^{-4} M

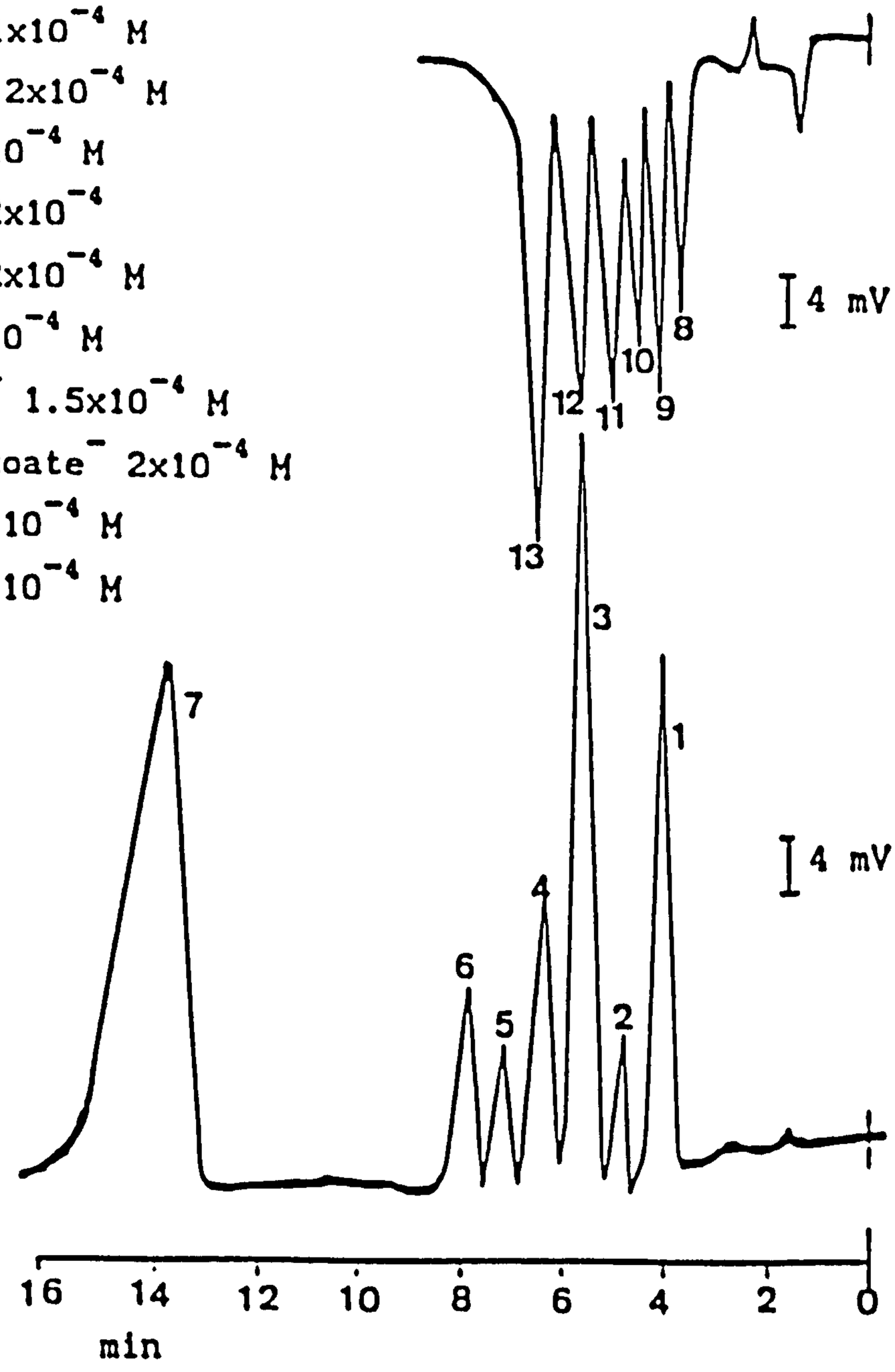
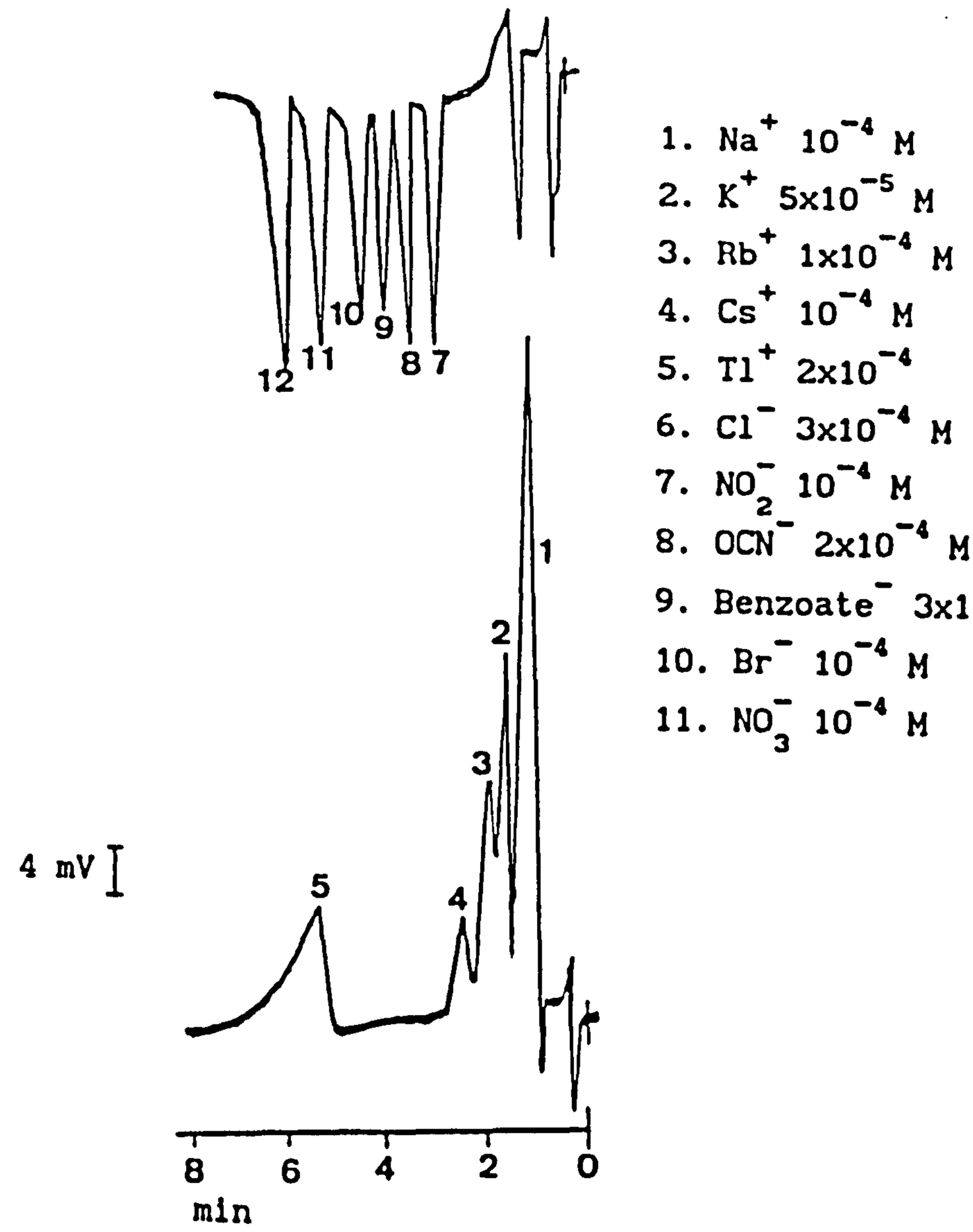
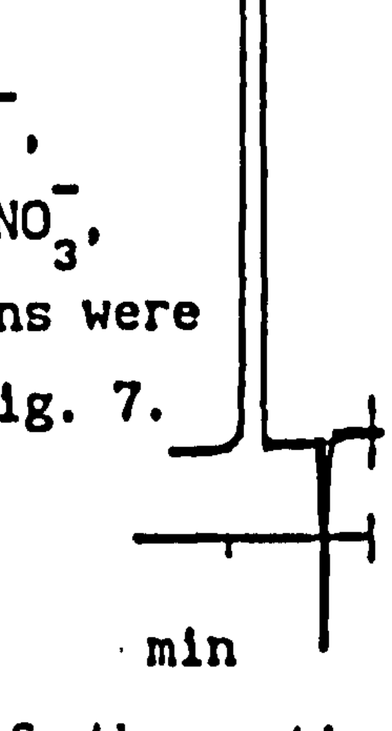


Figure 6. Independent potentiometric detection of anions and cations including thallium (I) cation using two ion selective electrode at the end of two column in tandem, eluent: 1.4 mM $MgSO_4$, flow-rate: 1.2 ml min^{-1}, columns: Dionex HPIC-AS4A and CS3.



1. Na^+ 10^{-4} M
2. K^+ $5x10^{-5}$ M
3. Rb^+ $1x10^{-4}$ M
4. Cs^+ 10^{-4} M
5. Tl^+ $2x10^{-4}$
6. Cl^- $3x10^{-4}$ M
7. NO_2^- 10^{-4} M
8. OCN^- $2x10^{-4}$ M
9. $Benzoate^-$ $3x10^{-4}$ M
10. Br^- 10^{-4} M
11. NO_3^- 10^{-4} M

Figure 7. The cation selectivity charecterictics of the anion exchange resin. Independent separation and detection of anions and cations using two ion selective electrode at the end of HPIC-AS4A anion exchange column only, eluent: 0.6 mM $MgSO_4$, flow-rate 1.5 ml min^{-1}.



1. Cl^-, NO_2^-, CNO^-, $Benzoate^-$, Br^-, NO_3^-, the concentrations were as the same in fig. 7.

Figure 8. The anion selectivity charecterictics of the cation exchange resin, the anion selective electrode was used at the end of HPIC-CS3 cation exchange column, the other conditions were as in fig. 7.

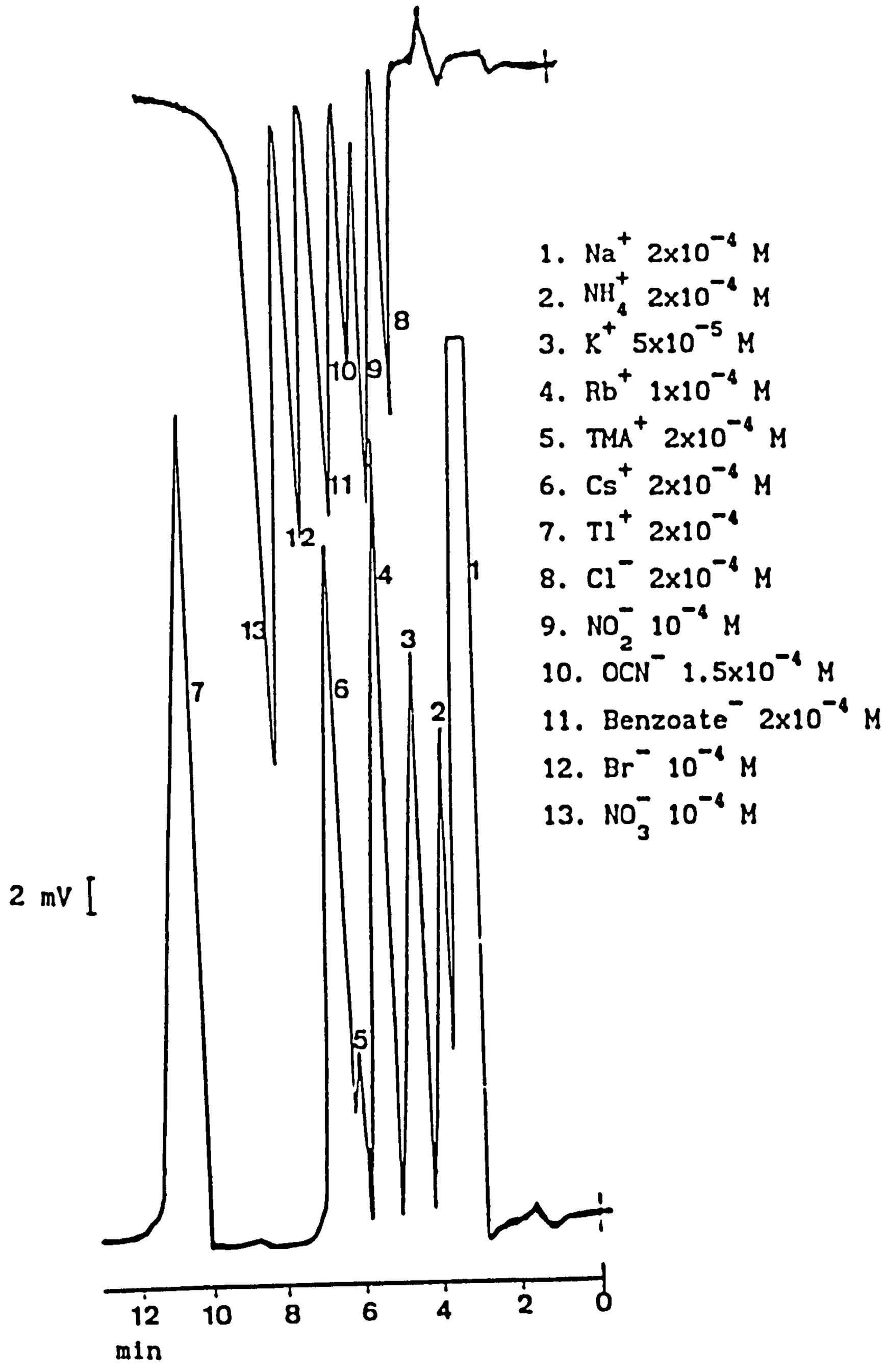


Figure 9. Independent separation and detection of anions and cations using three columns in series, combination of the columns and electrodes was as (HPIC-CS3 + CSE + HPIC-AG4A and AS4A + ASE), eluent: 1.4 mM $MgSO_4$, flow-rate: 1.1 ml min^{-1}.

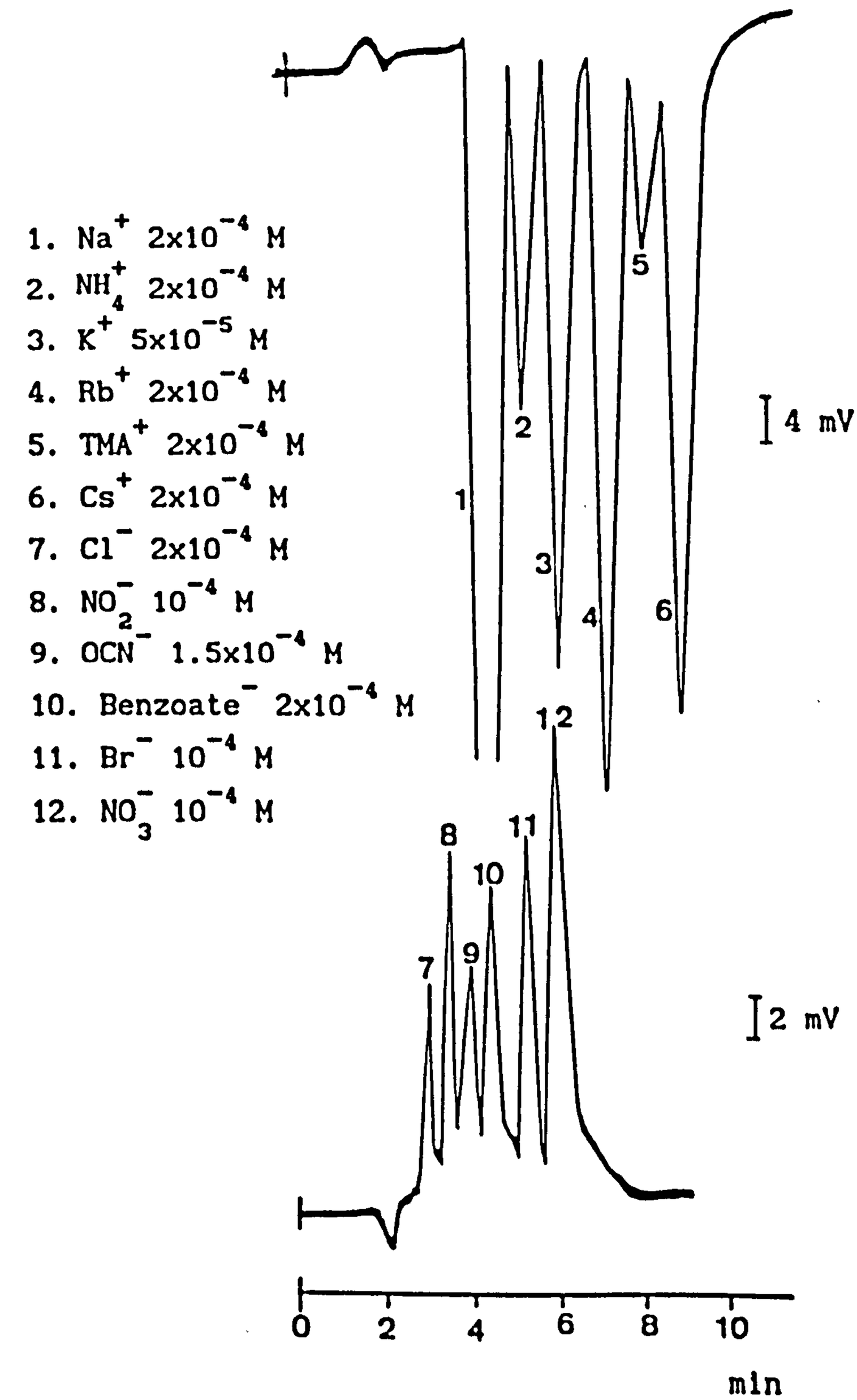
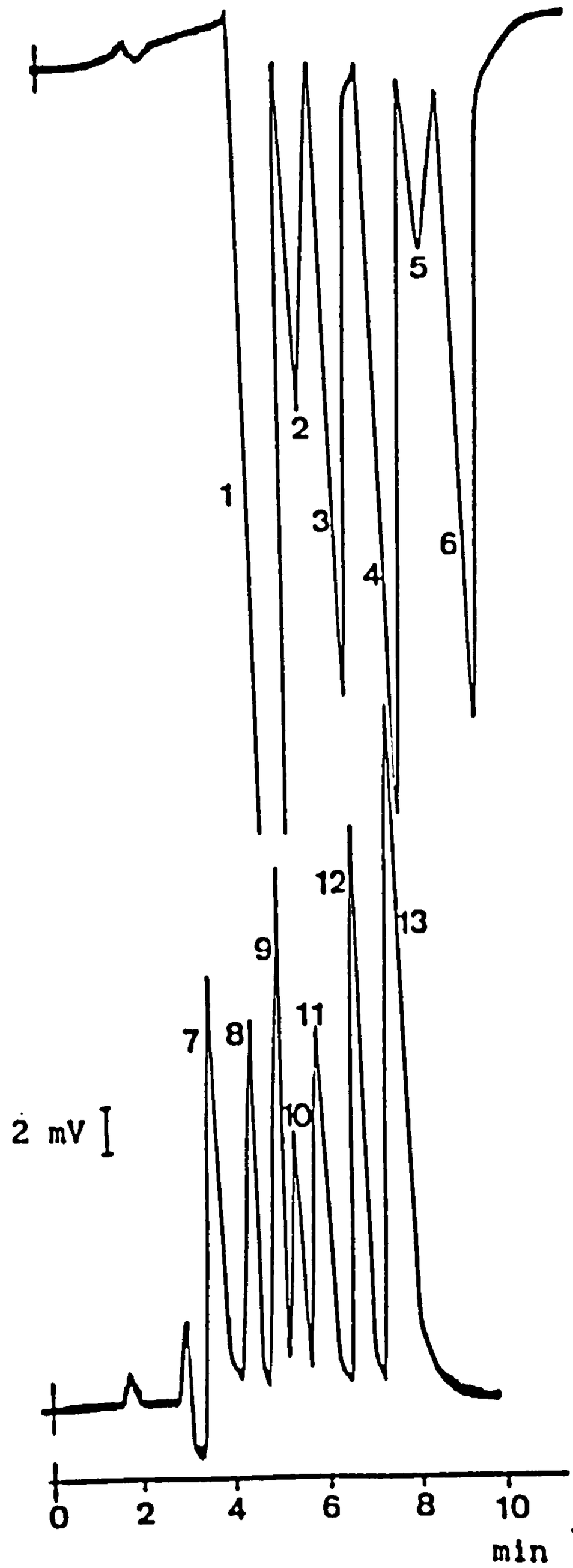


Figure 10. Independent separation and detection of anions and cations using three columns in series, the combination was as (HPIC-AG4A and AS4A + ASE + HPIC-CS3 + CSE), the other conditions were as in fig. 9.



1. Na^+ $2x10^{-4}$ M
2. NH_4^+ $2x10^{-4}$ M
3. K^+ $5x10^{-5}$ M
4. Rb^+ $2x10^{-4}$ M
5. TMA^+ $2x10^{-4}$ M
6. Cs^+ $2x10^{-4}$ M
7. propionate$^-$ $4x10^{-4}$ M
8. Cl^- $2x10^{-4}$ M
9. NO_2^- 10^{-4} M
10. OCN^- $1.5x10^{-4}$ M
11. Benzoate$^-$ $2x10^{-4}$ M
12. Br^- 10^{-4} M
13. NO_3^- 10^{-4} M

Figure 11. Independent separation and detection of anions and cations using three columns in series, the combination was as (HPIC-AG4A and AS4A + HPIC-CS3 + ASE + CSE), the other conditions were as in fig. 9.

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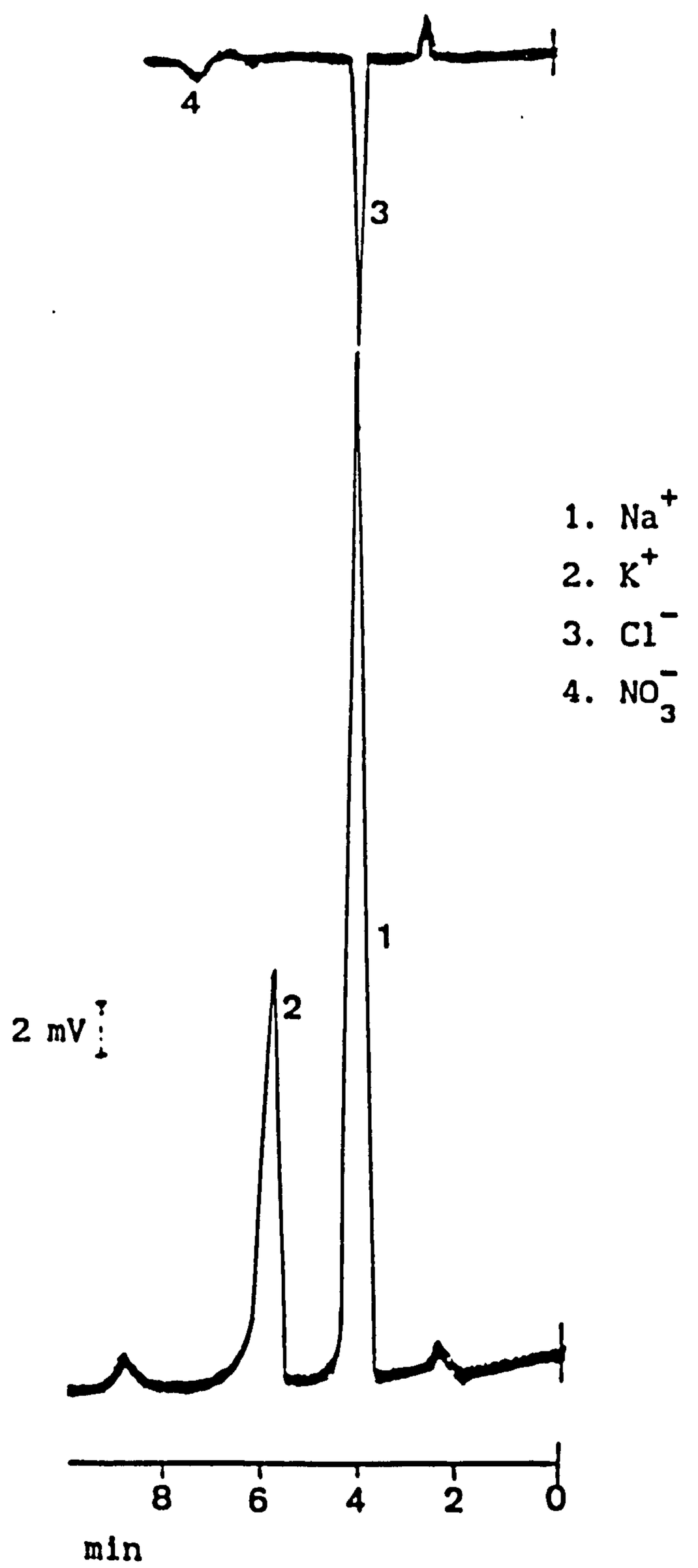


Figure 13. Simultaneous determination of anions and cations in river water, the other conditions were as in fig. 5.

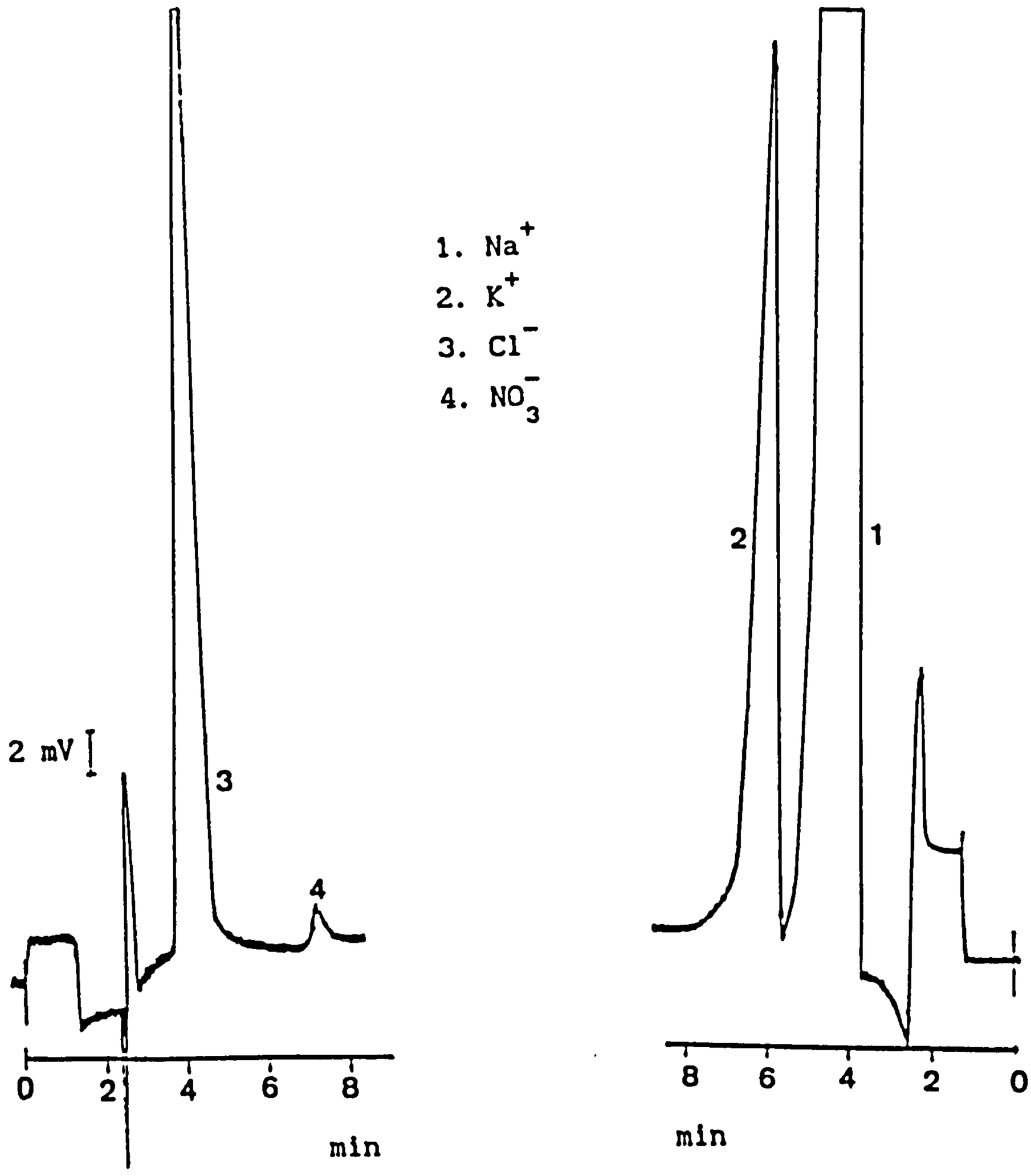


Figure 14. Simultaneous determinations of anions and cations in sea water diluted one hundred times, the other conditions were as in fig. 5.

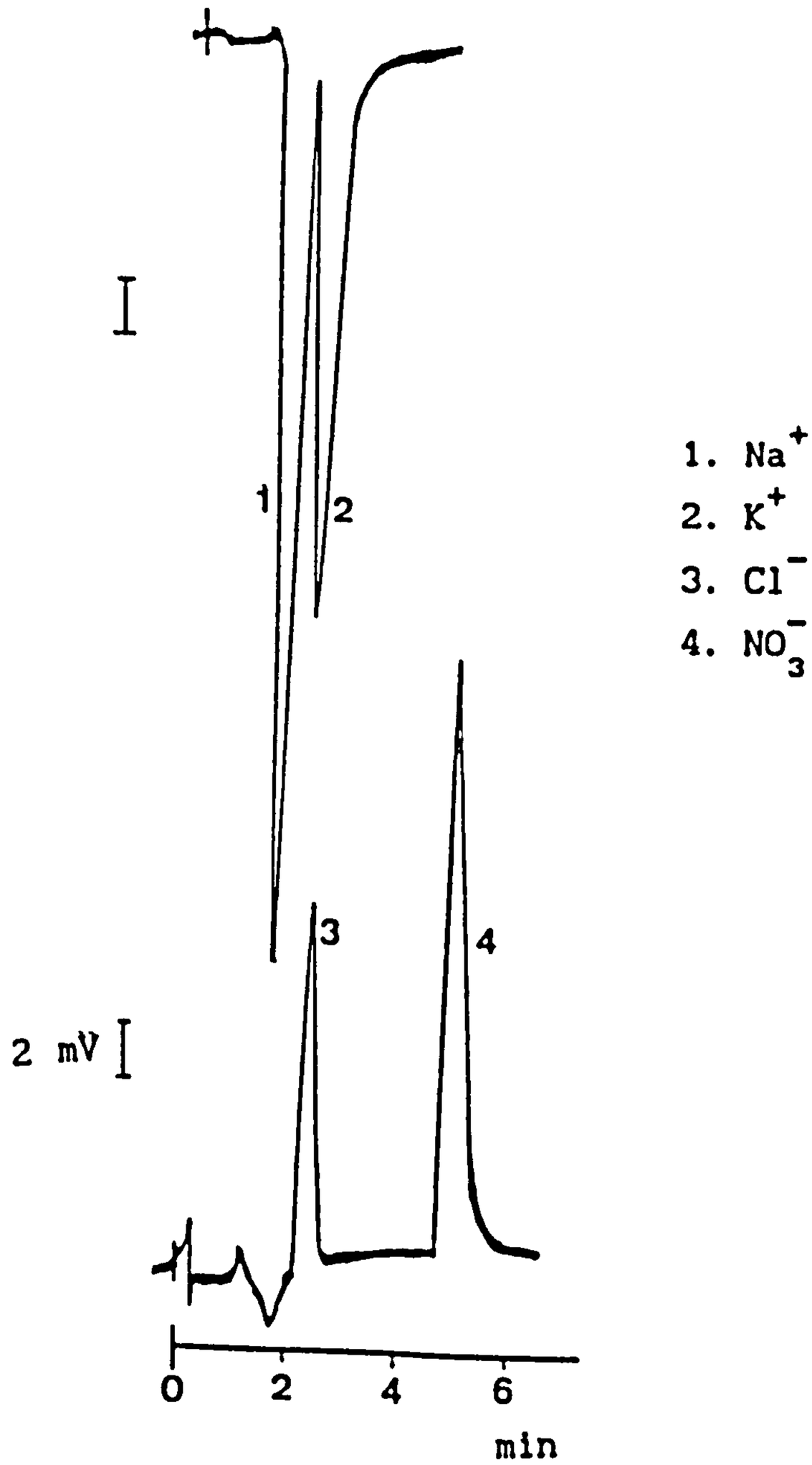


Figure 15. Simultaneous determination of anions and cations in drinking water using the anion and the cation selective electrodes at the end of HPIC-AG4A and AS4A columns, eluent: 1.4 mM $MgSO_4$, flow-rate: 1.2 ml min^{-1}.

10.8 REFERENCES

1. Gan D C. and Tarter J G., *J. Chromatogr.*, 1987, 404, 285.
2. Jones V K., Frost F A. and Tarter J G., *J. Chromatographic Sci.*, 1985, 23, 442.
3. Iskandarani Z. and Miller T E., *Anal. Chem.*, 1985, 57, 1591.
4. Tarter J G., *J. Chromatogr.*, 1986, 367, 191.
5. Jones V K. and Tarter J G., *Analyst*, 1988, 113, 183.
6. Brown D M. and Pietrzyk D J., *J. chromatogr.*, 1989, 466, 291.
7. Yamamoto M., Yamamoto H. and Yamamoto Y., *Anal Chem.*, 1984, 56, 832.
8. Pietrzyk D J. and Brown D M., *Anal. Chem.*, 1986, 58, 2554.
9. Shintani H. and Ube S., *J. Chromatogr.*, 1985, 344, 145.
10. Small H. and Miller T E., *Anal. Chem.*, 1982, 54, 462.
11. Slais K, *J. Chromatogr.*, 1991, 540, 41.

CHAPTER 11

11. SIMULTANEOUS DETERMINATION OF SODIUM, POTASSIUM AND CHLORIDE IN BSA PLASMA BY ION CHROMATOGRAPHY WITH POTENTIOMETRIC DETECTION

11.1 EXPERIMENTAL

11.1.1 Chemicals and Bovine Serum Albumin(BSA) Plasma Samples

Sodium chloride, potassium bromide and copper sulphate were of analytical reagent grade (BDH chemicals). BSA plasma samples at three concentration levels were supplied by Euro-Trol (Wageningen Holland) in 2 ml syringes and processed for the determination of the ions.

11.1.2 Ion Chromatographic System

The HPLC, ion selective electrodes and columns were identical with those described in chapter 10. The configuration of columns and detectors used was as follows: [Dionex IonPac-AS4A + IonPac-CS3 + anion selective electrode + cation selective electrode].

11.1.3 Preparation of Standard Sample Solution for Routine Calibration

A standard sample solution incorporating 0.05 mol dm^{-3} of sodium, 2 mmol dm^{-3} of potassium and 0.0502 mol dm^{-3} of chloride ions was prepared in deionized water and the solution was further diluted 2, 4, 8, 16 and 32 fold. The solutions were injected into the chromatographic system to obtain calibration curves for Na^{+}, K^{+} and Cl^{-}. The calibration curve obtained is shown in figure 1.

11.1.4 Filtration

An immersible, vacuum operated, dispersible filtration unit used was provided from Millipore, which contains immersible CX-30 ultrafilters (30.000 molecule weight cut-off) and immersible filtration components. All components other than the ultrafilters were polyethylene and polystyrene plastics. Filter units contained a glycerol and thimerosal solution to prevent drying and bacterial

contamination during storage.

A basic filtration was made using test tubes and vessels (fig.2). The ultrafilter was lowered into the plasma sample solution in the vessel to be processed and connected to a vacuum pump. Filtrate passes through the membrane and flows into the test tube. After the desired amount of filtrate was attained, the vacuum was broken and the filtrate was collected from the tube and further processed.

11.1.5 Determination of Free Monovalent Cations and Chloride in Non-filtered BSA Plasma Sample Level B

10, 30, 60 and 120 fold-diluted samples were directly injected into the chromatographic system. The concentration of each ion was calculated from the calibration curve obtained with the standard sample solution. The chromatogram shown in figure 3 was achieved with the sample diluted 60 fold. It can be seen that the resolution of sodium from potassium was satisfactory as the concentration of plasma sodium is about 300 times that of plasma potassium.

11.1.6 Determination of Free Monovalent Cations and Chloride in Filtered BSA Plasma Sample Level B

The determination was made for filtered samples in similar manner to that of non-filtered. The chromatogram shown in figure 4 was achieved with the sample diluted 60 fold. The concentrations of the ions found in the blood plasma sample type B, before and after filtration, are shown in table 1 for sodium, potassium and chloride.

11.1.7 Influence of Dilution

Plasma samples (before and after filtration) were diluted 10, 30, 60 and 120 fold and further processed. A non-linear decrease in the concentrations monovalent cations and chloride was found by the dilution of the non-filtered sample(fig. 5). But a linear decrease in the concentrations of sodium and potassium was found by the dilution of the filtered sample(fig. 6). This indicated that no liberation, or limited liberation, of the monovalent cations and

chloride by dilution of filtered sample from the ions complex was found.

11.1.8 The Coefficient of Variation

Two randomly chosen BAS plasma samples at level B (filtered and non-filtered) were split into diluted portions 10, 30, 60 and 120 fold. In each sample, the monovalent cations and chloride concentrations were determined.

The average sodium concentration was 0.156 mol dm^{-3} with a standard deviation 0.025 mol dm^{-3} in non-filtered and 0.131 mol dm^{-3} with a standard deviation 0.006 mol dm^{-3} in filtered sample.

The average potassium concentration was 4.45 mmol dm^{-3} with a standard deviation 0.18 mmol dm^{-3} in non-filtered and 4.32 mmol dm^{-3} with a standard deviation 0.06 mmol dm^{-3} in filtered sample.

The average chloride concentration was 0.113 mol dm^{-3} with a standard deviation 0.011 mol dm^{-3} in non-filtered and 0.105 mol dm^{-3} with a standard deviation 0.007 mol dm^{-3} in filtered sample.

The coefficient of variation for sodium was 16% for non-filtered and 4.6% for filtered sample.

The coefficient of variation for potassium was 4% for non-filtered and 1.4% for filtered sample.

The coefficient of variation for chloride was 9.7% for non-filtered and 6.7% for filtered sample.

11.2. DISCUSSION

The differences in the concentrations of monovalent cations and chloride between findings in this study and Euro-Trol are insignificant. The main deviation was obtained when diluted BAS plasma samples were used without filtration(fig 5).

A comparison of results obtained with 30 fold diluted samples is given in table 2 for different sample levels. The method is superior to the conventional methods for the determination of sodium potassium and chloride. There is no interference in the detection from other ions.

Table 1. Sodium, potassium and chloride concentrations remaining in identical BAS plasma samples (level B) before and after filtration.

sample*	sodium conc. (mol dm^{-3})	
	non-filtered	filtered
1	0.170	0.134
2	0.134	0.138
	potassium conc. (mmol dm^{-3})	
	non-filtered	filtered
1	4.48	4.33
2	4.42	4.30
	chloride conc. (mol dm^{-3})	
	non-filtered	filtered
1	0.110	0.108
2	0.115	0.103

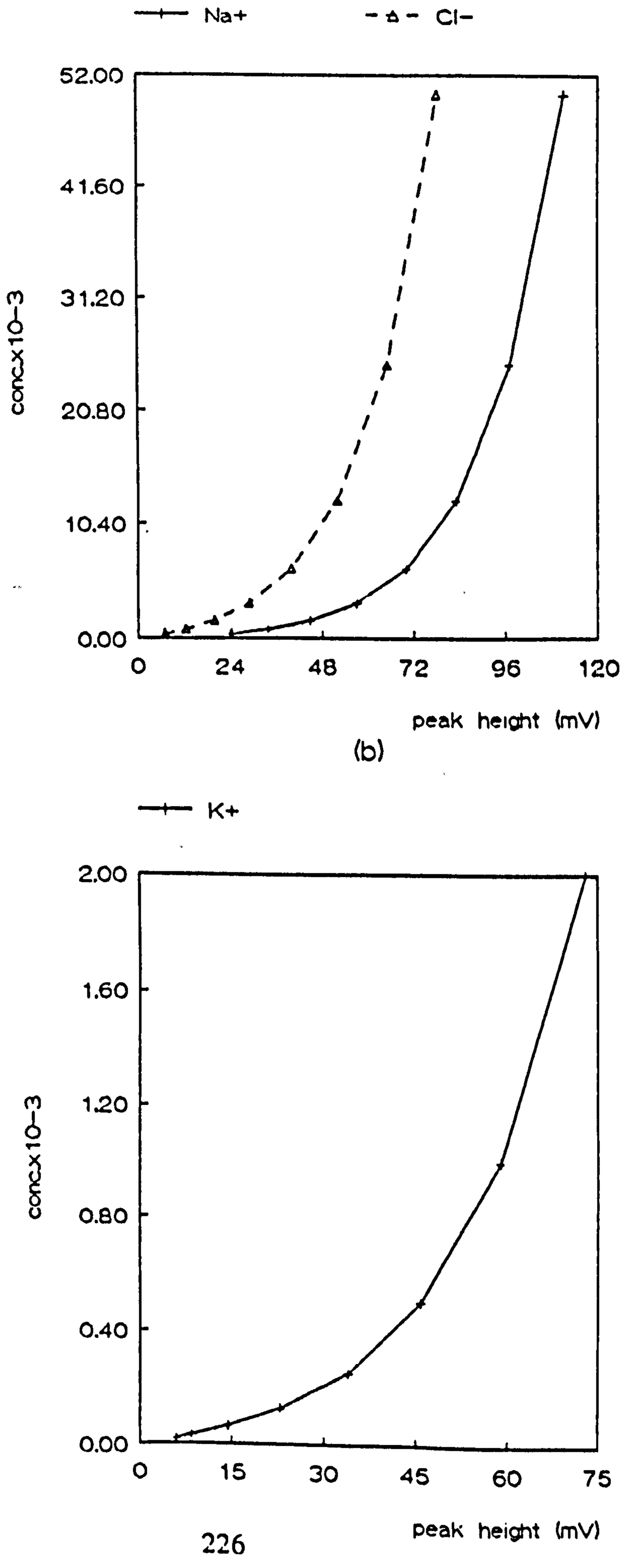
* each value was calculated from the average of concentrations in 10, 30, 60 and 120 fold diluted samples after three injections of each diluted sample.

Table 2. Comparison of the results obtained from 30 fold diluted samples with previous findings by Euro-Trol.

	sample level (A)	
	non-filtered*	Euro-Trol
sodium(mol dm^{-3})	0.093	0.119
potassium(mmol dm^{-3})	1.95	2.97
chloride(mol dm^{-3})	0.78	0.81
	sample level (B)	
sodium(mol dm^{-3})	0.16	0.139
potassium(mmol dm^{-3})	4.45	4.43
chloride(mmol dm^{-3})	0.11	0.10
	sample level (C)	
sodium(mol dm^{-3})	0.12	0.16
potassium(mmol dm^{-3})	5.15	5.94
chloride(mol dm^{-3})	0.13	0.12

* The data were obtained after three direct injections except sample level B which was the average of concentrations in two different samples after three injections of each.

Figure 1. Calibration curves for the monovalent cations and chloride obtained for each peak height plotted against concentration.



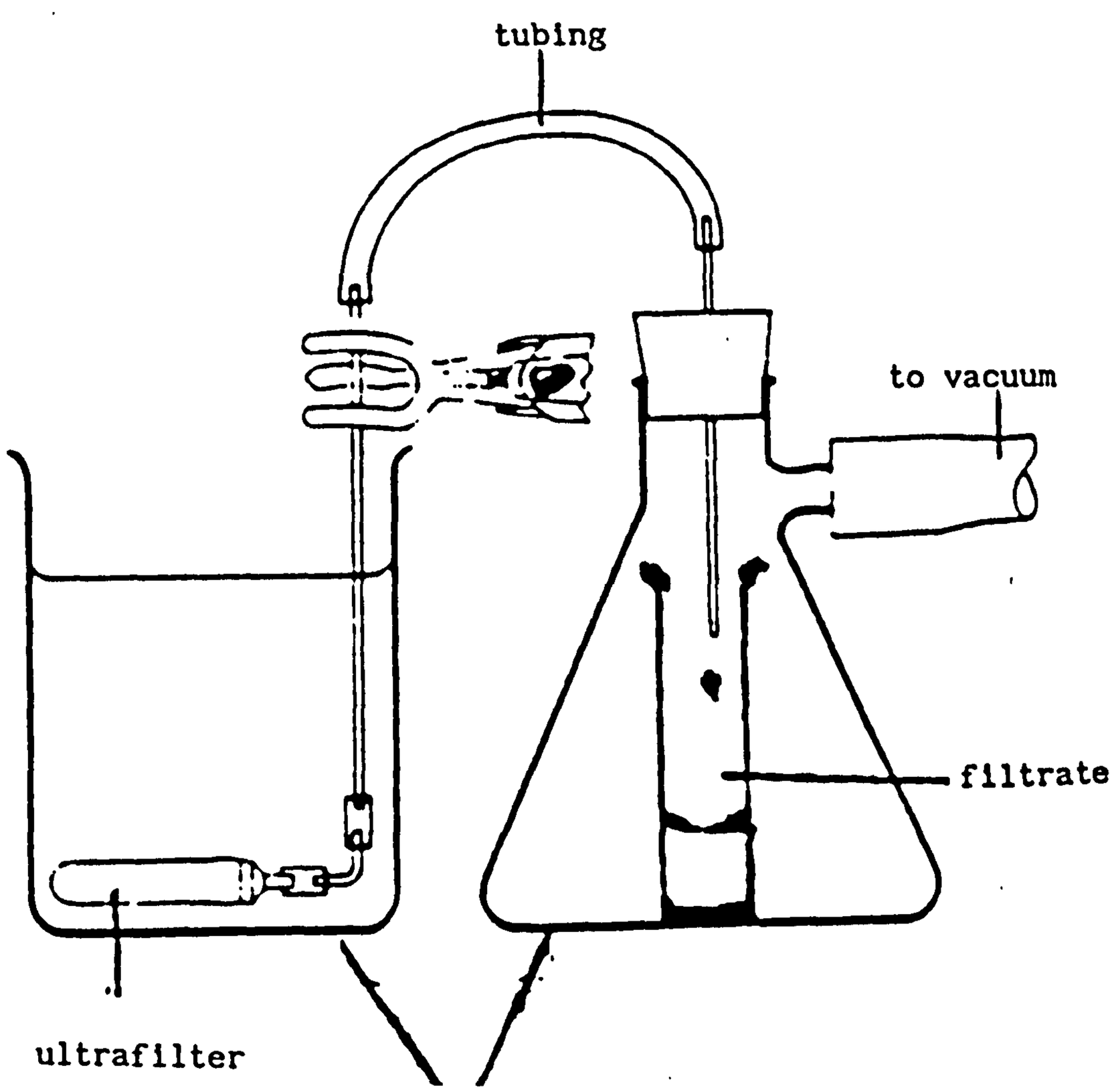
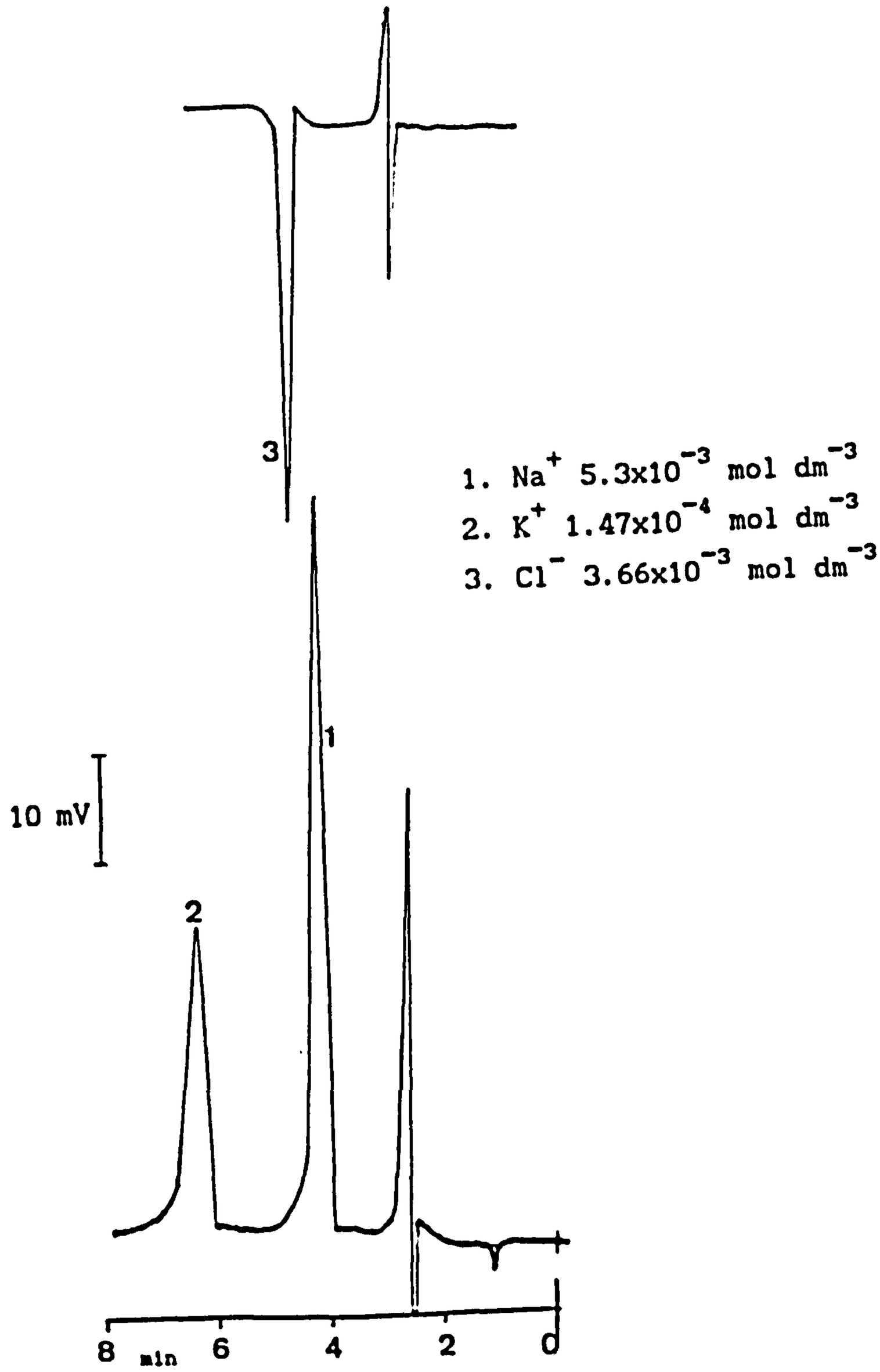
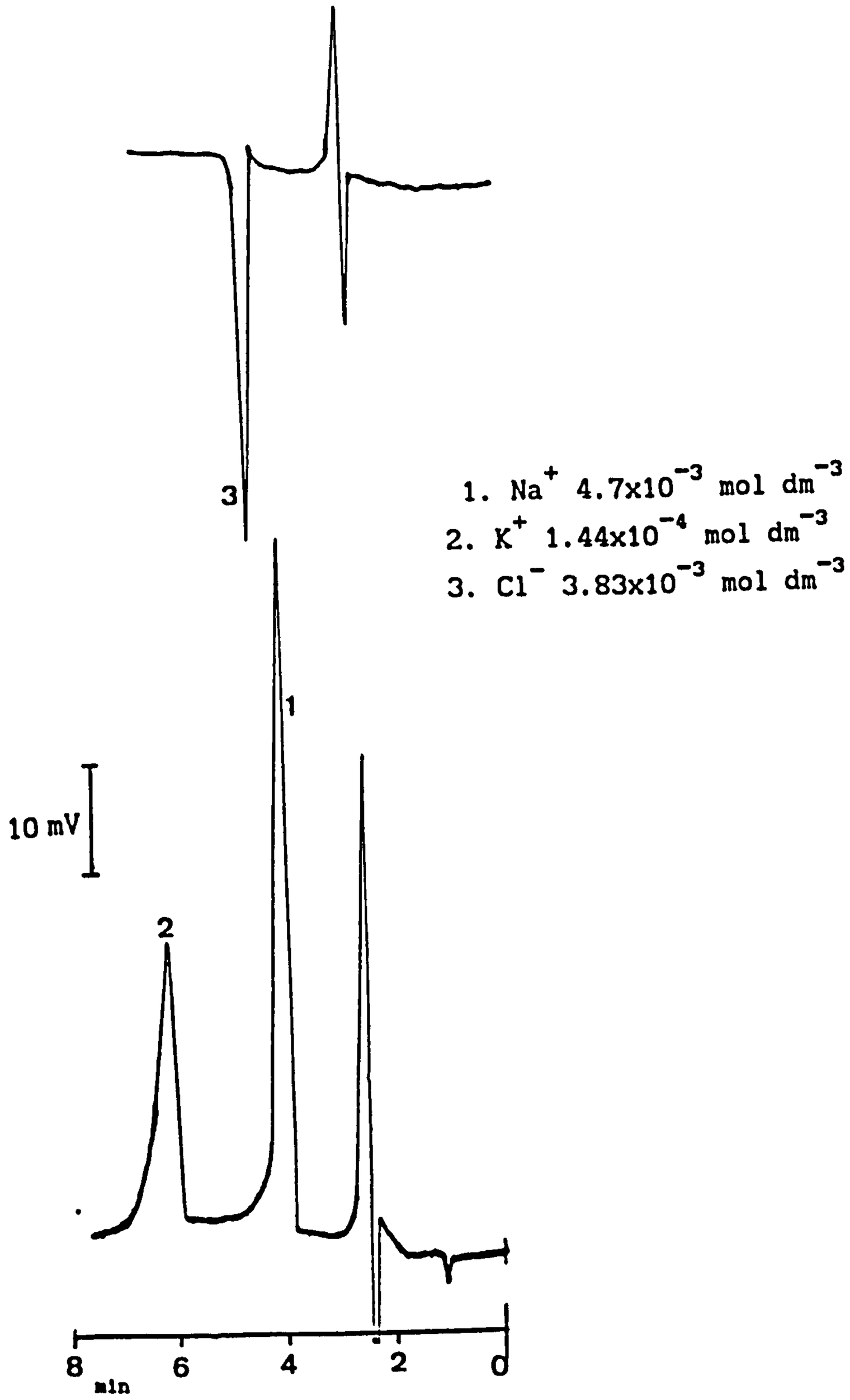


Figure 2. A schematic diagram of the filtration system used.



1. Na^+ 5.3×10^{-3} mol dm^{-3}
2. K^+ 1.47×10^{-4} mol dm^{-3}
3. Cl^- 3.66×10^{-3} mol dm^{-3}

Figure 3. Simultaneous determination of the monovalent cations and chloride in the plasma diluted 60 fold. Eluent: 0.6 mmol dm^{-3} $CuSO_4$, columns: IonPac-AS4A and -CS3 analytical, flow-rate: 1.2 ml min^{-1}, injection: 20 µl.



1. Na^{+} $4.7x10^{-3}$ mol dm^{-3}
2. K^{+} $1.44x10^{-4}$ mol dm^{-3}
3. Cl^{-} $3.83x10^{-3}$ mol dm^{-3}

Figure 4. Simultaneous determination of the monovalent cations and chloride in the plasma diluted 60 fold after the filtration. The other conditions were as in figure 3.

Figure 5. The effect of dilution on the free concentrations of the monovalent cations and chloride in the non-filtrated plasma sample.

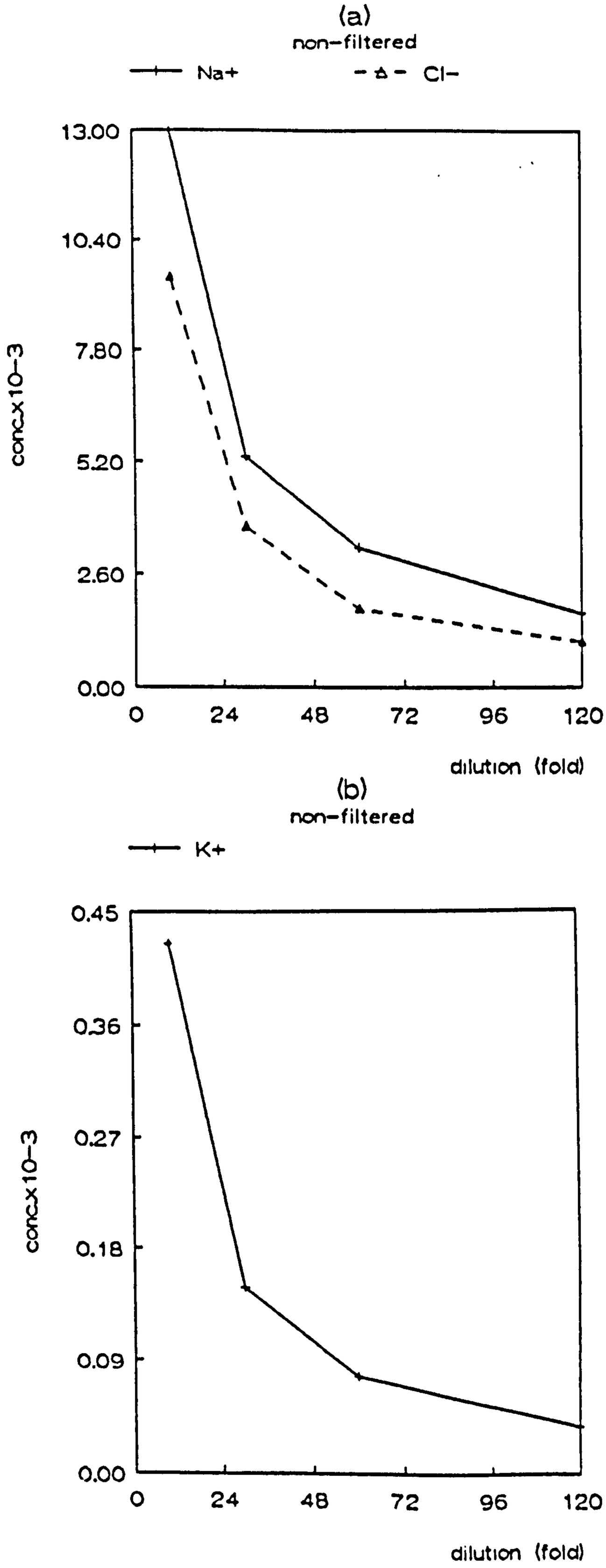
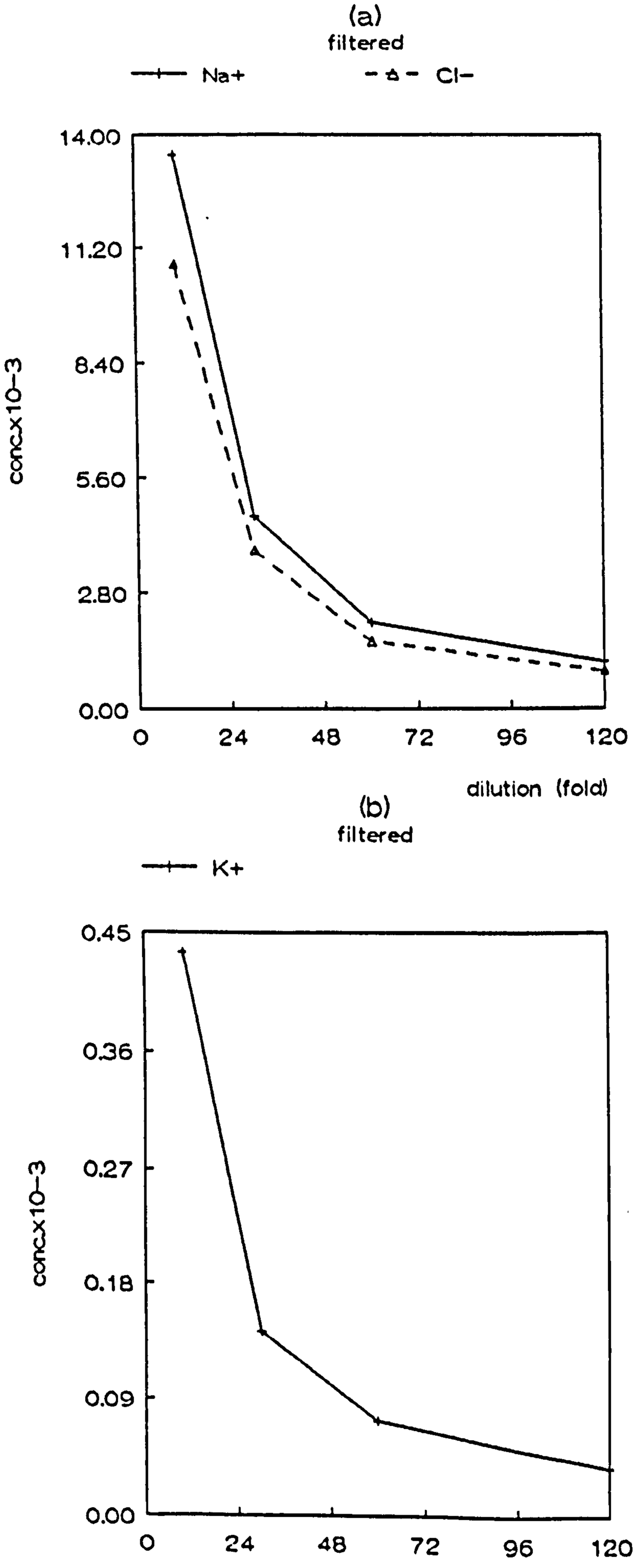


Figure 6. The effect of dilution on the free concentrations of the monovalent cations and chloride in the filtrated sample.



CHAPTER 12

12 CONCLUSIONS

12.1 INTRODUCTION

Ion selective electrodes have found wide application in flow injection analysis as the design of low dead volume cells and production of high selective electrodes is possible. Apart from special applications, ion chromatographic analysis systems are employed with detectors having more general response. Therefore the number of applications of potentiometric detection in ion chromatography is increased by the use of ISEs for which the response is not limited to a few number of ions.

On the other hand, in any liquid chromatographic technique, the eluent composition provides greatest flexibility for manipulating the retention of solutes in order to achieve desired separation and detection. Therefore attention must be given to selecting suitable eluents for potentiometric detection and then chromatographic separation.

For a successful potentiometric detection, a suitable flow-cell must be designed to accommodate the indicator and the reference electrodes. The performance of a detector can be assessed by the volume constant, or cell volume, in which solute remains for a time. Considering the dependence of the detection on the dispersion, the sensitivity of the indicator electrode can be greatly decreased with large volume cells and connector tube(s) that are part of the tubing.

The above considerations have been taken into account in presenting this thesis. A variety of flow-cells as designed for efficient potentiometric detection of ions of interest in ion chromatography. A tubular flow-through and all solid-state contact tubular liquid membrane electrode cells having 2 µl dead volume were found more efficient for chromatographic measurements. The performance characteristics of the latter were much better than other kinds. A very thin membrane can be coated on to the

solid surface so decreasing the response time and increasing sensitivity.

Selectivity and detection limit studies by the mixed solution method and flow-injection system have shown that membrane ion selective electrodes based on PVC can be used for more general detection of anions and cations in an ion chromatographic system. Using solutions with low background potential as eluents allow anions and cations to be detected at very low concentration levels. Reproducibility was high when a suitable detection cell constructed and Nernstian response was possible at ppm levels of standard sample solutions.

The determination of response time and an evaluation of the factors affecting the determination was examined in flowing conditions for chromatographic measurements. From the response time of the electrode to 10^{-5} M of primary ion in 20 µl volume, it can be concluded that smaller volumes of sample might be used for detection without high loss of a sample ion at low flow-rates as low as 1 ml min^{-1}. Decreasing detection volume down to 1 µl might reduce the retention times of ions in the analytical column leading to shortening of analysis time, because the retention time differences between ions in the column should be, at least, equal to response time of the electrode determined experimentally by the new definition. When flow-rate increases, the response time of the electrode decreases but the response volume increases. At high flow-rates, the response time was independent of the activity of the ion, namely, the effect of the activity on the response time was to be a minimum. The response time was decreased for low activities rather than for high activities of the solute ion when flow-rate is increased. Consequently the response time measurements in continuous flow systems could give the possibility of obtaining and comparing some useful parameters on electrode properties, such as; experimental detection limit and volume, selectivity, calibration etc., and also on chromatography and FIA, flow-rate choice, composition of eluent, optimum retention time differences between ions in the analytical column etc.

When potentiometric detection is employed in flow injection and

chromatographic analysis, the sample dispersion is an existing problem as there is no a comprehensive study. In the study on the contribution and influence of the sample dispersion on the sensitivity of tubular liquid membrane bromide selective electrode based on PVC, 10^{-5} molar solution of primary ion and deionized water as carriers in a flow-injection system was chosen to generalize the data for either ion chromatographic or flow injection analysis as the both deionized water and primary ion are main components of the eluents used in both analysis systems. The method was simplified due to the injections of the solutions dispersed beforehand by eliminating a dispersion coil or cell used in other experiments.[1] From the results, it can be concluded that the sensitivity of the electrode is completely dependent on its response time rather than flow-rate or dispersion. The highest sensitivity was obtained when a sample passes the electrode surface just within the electrode response time in flowing conditions. Whilst lower response volume caused the loss of the response and tailing, higher response volume caused broadening of the peak shape. Consequently peak broadening and tailing cause sampling rate limitations in flow injection analysis and poor resolution in chromatography. Hence the optimization of injection volume, flow-rate and tubing length depend on the dispersion and the response time of the electrode, which must be investigated in flowing conditions.

The future of potentiometric detection with ion selective electrodes in ion chromatography might lie in the availability of different classes of eluents. With the utilization of different classes of eluents, more efficient separations and sensitive detection might be obtained. Consequently, a good separation and sensitive potentiometric detection with the ion selective electrode as detector of seven monovalent anions including I^- and SCN^- was achieved using phosphate as eluent on Dionex analytical and guard columns. The eluent was capable of removing the strongly retained anions in 13 minutes, however the retention times for I^- and SCN^- were shorter than those obtained with other eluents used with such chromatographic columns. The high

sensitivity of the electrode allowed most anions detected to be at sub-ppb levels.

The electrode with cell volume 2 µl was applied with phosphate and hydrogen phosphate as eluents at low concentrations for potentiometric detection of eleven inorganic and organic monovalent anions in single ion chromatography employing ion exchange technique. This is a "monovalent anion detection method" as the selectivity of the electrode towards monovalent anions results in some other anions being undetected unless the concentration of those other anions is higher than 10^{-3} molar. It takes only seven minutes to complete the separation with good resolution. The use of the all solid-state contact bromide selective electrode as detector offers the best simultaneous sensitivity toward all anions when compared with other detection methods. The method might be very reliable for determination of ion content in many sample types, i.e. waste water, soils, blood, drinking water, foods, etc., as there is no interference from many other ions. Applications to river, sea, and drinking water samples show that common monovalent anions i.e. Cl^-, NO_3^-, could easily be detected without any further sample preconcentration. With an optimized system, trace analysis of many anions in most sample types might be done using the method developed here.

Suppressed ion chromatography with phosphate and hydrogen phosphate as eluents might be applied as the separation of the eleven anions was achieved using low concentrations of the eluents in non-suppressed ion chromatography with potentiometric detection. In this study, the eluents form phosphoric acid with the suppressor which produces hydrogen ions. Phosphoric acid exists unassociated in the solution since it is a stronger acid. Hence the background conductivity increased by hydrogen ions emerging within the solution. Species with low dissociation (e.g. organic anions and nitrite) emerged as negative peaks within the chromatogram whilst others were positive. This is direct and indirect detection in suppressed ion chromatography since both mechanisms exist in one run. The intensity of negative peaks could be diminished by decreasing of the regenerant flow-rate.

Chromatography requires as much as possible separation of ions in one run to diminish interferences to the determinations. The idea was that the anions, which were separated by phosphate and hydrogen phosphate and detected with the electrodes, might have been separated by the carbonate/bicarbonate buffer as eluent. So, utilization of this buffer composition offered the determination of eleven anions in suppressed ion chromatography with conductivity detection. Also the use of low concentration of the buffer brought more sensitive detection as the background conductivity decreased. Resolution was good and the baseline was better. One advantage of this detection method over potentiometric detection is that the number of anions separated could be increased since conductivity detection shows more general response than potentiometric detection, hence, 13 anions could be detected, but the retention times of phosphate and sulfate were unacceptably long.

Liquid membrane potassium selective electrodes with copper and magnesium salts as eluents can be used for a highly sensitive and selective detection of monovalent inorganic and organic cations and efficient separation in ion chromatography. The method is simple and eight minutes is needed to complete the separation with a good resolution. Lower detection limits allows trace detection of many cations. Applications to different sample matrices show the capability and reliability of the method developed. Sodium and potassium determinations in raw materials are especially difficult, since high concentrations of salts must be used so anions interfere with detection of early eluted cations such as sodium. The advantage of the method is that the detector was selective only to monovalent cations so various sample matrices e.g., blood, urine, environmental, might be introduced without any sample pretreatment.

The over-all efficiency of ion chromatographic techniques is achieved in the simultaneous detection and separation of common anions and cations in one run. The method developed here is an intelligent one which determines fourteen inorganic and organic common monovalent anions and cations simultaneously in a short

time. The base line noise was the only restriction to bring the simultaneous detection limits down to ppt levels. Previous reports[2-3] indicated that a background level of an electroactive species should be included in the eluent to give a stable electrode potential, but it was found that the membrane ion selective electrodes used, required no addition of primary ion to the eluent when the eluent exhibits small affinity towards the membrane, and serve as a baseline supporting electrolyte. If a pump is used which supplies a constant pressure of eluent, there should be no streaming potentials.

The method developed here has the following advantages;

-copper and magnesium sulphate as eluents lead an efficient independent separation of anions and cations in one run on Dionex low capacity anion and cation exchange columns used in series.

-the detectors allow simultaneous detection of most anions at ppb levels without any interference by each other.

-as the method covered most common anions and cations detected in a short time, there is no requirement for another system for determination of cations or anions in analytical laboratories.

-it can be applied with other analytical columns to allow anions and cations to be separated independently, so eliminating a second column and reducing the overall separation time so more ions may be determined,

-many sample types can be applied without any sample pretreatment, especially samples, such as blood, with organic contaminants which interfere with the detection of the common ions in other detection systems, e.g. conductivity,

-requires simple eluents such as copper or magnesium sulphate,

-in the case of copper as eluent, indirect photometric detection could be applied with potentiometric detection, or indirect photometric detection could be applied with cation suppressed conductivity detection,

-easy construction and use,

-routine identification and calibration,

-direct determination of many common anions and cations,

-cheap,

-rapid analysis, needing only 10 minutes or less to complete determination of a group of fourteen anion and cation,
-high sensitivity, reproducibility and resolution,
-a wide variety of samples can be studied without preconcentration,
-it can provide useful information about selectivity characteristics of commercial columns on the market or in home made,
-and the method could be applicable to other commercial columns
The simultaneous determination of sodium, potassium and chloride in BAS plasma indicates that the dilution of samples non-filtered gives rise a liberation of the free ions from ion complexes. This was not the same for filtered samples. The method can be easily applied to many difficult sample matrices where reproducibility and routine calibration are prime importance.
Finally, potentiometric detection with ion selective electrodes is the most capable and promising method of determining ppm and ppb levels of common anions and cations simultaneously.

12.2 FUTURE WORK

Future work should concentrate on simultaneous detection of a wide range of anions and cations. For this, an electrode array must be designed and suitable solutions must be found for sensitive detection and efficient separation. The possibility of simultaneous determinations using ion selective electrodes as detectors with other commercial columns has to be explored. This may soon bring the use of commercial potentiometric detectors into the chromatography market. Experiments on simultaneous determinations with the computerized system (appendix C) have to be improved and adapted to the chromatographic measurements. Another development should be the preparation of new, efficient, miniaturized columns for trace analysis since the performance of the detection method is high in more diluted solutions as eluents.

12.3 REFERENCES

1. Trojanowicz M. and Frenzel W., Z. *Anal. Chem.*, 1987, 328, 653.

2. Jyo A., Mori K. and Ishibashi N., *Bull. Chem. Soc. Jpn.*, 56, 1983, 3507.

3. Butler E C V. and Gershey R M., *Analytica Chimica Acta*, 164, 1984, 153.

APPENDIX A.

Distillation of tetrahydrofuran (THF):

To the THF are added 3 μg of benzophenone and *ca.* 1 g potassium metal. Prior to distillation, the still glassware should be flushed through with nitrogen gas (dried over NaOH) to remove water from the apparatus. This is necessary as water forms an azeotrope with THF. A nitrogen flow is not used during distillation, as this would remove the THF vapour.

Potassium effectively removes peroxides in THF; the formation of peroxides leads to an extremely unstable mixture. Benzophenone acts as an indicator by forming complexes with potassium and thus showing the extent of the reaction. During the process quinoline, a peroxide stabilizer in the THF, is also removed.

In order to reduce peroxide build up, the distillate should be stored under nitrogen and the container wrapped in aluminium foil to protect from light.

APPENDIX B.

1. Preparation of Thermal Electrolytic Silver / Silver Chloride Electrodes:

For each electrode, a *ca.* 40 mm strip of platinum wire, fused to a glass tube (100 mm length x 5 mm diameter), was cleaned using

i) concentrated nitric acid

ii) distilled water

iii) acetone

The platinum was then coiled into a small, compact helix and coated with a thin layer of silver oxide and placed in a pre-heated furnace at 450^{0}C for 40 min. This procedure was repeated three times, thereby reducing the likelihood of cracks in the silver coating.

The electrodes were chloridised by anodising against a platinum foil cathode of 10 mA for 7-8 min for one electrode (or 12 mA for 8 min for two electrodes, connected in parallel)

To reduce the bias potential, the completed Ag/AgCl was placed overnight in 0.05 mol dm^{-3} hydrochloric acid. The potentials of the batch bias potentials were then intercompared and individual electrodes differing from the average potential of the group by > 0.1 mV discarded. Insufficient washing of silver oxide during preparation (see below) may lead to a slow electrode response and a high bias potential. This can often be remedied by heating the electrode for 2 h in distilled water at 50 to 60^{0}C.

2. Preparation of Silver Oxide:

To 338 g of silver nitrate dissolved in three liters of deionized water, dropwise add a solution of 80 g sodium hydroxide dissolved in 400 ml water. The silver nitrate solution should be stirred vigorously throughout and the final precipitate should contain a slight excess of silver. This precipitate should be washed thirty to forty times with distilled water until the conductance of the washings is constant.

APPENDIX C

THE FIGURES SHOW POTENTIOMETRIC DETECTION OF CATIONS IN ION CHROMATOGRAPHY USING A COMPUTERIZED SYSTEM DEVELOPED HERE

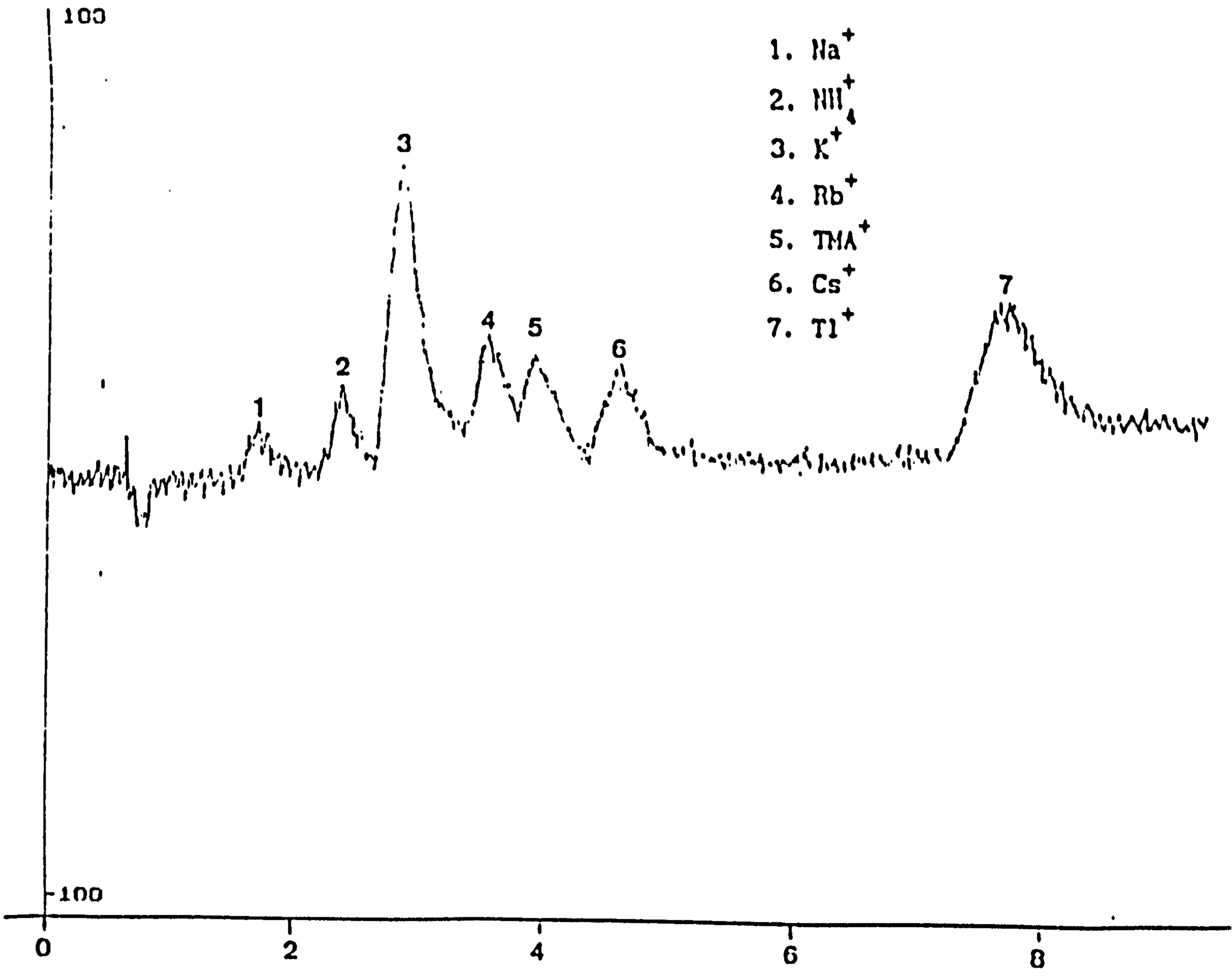
The operating conditions

Column: IonPac-CS3 cation-exchange

Eluent: 1.5 mmol dm^{-3} magnessium sulphate

Flow-rate: 1.4 ml min^{-1}

Injection: 20 µl of $1.3x10^{-5}$ mol dm^{-3} standard solution of each cation except TMA^+ which was $2.6x10^{-5}$ mol dm^{-3}



The operation conditions

Column: IonPac-CS3 cation-exchange

Eluent: 0.05 mmol dm^{-3} Ce^{3+} + 10 mmol dm^{-3} Li^{+}

Flow-rate: 1.3 ml min^{-1}

Injections: 20 µl of 10^{-4} mol dm^{-3} standard solution of each cation