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Review

Determination of inorganic ions of clinical interest: state-of-the-art and trends

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Abstract

An overview is presented of techniques, analysers and methods currently available for the determination of inorganic ions of interest in clinical laboratories; methods include those based on activation and inhibition of enzymatic reactions by these target analytes. The foreseeable trends in this area are also discussed.

Keywords: Clinical electrolytes; Inorganic ions; Review

1. Introduction

The so-called "electrolytes" in clinical chemistry are essential components of living matter. Their functions in the organism are multiple; almost all metabolic processes depend on, or are affected by, the activity of one or several of these electrolytes. Certain cations such as sodium, potassium, calcium and magnesium together with anions such as chloride, phosphate, bicarbonate and sulphate are among those found at higher concentrations in living matter. The most important functions of these electrolytes are: to maintain suitable hydration and osmotic pressure; and to participate both as cofactors in enzymatic reactions and as redox agents in electron-transfer reactions. In addition, the different concentrations of these species in intra- and extracellular fluids regulates both membrane potentials and normal functioning of nervous and muscular tissues. Because of the nature of these functions of clinical electrolytes, abnormal concentrations of these species are either the cause or the consequence of a wide variety of metabolic disorders. For these reasons, the determination of these species in biological fluids (e.g. plasma, serum, urine and sweat) is of paramount importance both for the diagnosis and monitoring of pathological states of individuals.

The concentrations of Na^+, K^+, Cl^- and HCO_3^- are usually determined as screening tests in medical emergencies. These parameters provide information on the hydroelectrolytic balance of the patient and enables derived parameters such as osmolality and anion gap to be calculated; this allows an orientative diagnosis so that further complementary tests can be requested for a definitive diagnosis. Calcium, magnesium and phosphate ions in man are in lower concentrations than the other electrolytes; thus their contributions to hydroelectric balance are smaller. Nevertheless, knowledge of their concentrations in serum and urine samples is of clinical value.

Owing to the clinical importance of the concentration of electrolytes and the urgency of

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their determination, clinical laboratories must provide very accurate results of these analyses as quickly as possible. The number of analyses of these electrolytes to be performed in a clinical laboratory makes mandatory the use of automatic methods of analysis that are capable of providing a suitable and prompt response to a request for these tests.

The great variety of automatic methods for the determination of electrolytes permits the demands of large medium and small hospitals to be satisfied. The rapid development of new methods over the last few years calls for a review of the instrumental techniques, types of analysers and methods currently available as well as the most important trends in this field.

2. Analytical techniques

There are three basic aspects to be taken into account in selecting an analytical technique for the development of methods for the determination of electrolytes in clinical samples: number of target analytes to be determined per sample; type and volume of the sample; and number of samples to be analysed. In turn, these aspects condition the analytical process to be developed, the technique design to be implemented and last, but not least, the costs to be entailed in setting up a clinical laboratory.

The analytical techniques used in the development of methods for the determination of electrolytes can be divided into four categories: optical techniques, both molecular and atomic; electroanalytical techniques, mainly direct and indirect potentiometry based on the use of ion-selective electrodes (ISEs); analytical separation techniques, such as chromatography or isotachophoresis, coupled on-line with different detection techniques; and optical sensors, either optic-fibre or flow-through integrated sensors [2,3].

A comparative overview of the present usage of analytical techniques for the determination of these species is shown in Fig. 1. A predominance of potentiometric and atomic techniques is clear from this figure.

2.1. Optical techniques

Atomic techniques

Among atomic techniques those based on emission are the most commonly used at present for multicomponent analysis. Although flame atomic emission spectrometry (FAES) was predominantly used in the last decade [4–9], the development of inductively coupled plasma (ICP) and direct current plasma (DCP) sources have led to the use of sequential or multichannel detectors for the determination of Na, K, Mg, Ca and P [10–30]. Atomic absorption spectrometry either aided by flame atomization [31–35] or graphite furnace [36] devices has been frequently used for the determination of alkaline and alkaline-earth species in biological fluids.

Recently, several modes of atomic fluorescence spectroscopy (AFS) with [37,38] or without [39] ICP excitation have been used for the determination of Na, K, Mg and Ca, while laser-induced AFS with microwave-induced plasma atomization has been used for the determination of Na [40]. Although these techniques have been used in general with aqueous samples, their sensitivity and selectivity suggest that they will be used successfully for the determination of these species in clinical samples. Owing to the costs (for both acquisition and maintenance) of atomic emission spectrometers (with the exception of flame spectrometers), these methods have been used chiefly as reference techniques for comparison with more common techniques. The coupling of atomic spectrometers with clinical ion-analysers is not an easy task; probably the greatest potential in this respect lies with the coupling of AFS [12] and atomic absorption spectroscopy (AAS) [34,41] with continuous unsegmented-flow techniques for flow-injection analysis.

Molecular techniques

The limited use of molecular techniques for the determination of electrolytes (especially Na and K) as compared with atomic and electroanalytical techniques is a consequence of their very limited capability to form coloured or flourescent compounds. Over the last few years, the aim of research involving optical-molecular techniques for the determination of inorganic species of clinical interest has been focused on two directions: first, searching for new reagents capable of yielding coloured or fluorescent compounds, mainly complexes, with the target analytes; and, second, the development of methods based on both activation and inhibition of enzymatic reactions whose reaction products can be monitored by spectrophotometry, spectrofluorimetry of (bio)chemiluminometry. Among cations, calcium is the

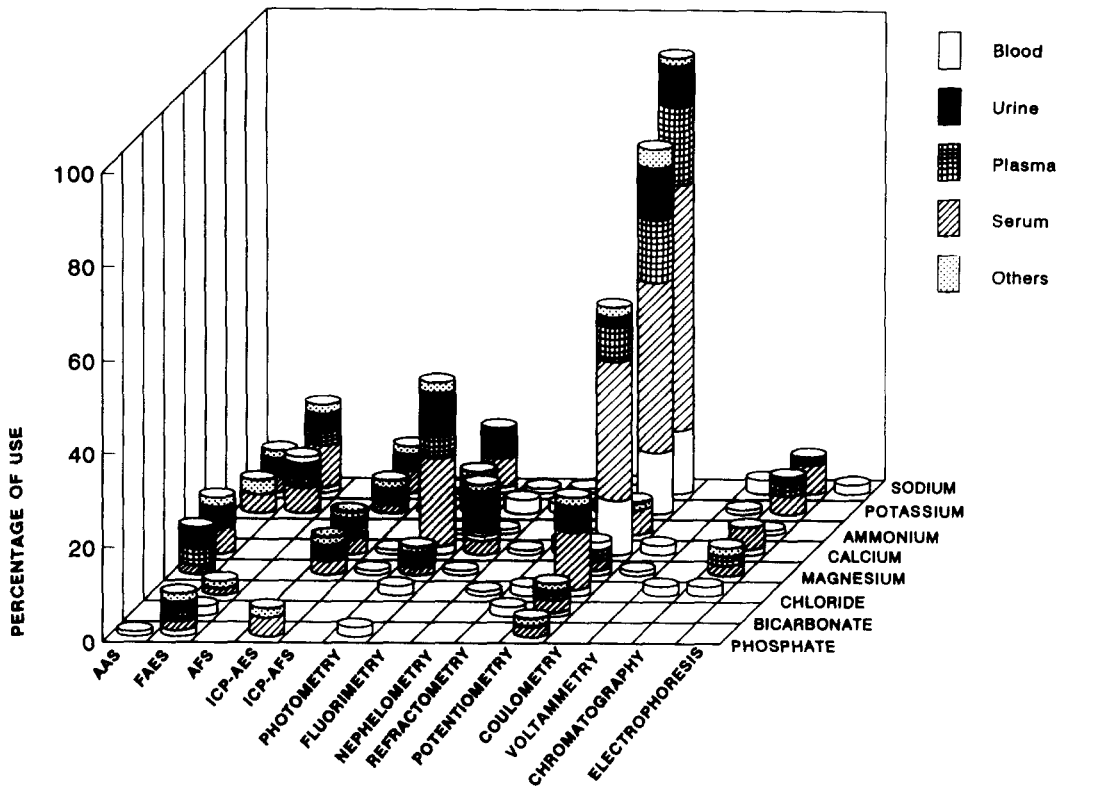

Fig. 1. Three-dimensional plot of the frequency of use of the analytical techniques for the determination of clinical eletrolytes and samples in which they have been determined.

electrolyte most prone to complex formation as shown by the use of spectrophotometry [42–50] and spectrofluorimetry [51–64] (Fig. 1). The second use of spectrophotometry corresponds to magnesium ions [45,65–67] and phosphate ions [48]. The use of spectrophotometry for the determination of sodium and potassium ions has been promoted by the use of crown ethers [44,68–70] and mainly by their activating of inhibitory effect on enzymatic reactions [71–87].

2.2. Electroanalytical techniques

Optical techniques have traditionally had a more extensive use in clinical laboratories than electroanalytical techniques, but this fact is not true for monovalent cations (Fig. 1). A 2:1 ratio of electroanalytical to optical techniques has been found for sodium (60/34%) and potassium ions (62/32%) whereas for calcium the ratio is 2/3 (41/56%) and 1/3 for magnesium (15/76%). Several reviews from the last decade [5,88–94] show the extensive use of electroanalytical techniques for the determination of clinical electrolytes. The development of new ISEs has given potentiometry an outstanding place in clinical laboratories, chiefly for the determination of sodium and potassium ions [89,95–113], and to a lesser extent for the determination of calcium [114–116], magnesium [117], chloride [117–121] and bicarbonate [107,117,119].

Two of the drawbacks entailed in the use of ISE-potentiometry in clinical laboratories are: the type of information these sensors provide (activity instead of concentration of the target analytes [103,122]; and the fact that the values obtained by direct potentiometry (undiluted samples [123]) are always higher than those provided by flame spectrometry used as a reference technique [103,104,107]. In addition, a series of factors that influence or condition activity measurement of the target analytes are: aqueous content of the sample [103,124,125], which is in turn a function of the dilution it requires; changes in the liquid junction potential, which is a consequence of the type of liquid junction used [92,99,126–128]; differences of activity coefficients due to the ionic strength of the medium [103,126]; and interactions between the sample matrix and electrodes

[129–132], especially from proteins [133–135] and heparin [136] usually present in blood and plasma, respectively.

Improvements in the design of ISEs are focused on avoiding or minimising these effects in order to enhance both selectivity and stability of these probe sensors, thus making feasible prior sample dilution [105,121,137]. The comparison of indirect potentiometry with more usual techniques such as FAES and AAS causes serious shortcomings to inexperienced clinical analysts. Despite this problem and others from proteins [133–135], mechanical resistance and interference from protons, glass-selective membranes for sodium ions have endowed ISEs with higher stability and mechanical resistance. The use of polymeric membranes modified with different agents (e.g. bis-(1-butyl-pentyl)adipate [100,117,141–143] and of ceramic membranes [111] has improved the mechanical resistance of these probes and new ionophore species have contributed significantly to improve their selectivity. Liquid valinomycin membranes enable the selectivity to be enhanced in potassium determinations [117, 138]. Polymeric membranes have been prepared using methylmonensin [117,144] calixarene derivatives [145–147] and *N,N,N′,N′*-tetracyclohexyl-1,2 phenylnedioxydiacetamide with or without neutral carriers [100,128,148]. Liquid membranes for sodium ions based on crown ether derivatives and on ceramic Nasicon membranes [142,149,150] have also been proposed.

Recent developments in calcium selective electrodes entail polymeric membranes modified with ionophores such as tetrakis-(4-chlorophenyl)borate and neutral carriers [103,151], with bicyclo polyetheramide [152] and with organophosphorus derivatives [153] and neutral exchangers [120].

Chloride ion has been determined in biological fluids using two main types of ISE: those based on liquid membranes involving quaternary ammonium salts [121,139,140]; and polymeric membranes impregnated with trioctyl ammonium chloride [154]. In contrast, bicarbonate ions in biological samples have chiefly been determined by gas-based selective electrodes, the concentration of the target analyte being calculated by the Henderson–Hasselbalch equation [155–158]. Ion-selective electrodes for carbon dioxide based on polymeric membranes impregnated with quaternary ammonium exchangers [89,110,117,159,160] have also been reported.

The development of ion-sensitive field effect transistors (ISFETs) and chemical-sensitive field effect transistors (ChemFETs) [115,161–163] has enabled the miniaturization of potentiometric probes for multicomponent analysis [115,162,163], which in turn has opened the door for in vivo monitoring.

It is worth noting the scant use in this field of other electroanalytical techniques such as coulometric titrations with amperometric determination of the end-point, which is the basis of the reference AACC method for chloride [164]. Use of polarography has been performed in the determination of clinical electrolytes. Single-sweep polarography has been used for the determination of calcium in serum (monitoring potential -0.65 V vs. SCE) [165] and oscillographic polarography for the sequential determination of calcium and magnesium ions [166]. Owing to the lack of selectivity, conductimetry has only been used when coupled with high-discrimination separation techniques such as chromatography [167–169].

2.3. Separation techniques

The development of continuous separation techniques over the last two decades [170] has endowed clinical analysis of inorganic ions with the required selectivity. The greatest contribution in this respect has been made by ion-chromatography, capillary electrophoresis and isotachopheresis. The main application of ion-chromatography is the sequential use of a cationic column for the determination of sodium, potassium, ammonium, magnesium and calcium ions, and an anionic column for the determination of chloride and bicarbonate ions. Conductimeters [168,169,171,172], spectrophotometers [173], both types of instruments [168,169,171], and potentiometers with ISEs [174] have been coupled to ion-chromatographs. Special mention must be made of an ion-pair chromatograph coupled to a fluorimetric detector for the indirect determination of sodium, potassium and ammonium ions [175]. Gel-filtration/fluorimetry for the determination of calcium ions [176] and HPLC-post column derivatization with *o*-cresolphthalein complexone have also been used for the determination of calcium and magnesium.

Capillary electrophoresis [178] and isotachophoresis [179] applied to the determination of sodium and potassium ions can be the starting point for an extensive use of these techniques

in the field of clinical inorganic-ion analysis. Batch separation techniques such as liquid–liquid extraction have only been occasionally used [70,180,181]. On-line, continuous liquid–liquid extraction could be a successful way of automatizing the determination of these ions in clinical laboratories. An example of the potential of these arrangements is the capillary-flow sensor for the determination of potassium in urine which has been coupled to segmented (CFA) and unsegmented (FIA) analysers [182].

2.4. Sensors

One of the most significant contributions to analytical chemistry of today is the development of (bio)chemical sensors [2,3] as they endow clinical instruments with the suitable degree of miniaturization, in addition to automation.

Probe-type electroanalytical sensors (ISEs, ISFETs and ChemFETs) are well known and they have been discussed in Section 2.2. For this reason, only optical sensors, both probe and flow-through types, are the subject matter of this section. These sensors are based on immobilization on the detection point of an agent which converts this into an active zone and gives rise to a chemical or biochemical reaction. Two types of sensors can be distinguished depending on whether the material is transiently or permanently immobilized on the sensing microzone: chemical and biochemical sensors. The name "biosensors" to designate the latter depends on the definition to be adopted; the authors working in this field consider as biosensors those devices in which either one or both the immobilized species and the analyte are of a biological nature [2].

An optic-fibre sensor designed for the determination of potassium ions belongs to the probe type [183]. It is based on the immobilization of valinomycin bound to dicarbacyanine at the end of the optic-fibre. The technology involved in the immobilization process is similar to that used for the development of ISEs based on PVC polymeric membranes. A flow-through sensor for potassium uses valinomycin/fluorescein immobilized on the walls of the flow cell via polymeric membranes [184].

Selective optrodes for the determination of sodium ions in plasma have been based on the immobilization of selective neutral ionophores combined with a proton-selective ionophore [185]. A similar principle has been used in the development of a sensor for calcium ions [186,187].

In a wide sense, biosensors for clinical electrolytes can be based on activation or inhibition of an enzymatic reaction taking place at the detection point. Biosensor flow-through systems, which can be the basis for these biosensors, have been recently reported for the determination of sodium [188], potassium [189,190], magnesium [191,192], ammonium [193], phosphate [194] and bicarbonate ions [195].

3. Analysers

Without doubt, clinical analysis has been the area of analysis in which automation of the analytical process has attained a higher degree of sophistication [1]. Aspects such as number of samples to be processed, multicomponent analysis, urgency in obtaining reliable results in short time, and the clear necessity for avoiding or minimizing human participation in order to avoid errors, created a suitable frame to pursue and achieve these goals. At present, clinical analysers offer a high degree of sophistication and automation; nevertheless, and despite the "flashes" of commercial catalogues, these analysers do not provide full automation of the analytical process.

The design of new analysers based on different batch, segmented and unsegmented modes, is the target research of a pleiade of scientists. Two drawbacks hinder the diffusion and commercialization of the new approaches: first, they are infrequently published in clinical journals which show a clear preference for methods developed with commercial, well-established analysers, which in turn provide very scarce versatility; and, second, manufacturers are not prone to change the usual production.

Both the number of species to be determined (which in turn allows a basic classification into mono- and multiparameter analysers) and the information required play a decisive role in the design of commercial analysers.

The design and commercialization of new analysers in the last ten years have been focused on the automation of one or several steps of the analytical process: sampling and sample treatment (i.e. introduction of the sample into the analyser and conditioning); measurement and transduction of the analytical signal, where the development of microelectronics has played

a crucial role, helping to enhance the selectivity and sensitivity achievable by analysers; and data acquisition and treatment. The astonishing development of computer science allows easy data manipulation and a better interpretation of the results. The joint use of microelectronics and computer science supports control and calibration systems in clinical analysers.

The detection techniques most commonly use in clinical analysers for the determination of electrolytes are spectrophotometry and potentiometry (ISEs). The increased degree of automation is used in commercial kits in autoanalysers in order to increase speed and simplicity but it also dramatically increases the cost of the analysis. Several reports in clinical journals are devoted to the comparison of both analysers and kits [67,196–198].

The use both in routine and research of segmented-flow analysers reached a peak in the past two decades; however, these analysers now appear to be used only as reference analysers for newer systems [199,200]. Innovative research has been developed using non-commercial flow systems, such as flow-cytometry coupled to fluorimetric detection [62], or the coupling of completely continuous-flow manifolds to separation techniques [201] and ion-selective analysers [153,202,203]. The drawbacks entailed in coupling atomic techniques to the design of new electrolyte analysers can be circumvented using unsegmented-flow analysers (namely, flow injection analysis, FIA) as shown in arrangements such as FIA-AFS [12], and FIA-AAS [34,41], among others.

Batch analysers are most commonly used in clinical laboratories for the determination of inorganic species, with a clear predominance of those based on ISEs (93%) compared with photometric analysers (5%).

Special mention must be made of multiparameter analysers based on dry-chemistry technology with reflective or electroanalytical detection. Among these analysers the Kodak Ektachem is extensively used in clinical laboratories for the determination of sodium, potassium, calcium, chloride, magnesium and phosphate ions [204–209].

4. Methods

Methods for the determination of clinical electrolytes can be classified according to whether or not they require a (bio)chemical reaction.

4.1. Methods without (bio)chemical reaction

The most common method for the clinical determination of sodium and potassium uses FAES with Li or Cs as the internal standard and makes a prior 1:200 sample dilution. Improvements on this method have been adressed to achieve greater automation [6,8] and to develop the steps prior to measurement using a flow-injection manifold on-line with the detector [41,201]. An unusual method for the determination of sodium is based on neutron activation [210].

ISEs provide the second most used alternative in clinical laboratories for the determination of sodium and potassium. The usual procedure involves a 1:200 sample dilution before the potentiometric measurement, which is improperly called indirect potentiometry. FI manifolds have also been proposed in which arrays of ISEs allow simultaneous determination of several electrolytes [211] and the use of miniaturized ISEs [112]. Nevertheless, the most important contribution, especially for in vivo monitoring, is made by potentiometric methods without sample dilution [99,107,112–216], despite the problems associated with comparing the results with those provided by other methods such as those based on AES or even potentiometric methods with prior dilution. A question difficult to answer is which method — with or without dilution — provides results more closely related to the physiological state of the patient. A shortcoming of the undiluted sample method is the design of a suitable procedure for calibration.

Methods based on capillary electrophoresis [178,179], ISFETs, ChemFETs [115,214], and ion-chromatography [168,169,174] for clinical electrolytes are relatively recent, and their use in clinical laboratories is still scant.

4.2. Methods involving (bio)chemical reactions

Methods for clinical electrolytes involving derivatization reactions can be divided into two categories depending on the nature of other components of the reaction: derivatization methods; and enzymatic methods.

Methods based on chemical derivatization

These are specially abundant for calcium and are usually based on complex formation.

Ligands such as *o*-cresolphthalein complexones [42–44], arsenaze III [45,46] and chlorophosphonaze III [47,48] have been reported for the individual determination of this analyte, as well as for joint determinations [47,48].

Of special interest is the photometric method for the determination of protein-bound calcium based on solid-phase retention of the analyte in calsequestine immobilized on Sepharose 4B, reaction with ruthenium red and monitoring a 533 nm [49]. In addition, a method for calcium based on tribromoarsenaze and monitoring at two wavelengths allows the enhancement of selectivity in the presence of iron and aluminium ions [50]. Development of new fluorophores such as Quin-2 [51,52], Fura-2 [53–55], Fluor-3 [56,57] and indol-1-acetoxymethyl ether [58,59] has made feasible the development of fluorimetric methods to be implemented in living cells for speciation of intra- [51,52,54,58–60] and extracellular calcium [55–58]. Methods based on flow-cytometry [56,62] and video image processing [61] using a reaction with Fura-2 and fluorimetric detection for ion-free intracellular calcium have been reported, as well as others based on complex formation with calceine [63,64].

Complex formation has also been extensively proposed for the photometric determination of magnesium and phosphate based on the use of calmagite [65,66] or magneson [45,67] and ammonium molybdate [48], respectively.

Potassium–crown ether complex formation with subsequent liquid–liquid extraction into an organic solvent and spectrophotometric detection [70] was the starting point of colourimetric methods for potassium, which gave rise to the commercialization of water-soluble chromogenic ionophores such as Chromolyte^{R} (Technicon) used at present for the determination of potassium and sodium ions [69].

Special mention must be made of methods involving chemical reagents immobilized on the end of an optic-fibre or on the walls of a flow-cell (probe-type and integrated flow-through chemical sensors, respectively [2]), with a very promising future for in vivo measurements and miniaturization/automation, in general.

Enzymatic methods

These endow clinical electrolyte determinations with suitable selectivity. Both inhibitory and activation effects of inorganic ions on enzymatic reactions are the basis of these methods. After the enzymatic methods for sodium and potassium reported by Berry and co-workers in 1988 [72–74], others for sodium [75,76,188], magnesium [77–83,191,192], phosphate [84,194], chloride [85] and carbon dioxide [86,87] based on the use of biocatalysts have also been proposed. The most usual method for phosphate has been based on the activation of glyceraldehyde-3-phosphate dehydrogenase [215], phosphoglucomutase [216, 217] and nucleoside phosphorylase [218,219] with photometric detection. In addition, nucleoside phosphorylase and xanthine oxidase immobilized and located in a flow-injection manifold would have been used for the amperometric determination of phosphate [220]; a xanthine oxidase–nucleosidephosphorilase–peroxidase system immobilized and located in a flow-injection manifold coupled to a fluorimetric detector has been used for the same purpose.

Activation of kinases has been the principle of the enzymatic determination of magnesium. Hexokinase [77], glucokinase [79], and glycerol kinase [78] have been used together with other coupled reactions to yield a coloured (use of β-galactosidase [67,191]) or luminescent (use of lucipherase [221]) monitored product formed in an unsegmented-flow manifold.

Less numerous are enzymatic methods for other clinical electrolytes such as: that reported for bicarbonate using phosphoenol pyruvate carboxylase [87,195,222]; that for calcium with phospholypase D [223]; and that for sodium with β-galactosidase [72,74,188]. Enzymatic methods have also been proposed for potassium based on its activation effect on pyruvate kinase [73,74,189,190] and tryptophanase [223].

4.3. Dry-chemistry reagents

The use of dry-chemistry methods in clinical laboratories is now increasing as a result of easy handling and acceptable precision [224–226]. These anhydrous reagents are used for the determination of enzymes, metabolites and electrolytes. The methods for electrolytes are based on selective membranes and potentiometric or reflectometric detection.

Dry-chemistry methods with potentiometric monitoring [227,228] use two electrolytic hemicells with an upper layer of selective membrane: methyl monesine for sodium; valinomycine for potassium; quaternary ammonium resin for carbon dioxide; and a protective layer of cellulose acetate for chloride which minimizes interference from other halogens. Kodak Eastman

has contributed noticeably to the development of these reagents [229,230]. Measurements are implemented in undiluted samples: thus the results are comparable with those obtained with conventional ISEs based on the same methodology.

Reflective monitoring is used in dry-chemistry methods for potassium [231], sodium and chloride in urine [232], and also for calcium, phosphate and magnesium [223,234].

5. Biological samples

The biological fluids in which clinical electrolytes have been determined and their relative abundances are shown in Fig. 1.

6.1. Techniques

In addition to the evolution of detection techniques used at present for the determination of clinical electrolytes, other very sensitive and selective detection techniques such as adsorptive stripping voltammetry will be employed in order to obtain lower determination levels, which will enable the use of more diluted samples.

Miniaturization, one of the most powerful trends of today's analytical chemistry, will play one of its more important roles in the design of sensors for individual determinations, but especially for multideterminations. The principle behind these sensors could be ISFET and ChemFET technologies in single or array configuration; or optical probe-type and flow-through integrated (bio)chemical sensors aided by integrated microcircuits [235]. The final goal of miniaturization is twofold: in vivo measurements; and/or decrease of the sample volume.

A major use of continuous separation techniques will be focused on chromatography coupled on-line with commercial analysers, as well as extensive use of isotachophoresis and capillary electrophoresis. Other non-chromatographic continuous separation techniques such as liquid–liquid extraction can help optical molecular techniques to achieve suitable selectivity.

6.2. Methods

In dealing with clinical methods for electrolytes, the development of (bio)chemical methods involving enzymes and immunoassays is foreseeable. Conventional methods and also those based on the use of commercial kits with biochemical reagents in solution, especially those based on dry-chemistry, will be prominent in the future of electrolyte analysis.

6.3. Analysers

A starting point for changing the present situation of commercial analysers is to pay more attention to research and development. Reports of advances must be published in clinical journals in order to be more available for personnel working in clinical laboratories; these workers, in turn, must be open to new designs capable of circumventing "chronic" problems in the area of electrolyte analysis.

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