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Ion sensors: current limits and new trends

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Abstract

The current status of ion sensors in their main application, clinical chemistry, is highlighted. The reasons for the practical success of sensors in this particular area are discussed together with the expected influence of novel technical possibilities for the next generation of clinical ion analyzers. A series of recent research results, including the improvement of lower detection limits, the establishment of selectivities that are much better than reported so far, and new types of reference electrodes will very likely open up numerous new fields of applications of these sensors. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

Ion-selective electrodes (ISEs) are the chemical sensors of longest history and probably still the largest number of applications. More recently, optical sensors based on partitioning of the analyte between the sample and the bulk of the sensing film (bulk optodes) exhibited a rapid development. Given the closely related detection mechanism, many of the characteristics such as selectivities and detection limits are comparable for both types of transduction [1]. Also the possibility of miniaturization is similar with the

two transduction techniques. The recent advances of fluorescence based bulk optodes [2] have led to dimensions comparable to those of ion-selective microelectrodes introduced decades ago [3] for activity measurements in cells or, in some cases in cell nuclei. So far, ISEs have been described for about 60 analytes, about twice as many as optodes [4]. However, it would be a relatively straightforward task to develop bulk optodes for the remaining 30 analytes on the basis of their potentiometric counterparts. As to ruggedness and response time, especially at submicromolar activities, ISEs have clear advantages.

This review article summarizes the current status with emphasis to blood electrolyte measurements, the most advanced routine application, current research and development for improving ruggedness and transfer ISEs to other fields of application, and finally

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presents very recent research results, likely to open up new dimensions in potentiometric ion sensing.

2. Current status

With billions of measurements performed each year with corresponding instruments in nearly every hospital all over the world, the largest area of routine application of ISEs today is clinical analysis [5–7]. There are two basic reasons behind this success. First, clinical demands for the assay of ions (electrolytes) nearly perfectly meet the possibilities offered by ISEs. Electrodes are available for the most important clinically relevant ions (Na^+ , K^+ , Cl^- , Ca^{2+} and H^+) and can be directly used in biological fluids such as whole blood, serum, plasma, and urine. The activities of the free, ionized fractions are in the mmol l^{-1} range (except H^+ ; blood pH being close to 7.4) and thus conveniently accessible with ISEs satisfying the required (within and between-day) accuracy and precision (Table 1). There is a straightforward combination with other electrochemical sensors for the blood gases O_2 and CO_2 and for metabolites such as glucose and urea. A direct, fast and non-expensive measurement is possible and maintenance-free, ready-to-use ion sensors with long life time are available. The second reason of success is market related. Since determination of electrolytes is the most frequent request in general clinical analysis (Fig. 1) the relatively low production costs of ion sensors coupled with the requested volume create an opportunity for a decent return on investment. The market for in vitro diagnostics by 1992 reached about 13 billion US \$ per

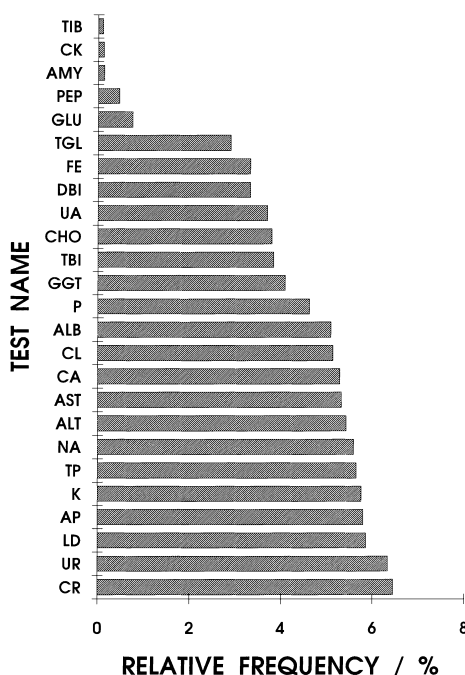


Fig. 1. Relative frequency of analytes determined in blood samples. Abbreviations: CR, creatinine; UR, urea; LD, lactate dehydrogenase; AP, alkaline phosphatase; K, potassium; TP, total protein; NA, sodium; ALT, alanine transferase; AST, aspartate transferase; CA, calcium; CL, chloride; ALB, albumin; P, inorganic phosphate; GGT, gamma globulin transferase; TBI, total bilirubin; CHO, cholesterol; UA, uric acid; DBI, direct bilirubin; FE, ferritin; TGL, triglyceride; GLU, glucose; PEP, phosphoenol pyruvate; AMY, amylase; CK, creatinine kinase; TIB, total iron buffer.

year, of which the volume for ion-sensor based analyzers is estimated to be close to 800 million dollars [8]. This sum is by far larger than any other turnover in other application of ISEs, such as environmental and process control applications.

Because of the general demand of quality assurance and traceability, analytical and clinical chemists gathered in committees of the International Federation of Clinical Chemistry (IFCC) to work on recommendations on 'sampling, measurement and reporting' of all analytes covered by sensors. Unfortunately, besides general recommendations on the use of ion sensors for measurements in blood [9], guidelines are so far only available for pH measurements [10]. Although corresponding recommendations are very advanced in other cases, e.g., for Na^+ and K^+ [11], their publication is still delayed due to the lengthy process of an IFCC approval.

Table 1
Reference intervals and required precision of analysis for major ionized fractions of electrolytes in serum and urine

	Serum (mmol l^{-1})	CV (%) ^a	Urine (mmol day^{-1})
Na^+	135–145	<1	40–220
K^+	3.6–5.4	<1	35–85
Ca^{2+}	1.17–1.29	<2	3–8
Mg^{2+}	0.55–0.67	<2	2.5–8.5
Cl^-	97–107	<1	85–170

^a Required within-day precision reported as coefficient of variation. The required limits for between-day precision and accuracy are <2% and <3% for single and double charged ions, respectively.

3. Recent developments

The aim of sensor research for many years was to produce rugged, miniaturized, solid-state devices, with rapid, sensitive response and exquisite selectivity that could be mass produced at low cost. This would open a whole array of applications which would lead to the development of a very successful commercial sector associated with sensor manufacturing and applications. The reality is that, aside from physical transducers and certain gas sensors, the only real success has been in the blood electrolyte/gases market. The approach was to reproduce the success of the electronics industry through the adoption of 'system integration' concepts. This requires the manufacturer to address all issues related to a particular application. For example, in blood analysis, the 'integrated sensing system' must be able to measure the required blood parameters (see above) under controlled conditions (35°C). Furthermore, the array of sensors must be exposed in a very reproducible manner to the sample, and after measurements, the array must be conditioned in a reproducible way before the next sample is introduced. Hence, the design must integrate sensor array fabrication with sample and reagent handling. The need for reduction of waste and sample volumes, and the increasing demand for point-of-care analysis (i.e. distributed measurement rather than centralized) has driven the development of these miniaturized sensor arrays.

One of the main targets for those involved in ion-sensor fabrication has been to eliminate the liquid internal filling solution used in conventional bench-type ion-selective electrodes, as it is difficult to fabricate miniaturized versions of these sensors in a reproducible manner if the liquid has to be trapped inside the electrode. This goal has been achieved through the use of materials such as hydrogels doped with the appropriate salt, i.e. one which can decouple charge transfer between the internal PVC-membrane boundary and the internal Ag/AgCl reference electrode [12]. These 'pseudo' solid-state sensors are more compatible with techniques employed in the electronics/semiconductor industry such as thin/thick film deposition techniques, drop-on-demand liquid handling and the use of photo-polymerizable polymers [13]. We are now at a stage where, for the first time, arrays of very reproducible potentiometric sensors are

Central channel with sensor array

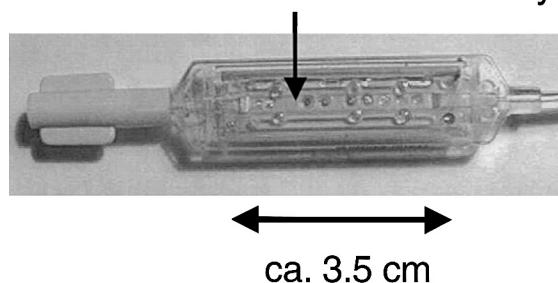


Fig. 2. Sensor array flow cell manufactured by the SenDx Corporation, Carlsbad, CA. The array contains solid-state PVC-membrane electrodes for Na^+ , K^+ , Ca^{2+} and pH which are visible as small features in the flow channel.

being mass produced in a miniaturized format. Typically, the array is packaged into a flow cell to enable sample and reagent handling to be integrated and controlled (Fig. 2). The development of new surface analysis techniques such as variable pressure scanning electron microscopy [14] means that non-conducting samples such as soft ion-selective PVC membranes can be directly imaged even when hydrated [15], without any sample preparation (Fig. 3). Coupled with scanning techniques such as energy dispersive spectroscopy (elemental distributions) and atomic force microscopy (atomic scale imaging), effects such as surface fouling and swelling may be conveniently studied, and the membrane composition optimized.

A number of factors need to be considered to implement a technological innovation in practice. An intrinsic advancement in sensor technology as discussed above is a prerequisite for the advancement of commercial instruments. However, both producers of clinical analyzers and their customers are at present pushed to rationalize the budgets, and look for *added value* (see also Table 2). For the end user the total cost per analysis is decisive. This means that the cost reduction of a technological advancement may sometimes be overshadowed by the overall cost of the instrument, consumables, service and labor. In this way the inventor is a part of a comprehensive process of generating not only good quality analytical results but a substantially positive *added value* to market sectors of his/her firm and a medical laboratory or site [8]. In recent years, e.g., technological advances have made it possible to perform all relevant blood ion

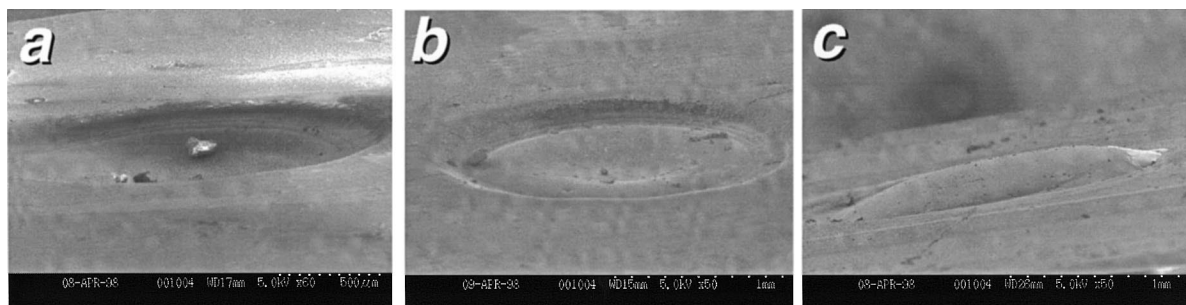


Fig. 3. Variable pressure scanning electron micrographs of the SenDx solid-state PVC-membrane ISEs: (a) a newly made unconditioned membrane; (b) a sodium membrane which has been left to hydrate for one day; (c) a calcium membrane which has been hydrating for 11 days. The micrographs were obtained with no sample preparation using a Hitachi 3000 series instrument [15].

analyses at the bedside of clinical patients by the use of disposable integrated sensor cartridges. Such rapid assays are however currently more expensive than those performed in centralized clinical laboratories. The future will show to what extent analysis speed, overall cost, and patient health will determine the market share of each method.

Table 2
Factors involved in the development of clinical analyzers

Marketing factors (784.5 M US \$ in 1996)

Return on investment
Customer satisfaction

Goal oriented factors

Place of analysis
 Bedside (portable system)
 Central laboratory (random access analyzer)
Cost per analysis
Throughput
User friendliness

Technology driven factors

R&D power
Ability of implementation
Manufacturing possibilities
Type of analyzer
Customer interface

Country dependent factors

Economical situation
Social security system
Organization of health care
Tradition
 Total CO₂/pCO₂
 Ionized calcium/total calcium
Quality control and homologation
e.g., ISO 9000, FDI

4. Applications beyond clinical chemistry

While the ‘integrated systems’ concept has been realized for the blood analysis market, it has not been adopted for applications in other areas such as environmental monitoring. There are several reasons for this:

1. Potentiometric sensors for the species of interest (e.g., heavy metals, organic waste, various inorganic and organic anions) either do not exist or do not meet the required specifications in terms of selectivity and/or limits of detection. The emerging possibility of lowering the limit of detection [16] of these devices by at least three orders of magnitude (see below) is therefore a major breakthrough, as it will bring applications (e.g., analysis of lead in drinking, surface or waste water) within the scope of potentiometry for the first time.
2. The market is relatively fragmented in that each industry has a different ‘wish list’ in terms of the species to be monitored and the concentration range of interest. This is in contrast to the blood analysis market where the composition and concentration range is relatively constant, and the specifications therefore can be precisely defined.

However, with better lower limits of detection, the ability to mass produce miniaturized sensor arrays, the gradual improvements in the characteristics of ionophores, and the optimization of membrane components, there are great commercial opportunities for companies willing to get involved in adapting the technology. For example, potentiometric sensor arrays

provide the only route to simultaneous analysis of mixtures of cations and anions with a compact, portable, low-cost instrument. Capillary electrophoresis is the only other technique which can provide rapid analysis of anion/cation mixtures [17], but it requires the maintenance of a stable high-voltage, typically 20–30 kV, which makes it difficult to design a light-weight instrument capable of being used in a field situation. However, to realize the commercial potential of ion-sensor arrays, a closer working relationship between sensor manufacturers and university research centers is of critical importance.

Now that reproducible arrays of ion sensors are being mass-produced, and the characteristics of the individual sensors are becoming more predictable, the potential for developing ‘intelligent integrated sensing systems’ will begin to be exploited. For example, if sensors are made small enough, it will be possible to introduce redundancy in the array. This means that we could have, e.g., five or six replicate sensors for each analyte rather than one. Consequently, the user would receive the mean and standard deviation of six independent determinations of the analyte in each assay. Faulty devices could be identified by prediction polling and switched out of the array, and the presence of interferents identified and their effect on the signal estimated and compensated. It should be stressed that this utopia could only be realized if certain conditions exist. The failure rate in manufacturing would have to be extremely low, and the devices very reproducible, in order to simplify calibration.

Ion-selective electrodes are a mature technology. Given the emergence of potentiometry as a technique capable of rapid analysis of cation/anion mixtures at low concentration levels, the ability to mass produce micro-dimensioned arrays of these sensors, and the availability of powerful surface characterization and visualization methods, we can expect to see a significant increase of research activity in this area.

5. New trends

Recent research has shown that a significant number of potentiometric ion sensors show extremely high ion selectivity. Interestingly, the relatively high detection limit around the micromolar range prevented the measurement of the true ion selectivity with routine

methods. Modified methods have shown that ion selectivities are sometimes better by 6 or more orders of magnitude than traditionally reported [16,18]. In practice, this means that on the basis of their ion selectivity, ISEs may indeed be used for trace level analysis. This insight has formed the basis of understanding and improving the detection limit of this widely established class of ion sensors.

Very recently, phenomenal improvements in the detection limit of ISEs have been reported [16]. It has been shown that transmembrane ion fluxes may perturb the local ion concentration at the surface of the membrane [19]. Since ISEs are sensitive to ion concentration changes at the phase boundary, such an accumulation of ions makes it impossible to measure dilute samples. The origin of such ion fluxes are manifold, as both codiffusion and counterdiffusion processes can be relevant [20,21]. Pb^{2+} ISEs with picomolar level detection limits have been realized by adding a chelator to the back filling solution of the membrane, inducing a small ion flux away from the sample in direction of the inner side [16]. While research continues to fully understand and optimize such intriguing systems, it has been clearly shown that potentiometric sensors can be used for trace level analysis (Fig. 4). Apparently, ISEs will be able to favorably compete with most other analytical techniques capable of measuring extremely low analyte levels. Considering the unique response characteristics of these devices, this is an important contribution to analytical chemistry.

Ion fluxes in potentiometric membrane electrodes can also be beneficial in some cases. It has been shown that various levels of acid (such as acetic acid) in the back filling solution of ISEs may lead to substantial acid fluxes across the membrane [22]. This effectively acidifies the surface layer of the ISE membrane, even in relatively well buffered samples. This concept is one important example to show that potentiometry is not synonymous with equilibrium measurements. Substantial sample perturbations may be performed at the surface of such electrodes. An array of specially formulated pH sensors, e.g., may be used to determine the buffer capacity of the sample without having to perform dedicated pH titration experiments. Local acidification is also necessary in other cases, e.g., with anion sensors where hydroxide interference may occur, or with calcium sensors where the sample

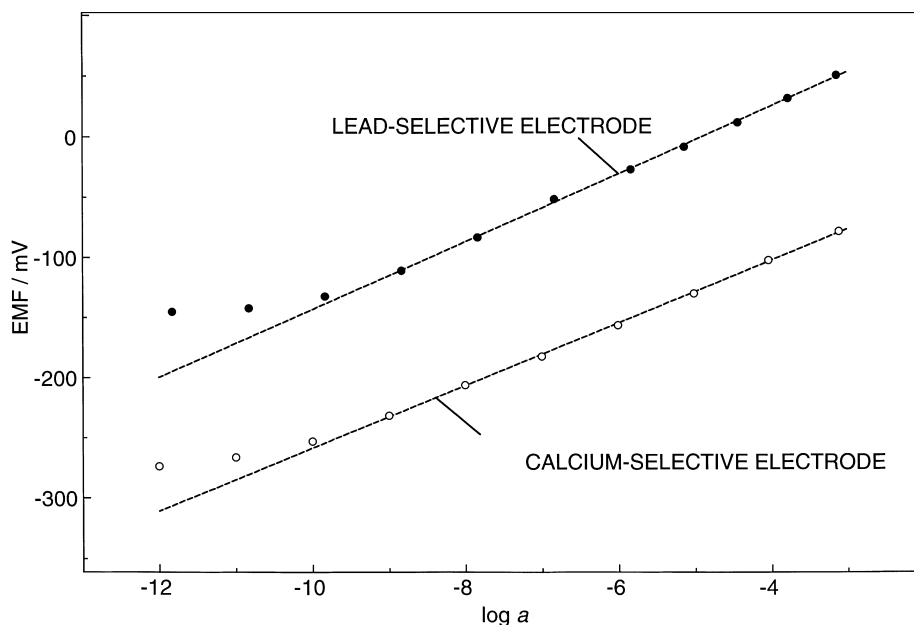


Fig. 4. Response of a Pb^{2+} - and a Ca^{2+} -ISE optimized for extended lower detection limits [21]. Pb^{2+} -ISE: 1.0 wt% 4-*tert*-butylcalix[4]arene-tetrakis(thioacetic acid dimethylamide, 0.8 wt% sodium tetrakis-[3,5-bis(trifluoromethyl)phenyl]borate (NaTFPB), 66.51 wt% bis(2-ethylhexyl)sebacate, and 33.28 wt% poly(vinyl chloride); inner filling solution: 10^{-2} M Na_2EDTA , 10^{-3} M PbCl_2 , and 2 M NaCl, at pH 4.5. Ca^{2+} -ISE: 1.3 wt% *N,N*-dicyclohexyl-*N',N'*-dioctadecyl-3-oxapentanediamide, 0.6 wt% NaTFPB, 65.3 wt% 2-nitrophenyl octyl ether, and 32.8 wt% PVC, membrane thickness: 3 mm, inner filling solution: 5×10^{-2} M H_2SO_4 .

needs to be acidified if total calcium is of interest. Dedicated membranes that effectively perturb surface concentration is a completely new research direction that may increase the number of applications where ISEs are used.

Surface concentration perturbations have also been exploited to make ISE membranes more biocompatible. The group of Meyerhoff, e.g., has incorporated nitric oxide releasing compounds within ISE membranes [23]. Upon contact with the sample, NO is released and decreases the extent of platelet adhesion to the membrane in platelet rich plasma samples. This concept is intriguing since the NO concentration is only high at the actual membrane surface, while the bulk sample perturbation is neglectably small. The group of Bakker has used specially formulated ISE membranes with high concentrations of a polyanionic anticoagulant such as heparin or liquid in the back filling solution to improve the biocompatibility of ISE membranes [24]. The ion flux from the inner membrane side leads to an elevated anticoagulant concen-

tration at the membrane surface, thereby effectively prohibiting fibrin formation at the ISE membrane. These are new, highly effective concepts to improve the characteristics of potentiometric ion sensors on the basis of non-thermodynamic approaches.

The groups of Meyerhoff and Yang [25] have established a novel ISE research area to determine a group of highly charged analytes such as heparin and protamine. These sensors also function in a non-thermodynamic fashion (see above), as pseudo steady-state ion fluxes determine their response. Consequently, at short measuring times, the electrode response apparently does not follow the Nernst equation which would only allow a non-useful slope of one millivolt or less per 10-fold analyte concentration change. While dedicated calibration and conditioning procedures are required that differ from those used with classical ion sensors [26], the determination of heparin in blood samples is now possible [25]. This shows that ISEs can be developed successfully for rather unusual analytes of practical importance.

Mass fabrication of ISE arrays is now routine. However, to date no reliable concepts are available to miniaturize the reference electrode in the same way as the sensing electrode. From a manufacturer's standpoint, it would be most convenient to use reference electrodes that are based on the same materials as the actual sensing membranes. Unfortunately, much of the work in this direction has not been performed critically enough as most of the empirical solutions that have been offered show severe principal drawbacks that will prohibit the wide use of these ideas (cf. [27]). An interesting concept in this direction has recently been proposed in view of an application in whole blood analysis [28]. It is known that polyion sensors (see above) show a very small Nernstian slope after conditioning the membrane for long periods of time in polyion solutions [29]. This is the basis for a novel type of reference electrode in view of a use in blood samples that contain a polyionic anticoagulant. Indeed, preliminary experiments have shown that a properly conditioned membrane loses its intrinsic chloride response behavior completely [28]. This and alternative concepts for manufacturing reliable miniaturizable reference electrode will be an important research direction in the future.

6. Conclusions

The introduction of ISEs in clinical analysis over the past decades is a major success story as it led to the nearly full replacement of an existing technology by a new one. Such a process is rather unusual in the medical field which is known to be particularly conservative and demanding especially high reliability and full traceability. Since blood and serum are very complex analytes, it is somewhat astonishing that ion sensors are not much more widely used in other fields. Numerous new developments including sensor arrays, new reference electrodes, polyion sensing, new ionophores, miniaturization for liquid chromatography and capillary electrophoresis detectors [30], and dramatically improved lower detection limits, open up a manifold of new possibilities and are likely to open up exciting new research activities in the field of ion sensing. One of the promising new areas of application is trace heavy metal analysis in environmental and biological samples.

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