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Point Of Care Testing of Ionized Magnesium in Blood with Potentiometric Sensors - Opportunities and Challenges

Wei Zhang*

Siemens Healthcare Diagnostics, Norwood, MA, 02062, USA
***Corresponding author**
Wei Zhang, Ph.D.
2 Edgewater Drive Norwood
MA 02062, USA.
Tel: 781 269 3756
Fax: 781 269 3220;
Email: w.zhang@siemens.com

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Abstract

Magnesium assays are being increasingly requested in hospitals and clinical research institutions. The ionized form of magnesium (iMg) is regarded as the biologically active fraction. Ionophore-based potentiometric sensors can determine iMg and apply a selectivity correction for co-existing interfering electrolytes, e.g. Ca^{2+}. This technology has been used in several commercial blood analyzers for measuring iMg in whole blood samples. This report reviews the utility of iMg in clinical settings with emphasis on point of care (POC) application, potentiometric Mg^{2+} sensor technology and challenges and opportunities for future ionized magnesium assays.

Keywords: ionized magnesium; hypomagnesemia; sensors; blood gas analyzers point-of-care testing.

1. Introduction

Magnesium (Mg) is the fourth most common metal in the human body and is the major intracellular divalent cation that plays an important role in chemical and biochemical processes. Recent experimental, epidemiological and clinical investigations have increased awareness of the range of biological functions of this "forgotten mineral" which include hundreds of enzymatic reactions, ion channel stabilization, energy metabolism, and contraction of myocardial muscle cells. The benefits of Mg testing on critically ill patients in acute admission, medical therapy, or cardiac surgery have been recognized [1, 2, 3] to play a role in preventing the development of serious and potentially fatal complications, especially for patients with poor food intake, malabsorption disorders, hypokalemia, hypocalcemia, alcoholism, or for those taking diuretics or other drugs associated with hypomagnesemia [4]. It wasn't until the mid 1990's when Mg^{2+} ion-selective electrodes became available for testing ionized magnesium (iMg) in blood samples that the use of iMg as a clinical diagnostic parameter began to be

extensively investigated. Many reviews have been published concerning Mg and iMg in general biological processes and specific pathological applications [5, 6]. G. Sanders et al. [7] published a special review on iMg and disease, which classified six major clinical situations related to Mg: renal disease, hypertension, preeclampsia, diabetes mellitus, cardiac disease, and the administration of therapeutic drugs. In 2000, Saris et al. [8] reviewed the role of iMg in clinical medicine, nutrition and physiology and summarized current analytical technologies for iMg and total magnesium (TMg) assays.

The human body contains 1000 mmol (24 g) of Mg of which ca. 60 % is present in bone. The remaining 40 % is nearly equally distributed between skeletal muscle and other tissues like heart and liver. Only 1 % of total body Mg is extracellular and its concentration is equal to the Mg concentration in the vascular compartment [9]. Mg (intra- and extracellular) exists in three states: (a) a free, ionized fraction (the physiologically active form); (b) complexed to anions, e.g., phosphate, bicarbonate, and citrate; (c) protein bound (mainly to albumin), the fraction which is non-ultrafilterable. The equilibrium between free and bound magnesium is influenced by temperature, ionic strength, pH and other ions competing with Mg for protein binding sites and chelators. Under normal physiological conditions in extracellular fluid, free iMg comprises 59-72 % of TMg, protein bound 23-31 % and the remaining 5-11 % is complexed with anions. These three fractions of Mg are in equilibrium with each other. The iMg fraction has major functional physiological roles including nerve conduction, skeletal, cardiac or uterine muscle contraction, as an ion channel adjuster, and serves as a cofactor for hundreds of enzymes. The iMg reference range is 0.45 to 0.66 mmol/L. Over the years, many different methods have been developed for determining Mg and its fractions (TMg and iMg) in blood samples (whole blood and serum/plasma). In 1934, the first colorimetric method for measuring Mg in biological fluids was described by Hirschfelder and Serles, [10] and this approach is still in use today. Other methods include enzymatic [11, 12], fluorometry (13), flame-emission spectroscopy and atomic absorption spectroscopy (AAS) [14] based methods. The latter approach has been proposed as the reference method for the determination of iMg in human serum/plasma [15]. Alternatively, in situ analysis can be carried out by X-ray fluorescence [16], and ^{31}P or ^{25}Mg-NMR spectroscopy is used for measuring intracellular Mg concentrations [17]. Although most of these methods are available clinically for determination of TMg in serum, plasma, urine, cerebral spinal fluid and other body fluids, none of them can determine iMg [9]. Until the development of Mg^{2+} ion selective electrodes (Mg-ISEs), the only method for assessing iMg in biological samples was ultrafiltration. While this process is capable of measuring free Mg^{2+}, it faces multiple problems including pH control and the need for a large sample volume. Additionally, the Mg^{2+}-anion complexes (bicarbonate, phosphate, citrate, etc) are filterable, testing an ultrafiltrate does not exclude Mg^{2+}-anion complexes when attempting to measure only iMg.

With increasing knowledge around the importance of Mg in biological processes, new iMg sensors are expected to meet clinical diagnostic requirements, especially POC applications where rapid results (< 120 s), small sample volumes (< 150 μL), and easy to use instruments requiring minimal maintenance are essential [25, 26].

2. IMg testing for point of care diagnostics

Although iMg is the most useful indicator for evaluating Mg status in blood, in most cases only TMg is measured in clinical laboratories. Measurements are made in plasma or serum by AAS or colorimetry. The TMg reference range in plasma and serum is 0.66 - 1.06 mmol/L. Although TMg assays are commercially available in many central lab analyzers, e.g. Cobas® (Roche), Advia® (Siemens), Architect® (Abbott) and Synchron® (Beckman Coulter), they are not easily applied to POC testing applications, e.g., testing in ED, OR, ICU and ambulance environments. Recent clinical studies [3] demonstrated the association of hypomagnesemia and mortality rates [6] in ICU patients experiencing hemodialysis [18], type 2 diabetes

[19], cardiovascular diseases [20], cardiovascular deaths, and medical surgical intensive care [21]. Monitoring and controlling iMg may be more relevant in patient care than TMg, especially when an abnormal protein concentration can be expected, as is often seen for critically ill patients [22, 23]. Notably, a weak correlation has been found between iMg and TMg in blood samples of patients requiring evaluation of their Mg status [24].

In heart disease patients, Mg deficiency contributes to coronary vasospasm, arrhythmias, fibrillation, infarction, and sudden death [5]. A study on Mg intervention during cardiopulmonary bypass operations [27] showed that intraoperative correction of iMg is associated with a reduction in postoperative ventricular arrhythmia and maintenance of an uninterrupted sinus rhythm. In this study, the iMg concentration was monitored using an ISE and adjusted by administering $MgSO_4$ during open-heart surgery. An investigation on patients with traumatic brain injuries [28] indicated that blood iMg significantly declined by 29 % to sub-therapeutical levels at 30 min post-injury and remained depressed for the next 24 hr. In contrast, no change was observed for blood TMg which remained constant for the duration of the study. POC testing on iMg rather than TMg has been proposed to be a predictor of the long-term neurobehavioral outcome following head injury and ischemia stroke [29, 30, 31]. Clinical trial results [32, 33, 34] suggest a benefit for Mg therapy for acute stroke patients in the ambulance or ED within the first two hours of the onset of stroke symptoms. Mg monitoring is also advocated in preeclampsia [35, 36], in a condition reported to be linked with hypomagnesemia [37, 38, 39, 40] and which occurs in 5-7 % of pregnancies in Europe and the USA. Magnesium sulfate treatment has been used successfully for more than 80 years to minimize the increased vascular reactivity, hypertension, cerebral ischemia, premature labor, and convulsions that are associated with this condition. Very recently, Apostol et al. [41] reported findings suggesting that the ratio of iCa:iMg is a crucial diagnostic parameter for prevention of vascular and neurological complications in preeclampsia-eclampsia patients. In critically ill patients, hypomagnesemia is frequently found upon admission to the ICU, a typical POC testing location, and Soliman et al. [3] reported a correlation between the onset of ionized hypomagnesemia during ICU stays and high morbidity and mortality rates. This work noted that iMg is a stronger predictor for hypomagnesemia outcomes compared to TMg.

The current low level iMg assay requests in clinical practice is partially attributable to poorly defined clinical parameters and interpretative guidelines of iMg relative to total Mg as well as the lack of reliable and convenient methods for measuring iMg compared to TMg.

3. Potentiometric iMg sensors in clinical analyzers

3.1. Ionophores and selectivity

All commercial iMg sensors are based on neutral ionophore-based potentiometric Mg^{2+} sensors. The features of the ionophore determine the Mg-ISE performance (sensitivity, selectivity against interference, response time, and use lifetime). Development of Mg^{2+} ionophores started in the 1980s and the exploration for selective ionophores in Simon's group at ETH Zurich led to identification of more than 50 compounds [42].

The charge, radius, polarity and the polarizability of Mg determines the nature of its interaction with the ionophore (Table 1). Mg^{2+} has highest charge density among common cations and is preferentially complexed in an aqueous environment by anions with a high charge density, e.g., ATP^{4-}, oxalate, malonate. The octahedrally-coordinated water of hydration is more difficult to displace for Mg^{2+} than Ca^{2+} and other alkaline earth cations. The major challenge in Mg^{2+} ionophore development is to obtain selectivity for Mg^{2+} over all other cations. Mg^{2+} has a higher free energy of hydration (1898 $kjmol^{-1}$) than other mono- and divalent cations. To interact with Mg^{2+} in aqueous samples, the ISE membrane has to compensate for this high $\Delta G_{hydration}$ through the strength of the ionophore binding with Mg^{2+} and through the subsequent transfer of the Mg^{2+}-ionophore complex to the electrode membrane solvent.

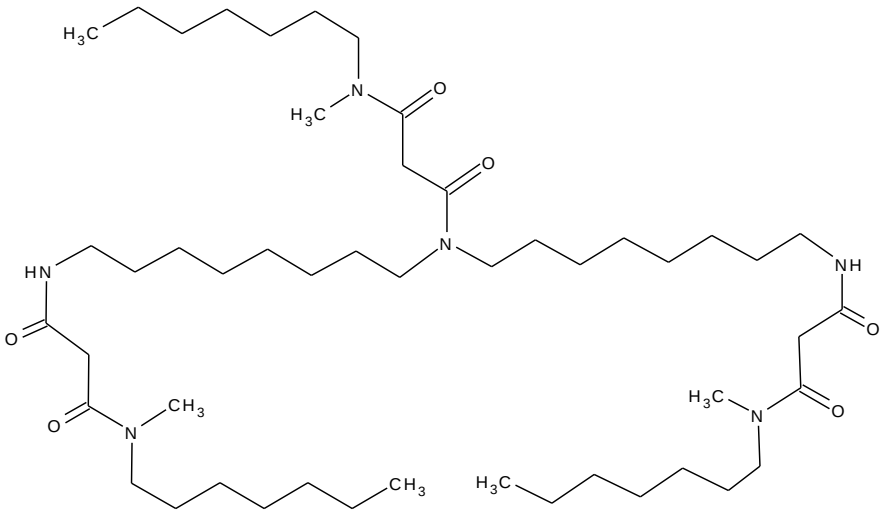
Table 1. Alkali and alkaline earth cations [43]

Cation	Ionic radius (Å)	Hydration number	Surface charge density (z Å^{-3})	Polarizability (Å^3)	$-\Delta G_{hydration}$ (kjmol^{-1}, 25 ℃)
Li^+	0.76	6	0.13	0.03	510
Na^+	1.02	6	0.08	0.3	412
K^+	1.38	6	0.04	1.1	336
Mg^{2+}	0.72	6	0.26	0.2	1898
Ca^{2+}	1.12	8	0.14	0.9	1584

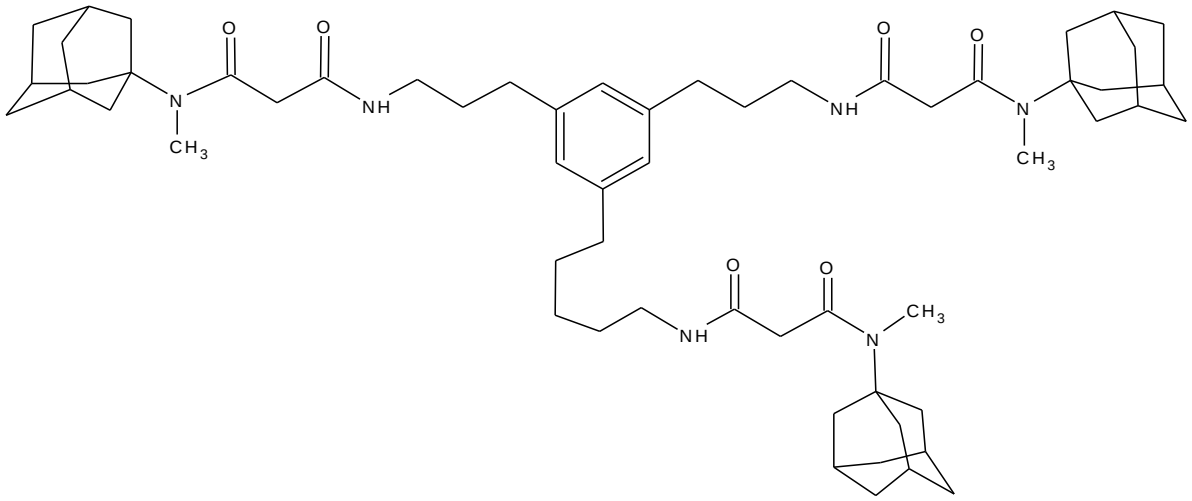
For Mg^{2+}, the binding strength of various ligands is ester < ether < phosphate < amide < amine, which is based on the donicity values of simple organic molecules in Lewis acid-base theory [44]. On this basis several types of Mg ionophores were designed and developed (Figure 1). Most Mg ionophores contain (i) diamide side chains, which offer selective binding sites for alkaline earth cations (e.g., Mg^{2+}, Ca^{2+}) against alkali cations (e.g., Na^+, K^+); (ii) lipophilic subunits at the terminals of the side chains, which offers adequate lipophilicity for the Mg-ionophore complex; (iii) stereo-structured side chains with spacer units designed to maximize the selectivity for Mg^{2+}.

Modern potentiometric sensors based on polymer membranes exhibit well-defined ion-exchange properties, and the sensor response may in many cases be quantitatively described by the phase boundary potential model of the Galvanic potential between the aqueous sample and the membrane phase [45]. This model has been used to relate the selectivity coefficients of potentiometric magnesium sensors to underlying thermodynamic parameters including complex formation constants and stoichiometry [46]. Since the stoichiometry of ionophore:cation complexes may be different for Mg^{2+} and Ca^{2+} (the main interfering cation to Mg^{2+} in blood sample analysis), Mg^{2+} and Ca^{2+} can be differentiated in their potentiometric responses by adjusting the concentration of the lipophilic anion salt in the sensor membrane formulation [47, 48]. For example, tripodal structured Mg^{2+} ionophores, or tris(malondiamides), e.g. ETH 7025, ETH 3832, ETH 5506 and ETH 5504, form 1:1 complexes with Mg^{2+} and 2:1 complexes with Ca^{2+}. The modeling of mass balance and electrostatic balance in the interfacial exchange of cation and cation-ionophore complexes leads to the optimal theoretical molal ratio of 1.5 (lipophilic sites:ionophore) for the best selectivity of Mg^{2+} over Ca^{2+} [46], which has been verified experimentally. The most selective ionophores in this class are ETH 5506 and ETH 5504 with selectivity coefficients against Ca^{2+} of $10^{-2.3}$ (Separate Solution Method, or SSM). In the mid 1990s, a group of double-armed, diazacrown ether based ionophores were developed by Suzuki et al. [49] which provide a hydrophilic space for cations to fit inside the ring cavity. The optimal lipophilic sites:ionophore molal ratio is 1.0 which indicates that these ionophores form Mg^{2+} and Ca^{2+} complexes of the same stoichiometry (2:1). Among these ionophores, K22B5 has the highest selectivity against Ca^{2+} of $10^{-2.6}$ (SSM). However, this ionophore has a lower lipophilicity ($\log P_{TLC}$ = 3.5 ± 0.6) compared to ETH ionophores ($\log P_{TLC}$ = 8.48 ± 0.98) [46], leading to their leaching from the sensor membrane which adversely affects both the sensor's use lifetime and shelf lifetime.

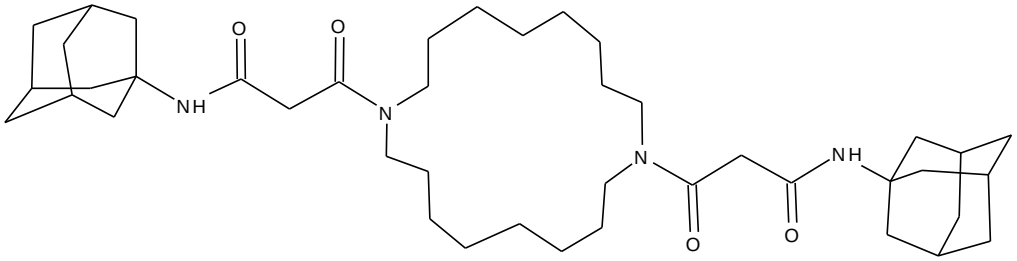
However, even sensors which use the best performing Mg ionophores, e.g., ETH 5506, K22B5, struggle to meet the clinical requirements [50, 51, 52] of selectivity against the major interfering cations, i.e., Ca^{2+}, Na^+ and K^+ (Table 2).



ETH 7025



ETH 5506



K22B5

Figure 1. Mg^{2+}-selective ionophores

Table 2. Normal adult clinical ranges of the main cation electrolytes in blood and the required selectivity coefficients assuming a 1% allowable error. The calculations are based on the traditional Nicolsky-Eisenman equation. [50)

	Reference interval (mmol/L plasma)	Required selectivity coefficient for iMg (without calibration*)	Required selectivity coefficient for iMg (with calibration*)
Na^+	134 – 143	-3.9	-2.7
K^+	3.3 – 4.8	-1.0	-0.5
Ca^{2+}	1.00 - 1.26	-2.5	-1.7
Mg^{2+}	0.46 - 0.66	--	--

For direct measurement of blood with iMg sensors, selectivity against Ca^{2+} is the major challenge since extracellular Ca^{2+} concentrations (1.0-1.26 mmol/L) are much higher than Mg^{2+} concentrations (0.46-0.66 mmol/L). This requires an iMg sensor with a high Mg^{2+} selectivity over Ca^{2+} to ensure sufficient accuracy in reporting iMg, especially for low Mg^{2+} samples (hypomagnesemia). Inaccuracy in iMg measurements is minimized if the sensor is calibrated with calibrators containing physiological concentrations of the interfering compounds (Ca^{2+}, K^+ and Na^+). Additional inaccuracies can be caused by variations in the activity of the interfering species. This interference can be minimized by chemometric correction of the analyte signal based on measurements of the activity of the cations. However, the selectivity of each sensor must be sufficiently high in order to avoid the propagation of errors.

3.2. Challenges of iMg sensors in blood analyzers

Since the 1990s, three diagnostic companies have commercialized Mg^{2+} selective electrodes for iMg assays in blood, serum or plasma. The platforms are KONE Microlyte 6 (Kone Instruments, Espoo, Finland); AVL 988/4 (AVL Medical Instruments (now Roche Diagnostics), Graz, Austria), and Nova CRT, CCX (Nova Biomedical, Waltham, MA, USA). Nova's iMg sensor is the only one currently available in commercial products.

Although iMg sensors in these commercial analyzers meet the general requirements for blood assays in normal clinical situations, some performance issues have raised concern. One study showed different iMg reference intervals across the three platforms [53] with KONE and AVL showing the closest agreement (likely because of similarities between their ionophores and electrode designs (AVL: ETH 7025; Kone: ETH 5220). Nova's iMg results were 0.11 mmol/L higher than AVL and KONE and different selectivity patterns against Ca^{2+} and monovalent cations were observed between the NOVA and AVL iMg sensors [54, 55].

The AVL 988/4 analyzer was susceptible to the so-called "silicone interference" which can devastate iMg results [55]. This effect arises when blood is collected in BD Vacutainer plastic tubes containing clot activator and silicone as the stopper lubricant (serial no. 367820) as opposed to using a BD glass tube with glycerol lubricant on the stopper. The silicone substance and clot activator from the Vacutainer tube are believed to affect the iMg sensors surface lipophilic characteristics. A working hypothesis was made that one or several molecular layers of the extremely non-polar silicone material could form an additional barrier for the transition of the very polar magnesium ion to the membrane phase, where other less polar ions, such as calcium and sodium would transit the interface between solution and membrane more easily and, therefore, are preferred. The membrane would sense more interfering ions than magnesium ions.

NOVA's iMg sensor showed an overestimation of Mg^{2+} at very low Mg concentrations (TMg < 0.35 mmol/L), and in samples containing a low TMg/TCa ratio of 0.16 [56]. This could be due to an improper chemometric correction of the Ca interference on the Mg electrode, non-linearity, or inadequate calibration. The failure of the product to correctly measure severe hypomagnesemic samples "erodes its diagnostic usefulness". Furthermore, such iMg sensors exhibit significant interference from thiocyanate (SCN^-) an analyte is commonly found in smoker's blood [57]. The AVL iMg sensor was not affected by the presence of SCN^-. The authors speculate that the NOVA interference by SCN^- may result from the absence of anionic sites in the membrane of the Nova iMg sensor, in which anion species like SCN^- may be easily exchanged. The addition of lipophilic salts (e.g., tetraphenyl borate) may minimize the effect of SCN^- and indeed some improvements on the Nova iMg sensor performance have been reported, especially at higher blood iMg concentrations [58].

A number of studies have reported reliable results between three analyzer types, while others have shown marked analyzer-specific variations [59, 60). Such variations and discrepancies have partially contributed to the lack of agreement of clinical interpretations regarding ionized magnesium results. Nevertheless, the availability of Mg^{2+}-selective electrodes does now make it possible to directly measure blood iMg for *in vitro* diagnostics and clinical investigations. Clearly, the clinical demands of blood iMg assays require further development of iMg sensors with high reliability, fast response, easy maintenance, and long use lifetimes.

Currently, there is no reference method for determining the iMg in blood [54, 55]. Several authors [54, 61, 62] have suggested using a combination of ultrafiltration and AAS as a reference method, however, these ultrafiltration methods measure "free" Mg, not iMg since anion-complexed Mg is also contained in the ultrafiltrate. One possible reference method for iMg is currently in development [63].

3.3. Other factors affecting iMg sensors

Since current iMg sensors cannot provide adequate selectivity for Mg^{2+} over Ca^{2+} and/or Na^+ in the presence of pathophysiological electrolyte concentrations, chemometric corrections are needed to compensate for these interferences. In addition to iMg, on-board sensors and calibration are required for Ca^{2+}, Na^+, and K^+ at a minimum. Most calculations around calibrations and sample testing are based on the empirical Nicolsky-Eisenman equation [64], in which the sensor signal (mV) is proportional to the logarithm of the primary ion activity. Improvements have been made to the original equation to enhance its application to real-life applications [65, 66]. These chemometric algorithmic correction calculations [67] are based on the response slope, intercept and selectivity obtained from calibration procedures.

IFCC guidelines [68] recommend a blood iMg assay result to be normalized to pH 7.4 where the pH of the sample is measured simultaneously [69]. It is well known that as sample pH increases, the free Mg^{2+} concentration decreases, due to enhanced binding of Mg^{2+} with proteins (mainly albumin). In the ultrafilterable fraction, pH affects the binding of anions e.g. bicarbonate, lactate and phosphate to Mg^{2+} in a similar way to that seen for Ca but to a smaller extent [23, 73]. These effects have been studied exhaustively but discrepancies in results between analyzer types have been observed [70, 71, 72, 74, 75].

In automated blood gas analyzers, surfactants can be present in the calibration rinse/wash, and quality control reagents [76]. Nonionic surfactants based on poly(ethylene oxide), e.g., Brij 35 and Triton X-100, are commonly used. Malinowska et al. [77, 78] found that this type of surfactants can introduce a significant positive signal bias (> 100 mV) to iMg sensors (using ETH 7025 as the ionophore), an effect not observed for iCa sensors and a dramatic loss of selectivity for Mg which renders direct iMg measurement virtually impossible. The mechanism may be due to the formation of a thin surfactant film on the sensor surface that alters the electrode partitioning and ion-exchange pattern [79]. This surfactant mechanism may also play a role in the observed interference by silicone on AVL iMg sensors discussed in Section 3.2 Accordingly, IFCC's guidelines for measuring iMg in blood [68], recommend avoiding polyethylene oxide based materials in calibrants or QC standards. Alkyl-N-

methylglucamide-based nonionic surfactants, e.g., MEGA 8®, may cause less interference but suffer from an increased cost as well as low foaming features. Recently, a proprietary surfactant based on acetylenic diols (AD) was investigated for iMg sensor applications (ETH 5506 as ionophore). But the iMg sensor showed a "memory effect" [80]. Studies on another proprietary nonionic surfactant based on poly(ethylene oxide) [81] indicate that in a narrow concentration range, the surfactant can be used in calibration reagents giving an acceptable selectivity and sensitivity, and a fast and stable response over clinically relevant iMg concentrations.

4. Conclusion

Increased recognition of the clinical importance of iMg over the last 30 years has resulted in greater demand for reliable iMg testing in the POC diagnostics field. The development of neutral ionophore based potentiometric sensors has made it possible to directly measure iMg. Interference from other cations, notably Ca^{2+}, can be corrected by chemometric calculations with selectivity coefficients obtained from concomitant calibrations. Current commercial iMg sensors still face multiple challenges in reporting accurate and precise results for hypomagnesemia samples, in addition to being practical for routine POC use by combining adequate use lifetimes with easy operation and minimal maintenance. A standard reference method for iMg in blood is still required. These challenges have to date limited the utility of iMg testing in clinical settings and have hindered iMg academic investigations. With the advances in sensor technology and analyzer automation that have occurred in recent years, future iMg sensors are expected to be more reliable and practical for widespread adoption of iMg testing in POC applications.

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