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Characterization of disposable optical sensors for heavy metal determination

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ABSTRACT

This paper presents the development, characterization and quality control of analytical methods based on the use of disposable optical sensors for determination of heavy metals. Chromogenic reagents such as 1-(2-pyridylazo)-2-naphthol, (2-pyridylazo)resorcinol, Zincon, Ferrozine, and Chromazurol S were used to develop optical sensors of heavy metal ions found as contaminants in pharmaceutical substances and products, such as Zn(II), Cu(II), Ni(II), Fe(II), and Fe(III). The chromogenic reagents were immobilized in polymeric membranes by spin-coating from cocktails containing all reagents needed. The methods were prevalidated using a comprehensive quality control strategy based on a system of mathematical/statistical testing and diagnosis of each prevalidation step. This system involved characterization of analytical groups; checking of two limiting groups; testing of data homogeneity; recognition of outliers; and determination of analytical functions, limiting values, precision and accuracy. The prevalidation strategy demonstrated the reliability of the proposed method and pointed out some limitations. Combining the optical sensors with multicomponent linear regression allowed simultaneous determination of multiple metals in synthetic mixtures with different compositions. Good agreement between experimental and theoretical amounts of heavy metals in the mixtures was obtained for the majority of sensors and metals. Even better agreement was obtained between the experimental and theoretical total amounts of metals in the mixtures. The proposed analytical methods were successfully applied to the determination of zinc in pharmaceutical preparations of insulin and the determination of metal mixtures in a commercial nasal spray of isotonic seawater. The reliable and sensitive individual optical sensors developed in this study may be useful for designing a multimembrane optical tongue that with appropriate further optimization can be used for screening heavy metals in various matrices.

1. Introduction

Heavy metals are ubiquitous and monitoring them is important in various fields because of their effects on ecosystems and living organisms [1–5]. For example, an important part of drug quality control is detecting and determining heavy metals. Bulk drug substances and their intermediates can be contaminated by metals in many ways, such as from raw materials, reagents, and solvents; from electrodes, reaction vessels, storage containers, plumbing and other equipments used in synthesis; or from exposure to air-borne particles. Metal catalysts and metal reagents, are substances used in the synthesis of the drug substance or an excipient used in a medicinal product, and, therefore, can also introduce metals into drug preparations [6,7]. Although evaluated for their potential risk to human health and placed into one of three classes (metals of significant, low, and minimal safety concern), some of them, such as Fe, Zn, and Cu are also important in human nutrition.

For over a century [7], drug quality control departments have relied on the heavy metal limit test recommended by most pharmacopoeias [8–11]. Besides the heavy metal limit test, European Pharmacopoeia [8] prescribes determination of specific metals in drug substances, such as copper in ascorbic acid, zinc in insulin, and nickel in polyols. The heavy metal limit test is based on sulfide precipitation in a weakly acidic medium and comparison with a lead solution. The test can be easily transferred from one laboratory to another and does not require expensive instrumentation or highly trained laboratory personnel. However, it suffers from several disadvantages. It requires subjective visual interpretation, large amounts of sample, and usually a heating or ashing step that causes losses of volatile elements. In addition, it does not provide any qualitative or element-specific information. Several attempts have been made to overcome these limitations [12,13], but no major improvements have been achieved. Thus, this standard method remains suitable for only a few elements, and it cannot specify the content of any particular metal ion but only the overall content of ions [12].

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Recently, as another alternative to the pharmacopoeial heavy metal limit test, a simple and sensitive methodology was proposed for simultaneous determination of metals in mixtures using solid-phase spectrophotometry [14]. This methodology was successfully applied to the determination of several heavy metals as impurities in pharmaceutical substances.

Several investigators have suggested replacing the heavy metal limit test with more sophisticated analytical methods such as ICP-MS to test drug substances, intermediates, and raw materials [15–18]. ICP-MS allows sensitive, rapid and automated multi-elemental analysis with a minimum of sample size and elemental interferences. In spite of the promise offered by this technique, the heavy metal limit test remains in use, mainly due to the lack of an alternative that provides the required information with comparable ease and simplicity.

In the past several years, the use of optical sensors for heavy metal analysis has increased because they are simple and inexpensive, and they allow remote and continuous monitoring [2,19]. These so-called optodes for metal ion determination can be fabricated by employing different types of reagents, such as chromogenic, fluorescent, and ionophoric compounds and enzymes. Numerous optical sensors based on chromogenic reagents have been described, such as xylenol orange [20], dithizone [21], chromeazurol S [22], PAN [23–25], PAR [25,26], TAR [27], Br-PADAP [28–30], ACDA [31], NN [25], and pyrocatechol violet [32]. Although some of these reagents are selective for certain metals under specific conditions, most of them show low selectivity.

Electronic tongues are a special case of optical sensors consisting of an array of sensors exhibiting different selectivities; pattern recognition systems analyze their responses to determine the profile of analytes present in the sample [33]. These tongues have been prepared using sensors based on a variety of principles, including tin oxide catalysis, conducting polymers, acoustic waves, quartz crystal microbalance, ion-selective field effect transistors (FET), ion mobility spectrometry, and mass spectrometry techniques such as atomic pressure ionization (API) and proton transfer reaction (PTR). Tongues have also been prepared using sensors based on optical techniques, principally fiber optics and fluorescence [34]. In optical tongue systems, the sensors should present low selectivity or high cross-sensitivity, and they should have reproducible analytical characteristics. In addition, the systems should be relatively inexpensive and portable. Ideally the sensors should be more sensitive and more robust, which is contradictory to a certain degree, since the more sensitive a sensor is, the less robust it becomes [35]. One solution is to use disposable sensors that are not integrated into the device.

We are developing an approach to analyze heavy metals which are important in pharmaceutical practice (such as Zn, Cu, Ni, Fe (III), and Fe(II)) using an optical tongue that consists of an array of membranes containing conventional chromogenic reagents immobilized on a transparent support. At present, metals are selected according to EMEA [6] classification for metallic residues in drug substances and excipients, where besides platinoids, Ni (Class 1), Cu (Class 2), Zn and Fe (Class 3) are metals with potential toxic effect. It may even be possible to extend the use of optical tongue to the analysis of other metals, such as Pb, Cd, Hg, Co, As, and Mn. As a first step, we present here the development and characterization of individual, disposable optical sensors that could be assembled into a disposable multimembrane sensor for simultaneous determination of heavy metals. The analytical quality of the individual membranes was assessed using a comprehensive prevalidation strategy [36]. In addition, the sensors were tested using synthetic metal mixtures and real pharmaceutical preparations; optical response was processed using powerful multicomponent analysis [37]. This analytical system may be a valuable alternative to the present pharmacopoeial heavy metal limit test, and it may prove useful for the determination of heavy metals in different matrices.

2. Experimental

2.1. Reagents and materials

Working standard solutions of zinc, copper, nickel, and iron(III) ($100\,\mu g\,mL^{-1}$) were prepared by appropriate dilution of the standard stock solution of each metal ($1000\,\mu g\,mL^{-1}$) (Sigma–Aldrich Química S.A., Madrid, Spain) acidified with concentrated HNO_3 (Sigma). Iron(II) stock solution ($100\,\mu g\,mL^{-1}$) was prepared by dissolving ammonium iron(II) sulfate hexahydrate (Panreac, Barcelona, Spain) in water acidified with concentrated HNO_3. Solutions of lower concentrations were prepared by dilution with water.

Sensor films were prepared using polyurethane hydrogel D4 (Tyndale Plains-Hunter, Lawrenceville, NJ, USA) and the following reagents, were all purchased from Sigma–Aldrich (Madrid, Spain): high molecular weight polyvinyl chloride (PVC), o-nitrophenyloctylether (NPOE), tributyl phosphate (TBP), potassium tetrakis(4-chlorophenyl)borate (TCPB), hexadecyltrimethylammonium bromide (HTMAB), benzethonium chloride (BTC), cellulose acetate (CA), and tetrahydrofuran (THF). Sheets of Mylar-type polyester (Goodfellow, Cambridge, UK) were used as a support.

The following chromogenic reagents were purchased from Sigma: 1-(2-pyridylazo)-2-naphthol (PAN), 1-10-phenanthroline, 4,7-diphenyl-1,10-phenanthroline, 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-4′,4″-disulfonic acid sodium salt (Ferrozine, FER), (Z)-5-((3-carboxy-5-methyl-4-oxocyclohexa-2,5-dien-1-ylidene)(2,6-dichloro-3-sulfophenyl) methyl)-2-hydroxy-3-methylbenzoic acid (Chromazurol, CS), 2-carboxy-2′-hydroxy-5′-sulfoformazyl-benzene monosodium salt (Zincon), 3,3′-bis[N,N-bis(carboxymethyl)aminomethyl]-o-cresolsulfonephthalein disodium salt (xylenol orange, XO), 8-hydroxyquinoline, tetraphenylporphyrine, 1,8-dihydroxynaphthalene-3,6-disulfonic acid disodium salt (chromothropic acid disodium salt), salicylhydroxamic acid, and dimethylglyoxime. In addition, the chromogenic reagents 1,2,4-trihydroxy-9,10-anthracenedione (purpurin) and 4-(2-pyridylazo)resorcinol (PAR) from Fluka (Madrid, Spain), and 1,2-dihydroxy-9,10-anthracenedione (alizarine) from TCI (Zwijndrecht, Belgium) were used.

All chemicals were of analytical-reagent grade, and reverse osmosis-purified water (Milli-RO 12 plus Milli-Q station, Millipore) was used throughout.

2.2. Preparation of disposable membranes and measurement set-up

The following cocktails were prepared in order to make heavy metal-sensing membranes: (a) PAN cocktail (133.7 mmol of PAN/kg of PVC), containing 30.0 mg PVC, 60.0 mg NPOE, and 1.0 mg PAN dissolved in 1 mL of freshly distilled THF; (b) PAR cocktail (38.7 mmol of PAR/kg of D4), containing 240.0 mg D4 polyurethane hydrogel, 2.0 mg PAR, and 10.3 mg TCPB dissolved in a mixture of 3.6 mL of ethanol and 0.4 mL of water; (c) Zincon cocktail (43.3 mmol of Zincon/kg of D4), containing 100.0 mg D4 polyurethane hydrogel, 2.0 mg Zincon, and 10.0 mg BTC dissolved in a mixture of 3.0 mL of ethanol and 0.2 mL of water; (d) FER cocktail (38.9 mmol of FER/kg of D4), containing 100.0 mg D4 polyurethane hydrogel, 2.0 mg FER, and 2.0 mg TCPB dissolved in a mixture of 3.0 mL of ethanol and 0.2 mL of water; and (e) CS cocktail (35.5 mmol of CS/kg of D4), containing 100.0 mg D4 polyurethane hydrogel, 2.0 mg CS, and 10.0 mg HTMAB dissolved a mixture of 3.0 mL of ethanol and 0.2 mL of water.

To prepare membranes, the different cocktails (20 μL) were placed individually on polyester sheets (14 mm × 4 cm × 0.5 mm thick) and subjected to spin coating. The sensing zone on each membrane was a circle 7 mm in diameter. The membranes were stored in a closed container at room temperature to enable slow solvent evaporation and then kept in a dark place until use to avoid photodegradation.

The response of PAN and Zincon membranes was evaluated using 8 mL of test solution together with 2 mL of 2 M ammonia buffer solution (pH 9.5); the response of PAR, FER and CS membranes was evaluated using 2 mL of 2 M acetic acid buffer (pH 4.0). Test solutions were prepared either of individual metals at concentrations ranging from 1.0 to 10.0 $\mu g\,mL^{-1}$, or of mixtures of 2–5 metals at individual concentrations of 1.0–5.0 $\mu g\,mL^{-1}$. The mixtures of test and buffer solutions were placed in polyethylene tubes (10 cm × 1.5 cm), a disposable sensor was inserted, and the tube was capped. The samples were shaken for 10 min on a vibrating agitator at 800 oscillations per minute at room temperature. The sensors were then removed from the tubes, residual solution was dried from the membrane surface, and membrane absorbance was measured using a Hewlett-Packard diode array UV/Vis spectrophotometer (Model 8453; Norwalk, CT, USA) equipped with a custom-made membrane holder. The holder was an iron block painted matte black and measuring 44 mm high and 12 mm wide [38]. This cell holder enables measurements of a zone of constant diameter that is smaller than the diameter of the sensor's active zone, which avoids noise due to variation in the surface area of the membrane active zone. All sample and blank measurements were carried out against a clear Mylar polyester strip, at room temperature (20 ± 3 °C). The membranes were not conditioned before use.

Absorbance measurements were collected using Chemstation software (Hewlett-Packard). Data were analyzed using the Statgraphics software package (version 6.0; Statpoint Technologies, Warrenton, Virginia, USA).

2.3. Prevalidation study

A comprehensive prevalidation strategy [36] was carried out to diagnose the accuracy and reliability of the procedures using the PAN, PAR, Zincon, FER, and CS membranes (*Me-sensor* systems). Prevalidation experiments were based on 24 measurements divided into six analytical groups (6 standard solutions of metal *j*). Each analytical group comprised 4 replicate experiments (*i*). For each measurement of the standard, the corresponding blank solution was also measured (24 blank measurements). Working solutions of heavy metals were prepared by appropriate dilution of the standard stock solution of each heavy metal ($1.0x_U = x_1 = 10.0\,\mu g\,mL^{-1}$, upper end of analyte range; $0.8x_U = x_2 = 8.0\,\mu g\,mL^{-1}$, $0.6x_U = x_3 = 6.0\,\mu g\,mL^{-1}$; $0.4x_U = x_4 = 4.0\,\mu g\,mL^{-1}$; $0.2x_U = x_5 = 2.0\,\mu g\,mL^{-1}$; and $0.1x_U = x_6 = x_L = \mu g\,mL^{-1}$, lower end of analyte working range), and measurements were carried out according to the procedure described in Section 2.2. Blank solutions were prepared and absorbance was measured in the same way, but without analyte. The data were analyzed using descriptive and prognostic statistics to assess measurement quality at the lower end of the analytical working range, assess data homogeneity, determine calibration and analytical evaluation functions, detect outliers, and estimate limiting values, precision and accuracy.

2.4. Method for multicomponent analysis of heavy metals

A combination of sensors (PAN, PAR, Zincon, FER, and CS sensors) and a chemometric algorithm of multicomponent analysis by multiple linear regression (Metal-Sensor Multicomponent Analysis, *MeSeMA*) was used as a valid methodological approach for the simultaneous determination of heavy metals in a synthetic mixture without previous concentration or separation.

Table 1
Characteristic wavelengths of sensing systems.

Sensor	Characteristic wavelengths, λ (nm)						**K**-matrix
	Zn(II)	Cu(II)	Ni(II)	Fe(II)	Fe(III)	Range	
PAN	560	563	573	–	–	520–600	3 × 10
PAR	520	530	540/580	–	720	490–720	4 × 12
Zincon	–	624	675	–	–	580–740	2 × 11
FER	–	–	–	560	–	560–610	1 × 6
CS	–	–	–	–	680	600–710	1 × 4

2.4.1. Establishment of the molar absorptivity matrix ***K***

The first step in the multicomponent analysis is the establishment of the absorptivity matrix **K**, the elements of which are the absorptivities of *m* components (metals) at *n* wavelengths. These elements can be obtained from the spectra of the pure components. Therefore, a concentration-normalized spectrum of each system containing 10 $\mu g\,mL^{-1}$ of a particular metal was recorded in the corresponding wavelength range (Table 1). The elements of the **K** matrix were treated as the independent variables, and the number of wavelengths was equal to or greater than the number of components. Matrix elements were apparent molar absorptivities ε obtained at 4, 7, 9, 10 and 11 wavelengths from recorded spectra of each metal for CS, FER, Zincon, PAN, and PAR membranes. The order of the matrix ($m \times n$) used for multicomponent analysis depended on the number of metals in the mixture and ranged from 1 × 4 (CS sensor) to 4 × 12 (PAR sensor).

2.5. Determination of metals in synthetic mixtures

The *MeSeMA* methodology was used to determine total and specific amounts of metals in synthetic mixtures with different compositions and metal amounts. The following systems were investigated: (a) PAN sensor with a mixture of Zn, Cu, and Ni; (b) PAR sensor with a mixture of Zn, Cu, Ni, and Fe(III); (c) Zincon sensor with a mixture of Cu and Ni; (d) FER sensor with a mixture of Zn, Cu, Ni, and Fe(II); and (e) CS sensor with a mixture of Zn, Cu, Ni, and Fe(III). The concentrations of individual metals in the mixtures varied from 1.0 to 5.0 $\mu g\,mL^{-1}$. Calculations were performed using the appropriate absorptivity matrix **K** and a set of equations for multiple linear regression [37,39]. To perform faster calculations, *SPIS* software for multicomponent analysis of mixtures by multiple linear regression was used [14].

2.6. Determination of zinc in pharmaceutical preparations of insulin

Insulin preparations were obtained from the Croatian Agency for Medicines and Medical Products, which had received them from various pharmaceutical manufacturers for mandatory routine testing. The following preparations were obtained: Humalog®, Humalog Mix25®, Humalog Mix50®, and Humulin M3® (Eli Lilly, USA); Insulatard® Penfill® (Novo Nordisk, Denmark); and Lantus® Solostar® (Sanofi Aventis, France). Each preparation was shaken gently, and a volume containing 200 IU of insulin was diluted to 25 mL with 0.01 M hydrochloric acid. A series of samples were prepared by adding known amounts of zinc to 1 mL of the insulin dilutions prepared in the previous step. The procedure described in Section 2.2 was applied and zinc content was determined in spiked samples according to the *MeSeMA* methodology.

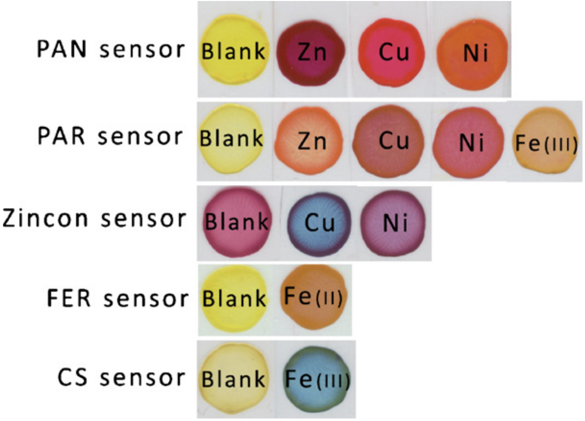

Fig. 1. Optical sensor before (blank) and after the reaction with metal.

2.7. *Determination of metals in isotonic seawater*

A series of samples were prepared by adding known amounts of metals (Zn, Cu, Ni, and Fe(III)) to 8 mL of a commercial nasal spray of isotonic seawater (Sterimar®, Sodibel-Laboratoires, Fumouze, France). The procedure described in Section 2.2 was applied to spiked samples and metal content was determined according to the *MeSeMA* methodology.

3. Results and discussion

3.1. *Selection of the reagents*

The goal of this study was to prepare a set of heavy metal-sensing membranes for the analysis of mixtures of Zn(II), Cu(II), Ni(II), Fe(II), and Fe(III) ions based on absorbance measurement. Fifteen different chromogenic reagents were studied (see Section 2.1) in different cocktails containing different types and amounts of membrane polymer, plasticizer, and lipophilic salt. Membranes were prepared from selective and non-selective chromogenic reagents in order to achieve different selectivity patterns for the heavy metals with no leaching over the entire concentration range studied.

Five membranes satisfying these criteria were selected: PAN, PAR, Zincon, FER, and CS (Table 1). These membranes gave a fast reaction, leaching of the reagents or reaction product was not observed, and they were easily immobilized onto the appropriate support (PVC or D4). PAN, PAR and Zincon showed non-selective behavior, reacting with several metal ions. In contrast, FER and CS were selective for Fe(II) and Fe(III), respectively. These five reagents yielded membranes that gave the greatest color change in the presence of analyte.

The composition of five selected membranes was optimized to (1) minimize leaching by varying the lipophilic salt, plasticizer, and membrane polymer; (2) maximize color intensity by varying the concentration of chromogenic reagent; and (3) minimize response time by varying plasticizer, membrane polymer, and the volume of cocktail used to prepare the membrane. The optimum compositions are indicated in Section 2.2. The optimal lipophilic salt/reagent ratio varied widely from 1:1 to 8:1.

3.2. *Optimization of experimental parameters*

The effect of pH on product formation and sorption on membranes was investigated in the range pH 3–12. Maximal response was obtained at pH 9.5 for PAN and Zincon membranes and at pH 4.0 for PAR, FER, and CS membranes. At pH values above 7.0, leaching of PAR into the solution was observed. The Zincon sensor was sensitive to Cu and Ni at basic pH, but it did not detect Ni at acidic pH. Longer reaction time (over 30 min) increased the optical response, especially for the PAN sensor, but leaching from the PAR membrane was observed for reaction times longer than 15 min. Therefore, a reaction time of 10 min was used for all sensing membranes.

3.3. *Color change of sensing membranes*

All the sensing membranes gave different color changes in the presence of different metals (Fig. 1). The PAN membrane gave a positive reaction in the presence of Zn, Cu, and Ni; the PAR membrane, in the presence of Zn, Cu, Ni, and Fe(III); and the Zincon membrane, in the presence of Cu and Ni. The FER and CS sensing membranes showed a highly selective response than the other three membranes; they gave color changes for Fe(II) and Fe(III), respectively.

In order to describe the sensing mechanism of our membranes, we studied the complexation between PAN in the membrane and Cu(II) in solution at pH 9.5. We assumed a 1:1 stoichiometry [40], which is usually observed when PAN is used as chromogenic reagent for different metals in ion-exchange materials [41–44], lipophilic salts [45] and adsorbents [46–48]. We therefore described the complexation with Eq. (1):

$$Cu^{2+} + X^{-} + \overline{HL} \leftrightarrow \overline{CuLX} + H^{+} \qquad (1)$$

This co-extraction equilibrium is characterized by a constant K_e that includes, for the ion pair involved, a stability constant, dissociation constant, distribution constant between aqueous and membrane phases of different species, and dissociation constant. The activity of the copper ion in the aqueous phase is related to the equilibrium constant K_e through a sigmoidal response function that

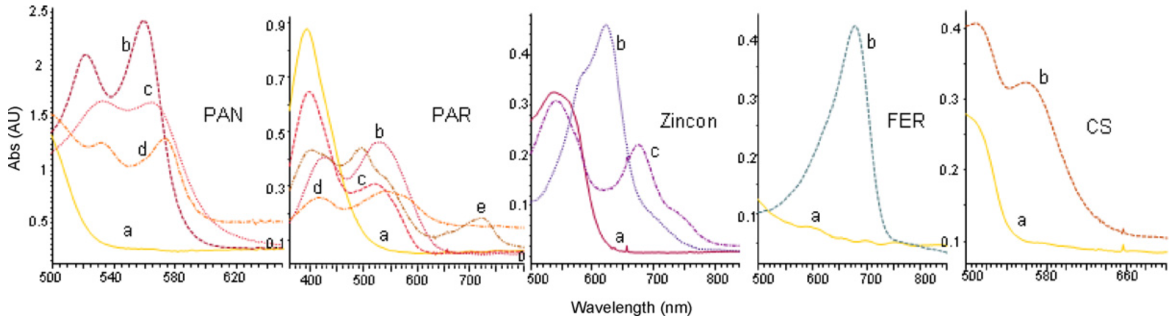

Fig. 2. Absorption spectra of sensors for heavy metals. **PAN** sensor at pH 9.5: (a) PAN (blank); (b) Zn–PAN product (10 mg mL^{-1} of Zn); (c) Cu–PAN product (10 mg mL^{-1} of Cu); (d) Ni–PAN product (10 mg mL^{-1} of Ni). **PAR** sensor at pH 4.0: (a) PAR (blank); (b) Zn–PAR product (10 mg mL^{-1} Zn); (c) Cu–PAR product (10 mg mL^{-1} Cu) (d) Ni–PAR product (10 mg mL^{-1} Ni); (e) Fe(III)–PAR product (10 mg mL^{-1} Fe(III)). **Zincon** sensor at pH 9.5: (a) Zincon (blank); (b) Cu-Zincon (10 mg mL^{-1} Cu); (c) Ni-Zincon (10 mg mL^{-1} Ni). **FER** sensor at pH 4.0: (a) FER (blank); (b) Fe(II)–FER product (10 mg mL^{-1} Fe(II)). **CS** sensor at pH 4.0: (a) CS (blank); (b) Fe(III)–CS product (10 mg mL^{-1} Fe(III)).

Table 2
Precision of the measurements of *Me-sensor* systems.

	s_r (%)								
	Zn–PAN	Cu–PAN	Ni–PAN	Zn–PAR	Ni–PAR	Cu–Zincon	Ni–Zincon	Fe(II)–FER	Fe(III)–CS
B	25.8–140.8	21.8–81.0	10.1–95.4	2.8–12.4	2.5–24.8	22.9–50.0	21.6–42.5	6.7–27.7	7.9–24.2
y	0.8–5.4	1.6–3.5	4.1–7.7	1.3–8.8	0.6–7.0	1.7–14.7	2.0–12.5	2.5–9.6	0.4–11.1
S	0.9–5.8	1.5–3.8	4.2–8.0	1.7–13.5	0.7–9.0	1.9–23.6	2.0–16.3	2.4–9.8	0.7–12.7
A	0.9–5.8	1.5–3.8	4.2–8.0	1.7–13.5	0.7–9.0	1.9–23.6	2.0–16.3	2.4–9.8	0.7–12.7

includes the experimental degree of uncomplexed chromogenic reagent α (Eq. (2)).

$$K_e = \frac{(1-\alpha)\cdot a_{\mathrm{H^+}}}{\alpha \cdot a_{\mathrm{Cu^{2+}}} \cdot a_{\mathrm{X^-}}} \qquad (2)$$

The good fit of experimental data to Eq. (2) ($R^2 = 0.9999$) supports the 1:1 stoichiometry, with a K_e of 1.2×10^{10}.

3.4. Spectral characterization of sensing membranes

The spectral characteristics of all complexes investigated are shown in Fig. 2. Substantial overlap of the spectra was observed, due to the low selectivity of PAN, PAR, and Zincon sensors. The complexes Zn–PAN, Cu–PAN, and Ni–PAN showed absorption maxima in the range of 520–580 nm. The absorption maxima of nearly all products on the PAR sensor were in the range of 470–600 nm. The absorption spectrum of Fe(III)–PAR differed significantly from that of the others, with a maximum at 730 nm. In the case of the Zincon sensor, the overlap between the spectra of Cu–Zincon and Ni–Zincon was less extensive. Of all the sensing membranes investigated, the FER and CS membranes showed the highest selectivity; they were selective for Fe(II) and Fe(III), respectively. Absorbance of the reagent blanks for nearly all sensors was negligible in the wavelength range where the reagent–metal complexes had their absorption maxima.

3.5. Prevalidation of sensor results

A comprehensive prevalidation strategy was carried out to diagnose the quality of *Me-sensor* systems and identify any limitations. Initial prevalidation data were as follows: amounts of metal ion (x) were within the working range from 1.0 to 10.0 μg mL^{-1}, absorbances were obtained from measurements of the blank (B) and the sample (y), and the corrected absorbance (S) was calculated

Table 3
The relationship between analyte and signal in *Me-sensor* systems.

	Least square method	Calibration function	Analytical evaluation function
Zn–PAN	$y = 0.1888x + 0.0559$ $r^2 = 0.9911$	$\hat{S} = 0.20x$ $s_M = \pm 0.088$	$\hat{x} = 5.06S$ $s_M = \pm 0.44$
Cu–PAN	$y = 0.1430x + 0.0262$ $r^2 = 0.9983$	$\hat{S} = 0.15x$ $s_M = \pm 0.031$	$\hat{x} = 6.81S$ $s_M = 0.21$
Ni–PAN	$y = 0.0575 + 0.0229$ $r^2 = 9916$	$\hat{S} = 0.06x$ $s_M = \pm 0.027$	$\hat{x} = 16.38S$ $s_M = 0.44$
Zn–PAR	$y = 0.0463x + 0.0069$ $r^2 = 0.9985$	$\hat{S} = 0.047x$ $s_M = \pm 0.0092$	$\hat{x} = 21.1S$ $s_M = \pm 0.194$
Ni–PAR	$y = 0.0464x - 0.0086$ $r^2 = 0.9982$	$\hat{S} = 0.045x$ $s_M = \pm 0.0103$	$\hat{x} = 22.1S$ $s_M = 0.227$
Cu–Zincon	$y = 0.0311x + 0.0136$ $r^2 = 0.9925$	$\hat{S} = 0.03x$ $s_M = \pm 0.014$	$\hat{x} = 30.2S$ $s_M = \pm 0.44$
Ni–Zincon	$y = 0.013x + 0.0061$ $r^2 = 0.9967$	$\hat{S} = 0.013x + 0.006$ $s_M = \pm 0.0035$	$\hat{x} = 77.0S - 0.43$ $s_M = 0.270$
Fe(II)–FER	$y = 0.020x + 0.0134$ $r^2 = 0.9973$	$\hat{S} = 0.02x + 0.0134$ $s_M = \pm 0.0049$	$\hat{x} = 49.7S - 0.639$ $s_M = \pm 0.244$
Fe(III)–CS	$y = 0.039x + 0.046$ $r^2 = 0.9904$	$\hat{S} = 0.039x + 0.046$ $s_M = \pm 0.018$	$\hat{x} = 25.5S - 1.07S$ $s_M = 0.459$

Table 4
Metrological characteristics of *Me-sensor* systems.

	Analyte amount (μg mL^{-1})						Random deviations		Systematic deviations		L_D (μg mL^{-1})	L_Q (μg mL^{-1})
Actual (μg mL^{-1}):	10.0	8.0	6.0	4.0	2.0	1.0	$s_{\hat{x}}$ (μg mL^{-1})	$s_{r\hat{x}}$ (%)	$\Delta\bar{x}$ (μg mL^{-1})	$\Delta\bar{x}$ (%)		
Found (μg mL^{-1})												
Zn–PAN	9.6	8.6	5.5	4.1	2.1	1.4	(±0.022)–(±0.489)	(±0.9)–(±5.8)	(−0.46)–(+0.57)	(−7.5)–(+40.8)	0.014	0.082
Cu–PAN	10.0	8.0	5.8	4.2	2.1	1.2	(±0.046)–(±0.264)	(±1.5)–(±3.7)	(−0.23)–(+0.23)	(−3.8)–(+22.7)	0.008	0.049
Ni–PAN	9.7	8.0	5.8	4.5	2.4	1.0	(±0.067)–(±0.624)	(±4.3)–(±8.0)	(−0.34)–(+0.50)	(−3.4)–(+18.2)	0.050	0.302
Zn–PAR	9.9	8.0	6.1	4.2	2.0	1.1	(±0.103)–(±0.220)	(±1.7)–(±13.5)	(−0.12)–(+0.20)	(−1.2)–(+19.8)	0.080	0.460
Ni–PAR	9.9	8.2	6.1	3.8	1.9	0.8	(±0.059)–(±0.291)	(±0.7)–(±9.0)	(−0.21)–(+0.18)	(−21.0)–(+2.2)	0.147	0.891
Cu–Zincon	9.6	8.2	6.1	3.9	2.4	1.3	(±0.187)–(±0.490)	(±1.9)–(±23.6)	(−0.38)–(+0.43)	(−3.8)–(+31.0)	0.592	3.601
Ni–Zincon	10.0	8.0	6.0	3.9	2.1	1.1	(±0.211)–(±0.354)	(±2.1)–(±23.0)	(−0.15)–(+0.09)	(−3.6)–(+4.2)	0.296	1.802
Fe(II)–FER	10.0	8.0	6.0	4.1	1.9	1.0	(±0.053)–(±0.467)	(±2.6–(±11.3)	(−0.08)–(+0.02)	(−3.4)–(+3.3)	0.019	0.118
Fe(III)–CS	10.2	7.5	5.7	4.3	2.5	0.7	(±0.061)–(±0.413)	(±0.8)–(±32.2)	(−0.49)–(+0.45)	(−29.7)–(+22.6)	0.043	0.261

Table 5
Content of specific metal in the mixture.

Sensor	Metal	Added (μg mL^{-1})	Found (μg mL^{-1})	SD[a] (μg mL^{-1})	RSD (%)	Recovery (%)	Matrix
PAN	Zn	3.00	3.20	0.071	2.21	106.7	Cu, Ni
		0.00	0.0	0.000	0.00	0.0	Cu, Ni
		1.00	1.06	0.058	5.41	106.7	Cu, Ni
	Cu	3.00	2.75	0.071	2.57	91.7	Zn, Ni
		4.00	4.33	0.141	3.26	108.3	Zn, Ni
		1.00	0.95	0.071	7.44	95.0	Zn, Ni
	Ni	3.00	2.85	0.212	7.44	95.0	Zn, Cu
		4.00	3.90	0.000	0.00	97.5	Zn, Cu
		5.00	5.05	0.354	7.00	101.0	Zn, Cu
PAR	Fe(III)	3.00	2.85	0.071	2.48	95.0	Zn, Cu, Ni
		2.00	1.80	0.566	31.43	90.0	Zn, Cu, Ni
		3.00	2.50	0.000	0.00	83.3	Zn, Cu, Ni
Zincon	Cu	3.00	3.10	0.000	0.00	103.3	Zn, Ni
		5.00	5.75	0.212	3.69	115.0	Zn, Ni
		1.00	1.00	0.000	0.00	100.0	Zn, Ni
	Ni	2.00	2.05	0.212	10.35	102.5	Zn, Cu
		4.00	3.40	0.212	6.24	85.0	Zn, Cu
		4.00	4.25	0.071	1.66	106.2	Zn, Cu
FER	Fe(II)	5.00	4.45	0.212	4.77	89.0	Zn, Cu, Ni
		2.00	1.75	0.212	12.12	87.5	Zn, Cu, Ni
		2.00	2.00	0.00	0.00	100.0	Zn, Cu, Ni
CS	Fe(III)	5.00	5.55	0.212	3.82	111.0	Zn, Cu, Ni
		3.00	3.40	0.00	0.00	113.3	Zn, Cu, Ni
		2.00	1.65	0.354	21.43	95.0	Zn, Cu, Ni

[a] Mean of three determinations.

as the difference $y - B$. The following membrane–metal systems were analyzed in the prevalidation: Zn–PAN, Cu–PAN, and Ni–PAN; Zn–PAR, Ni–PAR; Cu–Zincon, Ni–Zincon; Fe(II)–FER; and Fe(III)–CS.

The first step in calculating descriptive statistics of the prevalidation process was to characterize six analytical groups of all investigated systems by calculating average values and standard and relative standard deviations. These values were used to evaluate repeatability of the measurements as a part of precision (Table 2). Relative standard deviations of blank absorbances showed low precision of blank measurements in most systems. These fluctuations of the blank values may reflect variability in sensor quality or method's performance characteristics. Nevertheless, even in the worst cases, the fluctuations in blank values could be ignored because they were so small compared to the analyte signal. In contrast, relative deviations of sample measurements and corrected absorbances showed high precision for nearly all systems. The Me–PAN system showed the highest precision; deviations (s_{ry} from ±0.80% to ±5.40%, s_{rs} from ±0.87% to ±5.82%) satisfied the strict prevalidation criterion of s_r <±5% [36]. In most systems for which s_{ry} and s_{rs} were somewhat higher than ±5%, the offending deviations occurred with the smallest quantity of analyte (x_6).

A preliminary check of analytical groups 1 and 6, which limit the working range of the proposed method, indicated good quality of measurements and unambiguous distinction between analyte and blank signals in the group with x_6. The analyte signal in the Me–PAR and Me–Zincon systems was slightly different from blank signal, while the blank signal was significantly lower than analyte signal in the group with x_6 in the case of Me–PAN (AC = 158.7 for Zn, AC = 122.6 for Cu, and AC = 12.6 for Ni), Fe(II)–FER (AC = 21.4), and Fe(III)–CS (AC = 11.8). Additional checking of how well the systems resolved analyte and blank signals at x_6 showed excellent resolution in the case of Zn–PAN, Cu–PAN, and Fe(II)–FER; very good

Table 6
Content of total metals in mixtures.

Analytical system	Composition (μg mL^{-1})	Total amount (μg mL^{-1})		SD[a] (μg mL^{-1})	RSD (%)	Recovery (%)
		Added	Found			
PAN (Zn, Cu, Ni)	(3:3:3)	9.00	8.75	0.071	0.81	97.2
	(0:4:4)	8.00	8.20	0.212	2.59	102.5
	(1:1:5)	7.00	7.00	0.141	2.02	100.0
PAR (Zn, Cu, Ni, Fe(III))	(3:3:3:3)	12.00	13.4	2.263	16.89	111.7
	(0:5:5:2)	12.00	11.2	0.656	5.85	93.3
	(1:1:5:3)	10.00	13.7	0.990	7.23	137.0
Zincon (Zn, Cu, Ni)	(3:3:2)	5.00	5.15	0.071	1.37	103.0
	(0:5:5)	10.00	9.65	0.071	0.73	96.5
	(1:1:5)	6.00	5.80	0.141	2.44	96.7
FER (Zn, Cu, Ni, Fe(II))	(3:0:3:5)	5.00	4.45	0.212	4.77	89.0
	(0:3:3:2)	2.00	1.75	0.212	12.12	87.5
	(1:1:3:2)	2.00	2.00	0.000	0.00	100.0
CS (Zn, Cu, Ni, Fe(III))	(3:0:3:5)	5.00	5.55	0.212	3.82	111.0
	(0:3:3:3)	3.00	3.40	0.000	0.00	113.3
	(1:1:3:2)	2.00	1.65	0.356	21.43	95.0

[a] Mean of three determinations.

Table 7
Zinc in pharmaceutical preparations of insulin.

Sample	Amount of Zn (μg mL^{-1}) Added	Amount of Zn (μg mL^{-1}) Found	SD[a] (μg mL^{-1})	RSD (%)	Recovery (%)
Humalog®	0.00	0.20	0.002	1.42	–
	0.50	0.74	0.020	2.52	107.1
	2.00	2.30	0.020	0.89	104.7
	4.00	4.24	0.166	3.91	101.0
Humalog Mix25®	0.00	0.15	0.002	1.53	–
	0.50	0.66	0.093	1.40	101.5
	2.00	2.13	0.035	1.72	99.0
	4.00	4.17	0.240	5.37	100.3
Humalog Mix50®	0.00	0.16	0.005	4.92	–
	0.50	0.67	0.001	1.37	103.2
	2.00	2.21	0.093	4.45	102.8
	4.00	4.12	0.227	5.31	99.2
Humulin M3®	0.00	0.29	0.014	5.23	–
	0.50	0.84	0.029	4.05	109.8
	2.00	2.21	0.119	5.17	100.2
	4.00	4.12	0.013	0.32	99.8
Insulatard ®Penfill®	0.00	0.27	0.004	1.43	–
	0.50	0.77	0.036	4.68	101.0
	2.00	2.28	0.024	1.08	100.3
	4.00	4.26	0.000	0.00	99.6
Lantus® Solostar®	0.00	0.28	0.005	1.67	–
	0.50	0.77	0.005	0.66	103.7
	2.00	2.28	0.028	1.17	103.5
	4.00	4.26	0.245	5.79	98.9

[a] Mean of three determinations.

resolution in the case of Ni–PAN, Zn–PAR, and Fe(III)–CS; and good resolution in the case of Ni–PAR, Cu–Zincon, and Ni–Zincon.

The homogeneity check was carried out using simple analysis of variance (ANOVA) and Bartlett's test. ANOVA of blank values indicated high homogeneity in all systems except Ni–PAN and Ni–Zincon. However, although the blank values showed high homogeneity, high values of s^2_{Bw} and s^2_{Bb} gave a small ratio value, suggesting that random and systematic errors were present. According to auxiliary criteria [36], the influence of inhomogeneity of blank values can be considered negligible if the inhomogeneity is small in relation to the corresponding analyte values obtained at x_1, and if sr_{BN} does not exceed ±50%. These criteria were satisfied in the case of Zn–PAN and Cu–PAN, and nearly so in the case of Ni–PAN and Fe(II)–FER, and each y value was corrected with a grand blank mean. In contrast, although ANOVA of blank values showed homogeneity in the case of Zn–PAR, Ni–PAR, Cu–Zincon, and Fe(III)–CS, the inhomogeneity was not small in relation to the corresponding analyte values, so the influence of the blanks on the results could not be neglected. Bartlett's test was used to test the homogeneity of s and s_r values for y, S, A, and apparent mass ($\hat{x}$) values for the different analytical groups. The test indicated that most values of s and s_r in all systems were almost homogeneous, homogeneous, or strongly homogeneous. Inhomogeneous s_r values were obtained only for gross (y) and corrected (S) values in the Zn–PAN system.

The relationship between analytical signal (absorbance) and analyte content (heavy metal quantity) was determined using three methods: preliminary linearity check, method of least squares, and systematic t-testing of the reality of constants (Table 3). A preliminary linearity check, applied to A values (measure of particular sensitivity, $A_n = S_n/x_n$) of limiting groups 1 and 6, showed that linear calibration functions were expected for Ni–PAN, Zn–PAR, Cu–Zincon, and Ni–Zincon. The method of least squares showed that correlation existed in all systems, with Cu–PAN, Zn–PAR, and Ni–PAR showing the highest correlation coefficients. Through systematic evaluation of analytical functions, constants were determined for the calibration and analytical evaluation functions over the entire analyte working range, and ideal analytical functions were found for most systems, except Ni–Zincon, Fe(II)–FER, and FE(III)–CS, which were characterized by linear functions with an intercept (Table 3). These analytical functions were used to test for outliers, determine limiting values, and evaluate apparent signal values ($\hat{S}$) and apparent quantities of heavy metals ($\hat{x}$).

Outlier testing was performed to check whether any measurement differed significantly from the others in the set of signals used to carry out calibration (S) and in the set of heavy metal quantities (x) used to generate the analytical evaluation function. The presence of regression outliers was checked by comparing $|S^*|$ and $|x^*|$ values with the t-values of the 95% and 99% confidence intervals. Only one outlier was obtained in each of the following systems: Zn–PAN ($2.807 > \left|S^*_6\right| > 2.069$), Ni–PAN ($2.807 > \left|S^*_{13}\right| > 2.069$, $2.807 > \left|x^*_{13}\right| > 2.069$), Cu–Zincon ($2.807 > \left|S^*_{20}\right| > 2.069$, $2.80 > \left|x^*_{20}\right| > 2.069$), and Fe(II)–FER ($2.807 > \left|S^*_{13}\right| > 2.069$, $2.80 > \left|x^*_{13}\right| > 2.069$). According to the prevalidation criteria [36], the data remain homogeneous despite these outliers. No outlier values were found in the other systems.

Limiting values, such as limiting signal value, limit of detection and limit of quantification were estimated using the analytical evaluation function and recommended concepts of limiting values [49,50]. For nearly all systems, estimated limiting values were significantly lower than the amount of metal at the lowest level of analyte (<1 μg mL^{-1}), with Me–PAN and Fe(II)–FER showing the lowest limiting values (Table 4). In the Me–Zincon system, the L_Q was slightly higher than 1 μg mL^{-1}; this could be due to the high fluctuation of blank values observed in the homogeneity testing.

The final calibration and analytical evaluation functions were used to determine apparent signal values ($\hat{S}$) and apparent masses of analyte ($\hat{x}$), respectively. The analytical evaluation function was also used to evaluate the precision and accuracy of the systems. Information on accuracy was further obtained by comparing actual (x) and observed ($\hat{x}$) amounts of heavy metal. The data structure for the systems is given in Table 4. The systematic deviations,

Table 8
Metals in isotonic seawater.

Sensor	Metal	Added ($\mu g\,mL^{-1}$)	Found ($\mu g\,mL^{-1}$)	SD[a] ($\mu g\,mL^{-1}$)	RSD (%)	Recovery (%)	Matrix
PAN	Zn	1.00	0.94	0.095	10.1	94.0	Cu, Ni
		1.00	1.07	0.042	4.08	104.0	Cu, Ni
	Cu	1.00	1.06	0.064	6.08	105.7	Zn, Ni
		2.00	2.10	0.085	4.16	102.0	Zn, Ni
	Ni	4.00	3.78	0.257	6.84	94.5	Zn, Cu
		1.00	0.98	0.021	2.20	96.5	Zn, Cu
	Total	6.00	5.78	0.336	5.82	96.3	
		4.00	4.05	0.106	2.62	101.1	
Zincon	Cu	1.00	1.02	0.010	0.98	102.0	Zn, Ni
		2.00	2.00	0.049	2.48	99.8	Zn, Ni
	Ni	5.00	5.05	0.028	0.56	101.0	Zn, Cu
		3.00	2.90	0.007	0.24	96.5	Zn, Cu
	Total	6.00	6.07	0.042	0.70	101.2	
		5.00	4.89	0.042	0.87	97.8	
CS	Fe(III)	2.00	2.04	0.042	2.04	102.2	Zn, Cu, Ni
		3.00	3.04	0.035	1.16	101.2	Zn, Cu, Ni

[a] Mean of three determinations.

a measure of accuracy, ranged from −29.7% to +40.8%. As could be expected, the lowest degree of accuracy in all systems was in the analytical group with the smallest amount of heavy metal (1 $\mu g\,mL^{-1}$), while in the other groups favorable accuracy was obtained. It is likely that large deviations between blank values and analyte values were the main cause of large systematic errors and therefore lower accuracy. The highest level of accuracy was obtained for Ni–Zincon (from −3.6% to +4.2%) and Fe(III)–FER (from −3.4% to +3.3%). Random deviations, a measure of precision of analytical systems, ranged from ±0.7% to ±32.2%. Most systems showed high precision, with Me–PAN showing the highest, based on the prevalidation criterion of $s_r < \pm 5\%$. In systems showing lower precision, such as Me–Zincon and Fe(III)–CS, unfavorable precision was obtained only in the analytical group with the smallest amount of heavy metal (x_6).

In summary, evaluation of repeatability of the measurements, homogeneity testing, reality of the linear analytical evaluation function, agreement of actual and found amounts of heavy metals, as well as random and systematic deviations showed good measurement quality. Very low limiting values indicated that the procedures were sensitive and could be successfully applied to the determination of heavy metal traces. A significant influence of blank dispersion was found to be a possible disadvantage in some systems. Me–Zincon was the only system where reliable measurement could not be made at the lowest level of the working range, because L_Q was somewhat higher than the analyte amount in x_6. For this system, the working range should be changed and the prevalidation procedure repeated.

4. Applicability of the *MeSeMA* procedure

4.1. Synthetic mixtures

New analytical procedures based on the use of disposable optical sensors were used to develop a sensitive, fast, and simple procedure for determination of heavy metals in mixtures without previous concentration or separation of analytes. The combination of sensitive sensors and the chemometric algorithm of multicomponent analysis by multiple linear regression (*MeSeMA* procedure) enabled determination of specific metals in synthetic mixtures, despite similarity in the spectral characteristics of the various analytes present. The results of the heavy metal screening are presented in Tables 5 and 6.

Due to the low selectivity of the PAN, PAR, and Zincon reagents, metal complexes sorbed onto the corresponding sensing membranes showed similar absorption spectra, with some differences in spectral characteristics and sensitivity. These small differences in absorption spectra were exploited by powerful multicomponent analysis to simultaneously determine the content of specific metals in metal mixtures. Metal mixtures for PAN and Zincon sensors contained Zn, Cu, and Ni in different ratios. The mixtures for the PAR sensor contained the same metals, as well as Fe(III). In contrast to the other reagents, FER and CS were selective for Fe(II) and Fe(III), even in the presence of the other metals.

An absorptivity matrix **K** was calculated for each metal mixture using the procedure described in Section 2.4.1. Then the procedure described in Section 2.5 was used to determine the content of particular metals in the mixture, as well as the total amount of metals. The good agreement obtained between experimental and theoretical amounts of individual metals and total metals in the mixtures confirmed the validity of the proposed method. The only case where the *MeSeMA* procedure could not determine the amount of particular metal in the mixture was the PAR system. Although the total amount of metals was predicted with acceptable precision, the prediction of specific metal amounts using PAR sensor was unsuccessful. Only the amount of Fe(III) could be predicted, since its spectrum differed greatly from that of the other metals in the mixture.

4.2. Pharmaceutical samples

The PAN sensor was used to determine zinc in pharmaceutical preparations of insulin, and the PAN, Zincon, and CS sensors were used to determine three metals in nasal spray of isotonic seawater (Tables 7 and 8). The European Pharmacopoeia [8] recommends that zinc be determined in pharmaceutical preparations of insulin, and that no more than 40.0 μg per 100 IU of insulin should be present. In all pharmaceutical samples of insulin, the amounts of zinc determined ranged from 0.15 to 0.29 $\mu g\,mL^{-1}$ (Table 7), all of which were above the limit of quantitation determined in the prevalidation procedure (0.082 $\mu g\,mL^{-1}$, Table 4). In the analysis of isotonic seawater, the amounts of zinc, copper, nickel or iron(III) were either below the limits of quantitation determined during prevalidation of the PAN, Zincon and CS sensors (Table 4), or they could not be determined.

In order to check the accuracy of the proposed method, recovery experiments for different amounts of metals were carried out. Highly satisfactory recoveries were obtained, ranging from 98.9% to 109.8% for insulin preparations (Table 7). Recoveries ranged from 94.0% to 105.7% for commercial nasal spray, showing that the PAN, Zincon, and CS disposable optical sensors could be successfully applied to the determination of metals (Table 8).

Multicomponent analysis based on the *MeSeMA* method for determination of total and specific heavy metals in mixtures may prove to be an important advance in the development of pharmacopoeial methodology. In contrast to official general methods for analysis of heavy metals, the proposed *MeSeMA* method enables identification of particular metal ions with a limit of determination lower than 5 mg L^{-1}, without the need to compare with lead solution. The proposed optical sensors are especially sensitive for zinc, copper, nickel, iron(II) and iron(III). With the proposed procedures, it is not possible to determine all heavy metals with the same success. However, by adjusting experimental conditions, it may be possible to determine a greater number of important heavy metals such as lead, cadmium, cobalt, and, with less sensitivity, manganese and mercury [14]. Furthermore, it may be possible to apply these procedures to the determination of other heavy metals important in pharmaceutical analysis, such as Ru, Rh, Pd, Pt, Ir, Au, Ag, As, Sb, and Bi.

5. Conclusions

We have designed five disposable optical sensors using nonselective (PAN, PAR, and Zincon) and selective (FER and CS) reagents for determination of zinc, copper, nickel, iron(II), and iron(III). By combining these sensors with powerful chemometric multicomponent analysis (*MeSeMA*), the levels of these individual metals can be determined simply and rapidly, without previous concentration or separation of analytes. A comprehensive prevalidation procedure confirmed that most sensors showed a linear relationship between analyte and signal, acceptable accuracy and precision and low limits of detection. At the same time, the prevalidation revealed some limitations of the proposed procedure, such as non-negligible influence of blank signals on measurements under certain conditions, and lower sensitivity of the Zincon sensor. The proposed sensors were used to determine the total and individual amounts of metals in various synthetic mixtures. Good agreement was obtained between experimental and theoretical amounts of heavy metals, showing that most of the optical sensors could be used for simultaneous determination of heavy metals. The agreement was slightly better for total amounts of metals in the mixtures, indicating that there is room for improvement in the proposed procedure. In the case of the PAR sensor, it was not possible to resolve the mixture of metal ions, which suggests the need for further optimization of the sensor and experimental conditions. When the method was applied to the determination of zinc in insulin preparations and determination of metals in isotonic seawater, recoveries ranged from 98.9% to 109.8% and from 94.0% to 105.7%, respectively. These results confirm the accuracy of the proposed procedures obtained during prevalidation.

This work describes the first step in our efforts to develop disposable multimembrane tongues for simultaneous testing of multiple metals in complex matrices. Our results suggest that the disposable optical sensors described here can be integrated into such tongues, though further optimization will almost certainly be necessary in order for all sensors to work well under identical experimental conditions. These sensors may prove to be an efficient and inexpensive alternative to the present pharmacopoeial heavy metal limit test. It may also be possible to integrate them into a portable device for *in situ* analysis of heavy metals in different matrices.

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References

[1] I. Palchetti, M. Mascini, M. Minunni, A.R. Bilia, F.F. Vincieri, J. Pharm. Biomed. Anal. 32 (2003) 251–256.
[2] I.M. Raimundo, R. Narayanaswamy, Sens. Actuators B 90 (2003) 189–197.
[3] D.W. O'Connell, C. Birkinshaw, T.F. O'Dwyer, Bioresour. Technol. 99 (2008) 6709–6724.
[4] E.E. Kostenko, M.I. Shtokalo, J. Anal. Chem. 59 (2004) 1158–1164.
[5] S.K. Yadav, S. Afr. J. Bot. 76 (2010) 167–179.
[6] European Medicine Agency, Committee for Human Medicinal Products, Guideline on the Specification Limits for Residues of Metal Catalysts, London, 2007.
[7] R. Ciciarelli, D. Jäkel, E. König, R. Müller-Käfer, M. Röck, M. Thevenin, H. Ludwig, Pharmacopoeial Forum 21 (1995) 1638–1640.
[8] E.D.f.t.Q.o.M., Council of Europe, European Pharmacopoeia, Strasbourg, 2006.
[9] British Pharmacopoeia Commission, British Pharmacopoeia, The Stationery Office, Norwich, UK, 2009.
[10] United States Pharmacopeial Convention, The United States Pharmacopeia, Rockville, USA, 2002.
[11] The Society of Japanese Pharmacopoeia, The Japanese Pharmacopoeia, Tokyo, 2001.
[12] K. Brozovic-Pohl, H. Altorfer, X. Perlia, Fres. J. Anal. Chem. 343 (1992) 348–351.
[13] G.E. Veeman, A. Bult, J.P. Franke, J.S. Faber, Pharm. Weekbl. 117 (1982) 8–13.
[14] J. Vukovic, S. Matsuoka, K. Yoshimura, V. Grdinic, R.J. Grubesic, O. Zupanic, Talanta 71 (2007) 2085–2091.
[15] J. Huang, X. Hu, J. Zhang, K. Li, Y. Yan, X. Xu, J. Pharm. Biomed. Anal. 40 (2006) 227–234.
[16] J.F. Kauffman, B.J. Westenberger, J.D. Robertson, J. Guthrie, A. Jacobs, S.K. Cummins, Regul. Toxicol. Pharmacol. 48 (2007) 128–134.
[17] A.S.R.K. Murty, U.C. Kulshresta, T.N. Rao, M.V.N.K. Talluri, Indian J. Chem. Technol. 12 (2005) 229–231.
[18] N. Lewen, J. Pharm. Biomed. Anal. 55 (2011) 653–661.
[19] I. Oehme, O.S. Wolfbeis, Mikrochim. Acta 126 (1997) 177–192.
[20] I. Klimant, M. Otto, Mikrochim. Acta 108 (1992) 11–17.
[21] W.A. Oliveira, R. Narayanaswamy, Talanta 39 (1992) 1499–1503.
[22] M. Ahmad, R. Narayanaswamy, Talanta 42 (1995) 1337–1344.
[23] M.J. Ayora Cañada, M.I. Pascual-Reguera, A. Molina-Diaz, Anal. Chim. Acta 375 (1998) 71–80.
[24] M. Ahmad, R. Narayanaswamy, Anal. Chim. Acta 291 (1994) 255–260.
[25] N. Malcik, O. Oktar, M.E. Ozser, P. Caglar, L. Bushby, A. Vaughan, B. Kuswandi, R. Narayanaswamy, Sens. Actuators B B53 (1998) 211–221.
[26] A.A. Ensafi, M. Fouladgar, Sens. Actuators B B113 (2006) 88–93.
[27] B. Kuswandi, M.N. Taib, R. Narayanaswamy, Sens. Actuators A 76 (1999) 183–190.
[28] Z.T. Jiang, Anal. Chim. Acta 392 (1999) 247–253.
[29] A.A. Vaughan, R. Narayanaswamy, Sens. Actuators B 51 (1998) 368–376.
[30] M.K. Amini, T. Momeni-Isfahani, J.H. Khorasani, M. Pourhossein, Talanta 63 (2004) 713–720.
[31] A.A. Ensafi, Z.N. Isfahani, IEEE Sens. J. 7 (2007) 1112–1117.
[32] I.M. Steinberg, A. Lobnik, O.S. Wolfbeis, Sens. Actuators B 90 (2003) 230–235.
[33] P. Ciosek, W. Wroblewski, Analyst 132 (2007) 963–978.
[34] A.K. Deisingh, D.C. Stone, M. Thompson, Int. J. Food Sci. Technol. 39 (2004) 587–604.
[35] M.C. Janzen, J.B. Ponder, D.P. Bailey, C.K. Ingison, K.S. Suslick, Anal. Chem. 78 (2006) 3591–3600.
[36] V. Grdinić, J. Vuković, J. Pharm. Biomed. Anal. 35 (2004) 489–512.
[37] D.L. Massart, B.G.M. Vanderginste, L.C.M. Buydens, S. de Jong, P.J. Lewi, J. Smeyers-Verbeke, Handbook of Chemometrics and Qualimetrics: Part A, Elsevier Science, Amsterdam, 1997.
[38] L.F. Capitan-Vallvey, M.D. Fernandez-Ramos, P. Alvarez de Cienfuegos, F. Santoyo-Gonzalez, Anal. Chim. Acta 481 (2003) 139–148.
[39] J.N. Miller, J.C. Miller, Statistics, Chemometrics for Analytical Chemistry, Pearson Education Limited, Essex, 2000.
[40] J.R. Paddock, A.T. Maghasi, W.R. Heineman, C.J. Seliskar, J. Chem. Ed. 82 (2005) 1370–1371.
[41] S.D. Kolev, T.J. Cardwell, R.W. Cattrall, L.d. Coo, Talanta 82 (2010) 1156–1163.
[42] F. Lazaro, M.D.L. de Castro, M. Valcarcel, Anal. Chim. Acta 214 (1988) 217–227.
[43] J.E. Madden, T.J. Cardwell, R.W. Cattrall, L.W. Deady, Anal. Chim. Acta 319 (1996) 129–134.
[44] S.B. Savvin, L.M. Trutneva, O.P. Shvoeva, V.K. Belyaeva, I.N. Marov, Zh. Neorg. Khim. 36 (1991) 393–399.
[45] N. Alizadeh, A. Moemeni, M. Shamsipur, Anal. Chim. Acta 464 (2003) 187–196.

[46] I.M. Maksimova, E.I. Morosanova, A.A. Kukhto, N.M. Kuz'min, Y. Zolotov, Zh. Anal. Khim. 49 (1994) 1210–1214.
[47] E. Morosanova, A. Velikorodnyi, Yu Zolotov, Fres. J. Anal. Chem. 361 (1998) 305–308.
[48] S.A. Morozko, V.M. Ivanov, J. Anal. Chem. 50 (1995) 572–578.
[49] ICH Harmonized Tripartite Guideline prepared within the International Conference on the Harmonization of Technical Requirements for the Registration of Pharmaceuticals or Human Use (ICH) Q2B, Validation of Analytical Procedures: Methodology, 1996. Available from: <http://www.ich.org/ich5q.html>.
[50] M. Thompson, S.L.R. Ellison, R. Wood, Pure Appl. Chem. 74 (2002) 835–855.