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.

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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.