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The chromatomembrane method used for sample preparations in the spectrophotometric determination of zinc and copper in pharmaceuticals

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Abstract

The chromatomembrane cell (CMC) was used to extract zinc and copper and to preconcentrate them for their separation from pharmaceutical preparations. The method performance was evaluated at the determination of the enrichment factor, the linearity of response, the reproducibility, the accuracy and the sensitivity. In practice a five-fold enrichment has been enough provided that sample sizes of 0.825 ml were introduced. Good linearities for zinc and copper ($r^2 > 0.99$) were observed under this condition. The relative standard deviation (<3%) proved the good reproducibility of the method. The accuracy has been verified using model solutions, which were prepared from a certified reference. Recoveries of 98.8, 99.5 and 100.3% were achieved with solutions containing 0.1, 0.3 and 0.5 μg ml^{-1} Zn(II), respectively. In case of copper 101.2, 99.6 and 100.6% recovery were obtained for 0.1, 0.4 and 0.7 μg ml^{-1} Cu(II), respectively. The detection limits (three-fold of the signal-to-noise ratio) were estimated at 0.04 μg ml^{-1} for both. The proposed method was applied on pharmaceutical preparations and the results were found to be in a good agreement with the contents guaranteed by the producers.
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Keywords: Chromatomembrane cell; Extraction of zinc and copper; FIA; Pharmaceuticals

1. Introduction

Copper has been used as a medicine since thousends of years whether for the treatment of chest wounds or the purifying of drink water. More recently, research has indicated that copper helps prevent inflammation in arthritis and similar diseases. Recent investigations are going on into antiulcer and antiinflammatory medicine as well as its application in radiology and for treating convolutions and epilepsy.

Although Cu^{2+} in water is hazardous to many aquatic organisms, minor amounts of it are needed in the diet for the activation of several enzymes. Thus, the monitoring of Cu is warranted because of the narrow window between essential and toxic concentrations. Zinc is an essential trace element for humans, animals, plants, and microorganisms. In the human body the zinc content amounts to 2–4 g. It is of great importance for all replications, gene expressions and for the metabolism of nucleic acids and different proteins [1].

Commonly, the analytical methods for the quantitation of zinc and copper are neutron activation analysis (NAA), atomic absorption spectrometry (AAS) and inductively coupled plasma mass spectrometry (ICP-MS), which all base on expensive and sophisticated instruments [2]. Inductively coupled plasma atomic emission spectrometry (ICP-AES) is one of the most used techniques for the determination of zinc. The most sensitive line for Zn is 213.856 nm. However, this line exhibits spectral interferences from elements like Fe, Ni, and Cu [3].

Along with the advancement of various physicochemical methods, pharmaceutical analysis showed a trend towards the use of automation of analytical assays carried out in appropiate control laboratories. At the same time, during its thirty years of existence, the flow injection analysis (FIA) technique became a versatile tool that contributed substantially to the development of automation in pharmaceutical analysis. The results from such advantages of FIA like the simplicity of instrumentation, high throughput capacity, reliable and inexpensive determinations, and the possibility of gathering a large body of analytical information [4].

In order to achieve accurate, reliable and sensitive results, extraction, preconcentration and separation are needed. Many

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extraction and preconcentration techniques for the determination of zinc and copper in FIA have been reported which include solid phase extraction (SPE), knotted reactor (KR), immobilization, ion exchange column, and liquid–liquid extraction. A great variety of complexing reagents, e.g. PAN [5–7], 5-Br-PADAP [3], PAR [8], Zincon [9], has been used for separating the distinct metals.

Liquid–liquid extraction (LLE), however, offers diverse feasibilities of analyte separation, and the extractant itself can be supplied directly to detection units like gas or liquid chromatography for instance. Drawbacks such as hazardous organic solvents have to be overcome by reducing sample sizes as much as possible. Flow injection spectrophotometry allows the extracted metal complexes to be detected either directly [10] or by using sophisticated techniques like MSFA-LLE (monosegmented flow analysis with liquid–liquid extraction) [11,12] and open-phase separators [13].

A number of organic reagents have been proposed for the photometric flow injection determination of zinc and copper. None of them, however, exhibits outstanding selectivity for these compounds. One of them is 1-(2-pyridylazo)-2-naphthol [PAN]. This reagent is practically insoluble in water, soluble in alkali with salt formation, and soluble in organic solvents to give yellow solutions with absorption maxima at 470 nm. The reagent does not absorb above 560 nm. PAN reacts with many metal ions to give intensively coloured chelate complexes, which can be extracted with CCl_4, CH_3Cl, benzene or diethylether. The divalent metal PAN complex has tetrahedral geometry at the metal ion and exists in two tautomer forms. It acts as a tetradentate ligand complexing with the metal through the hydroxyl oxygen atom, the pyridine nitrogen atom and one of the azogroup nitrogen atoms. Its Pd(II) and Co(II) complexes are green, while the other metal complexes vary in different shades of red.

More recently, a variety of nonclassical modes of performing LLE have been described which avoid several practical problems and beg of a critical reevaluation of the role of LLE in sample-preparation. Such nonclassical modes of LLE are [14] segmented flow techniques, single liquid drop techniques, unsupported liquid membrane techniques with three phases, and supported liquid membrane techniques. The interest in flow systems combined with membranes for substance separation is due to the simplicity of automation of extraction procedures.

Principally new ideas, however, are requested for the development of continuously working extraction devices, which need only smallest volumes of the organic phase. From this standpoint the liquid–liquid extraction with the chromatomembrane cell (CMC) is a promising technique, by which the three steps of the extraction procedure are combined in one small device. Fully automated processing is attainable, and the method is adaptable to the most conditions possibly given.

The chromatomenbrane method [15] is based on capillary effects that arise in hydrophobic porous media. Mass transfer between the flows of immiscible liquids or a liquid and a gas is accomplished in hydrophobic biporous PTFE. The fluxes of two phases move independently due to the presence of two types of pores in the PTFE significantly differing in their size. The macropores are selected so that the capillary pressure in them is negligibly small and does not hinder the transport of the polar liquid phase. In contrast, the micropores are so narrow that the capillary pressure prevents the polar liquid phase from penetration into them. At the same time the micropores must assure the substantial permeability of the biporous medium for the flux of the gas or the nonpolar liquid. The supply of the two phases into the CMC and their final separation occur by suitable placing of microporous PTFE membranes which prevent the throughflow only of the polar phase.

The principles of the chromatomembrane mass transfer have been used in FIA mainly for determining inorganic and organic substances in environmental samples. The CMC set-up was mostly coupled to photometric or chromatographic detection units [16–21]. Until now we do not have knowledge of the application of this method for the determination of zinc and copper in pharmaceutical preparations. Therefore, we feel encouraged to use the chromatomembrane method as a sample preparation step for the continuous spectrophotometric determination of these compounds in pharmaceutical preparations.

2. Experimental

2.1. Apparatus

The flow injection analysis system (Lambda FIAS-300, Perkin-Elmer GmbH, Bodenseewerk Überlingen) consists of three peristaltic pumps fitted with Tygon pump tubes, six-port electrically actuated selection valves and a Lambda 2 S Spectrophotometer (Perkin-Elmer) equipped with a 10 mm light path flowthrough cell with a volume of 18 μl. Data acquisition and device control were accomplished using a PC interface board. The FIAS software package obtainable from Perkin-Elmer for computer aided flow analysis, device control and data acquisition was used throughout. The flow system manifold includes Tygon tubing (0.95 mm i.d.), a replacement bottle, and a four-holes CMC, which consists of a rectangular PTFE block with macropores (250–500 μm) and micropores (1–2 μm). The dimension of the PTFE block was 17 mm × 10 mm × 8 mm giving a total volume of about 1.2 ml. The CMC was equipped with inlets and outlets for the both phases, its separation has been effected from two microporous PTFE membranes. The two phases have had a direction of 90° to each other. A PTFE tubing of 0.8 mm i.d. was used for connecting the different parts of the entire manifold. The zinc and copper PAN complexes were measured at 556 nm each at different pH (but not simultaneously).

2.2. Reagents, chemicals and samples

The Cu(II)-solutions with concentrations from 0.05 to 0.8 μg ml^{-1} were prepared by dilution of a stock solution of 10 μg Cu^{2+} in 0.2 M sulfuric acid.

A series of Zn(II)-solutions with concentrations from 0.05 to 0.6 μg ml^{-1} was prepared by dilution of a stock solution of 10 μg Zn^{2+} in 0.2 M sulfuric acid.

A 0.1% *m*/*v* (4×10^{-3} M) PAN (avocado) solution was prepared by dissolving the solid substance in ethanol.

The required buffer solutions at different pH (1.5, 3, 4, 4.5, 5, 6 and 7) were obtained by adding acetic acid or sodium hydroxide to a 0.5 M acetate buffer.

Unizink® 50 (Köhler Pharma GmbH), Zineryt® (Hermal), Zinkit® 3, 10, 20 (Wörwag Pharma), Zincfrin® (Alcon Pharma GmbH), Zinkorotat 20 (Ursapharm), Zink-D-Longoral® (Cassela-med GmbH Köln, Artesan Pharma GmbH Lüchow), Zinkoxid Salbe Law (Riemser Arzneimittel AG), Zinkoxid Emulsion Law (Riemser Arzneimittel AG), Zinkpaste Law (Riemser Arzneimittel AG) Zinksalbe Dialon® (Engelhard Arzneimittel), and Kupferorotat (Ursapharm) were used as pharmaceutical samples.

2.3. Analytical procedure

Fig. 1 shows the schematic diagram of Zn-PAN and Cu-PAN formation followed by extraction with chloroform and phase separation in the proposed flow system. Table 1 summarizes the device sequence for the determination of zinc and copper.

The automated analysis consists of three steps:

(a) Prefill: chloroform was pumped with 1.0 ml min^{-1} flow rate through the CMC. Its micropores were filled and the entire cell cleaned from possibly retained Zn and Cu complexes.
(b) The sample (0.8 ml min^{-1}), the buffer solution (0.8 ml min^{-1}) and the PAN (0.2 ml min^{-1}) were simultaneously supplied to the system. Sample and buffer passed the mixing coil first and reacted then with PAN in the reaction coil.
(c) The Zn and Cu complexes were extracted inside the CMC and eluted with chlororform (1.0 ml min^{-1}). The coloured complex was detected at 556 nm in the flow through cell and continuously monitored on the PC screen.

Thus, each complete cycle takes 6 min.

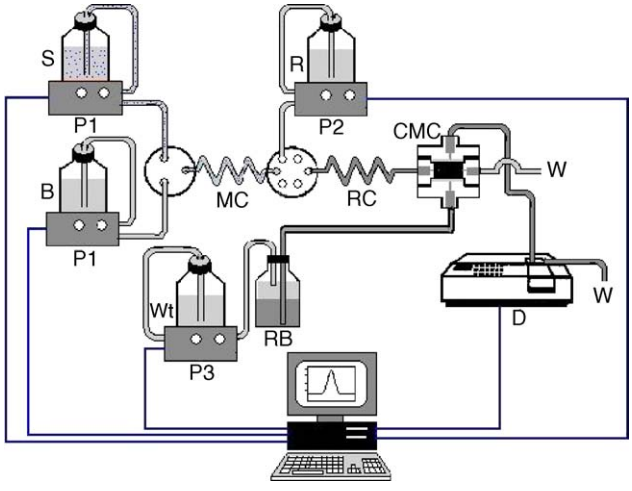

Fig. 1. Schematic diagram of the FIA for determination of zinc and copper: S, sample; B, buffer solution; R, reagent (PAN); CMC, chromatomembrane cell; P1, P2, P3, peristaltic pumps; MC, mixing coil; RC, reaction coil; V, six-ports valves; Wt, water; RB, replacement bottle filled with CH_3Cl; D, detector (spectrophotometer UV–vis Lamda 2S); W, wast.

3. Results and discussion

3.1. Optimization of the FIA system

3.1.1. Instrumental variables

The read time on the PC corresponds to the elution time, which depends on both, chemical variables (pH and PAN conc.) and system variables (flow rates, preconcentration time and coil lengths). The read time of the peak had to be sufficient for the highest complex concentation used for the calibration experiments. A short prefill-time should be included in order to avoid definitely any sample carry over. Thus, an elution time of 240 s was appropriate to guarantee linear calibration-plots up to 0.6 μg Zn per liter and 0.8 μg Cu per liter, respectively.

3.1.2. Chemical variables

PAN is somewhat soluble in water as a neutral molecule in pH range between 3 and 11. But the chelate formation is obstructed because of the hydrolytic instability of the metalions at pH > 8. The Zn-PAN complex shows high adsorbance at pH 6, the Cu-PAN complex at pH 4. Therefore, buffer solutions ranging between pH 4 and 6 were used in this study.

PAN concentrations were evaluated in the range from 2×10^{-4} to 10×10^{-4} M. Zinc shows a gradual increase in sensitivity as the reagent concentration increases, but is relatively constant from 8×10^{-4} M up. On the contrary, the PAN concentration is not the deciding factor in the system design for the determination of copper. Due to the baseline absorbance a PAN concentration of 4×10^{-4} M was preferred for the determination of copper.

3.1.3. Variables of the continuous flow system

The mixing coil (MC) in knotted reactor form was fixed at 90 cm in order to guarantee the mixing of sample and buffer solution. The preconcentration of the sample should be carried out either by changing the preconcentration time at a fixed flow rate of the sample or by changing the flow rate at fixed preconcentration time. The preconcentration time varied between 0.25 and 2.0 min for Zn and between 0.5 and 2.0 min for Cu each at fixed sample flow rates of 0.8 ml min^{-1}, which corresponds with enrichment factors from 1.27 to 10.2 assuming a final volume of 0.4 ml chloroform (that is the estimated volume of the micropores inside the CMC). One minute preconcentration time was preferred, so that the relation between signal level, elution time and sample throughput resulted in an optimum. Laying down this time the sample flow rate became varied between 0.6 and 1.3 ml min^{-1}, which corresponds to enrichment factors from 4 to 7.5. Because of leakages possible at higher flow rates all samples were supplied with 0.8 ml min^{-1} to the CMC.

Increasing flow rates of chloroform decrease the peak height, whereas, the peak area remains constant. So its flow rate was compromized to 1.0 ml min^{-1}.

The lengths of reaction coils were tested in the range 30–180 cm for Zn and 30–150 cm for Cu. Because of different formation rates of the two complexes a coil length of 150 cm was chosen for zinc and 90 cm for copper. Both elements were not determined simultaneously.

Table 1
Device sequence for the FIA system

Step	Time (s)	Pump 1 (ml min^{-1})	Pump 2 (ml min^{-1})	Pump 3 (ml min^{-1})	Valve	Read time (s)
Prefill	60	0.825	0	0.951	Inject	
1	60	0.825	0.18	0	Fill	
2	240	0	0	0.951	Inject	240

Table 2
Analytical figures of merit

Parameter	Zinc	Copper
Linear dynamic range (μg ml^{-1})	0.05–0.6	0.05–0.8
Function	$0.3515c + 0.0116$	$0.1411c + 0.0055$
Correlation coefficient (r^2)	0.9961	0.9968
R.S.D. ($n = 5$) (%)	2.7 (0.5)[a]	1.1 (0.6)[a]
Detection limit (μg ml^{-1})	0.04[b]	0.04[b]
Sampling frequency (h^{-1})	10	10
Enrichment factor	5	5
Volume of sample used (ml)	0.825	0.825

c: concentration of standard solutions.
[a] Concentration of Zn or Cu (μg ml^{-1}) at which R.S.D. was established.
[b] 3σ criterion.

3.1.4. Analytical figures of merit

According to the above-discussed conditions the calibration curves for zinc and copper were performed. The relationship between the absorbance and the Zn^{2+} concentration was found to be linear in the range from 0.05 to 0.6 μg ml^{-1} and in accordance with the equation $y = 0.3515c + 0.0116$ (y means the absorbance and c the Zn^{2+} conc.). The linear regression coefficient amounts to $r^2 = 0.996$ ($n = 5$).

The equation for calibration is $y = 0.1411c + 0.0055$ in case of Cu^{2+} with a linear range between 0.05 and 0.8 μg ml^{-1}, $r^2 = 0.997$ ($n = 5$). The time necessary for one complete cycle was 6 min that means the procedure has a throughput of 10 samples per hour in both cases. Table 2 summarizes the results.

Recoveries of 98.8, 99.5 and 100.3% were achieved with solutions containing 0.1, 0.3 and 0.5 μg ml^{-1} Zn^{2+}, respectively. In case of copper 101.2, 99.6 and 100.6% recovery were obtained for 0.1, 0.4 and 0.7 μg ml^{-1} Cu^{2+} concentration, respectively. The limit of detection (three-fold of the signal-to-noise ratio) is 0.04 μg ml^{-1} in both cases.

3.1.5. Determination of zinc and copper in pharmaceutical preparations

The samples were prepared by acid digestion: one tablet was dissolved in 8 ml of 50% (v/v) HCl and 2 ml of 50% (v/v) HNO_3 in a 100 ml conical flask. Gently warming on a hot plate completed the dissolving. Suspended particles, which might be present in the solution became removed on Millipore membrane filters (0.45 μm pore size). The samples were diluted with tridestilled water so that the metal ion concentrations came next to the calibration range.

The results were listed in Table 3. One should remark that the simplified digestion procedure as described above might not be adequate for the analysis of every pharmaceutical preparation.

PAN is a nonselective chelating reagent: The main interfering species are Cd^{2+}, Hg^{2+}, Ni^{2+} [11], Mn^{2+}, Co^{2+}, Fe^{2+} and Fe^{3+} [7]. These metal ions, however, are not present in the pharmaceutical samples analyzed with the proposed method and should not present a drawback to the use for this kind of determination. Therefore, it was not investigated how to avoid those interferences. Phoshates and organic matter in form of humic acids (5 mg l^{-1}), citrates, tartrates, sulfides, oxalates, fluorides (8 mg l^{-1}) and nitrates (20 mg l^{-1}) are not found to impair the formation of the Zn–PAN complex [22].

The main advantage of the proposed procedure is the flexibilty of the system. Preconcentration of the target compound can be carried out by changing either the flow rate of the sample or the preconcentration time. Although the analytical throughput of this method is relatively low, it still outclasses the FIA

Table 3
Determination of zinc and copper in pharmaceutical preparations

Pharmaceuticals	Form	Main content	Unit	Certified value[a]	Found[a]	Recovery (%)
Unizink® 50	Tablet	Zinc-DL-	mg/tablet	10	9.74	97.4
Zineryt®	Powder	Zinc	mg/g	68.59	65.88	96.0
Zinkit® 3	Tablet	$ZnSO_4 \cdot 7H_2O$	mg/tablet	3	2.93	97.8
Zinkit® 10	Tablet	$ZnSO_4 \cdot 7H_2O$	mg/tablet	10	9.73	97.3
Zinkit® 20	Tablet	$ZnSO_4 \cdot 7H_2O$	mg/tablet	20	19.58	97.9
Zincfrin®	Liquid	Zinc sulphate	mg/ml	2.5	2.43	97.1
Zinkorotat 20	Tablet	Zinkorotat $2H_2O$	mg/tablet	3.2	3.06	95.6
Zink-D-	Tablet	Zinc-D-	mg/tablet	6.54	6.26	95.6
Zinkoxid Salbe	Ointment	Zinc oxide	mg/g	80	77.08	96.3
Zinkoxid	Emulsion	Zinc oxide	mg/g	200	195.32	97.7
Zinkpaste Law	Paste	Zinc oxide	mg/g	160	155.37	97.1
Zinksalbe	Ointment	Zinc oxide	mg/g	80	77.44	96.8
Kupferorotat	Tablet	Cu(II)-orotate	mg/tablet	0.31[b]	0.31[b]	99.9

[a] As Zn: average of three measurements.
[b] As Cu: average of three measurements.

counterparts owing to the fact, that it uses cheap and readily available equipment; it needs reagents in the range of microliters, works fully automated and is simple to assemble and to operate.

4. Conclusions

The chromatomembrane cell has been successfully applied in the determination of zinc and copper in pharmaceutical preparations. The proposed procedure proves a good performance, which is indicated by several parameters, like enrichment factor, linearity of the calibration plot, reproducibility, accuracy and sensitivity of the method. The results are found to be in a good agreement each with the certified content.

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