The Analyst
FULL PAPER

Normal and differential demasking flow-injection manifold for the direct spectrophotometric determination of zinc(II) in biological materials and pharmaceutical formulations

Demetrius G. Themelis,* Paraskevas D. Tzanavaras, Aspasia A. Liakou, Harrisis D. Tzanavaras and John K. Papadimitriou

Department of Chemistry, Laboratory of Analytical Chemistry, Aristotle University of Thessaloniki, 54006 Thessaloniki, Greece. E-mail: themelis@chem.auth.gr

Received 20th July 2000, Accepted 15th September 2000
First published as an Advance Article on the web 25th October 2000

A normal and a differential demasking flow-injection (FI) manifold were developed and optimized for the spectrophotometric determination of microamounts of Zn(II) in biological materials and pharmaceutical formulations. The reported method is very sensitive, rapid, simple and it is based upon the reaction of Zn(II) with 2,2′-dipyridyl-2-pyridylhydrazone (DPPH) in a strongly basic medium to form a yellow-coloured complex (λ_{max} = 448 nm). By using the differential demasking manifold, the tolerance of the method to many cations was enhanced by a mean factor of 25. The obtained calibration graphs were linear in the range of 0–10 mg l^{-1} Zn(II), at a sampling rate of 120 injections h^{-1} in both cases. The precisions of both manifolds were very good (RSD = 0.6 and 0.8%, respectively) and the 3σ detection limits were quite satisfactory (c_L = 4 and 6 ng ml^{-1} respectively). The method has been successfully applied to the determination of Zn(II) in serum, human hair and pharmaceutical formulations with recoveries ranging between 98.0 and 101.6%. The obtained results were also in excellent agreement with flame atomic absorption spectrometry (FAAS), since the mean relative error was e_r = 0.9%.

Introduction

Zinc is an essential element in the nutrition of animals and man. It acts as a cofactor in numerous enzymes, and plays an important role in protein synthesis and cell division. It exerts a crucial influence on the maintenance of cell membrane stability and in the function of the immune system. It also constitutes an active ingredient in medical products intended for topical application. Zinc salts are used in ophthalmic solutions, in lotions intended for treatment of chronic skin diseases and are incorporated as astringents in various solutions.[1] Zinc deficiency effects may be severe. They range from impaired neuropsychological functions and wound healing to growth retardation, immune disorders and dermatitis. On the other hand, zinc can be toxic when exposures exceed physiological needs. After single or short-term exposure to concentrations of zinc in water and beverages between 1.0–2.5 mg l^{-1}, poisoning incidents with symptoms of gastrointestinal distress, nausea and diarrhea were reported. Additionally, pharmacological intakes of zinc have been associated with effects such as leukopenia and/or hypochronic microcytic anaemia and decreases in serum high density lipoprotein concentrations.[2]

It is, therefore, important to monitor the concentration of zinc and its salts in both biological materials and pharmaceutical formulations. For this reason, a simple, precise and accurate method, which allows the determination of the analyte with low operational cost and maximum sample throughput is required. Flow-injection (FI) analysis is a very effective technique for the automation of chemical analysis and has increasingly served the above purposes.[3] Several FI procedures have been reported for the determination of Zn(II) in biological materials and/or pharmaceutical formulations using FAAS[4–8] and ICP-AES[9,10] as detection systems. UV-Vis spectrophotometry is an advantageous alternative to the previously mentioned systems in terms of simplicity and low operational cost. The FI spectrophotometric methods that have been reported for these kind of samples employ solid phase spectrophotometry,[11,12] chemometrics,[13,14] the use of the ZINCON reagent,[14,15] pH gradients,[16] or the use of thiocyanate and malachite green in the presence of surfactants.[17]

The proposed method is very sensitive, simple, rapid, with a better dynamic range, and offers many significant improvements over the above FI spectrophotometric methods, *e.g.*, it does not require strict pH adjustment,[16] complicated procedures,[11,12] advanced chemometrics,[13,14] different masking agents for each interfering ion,[15] or even the use of an anion exchange column in order to enhance the selectivity.[11] Particularly, the two latter disadvantages were overcome by developing a FI masking–differential demasking technique, which offers significant selectivity enhancement for many cations.

The method is based upon the reaction of Zn(II) with 2,2′-dipyridyl-2-pyridylhydrazone (DPPH) in a strongly basic medium to form a yellow-coloured complex which is monitored spectrophotometrically at 448 nm.[18] DPPH was first synthesized in our laboratory,[19,20] and has been recently used for the FI spectrophotometric determination of Co(II) in cyanocobalamin samples[21] and Pd(II) in catalysts.[22] Although the developed normal manifold offers very satisfactory sensitivity, it is subject to interferences mainly from Cu(II) and Fe(III) which limit its application only to pharmaceuticals, where Zn(II) is the active ingredient and is found in high concentrations. In order to achieve the proper selectivity, and thus being able to determine Zn(II) in serum and human hair without any loss in sensitivity and sample throughput, a second FI manifold was developed based upon a differential demasking technique previously proposed by Platte and Marcy.[23] In this technique, heavy metals, including Zn(II), are masked by adding KCN to the sample. Chloral hydrate or formaldehyde is added after the

 DOI: 10.1039/b005875k

colour forming reagent in order to free the Zn(II) differentially and rapidly, without destroying the other metallic cyanide complexes, according to the reaction:

$$[Zn(CN)_4]^{2-} + 4\ \underset{\text{chloral hydrate}}{CCl_3CH(OH)_2} \rightarrow Zn^{2+} + 4\ \underset{\text{3-trichloro-2-hydroxypropionitrile}}{CCl_3CH(OH)CN} + 4\ OH^-$$

Since the differential demasking technique is basically a kinetic based technique, it is somehow 'ideal' for incorporation in a FI system, where the basic feature is that no chemical equilibrium of the reaction is required prior to measurement. By using this two-stage masking–differential demasking technique, we were able to enhance the selectivity of the determination by a mean factor of 25 for several cations, without considerable loss in sensitivity and sampling rate, and without using a different masking agent for each ion.

Experimental

Instrumentation

The FI system used was a Tecator 5010 analyzer (Tecator, Hõganas, Sweden) with a Tecator chemifold Type III manifold which is shown schematically in Fig. 1. The detector used was a Tecator 5023 FI star double-beam spectrophotometer, consisting of a 5032 detector controller and a 5023-011 spectrophotometer optical unit. The absorbance of the coloured Zn(II)–DPPH complex produced was monitored at 448 nm through a 1 cm path length flow cell. The flow system used was 0.5 mm id Teflon tubing throughout. Tygon pump tubes were used for delivering the aqueous solutions.

An Orion EA940 pH-meter (Cambridge, MA, USA) was employed for the pH measurements with absolute accuracy limits at the pH measurements being defined by NIST buffers.

A Perkin-Elmer Model 5100 PC atomic absorption spectrometer (Norwalk, CT, USA) was used to determine zinc(II) by using FAAS as the reference method.

Reagents

All chemicals were of analytical-reagent grade and were provided by Merck (Darmstadt, Germany), unless stated otherwise, and all the solutions were made up by doubly deionized water.

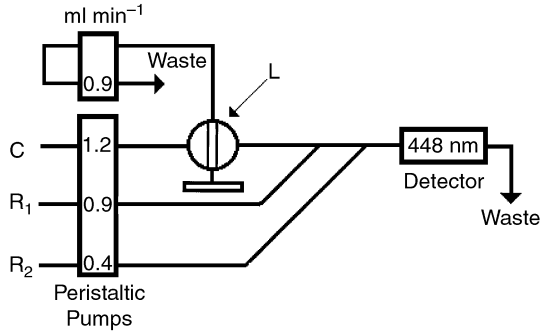

Fig. 1 Optimized normal and differential demasking FI setup for the determination of Zn(II). C, carrier stream (water for the normal setup and 5.1 g l^{-1} KCN–0.15% m/v ascorbic acid for the differential demasking setup); R_1, 2×10^{-3} mol l^{-1} DPPH reagent stream for both FI setups; R_2, 0.15 mol l^{-1} NaOH for the normal setup and 0.15 mol l^{-1} NaOH–0.25% v/v formaldehyde stream for the differential demasking setup; L, sample loop (130 μl).

DPPH was synthesized and identified as reported previously.[19,24] A standard stock 0.1 mol l^{-1} DPPH solution was prepared by dissolving the appropriate amount of the reagent in ethanol. This solution is stable for several months if kept in an amber-glass bottle.

The standard zinc nitrate stock solution [γ(Zn(II)) = 1000 mg l^{-1}] also contained 0.5 mol l^{-1} HNO_3 in order to prevent hydrolysis of the ions.

A standard stock solution of 0.15 mol l^{-1} NaOH was used for pH adjustment.

The masking agent was a mixture of 5.1 g l^{-1} KCN and 0.15% m/v ascorbic acid, while the demasking agent was 0.75% v/v formaldehyde in 0.15 mol l^{-1} NaOH. [**Caution**]: cyanide and formaldehyde are very toxic substances; in addition formaldehyde is carcinogenic. For these reasons, special precautions should be followed. The handling of these reagents is quite simple in FI systems, because FI is a closed system. The safe disposal of CN^- and formaldehyde was made by their oxidation to N_2, CO_2 and CO_2 respectively with the hypochlorite ion, maintaining the pH greater than 10 to avoid the formation of toxic cyanogen chloride.

Working solutions of Zn(II), DPPH and NaOH were prepared by dilution immediately before use.

Procedure for aqueous solutions

The FI setup used is depicted schematically in Fig. 1. The normal FI setup consisted of a carrier stream C (water) and two reagent streams (R_1, R_2) which were merged with the carrier stream. The R_1 reagent stream was 2×10^{-3} mol l^{-1} DPPH in 30% v/v EtOH. The R_2 reagent stream was 0.15 mol l^{-1} NaOH to adjust the pH. A 130 μl sample containing between 0 and 10 mg l^{-1} Zn(II) was injected directly into the carrier stream.

In the differential demasking FI setup, 130 μl of the sample containing 0–10 mg l^{-1} Zn(II) was injected directly into the carrier stream C, which was the masking agent. The carrier/sample stream which carried the masked analyte ($[Zn(CN)_4]^{2-}$) was then merged successively with the DPPH stream (R_1) and the NaOH–demasking agent stream (R_2).

In both manifolds the water-soluble Zn(II)–DPPH complex (λ_{max} = 448 nm) was formed on passage of the mixture through a 10 cm reaction coil. The cycle time was set to 30 s with 15 s cycle injection time. By using the cycle time of 30 s, 120 injections per hour were made.

The transient signal from the detector was recorded as a peak, the height of which was proportional to the zinc concentration in the sample, and was used for all measurements. The recorded peaks were sharp and the baseline was stable. Five replicate injections per sample were made in all instances.

Procedure for blood serum

After collection, approximately 12 ml of the blood samples were left to clot in centrifuge tubes at room temperature; 15 min later, the blood clot was detached carefully from the glass wall with a rod and centrifuged immediately at 3000 rpm. A 5 ml portion of the clear supernatant serum was pipetted off and treated with 5 ml of 1.2 mol l^{-1} trichloroacetic acid. The mixture was centrifuged at 3000 rpm, and the deproteinised supernatant was collected. The resulting solution was neutralized with 2 mol l^{-1} NaOH until a pH value of *ca*. 3 was reached and analyzed by using the procedure for aqueous solutions described for both manifolds.

Procedure for human hair

The hair samples were collected from healthy members of the laboratory by means of titanium oxide coated scissors. Each

sample was cut in *ca*. 1 cm long pieces and treated with 20 ml acetone for 10 min. After several washings with doubly de-ionized water to ensure complete removal of the organic solvent, the samples were treated three times with 1% v/v EXTRAN (MA-2, neutral, Merck), for 10 min. The samples were washed with doubly de-ionized water until all the detergent was removed, and dried at 60 °C for 12 h. An accurately weighed amount of the sample (*ca*. 0.5 g) was then treated successively with 20 ml *ca*. 14 mol l^{-1} HNO_3 and 2 ml 30% v/v H_2O_2, and after a period of 2 h, was heated almost to dryness. The resulting residue was treated with 1.0 ml *ca*. 14 mol l^{-1} HNO_3 and then heated gently until complete decolorisation. Finally, the resulting solution was neutralized with 2 mol l^{-1} NaOH, diluted to 100 ml with doubly de-ionized water, and was finally analyzed, using the procedure for aqueous solutions described above for both manifolds.

Procedure for pharmaceutical formulations

The selected pharmaceutical formulations were two commercially available ophthalmic solutions, and one anti-mycosis powder. The only pretreatment step employed for the ophthalmic solutions was the dilution of 2 ml of the sample with doubly de-ionized water to a final volume of 100 ml. The powder was firstly dissolved in 0.1 mol l^{-1} HCl, then neutralized with 1 mol l^{-1} NaOH, and finally diluted to 100 ml with doubly de-ionized water. The resulting solutions were analyzed using the above-described procedure for aqueous solutions for both manifolds.

Results and discussion

Preliminary studies

A two-line FI manifold, using either DPPH or NaOH as a carrier stream, showed that the Zn(II)–DPPH complex could be formed under flow conditions with an absorption maximum at 448 nm.

In this manifold the injection of the sample into the NaOH stream caused a considerable negative intercept of the resulted calibration curve, due to formation of complexes of the type $[Zn(OH)_4]^{2-}$ in the strongly basic medium which probably anticipated the more stable Zn(II)–DPPH complex.

In order to overcome the problem DPPH was used as a carrier stream. However, using this approach, the high ethanol content of DPPH caused serious disturbances of the base-line due to the 'Schlieren effect'.[25] The problem was solved adopting a three-line FI setup, shown in Fig. 1, with water as a carrier stream. Using this FI setup, stopped-flow experiments proved that the chemical reaction was instantaneous, since it reached 100% of the 'steady state' value within 2 s. This means that there is no need of an extra reaction coil to be used except for the possible shorter connections of the manifold.

Finally, in order to develop the differential demasking FI manifold, it was necessary to check whether this two-stage process could be carried out under flow conditions. The experiments showed that the masking–differential demasking process could be easily and very effectively incorporated in the normal FI setup without any changes in the initial design. Two main modifications were introduced in the batch technique proposed by Platte and Marcy.[23] The introduction of ascorbic acid in the KCN masking agent stream proved to be a very effective modification, since it increased greatly the selectivity regarding copper and iron, without affecting considerably the sensitivity of the determination. This was due to the fact that the cyanide complexes of Fe(II) and Cu(I) are much more stable than the Fe(III) and Cu(II) complexes. The other modification had to do with the selection of the demasking agent. Platte and Marcy proposed chloral hydrate as the demasking agent instead of formaldehyde, because, although formaldehyde demasked Zn(II) faster than chloral hydrate, it also demasked the other metals fast enough to cause interferences, requiring the measurement step to take place at much less than a minute after its addition. Taking full advantage of the kinetic nature of FI, we used formaldehyde as the demasking agent with very satisfactory results, since in the developed method the measurement step takes place only a few seconds after the mixing of the sample with formaldehyde. In this way, Zn(II) was demasked rapidly to react with the reagent, while the potential interferents were efficiently masked. By using chloral hydrate, the time needed for quantitative Zn(II) demasking and colour development was *ca*. 2 min, requiring the use of long reaction coils and, thus decreasing significantly the sampling rate.

Optimization of the normal FI manifold

The various chemical and FI variables of the normal FI system shown in Fig. 1 were optimized by using the univariate method at a fixed Zn(II) mass concentration of 5 mg l^{-1}. The absorbance was read in the flow-through cell at 448 nm.

The influence of the pH on the reaction was studied in the range of 10.01–12.39, by using the appropriate amount concentrations of NaOH and measuring the pH in the waste line. The DPPH stream was fixed at $c(\text{DPPH}) = 4.0 \times 10^{-4}$ mol l^{-1}. The experiments showed that maximum complex formation was achieved for pH values varying between 10.9–12.34. This 1.5 pH units 'window' is an advantage of the method, since no strict pH control is required. Thus, a pH of *ca*. 12 was selected as optimal.

Under the above optimal value for the pH, the effect of the amount concentration of DPPH was studied in the range of 1.0×10^{-4}–2.0×10^{-3} mol l^{-1}. The signal intensity increased non-linearly by increasing the amount concentration of the reagent, and leveled off at DPPH amount concentrations from 6.0×10^{-4} to 2.0×10^{-3} mol l^{-1}. A DPPH amount concentration of 2.0×10^{-3} mol l^{-1} was chosen as optimal because at this value the maximum linear range of the calibration curve was achieved. It also should be noted that up to 40% v/v ethanol in the reagent stream, did not have any effect upon the reaction and the stability of the base-line. A 30% v/v of ethanol was chosen as optimal, in order to eliminate any risk of blocking the flow system from precipitation of the reagent.

In order to proceed with the final system, the FI variables were studied under the above optimum chemical variables. The cycle time was set at 30 s with a 15 s cycle injection time.

The injection loop volume was varied from 30 to 330 μl, by changing the length of the sample loop in the injection valve. The peak heights increased non-linearly with increasing sample volume as it is inversely proportional to sample dispersion. A sample injection volume of 130 μl was selected as a compromise between sensitivity, the linear range of the calibration curve and the sampling rate.

The flow rate of the carrier stream is a very important variable in a FI determination, because it influences the dispersion of the sample zone and, thus, the sensitivity of the determination. The flow rate was studied at between 0.41 and 2.40 ml min^{-1}, keeping the pH at *ca*. 12. The peak heights increased non-linearly with increasing the carrier flow rate, showing a maximum at a flow rate of 1.2 ml min^{-1} which was chosen as optimal.

The flow rate of the DPPH stream was studied in the range of 0.40 and 1.60 ml min^{-1}, keeping the flow rate of the carrier and NaOH streams fixed at 1.2 and 0.9 ml min^{-1} respectively. The results showed that, increasing the flow rate of DPPH, a maximum sensitivity was obtained at 0.9 ml min^{-1}, which was then chosen as optimal. Above this value the peak heights were decreased due to dispersion effects.

The flow rate of the NaOH stream was studied in the range of 0.40–1.60 ml min^{-1}, keeping the carrier and DPPH streams fixed at 1.2 and 0.9 ml min^{-1} respectively. The results showed that increase of the flow rate of the NaOH stream between the selected range caused decrease of the absorbance due to dispersion effects. In order to achieve maximum sensitivity, a flow rate of 0.4 ml min^{-1} was chosen as optimal.

Optimization of the differential demasking FI manifold

The optimization of the differential demasking manifold, namely the concentrations of the KCN and ascorbic acid in the carrier stream and the concentration of formaldehyde in the NaOH stream, was performed by making a compromise between sensitivity and selectivity. For this reason, the experiments were carried out under a fixed mass concentration of Zn(II) of 5 mg l^{-1}, and three fixed Cu(II) mass concentrations corresponding to 5, 10 and 20 mg l^{-1}. The selection of copper was based on the fact that Cu(II) was the most critical and serious interference appearing in the normal FI manifold. The other optimal chemical and FI variables were those found for the normal FI manifold.

The effect of the mass concentration of KCN was studied in the range of 0.85–8.5 g l^{-1}, while keeping the formaldehyde content in the NaOH stream at 0.75% v/v. The results are shown in Fig. 2. As can be seen in Fig. 2, by increasing the mass concentration of KCN, the sensitivity of the determination decreased smoothly, while the absorbance caused by Cu(II), decreased dramatically. This means that the selectivity of the method is greatly enhanced. A KCN mass concentration of 5.1 g l^{-1} was chosen as optimal, as a compromise between sensitivity and selectivity. Under this KCN mass concentration the absorbance corresponding to 5 and 10 mg l^{-1} of Cu(II) was totally eliminated.

The effect of the addition of ascorbic acid in the KCN masking agent stream was studied in the range of ascorbic acid of 0–1.5% m/v, under the optimal value found for KCN. The results are shown in Fig. 3. As can be seen in Fig. 3, the addition of ascorbic acid in the masking agent had a marked positive effect upon selectivity. Up to 0.15% m/v concentrations of ascorbic acid the sensitivity was not affected, while the selectivity was greatly enhanced. Thus, a 0.15% m/v ascorbic acid concentration was chosen as optimal.

Under the optimal conditions found for the KCN and the ascorbic acid, the effect of the content of the demasking agent, namely formaldehyde, in the NaOH stream was studied between 0–1% v/v. The results are depicted in Fig. 4. The increase of formaldehyde concentration up to 0.25% v/v caused a dramatic increase in sensitivity, while the selectivity was not affected. Above 0.25% v/v of formaldehyde, the sensitivity leveled off, while the selectivity decreased. This happened because at high formaldehyde concentrations the copper cyanide complexes were destroyed more rapidly.

Finally, the effect of masking and demasking reaction times upon selectivity was checked by using reaction coils of different lengths right after the injection point and before the detector for the masking and demasking reactions respectively. The experiments showed that the demasking reaction is very fast, meaning that the use of an extra reaction coil is not required. Regarding the masking reaction time, the use of reaction coils with lengths varying from 30 to 100 cm, caused a moderate enhancement in selectivity, while at the same time the sensitivity was also seriously decreased from 25 to 40%, due to dispersion. Besides, a serious 50% decrease in sampling rate was also observed. Making a compromise among selectivity, sensitivity and sampling rate, and because the selectivity was already quite satisfactory for the analyzed samples, no reaction coil was used.

Features of the proposed method

Using the normal FI manifold and the optimal conditions described above, the recorded calibration graph for the determination of Zn(II) in aqueous solutions was linear in the range of 0.0–10.0 mg l^{-1}, and is described by the equation

$$A = (1.3 \pm 0.7) \times 10^{-3} + (231.0 \pm 1.5) \times 10^{-3}\ \gamma(\mathrm{Zn(II)})$$

where A is the absorbance as measured by the detector, and γ(Zn(II)) is the mass concentration of the analyte in the aqueous solution with a relative standard deviation of the slope of RSD = 0.6% (n = 8), a correlation coefficient of r = 0.99991 and a 3σ detection limit of 4 ng ml^{-1} (n = 10).

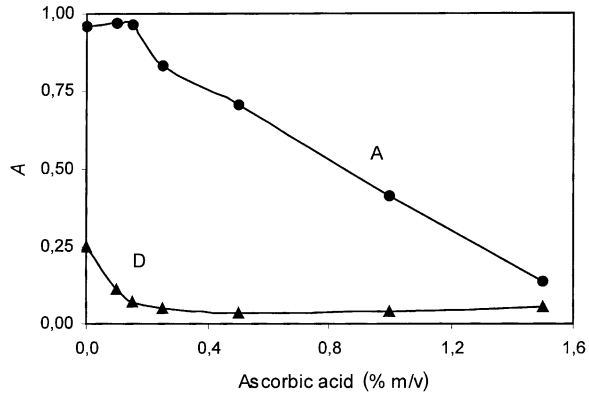

Fig. 3 Effect of ascorbic acid concentration upon the sensitivity and selectivity of the determination: A and D as in Fig. 2.

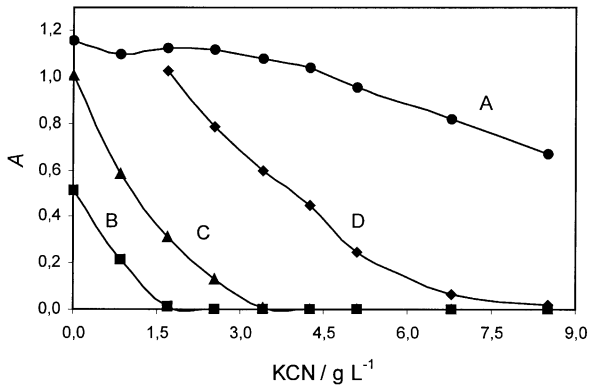

Fig. 2 Effect of KCN mass concentration upon the sensitivity and selectivity of the determination: A, 5 mg l^{-1} Zn(II); B, 5 mg l^{-1} Cu(II); C, 10 mg l^{-1} Cu(II); D, 20 mg l^{-1} Cu(II).

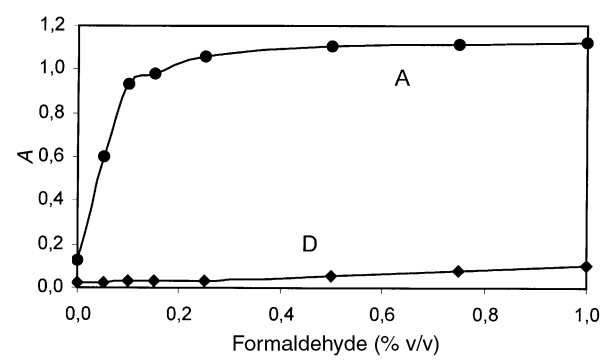

Fig. 4 Effect of formaldehyde concentration upon the sensitivity and selectivity of the determination: A and D as in Fig. 2.

The obtained calibration graph for the differential demasking FI manifold also extends between 0.0 and 10.0 mg l^{-1} Zn(II) and is described by the equation

$$A = (1.4 \pm 0.9) \times 10^{-3} + (217.8 \pm 1.8) \times 10^{-3}\ \gamma(\mathrm{Zn(II)})$$

with a relative standard deviation of the slope of RSD = 0.8% (n = 8), a correlation coefficient of r = 0.9999 and a 3σ detection limit of 6 ng ml^{-1} (n = 10).

All the standards were run in five replicate injections (n = 5), while for each detection limit calculation the blank was run in ten replicate injections (n = 10).

As can be seen in the two equations, there is a slight decrease in sensitivity when the differential demasking FI manifold is employed. The same phenomena was observed by Platte and Marcy.[23]

Interference study

The effect of many diverse ions upon the determination was examined under the optimum conditions described above for both the normal and the differential demasking FI manifolds. The criterion for interference was fixed at an e_r of less than ± 2% in the average absorbance signal taken for a Zn(II) mass concentration corresponding to 5 mg l^{-1}. Fig. 5 depicts a graphical comparison of the two developed manifolds upon the tolerance level of several cations. As can be seen in Fig. 5, the selectivity of the method is increased dramatically for many cations when the differential demasking manifold is employed. Quantitatively, the selectivity of the method is enhanced by a mean factor of 25 when the differential demasking technique is incorporated in a FI manifold over the normal FI mode, a fact that makes the method adequately selective for the determination of Zn(II) in serum and human hair. The following ions were studied in addition to those included in Fig. 5; 5000 mg l^{-1} of K^+, Na^+, NO_3^-, Cl^-, bicarbonates, urea and CH_3COO^-, 1000 mg l^{-1} of Ba^{2+}, PO_4^{3-}, F^-, oxalates and tartrates, 300 mg l^{-1} of Cr^{3+} and 130 mg l^{-1} of Ca^{2+} and Mg^{2+} did not interfere with either manifolds.

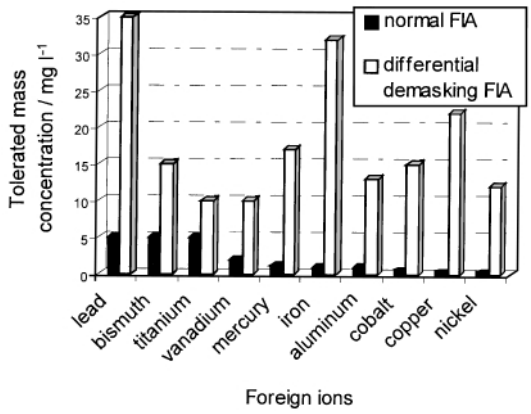

Fig. 5 Graphical comparison of the tolerance level for several cations for the normal and the differential demasking FI manifolds.

Determination of zinc in samples

The proposed method was applied to the determination of the analyte in serum, human hair and pharmaceutical formulations. The selected pharmaceutical formulations were two commercially available ophthalmic solutions (OCULOSAN™ and ZABYSEPT™) containing $ZnSO_4$, and an anti-mycosis powder (UNDEX™-R), containing zinc undecylenate. The results are listed in Table 1. The obtained recoveries for the differential demasking manifold were satisfactory for all samples and varied between 98.0–101.6%. The percentage recovery was determined by using the equation: percentage recovery = $c_{(found)} \times 100/c_{(added)}$, where $c_{(found)}$ is based on the net analyte signal for the 'spiked' sample. As it was expected while the normal manifold gave satisfactory results only for the pharmaceutical samples, where Zn(II) is in high concentrations and there are no serious interferences. Five replicate injections for each sample were made.

Accuracy of the proposed method

The accuracy of the developed method was checked by comparison of the results obtained by both the proposed manifolds to the values obtained by using FAAS as the reference method. The results are shown in Table 2. The calculated relative errors were between e_r = 0.37–1.7% for the differential demasking manifold, indicating the effectiveness

Table 1 Determination of Zn(II) in real samples by using the normal and the differential demasking FI manifolds

	Normal FI manifold			Differential demasking FI manifold		
Sample	Added[a]/mg l^{-1}	Found/mg l^{-1}	100 R[b]	Added/mg l^{-1}	Found/mg l^{-1}	100 R[b]
Serum I	—	1.96		—	1.14	
	1.0	3.70	125.1	1.0	2.09	98.0
Serum II	—	2.38		—	1.18	
	1.0	4.26	126.1	1.0	2.15	98.6
Serum III	—	1.69		—	0.89	
	1.0	3.44	128.2	1.0	1.92	101.6
Hair I	—	1.23		—	0.98	
	1.0	2.54	114.2	1.0	1.94	98.1
Hair II	—	2.16		—	1.55	
	1.0	3.84	121.7	1.0	2.59	101.5
Hair III	—	1.59		—	1.24	
	1.0	3.01	116.2	1.0	2.27	101.3
Oculosan™	—	1.58		—	1.58	
	1.0	2.60	100.9	1.0	1.59	100.6
Zabysept™	—	1.65		—	1.65	
	1.0	2.62	98.9	1.0	2.62	98.9
Undex™-R	—	6.32		—	6.32	
	1.0	7.25	99.1	1.0	7.27	99.3

[a] Mean of five results. [b] Percentage recovery.

Table 2 Comparison of the results obtained by the FI methods with those obtained by the reference FAAS method

	Zinc content found[a]				
Sample	FAAS	Differential demasking FI manifold	e_r[b] (%)	Normal FI manifold	e_r[b] (%)
Serum I	1.13[c]	1.14[c]	+0.88	1.96[c]	+73.4
Serum II	1.16[c]	1.18[c]	+1.7	2.38[c]	+105.1
Serum III	0.90[c]	0.89[c]	−1.1	1.69[c]	+87.7
Hair I	195.2[d]	196.5[d]	+0.66	210.6[d]	+7.8
Hair II	216.2[d]	215.5[d]	−0.37	242.0[d]	+11.9
Hair III	115.4[d]	114.2[d]	−1.0	126.1[d]	+9.2
Oculosan™	0.197[e]	0.195[e]	−1.2	0.195[e]	−1.2
Zabysept™	0.202[e]	0.203[e]	+0.61	0.204[e]	+0.99
Undex™-R	20.2[f]	20.5[f]	+1.5	20.1[f]	−0.49

[a] Mean of five results. [b] Relative error. [c] Zinc concentration in mg l^{-1}. [d] Zinc concentration in μg g^{-1}. [e] Zinc concentration in mg ml^{-1} $ZnSO_4$. [f] Zinc concentration as % m/m zinc undecylenate.

and accuracy of the proposed method for determining Zn(II) in the selected kind of samples, while the normal manifold gave accurate results only for the pharmaceutical products.

Acknowledgements

The authors thank Dr Haris Chatzikonstantinou, Director of Clinical Laboratory of the Hippocratic Hospital of Thessaloniki, Greece, for kindly supplying us with the blood samples.

References

1 The European Agency for the Evaluation of Medicinal Products, Veterinary Medicines Evaluation Unit, European Commission Joint Research Center, Bruxelles, Belgium, April 1997, www.eudra.org/vetdocs).
2 International Programme on Chemical Safety, Environmental Health Criteria for Zinc, International Zinc Association, Sept. 1996, www.ZincWorld.org.
3 J. Ruzicka and E. H. Hansen, *Anal. Chim. Acta*, 1975, **78**, 145.
4 A. S. Attiyat and G. D. Christian, *Clin. Chim. Acta*, 1984, **137**, 151.
5 S. L. Burguera, M. Burguera, A. M. de Abel de la Cruz, N. Anez and O. M. Alarcon, *At. Spectrosc.*, 1992, **13**, 67.
6 K. W. Simonsen, B. Nielsen, A. Jensen and J. R. Andersen, *J. Anal. At. Spectrom.*, 1986, **1**, 453.
7 B. F. Rocks, R. A. Sherwood, L. M. Bayford and C. Riley, *Ann. Clin. Biochem.*, 1982, **19**, 338.
8 R. A. Sherwood, B. F. Rocks and C. Riley, *Analyst*, 1985, **110**, 493.
9 C. W. McLeod, P. J. Worsfold and A. G. Cox, *Analyst*, 1984, **109**, 327.
10 H. B. Lim, M. S. Han and K. J. Lee, *Anal. Chim. Acta*, 1996, **320**, 185.
11 M. J. Ayola-Canada, M. I. Pascual-Reguera and A. Molina-Diaz, *Anal. Chim. Acta*, 1998, **375**, 71.
12 R. M. Liu, D. J. Liu and A. L. Sun, *Talanta*, 1993, **40**, 381.
13 O. Hernadez, F. Jimenez, A. I. Jimenez and J. J. Arias, *Analyst*, 1996, **121**, 169.
14 O. Hernadez, A. I. Jimenez, F. Jimenez and J. J. Arias, *Anal. Chim. Acta*, 1995, **310**, 53.
15 R. M. Liu, D. J. Liu and A. L. Sun, *Talanta*, 1993, **40**, 511.
16 S. Zhao, X. Xia, G. Yu and B. Yang, *Talanta*, 1998, **46**, 845.
17 S. G. Aggarwal and K. S. Patel, *Fresenius' J. Anal. Chem.*, 1998, **362**, 571.
18 G. S. Vasilikiotis and H. Alexaki-Tzivanidou, *Microchem. J.*, 1981, **26**, 519.
19 H. Alexaki-Tzivanidou, PhD Thesis, University of Thessaloniki, Greece, 1972.
20 G. S. Vasilikiotis, T. Kouimtzis, C. Apostolopoulou and A. Voulgaropoulos, *Anal. Chim. Acta*, 1974, **70**, 319.
21 D. G. Themelis, G. A. Zachariadis and J. A. Stratis, *Analyst*, 1995, **120**, 1593.
22 A. N. Anthemidis, D. G. Themelis and J. A. Stratis, *Anal. Chim. Acta*, 2000, **412**, 161.
23 J. A. Platte and V. M. Marcy, *Anal. Chem.*, 1959, **31**, 1226.
24 H. Alexaki-Tzivanidou, *Anal. Chim. Acta*, 1975, **75**, 231.
25 F. H. Bergamin, B. F. Reis and A. G. Zagatto, *Anal. Chim. Acta*, 1978, **97**, 427.