

## **6. Chromatomembrane Method as Sample Preparation for Spectrophotometric Determination of zinc and copper in Pharmaceuticals**

Since its existent, the principle of the chromatomembrane mass transfer has been used in flow injection analysis mainly for determining of inorganic and organic substances in environmental samples and coupled with several detection systems such as photometry, voltammetry, inductively coupled plasma-atomic emission spectroscopy, gas chromatography, ion chromatography, and high performance liquid chromatography with acceptable results. Several publications [98, 111, 113, 115, 133, 134] on the use of the CMC for extraction and pre concentration manifold in spectrophotometric determination of inorganic and organic compounds in environmental samples has been reported.

Copper in drinking water was determined based on its ability to form metal complex with dithizone [111]. A photometric detector measured the absorbance of the copper-dithizone complex formed. Photometric detection was also applied in combination with the CMC for the determination of  $\text{SO}_2$  in the air. An aqueous solution of  $\text{Fe}^{3+}$  and 1,10-phenanthroline was used as reagent, in which  $\text{Fe}^{3+}$  was reduced by  $\text{SO}_2$  forming the well known colour complex [111]. Another method on determination of  $\text{SO}_2$  was proposed by Sritharathikun et. al [134]. In this method,  $2 \text{ mg L}^{-1}$  of triethanolamine (TEA) solution was used as an absorbing solution. The amount of  $\text{SO}_3^{2-}$  in the absorbing solution was measured by spectrophotometry at 550 nm with a mixed reagent of pararosaniline and formaldehyde. The same absorbing solution was used for the indirect determination of  $\text{NO}_2$  in the air as reported by Wei et. al.[98, 133]. The amounts of  $\text{NO}_2^-$  in the absorbing solution were measured by a

diazotation-coupling reaction with sulfanilamide and N-(1-naphthyl) ethylenediamine. The detection was made by a light emitting diode (LED;  $\lambda_{\text{max}} = 525 \text{ nm}$ ) as a light source with a wavelength filter of 530 nm. Erxleben et. al. [113] reported photometric determination of phenol with FIA that includes a CMC for sample preconcentration based on the formation of the coloured phenol-complex which is produced by oxidation of 4-aminoantipyrine. The absorbance of the phenol complex was measured at 460 nm. Photometric determination of anionic surfactants was also studied [115]. The procedure was adopted from the Methylene Blue method. The ion-pair complex was preconcentrated and extracted in the CMC. Spectrophotometric detection was performed at 650 nm.

Several detection systems have been applied in the determination of pharmaceutical. Among them, spectrophotometry is the most widespread method for the detection of pharmaceuticals because of its versatility and low cost. Several examples on the use of spectrophotometry method for pharmaceuticals have been described in Section 2.5.

In this research, the chromatomembrane method was applied for the determination of zinc and copper in pharmaceutical preparations since, based on my knowledge, the application of this method has not been introduced for pharmaceuticals analysis. These facts open the possibilities to use the chromatomembrane method as a sample preparation step for spectrophotometric determinations of these compounds in pharmaceutical preparations.

## 6.1 Determination of zinc and copper

### 6.1.1 Introduction

Copper has been used as a medicine for thousands of years including the treatment of chest wounds and the purifying of drinking water. More recently, research has indicated that copper helps prevent inflammation in arthritis and similar diseases. The recent research is going on into anti-ulcer and anti-inflammatory medicine containing copper, and its use in radiology and for treating convulsions and epilepsy. Although there is not epidemiological evidence that copper can prevent arthritis, there have been claims that the wearing of copper bangles does alleviate the symptoms [135].

Although  $\text{Cu}^{2+}$  in water is hazard to many aquatic organisms, minute amounts of  $\text{Cu}^{2+}$  in the diet are needed for human, plant, and animal enzymes (serving as enzyme activators). Thus, the determination of  $\text{Cu}^{2+}$  in many samples is warranted by the narrow window of the concentration between essential and toxic.  $\text{Cu}^{2+}$  is known to catalyze lipid peroxidation, possibility forming free radicals. However,  $\text{Cu}^{2+}$  is usually incorporated into stable complexes within cells or vascular fluids.  $\text{Cu}^{2+}$  is essential for hemoglobine formation, carbohydrate metabolism, catecholamine biosynthesis, and the cross-linking of collagen, elastin, and hair ceratin [136].

Zinc is essential trace element for humans, animal, plants, and microorganisms; the zinc content in humans is 2-4 g. Zinc plays many

fundamental roles in all replications, gene expression and in the metabolism of nucleic acids and different proteins [137].

Monitoring zinc and copper in natural, environment, biological, pharmaceuticals and other real samples is very important. Commonly, the analytical methods for the quantification of zinc and copper are neutron activation analysis (NAA), atomic absorption spectrometry (AAS) and inductively coupled plasma-mass spectrometry (ICP-MS), which are all based on expensive and sophisticated instruments [138].

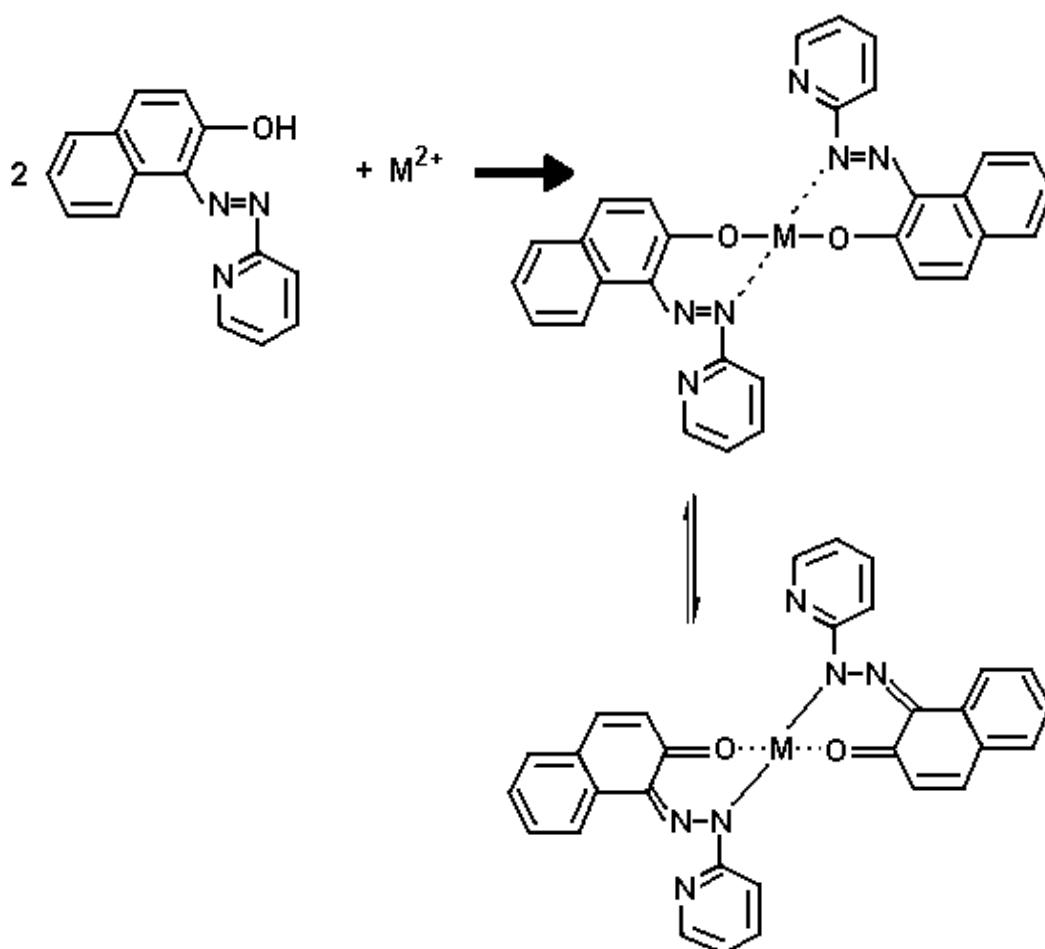
Inductively coupled plasma atomic emission spectrometry (ICP-AES) is one of the most used techniques for the determination of zinc. The most sensitive line for Zn is 213.856 nm. However, this line exhibits spectral interferences from elements such as Fe, Ni, and Cu [139].

In order to achieve accurate, reliable and sensitive results, preconcentration and separation are needed. Many preconcentration techniques for the determination of zinc and copper in FIA have been reported which include SPE, knotted reactor (KR), immobilization, ion exchange column, and liquid-liquid extraction. Hejazi et. al. [140] reported SPE of copper as their 1-(2-pyridylazo)-2-naphtol (PAN) complexes using polytetrafluoroethylene filter as solid phase. In this method, the mixtures of samples, buffer of acetic acid-sodium acetate solution, and PAN solution must be heated to boiling and then cooled to room temperature. Although the recovery percentages were acceptable, the extraction process was very tedious and time consuming. Impregnation of PAN on Ambersorb 563 for preconcentration of copper was also reported [141]. The preconcentration process was performed in a short glass column with an

inner diameter of 10 mm and a length of 100 mm, equipped with porous frits, was filled up with a suspension of 500 mg PAN impregnated Amborsorb 563 resin in water. Salonia et. al. [139] proposed 2-(5-bromo-2-pyridylazo)-5-diethylamino-phenol (5-Br-PADAP) as complexing agent for the determination of zinc in a knotted reactor. The zinc was retained as zinc-2-(5-bromo-2-pyridylazo)-5-diethylaminophenol complex at pH 9.2. An enrichment factor of 42 was obtained for the KR system with respect to ICP-AES using pneumatic nebulization. A flow-through solid phase spectrophotometric sensing device for the determination of zinc was developed [142] based on the reaction of this ion with PAN immobilized on a Dowex cation exchanger placed in the flow through cell. This method was able to measure 15-38 samples per hour. Preconcentration of zinc in AV-17 x 8 anion exchanger in the chloride form was used [143] for the determination of zinc with 4-(2-pyridylazo) resorcinol (PAR) as complexing agent. The procedure provides the determination of zinc in the concentration range from 5 to 100  $\mu\text{g L}^{-1}$  with RSD no higher than 25%. Ferreira [144] proposed to use an anion-exchange resin (Dowex 1-X8) for the preconcentration of zinc in which zinc chloro complexes were retained. Spectrophotometric determination was performed by addition of zincon (sodium salt of 2-carboxy-2'-hydroxy-5'-sulphoformazilbenzene). Liquid-liquid extraction methods were also widely used for the determination of zinc and copper. Copper was determined by flow injection spectrophotometry at 370 nm after extraction of copper(II) bis(acetylaceton)ethylenediiminate chelate into chloroform [145]. This method allowed to measure 20 samples  $\text{h}^{-1}$ . Monosegmented flow analysis liquid-liquid extraction (MSFA-LLE) was introduced [146] for determination of zinc and copper. The extracting efficiency of the organic phases was evaluated by using dithizone, PAN, diethyldithiocarbamate (DDTC) as

ligands for the metals. Typical sample frequency of 30 samples per hour was observed. Xylenol orange was used as complexing agent in spectrophotometric determination of zinc with a sequential injection system as reported by van Staden [147]. Thiosulphate was used as a masking reagent for copper(II) and lead(II). Liquid-liquid extraction in FIA using an open-phase separator for the spectrophotometric determination of copper was reported [148]. The phase separator was constructed from a 20 cm X 8 mm id glass tube. Extraction was performed using diethyldithiocarbamate in carbon tetra chloride. This method allowed 30 determinations per hour with detection limit of  $5 \mu\text{g L}^{-1}$ .

As already described, a number of organic reagents have been proposed for the photometric flow-injection determination of zinc and copper. However, none of them exhibits high selectivity for these compounds. One of them is PAN. PAN is practically insoluble in water, soluble in alkali with salt formation, and soluble in organic solvent to give yellow solutions with absorption maxima at 470 nm. The reagents does not absorb above 560 nm. PAN reacts with many metal ions to give intensively colored chelate complexes, which can be extracted with carbon tetra chloride, chloroform, benzene, or diethylether. Its palladium (II) and cobalt (II) complexes are green, while the other metal complexes are varies shades of red. PAN forms complexes with many divalent metals. The divalent metal-PAN complex has tetrahedral geometry at the metal ion and exists in two-tautomer form.



**Figure 6.1** Reaction of PAN with divalent metal ion

In this research, PAN was selected as a reagent for the determination of zinc and copper because of its availability, widespread practical testing, molar absorptivity of the analytical complex, and the rate of its formation in the aggregate. Besides, the reaction between zinc and PAN is well established and has been widely used for the determination of zinc in real samples.

## 6.1.2 Experimental

### 6.1.2.1 Preparation of Standard Solutions, Reagents, and Samples

Stock solution of copper(II)  $1000 \mu\text{g mL}^{-1}$  was prepared by dilution suitable amount of copper sulphat pentahydrate (Merck Darmstadt, Germany). Standard solution of copper(II) were prepared by dilution of stock solution. Aliquots (5 mL) of stock solution were diluted with 0.2 M of sulphuric acid in 500 mL calibrated flask to give the working stock solution of  $10 \mu\text{g mL}^{-1}$  of copper(II). A series of standard solutions (0.05, 0.1, 0.15, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7,  $0.8 \mu\text{g mL}^{-1}$ ) were prepared by appropriate dilution of working stock solution.

Stock solution of zinc(II)  $1000 \mu\text{g mL}^{-1}$  was prepared by dilution suitable amount of zinc sulphat heptahydrate (Merck Darmstadt, Germany). Standard solution of zinc(II) were prepared by dilution of stock solution. Aliquots (5 mL) of stock solution were diluted with 0.2 M of sulphuric acid in 500 mL calibrated flask to give the working stock solution of  $10 \mu\text{g mL}^{-1}$  of zinc(II). A series of standard solutions (0.05, 0.1, 0.2, 0.4, 0.5,  $0.6 \mu\text{g mL}^{-1}$ ) were prepared by appropriate dilution of working stock solution.

0.1% m/v ( $4 \times 10^{-3}$  M) of 1-(2-pyridylazo)-2-naphthol (PAN)(Avocado) was prepared by dissolving 0.1g of this substance with 100 mL ethanol in a calibrated flask. Different concentrations of reagent were prepared by appropriate dilution of reagent stock solution.



Acetate buffers were prepared by dissolving 56 g sodium acetate hydrate with 28.6 mL acetic acid 99% in 1 liter volumetric flask and fitted to the 1 liter mark with tridistilled water. The required buffer solutions at different pH (1.5, 3, 4, 4.5, 5, 6 and 7) were maintained by addition of acetic acid or sodium hydroxide

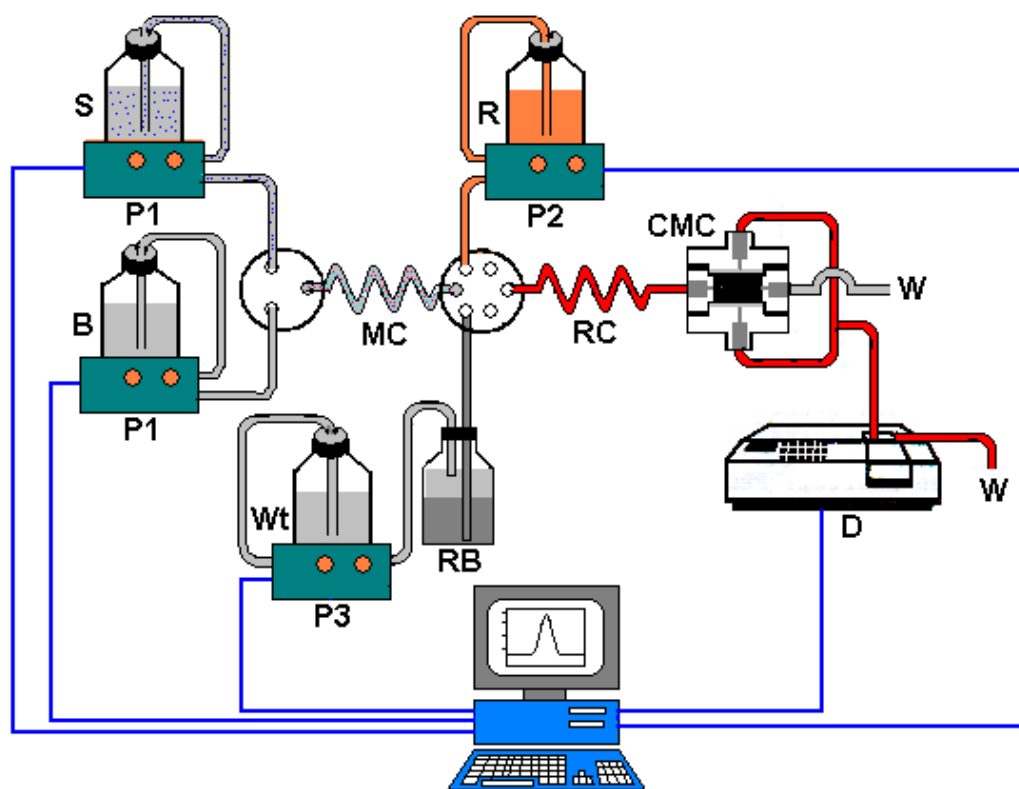
Chloroform and ethanol were purchased from Acros Organics (Schwerte, Germany). Tridistilled water was used throughout the experiment. All chemicals used were of analytical grade.

### **6.1.2.2 Apparatus**

The FIA system was assembled using the following equipment: Flow Injection Analysis System (Lamda FIAS-300, Perkin Elmer GmbH Bodenseewerk Überlingen) consist of three peristaltic pumps fitted with Tygon pump tubes, six-ports electrically actuated selection valves and a Lamda 2S Spectrophotometer (Perkin Elmer GmbH Bodenseewerk Überlingen) equipped with a 10 mm light path flow through cell with a volume of 18  $\mu$ L (Perkin Elmer). 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 included Tygon tubing (0.95 mm id), a replacement bottle, and a four-hole CMC. Connections between the various parts of the flow manifold were made with PTFE tubing of 0.8 mm id. Specification data of the CMC used in this experiment has been described in Section 4.1. Measurements of the zinc- and copper-PAN complexes were performed at 556 nm.

### 6.1.2.3 Analytical Procedure

The schematic diagram of the formation of the Zn-PAN and Cu-PAN complexes followed by extraction with chloroform and phase separation in the flow system is shown in Figure 6.2. Table 6.1 summaries the device sequence for the determination of zinc and copper.



**Figure 6.2** 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; D=detector (spectrophotometer UV-Vis Lamda 2S); W=waste

**Table 6.1** Device sequence for the FIA system

Step	Time (s)	Pump 1 (mL min <sup>-1</sup> )	Pump 2 (mL min <sup>-1</sup> )	Pump 3 (mL min <sup>-1</sup> )	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

The automatic analysis of the zinc and copper consisted of three steps:

- a) Prefill, chloroform was pumped with flow rate 0.951 mL min<sup>-1</sup> into CMC in order to occupy the micropore part of the CMC. The other function was to clean the rest of the sample in the tubing line and in the CMC in order to minimize the adsorption of the Zn-PAN and Cu-PAN complexes on the PTFE and minimize the carry over of the sample.
- b) Sample introduction, the sample (0.825 mL min<sup>-1</sup>), buffer solution (0.825 mL min<sup>-1</sup>), and PAN (0.18 mL min<sup>-1</sup>) were simultaneously pumped through flow injection system. The sample and buffer solution reached first the mixing coil. Then, the arriving of the sample-buffer mixture reacted with PAN in the reaction coil.
- c) Sample extraction and elution, the zinc-PAN and copper-PAN complexes were continuously extracted into chloroform in the CMC. Finally, the extractable compound was eluted with chloroform (0.951 mL min<sup>-1</sup>) and transported through the flow cell for detection of the coloured complex at 556 nm. The absorbance was continuously monitored on the screen of the PC (with the aid of the software package) and automatically saved on the hard disk. Thus, each cycle of analysis requires 360 second (6 minutes).

## 6.1.3 Results and Discussion

### 6.1.3.1 Optimization of the FIA System

#### *Instrumental variable*

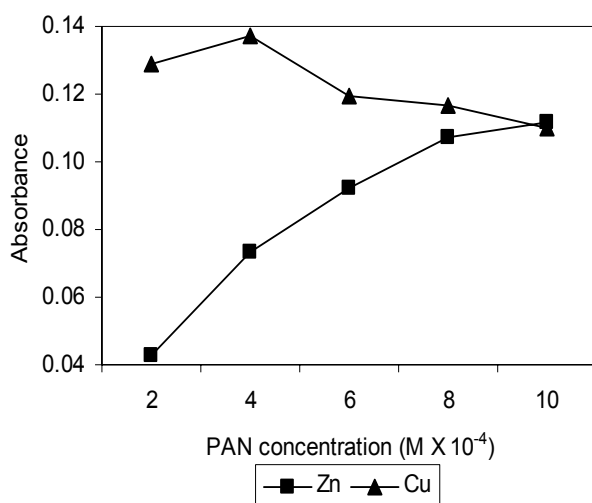
Read time of peak on the PC screen, which corresponds to the elution time, depended on the chemical variables (pH of the buffer solution and the concentration of the PAN solution) and the continuous flow system variables (flow rate of the sample and the eluting solution (chloroform), pre concentration time, and the length of reaction coil). The read time of the peak was optimized based on the highest concentration of standard solution of zinc and copper at optimum condition used in the experiments. Tailing of the peak was observed due to the high absorption of Cu-PAN and Zn-PAN complexes on the PTFE. Recent studies revealed that PAN can form a fairly stable 1:2 complex ( $M(\text{PAN})_2$ ) with Zn(II) and Cu(II) ions.  $M(\text{PAN})_2$  chelate has hydrophobic characteristic and retains on PTFE. The mechanism of the retention of the  $M(\text{PAN})_2$  on the PTFE should probably be molecular sorption and not mechanical entrapment, as was further investigated by Fang et. al.[149]. Thus, the relatively more time was required for eluting those complexes quantitatively from the CMC in order to minimize the carry over of the sample. However, the linear correlation between zinc and copper concentrations to absorbance was guaranteed until the highest concentration of standard solution of zinc ( $0.6 \mu\text{g mL}^{-1}$ ) and copper ( $0.8 \mu\text{g mL}^{-1}$ ) when 240 s of the read time was applied. Thus, the read time of 240 s was chosen for further experiments.

### ***Chemical variables***

The effect of the pH of aqueous samples on the extraction of Zn and Cu was studied in the pH range from 3 to 8 and 1.5 to 6 using acetate buffer. 6 M HNO<sub>3</sub> and 6 M NaOH were used for pH adjustment. PAN occurs as the neutral HR molecule between pH 3 and 11, which is partially soluble in water. It acts as a tetradentate ligand complexing with metal through the hydroxyl oxygen atom, pyridine nitrogen atom, and one of the azo group nitrogen atoms. In the pH lower than 3 the pyridine structure of PAN molecules is protonated and at pH higher than 7 the hydroxyl ion (OH<sup>-</sup>) competes with PAN for interacting with cations. Results of the experiments showed that at pH 4 Cu-PAN complex has a relatively high absorbance and Zn-PAN complex showed a high absorbance at pH 6. The result is similar with the result obtained by Ayora-Canada [142] from his research on continuous flow-through solid phase spectrometric determination of trace amounts of zinc. So a buffer solution of pH 4 and 6 were adapted for determination of copper and zinc. The suitable pH for the formation of the metal-ligand complex is quiet dependent on the type of metals, type of ligands, and type of the system used for providing the metal-ligand complex. The formation of the complex between zinc and xylenol orange was efficient at pH 5.9 [147]. Liquid-liquid extraction of zinc using PAN as complexing agent in monosegmented flow analysis (MSFA) system [146] showed a higher efficiency at pH>8 because the mechanisms governing the analyte (metal ion) transfer to the organic phase in MSFA includes its prior adsorption on the glass surface of the extraction tube as a consequence of higher ionization of the glass silanols. The formation of complex between copper and bis(acetylaceton)ethylenediimine was reported optimum at pH 5 [145]. The stability of the complex formation

between the salophen and Cu(II) ion could be reached at pH>4 because at lower pH, the nitrogen atoms of the salophen is protonated and reduce the stability of the complex [136]

Reagent concentration ranging from  $2 \cdot 10^{-4}$  to  $10 \cdot 10^{-4}$  M was evaluated. In case of zinc, the results show a gradual increase in sensitivity as the reagent concentration is increased. The peak profiles revealed that a concentration from  $8 \cdot 10^{-4}$  M gave a relatively constant sensitivity and this was chosen as the optimum reagent concentration for determination of zinc. In contrary, the PAN concentration is not a limiting factor in the system design for determination of copper. Within the range  $2 \cdot 10^{-4}$  to  $10 \cdot 10^{-4}$  M, the analytical signal is almost unaffected by variations of this

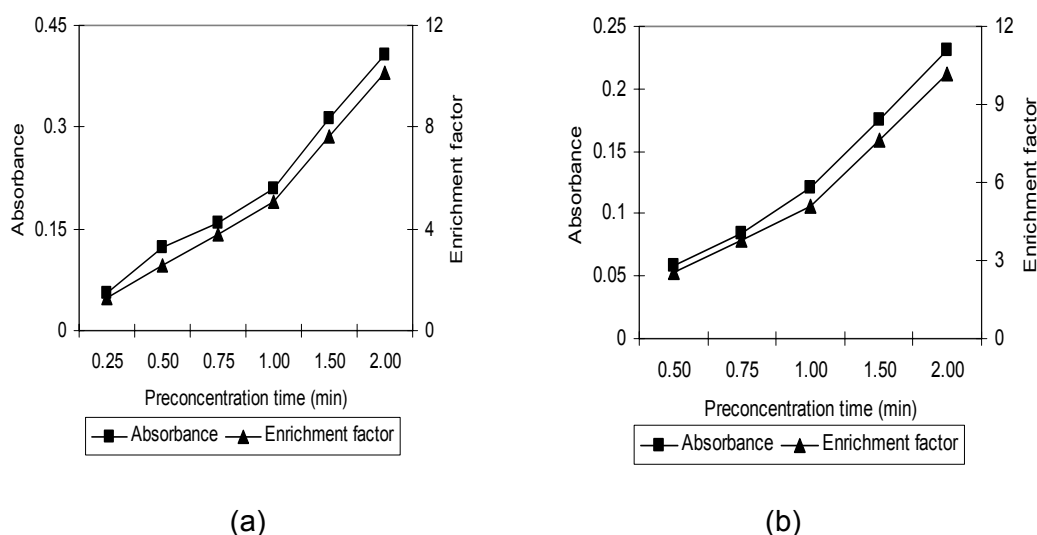


parameter. As the baseline absorbance is determined by the concentration and volumetric fraction of the chromogenic reagent, smaller amount of PAN are to be preferred. As a compromise, the reagent concentration was fixed at  $4 \cdot 10^{-4}$  M for the determination of copper.

**Figure 6.3** Effect of the PAN concentration

### **Continuous flow system variables**

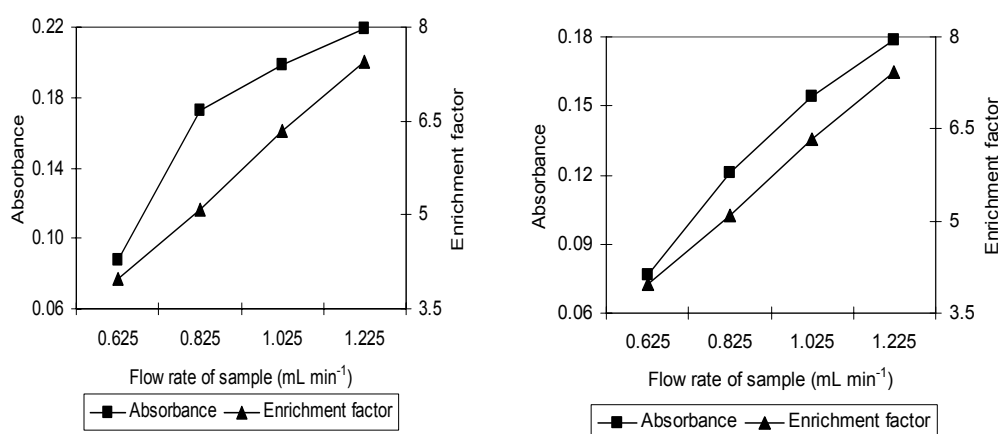
The mixing coil (MC) in knotted reactor form was fixed at 90 cm in order to guarantee the mixing between sample and buffer solution. The flow injection system was also designed that the preconcentration of the sample could be carried out in the system either by changing the preconcentration time with fixed flow rate of the sample or by changing the flow rate of the sample with fixed preconcentration time. The effect of the preconcentration time was studied in the range of 0.25 to 2 minutes for zinc and 0.5 to 2 minutes for copper in fixed flow rate of the sample  $0.825 \text{ mL min}^{-1}$ , which is correspond to enrichment factor of 1.27 to 10.2 with assumption that the end volume of the eluting organic compound (chloroform) is 0.36 mL (30% of the total PTFE block volume used in the CMC (see Section 4.1)). The results are shown in Figure 6.4.



**Figure 6.4** Effect of the preconcentration time on the analytical signal of the metal-PAN complexes and the enrichment factor  
(a) Zn (b) Cu

Fig. 6.4 shows a significant increase on the analytical signal of the both Zn-PAN complex and Cu-PAN complex. However, the application of the higher preconcentration time on the real analysis faced the disadvantages regarding with sample throughput and elution time. As described earlier, the high absorption of Cu-PAN and Zn-PAN complexes on the PTFE was observed which is indicated by tailing of the peak. The formed Zn-PAN and Cu-PAN complexes has hydrophobic characteristic and retains on PTFE. Thus, the preconcentration time should be chosen as compromise between higher analytical signal, sample throughput, and elution time. 1 minute of preconcentration time was preferred and could fulfilled the requirements.

The effect of the sample flow rate was studied in the range of 0.625 to 1.25 mL min<sup>-1</sup> for Cu in fixed preconcentration time of 1 minute, which is correspond to enrichment factor of 3.97 to 7.44 with the same assumption as already described earlier.

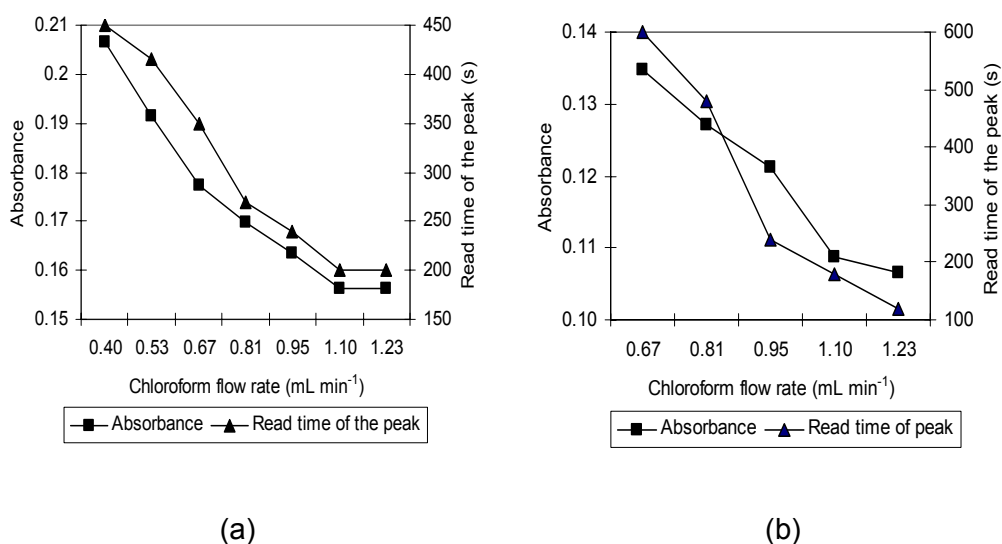


(a) (b)  
**Figure 6.5** Effect of the flow rate of the sample on analytical signal of the metal-PAN complexes and the enrichment factor  
(a) Zn (b) Cu



The results (Fig. 6.5) indicated that the increase of the flow rate resulted in both increasing the analytical signal and the enrichment factor because the amount of the zinc and copper ions that reacted with PAN was increased. In case of zinc, the significantly increase in slope between 0.625 and 0.825 mL min<sup>-1</sup> proved the optimum value of sample flow rate. However, the increase of flow rate produced also higher baseline value and reduced the stability of the CMC. At higher sample flow rate the leakage of the polar phase in the output for the non polar phase was observed. Therefore, the sample flow rate of 0.825 mL min<sup>-1</sup> was chosen as a compromise to ensure a lower baseline and higher stability of the CMC.

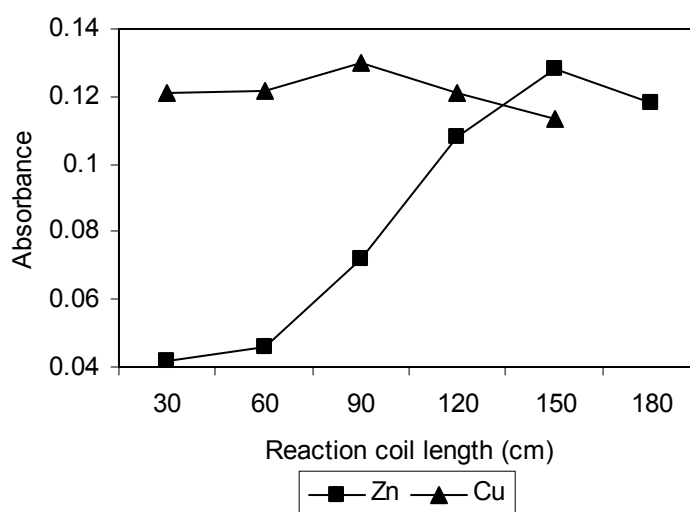
Since chloroform was used as an eluting solution for the zinc and copper complexes formed in the CMC, the flow rate of the chloroform is also an important factor in the flow injection system designed.



**Figure 6.6** Effect of the chloroform flow rate on analytical signal and the read time of the peak of the metal-PAN complexes  
(a) Zn (b) Cu

As can be seen in Fig. 6.6, the increase of the chloroform flow rate resulted in decreasing of the analytical signal of the Zn-PAN and Cu-PAN complexes due to the dissolution process. In contrary, increasing the flow rate of the chloroform significantly reduced the read time of the peak. Therefore, the chloroform flow rate of  $0.95 \text{ mL min}^{-1}$  was chosen as a compromise between a higher analytical response and the shorter time for analysis.

Reaction coil of different lengths (30-180 cm for zinc and 30-150 cm for copper determination) were tested to ensure good metal-PAN complexes formation. In case of zinc, the Zn-PAN complex formation could be reached when the length of the reaction coil was increased from 30 to 150 cm. Meanwhile the Cu-PAN complex formation was relatively faster than that of zinc so that variation of reaction coil length from 30 to 150 cm didn't affect the analytical signal. Thus, the reaction coil of 90 cm and 150 cm were chosen as an optimal value for the determination of copper and zinc.



**Figure 6.7** Effect of the reaction coil on the analytical signal of the metal-PAN complexes

The optimum conditions obtained are summarized in Table 6.2 and were used for constructing calibration curves of zinc and copper and the determination of zinc and copper in pharmaceutical preparations.

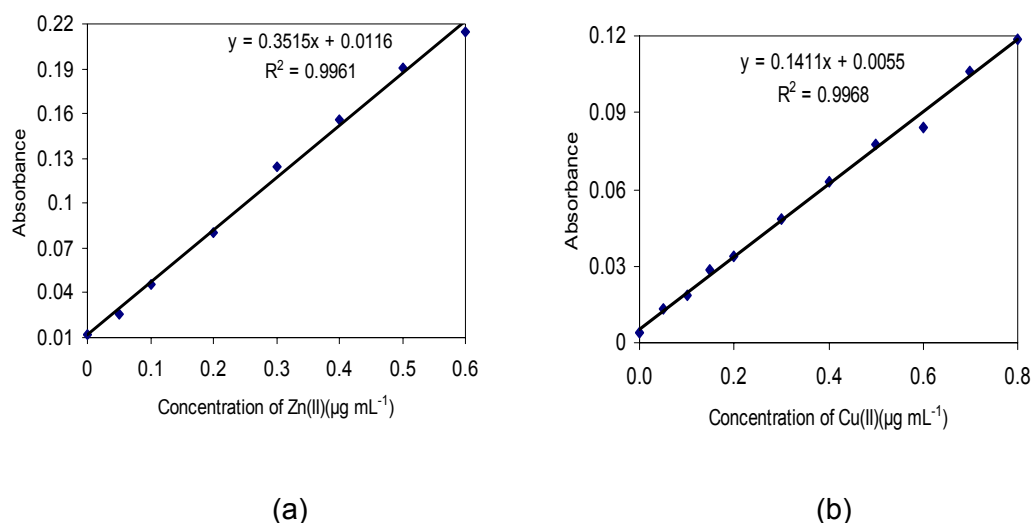
**Table 6.2** The optimum conditions for the determination of zinc and copper

Parameter	Unit	Zinc	Copper
Read time of the peak	s	240	240
pH	-	6	4
PAN concentration	M	$8.10^{-4}$	$4.10^{-4}$
Sample flow rate	$\text{mL min}^{-1}$	0.825	0.825
Eluent (chloroform) flow rate	$\text{mL min}^{-1}$	0.95	0.95
Preconcentration time	minute(s)	1	1
Reaction coil length	cm	150	90

### 6.1.3.2 Analytical Figures of Merit

In the above-established working conditions two calibration curves for zinc and copper were performed. Under the optimum conditions, the calibration curve of zinc was linear between 0.05 and 0.6  $\mu\text{g mL}^{-1}$  with the relationship between absorbance and zinc(II) ion concentration given by the relation  $y = 0.3515x + 0.0116$ , where y is absorbance and x is zinc ion concentration with a linear regression coefficient ( $r^2$ ) of 0.9961 ( $n=5$ ). Copper also shows the same behavior. The calibration curve of copper was linear between 0.05 and 0.8  $\mu\text{g mL}^{-1}$  with the relationship between absorbance and copper(II) ion concentration given by the relation  $y = 0.1411x + 0.0055$ , where y is absorbance and x is copper ion concentration with a linear regression coefficient ( $r^2$ ) of 0.9968 ( $n=5$ ). The experimental period for one complete analytical cycle was 6 minutes long giving an overall sampling rate of ten samples per hour. The system designed allowed an enrichment

factor as high as 5. The results are shown in Figure 6.8 and summarized in Table 6.3.



**Figure 6.8** Calibration curves of metal-PAN complexes  
(a) Zn (b) Cu

**Table 6.3** Analytical figures of merit

Parameter	Unit	Zinc	Copper
Linear dynamic range	( $\mu\text{g mL}^{-1}$ )	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
RSD (n=5)	(%)	2.7(0.5) <sup>a</sup>	1.1(0.6) <sup>a</sup>
Detection limit	( $\mu\text{g mL}^{-1}$ )	0.04 <sup>b</sup>	0.04 <sup>b</sup>
Sampling frequency	( $\text{h}^{-1}$ )	10	10
Enrichment factor	-	5	5
Volume of sample used	mL	0.825	0.825

c = concentration of standard solutions

<sup>a</sup> = concentration of Zn or Cu ( $\mu\text{g mL}^{-1}$ ) at which RSD was established

<sup>b</sup> =  $3\sigma$  criterion

The detection limit (three times the signal-to-noise ratio) was estimated  $0.04 \mu\text{g mL}^{-1}$ . The reproducibility of the proposed method was proven by five repetitive analysis cycles of each sample at the concentration of  $0.5 \mu\text{g mL}^{-1}$ (Zn) and  $0.6 \text{mg mL}^{-1}$ (Cu). The results showed that the proposed method has a good reproducibility, which is indicated by lower relative

standard deviation (<3%). The accuracy of the method was verified using model solutions prepared from certified reference solution (Merck, Darmstadt Germany). Spiking of the 0.1, 0.3, and 0.5 mg mL<sup>-1</sup> Zn(II) solutions gave the percent recovery of 98.8, 99.5, and 100.3 %. 0.1, 0.4, and 0.7 µg mL<sup>-1</sup> were tested for the determination of the Cu(II) accuracy. The percent recovery of 101.2, 99.6, and 100.6 % were obtained which indicated that the proposed method has a good accuracy.

### **6.1.3.3 Determination of zinc and copper in Pharmaceutical Preparations**

Unizink<sup>®</sup> 50 (Köhler Pharma GmbH), Zineryt<sup>®</sup> (Hermal), Zinkit<sup>®</sup> 3, 10, 20 (Wörwag Pharma), Zincfrin<sup>®</sup> (Alcon Pharma GmbH), Zinkorotat 20 (Ursapharm), Zink-D-Longoral<sup>®</sup> (Cassela-med GmbH Köln, Artesan Pharma GmbH &Co. KG Lüchow), Zinkoxid Salbe Law (Riemser Arzneimittel AG), Zinkoxid Emulsion Law (Riemser Arzneimittel AG), Zinkpaste Law (Riemser Arzneimittel AG), Zinksalbe Dialon<sup>®</sup> (Engelhard Arzneimittel), and Kupferorotat (Ursapharm) from local drugstore were used as pharmaceutical samples. The data of the pharmaceutical preparations used are presented in Table 6.4.

The samples were prepared through acid digestion [145]. One tablet was dissolved in 8 mL of 50% (v/v) HCl and 2 mL of 50% (v/v) HNO<sub>3</sub> in 100 mL conical flask. In case of liquid, a suitable volume of the sample was taken and treated as already described earlier. When the samples in powder or ointment form, a suitable sample was weighed and treated as already described earlier. The dissolution process was completed by gently warming on a hot plate in order to remove the matrix. The clear solution

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was cooled and transferred quantitatively to 100 mL calibrated flask, neutralized, and make up to volume with tridest water.

**Table 6.4** The data of the pharmaceutical preparations

Pharmaceuticals	Form	Main content	Certified value <sup>a</sup>	Unit
Unizink <sup>®</sup> 50	tablet	Zinc-DL-Aspartate	10	mg/tablet
Zineryt <sup>®</sup>	powder	Zinc acetate.2H <sub>2</sub> O	68.59	mg/g
Zinkit <sup>®</sup> 3	tablet	ZnSO <sub>4</sub> .7H <sub>2</sub> O	3	mg/tablet
Zinkit <sup>®</sup> 10	tablet	ZnSO <sub>4</sub> .7H <sub>2</sub> O	10	mg/tablet
Zinkit <sup>®</sup> 20	tablet	ZnSO <sub>4</sub> .7H <sub>2</sub> O	20	mg/tablet
Zincfrin <sup>®</sup>	liquid	Zinc sulphate	2.5	mg/mL
Zinkorotat 20	tablet	Zinkorotat.2H <sub>2</sub> O	3.2	mg/tablet
Zink-D-Longoral <sup>®</sup>	tablet	Zinc-D-gluconate. 3H <sub>2</sub> O	6.54	mg/tablet
Zinkoxid Salbe Law	ointment	Zinc oxide	80	mg/g
Zinkoxid Emulsion Law	emulsion	Zinc oxide	200	mg/g
Zinkpaste Law	paste	Zinc oxide	160	mg/g
Zinksalbe Dialon <sup>®</sup>	ointment	Zinc oxide	80	mg/g
Kupferorotat	tablet	Cu(II)-orotate	0.31 <sup>b</sup>	mg/tablet

<sup>a</sup> as Zn

<sup>b</sup> as Cu

Most of the solution after digestion process was clear. However, the filtration with a 0.45 µm pore size Millipore membrane filter was carried out to remove the suspended particles that might be present in the solutions. The appropriate volume of the samples was diluted so that the concentration of the targeted zinc and copper ions in the range of the concentration of zinc and copper standard solutions in the calibration curves. Then, the samples were introduced to the flow injection system.

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**Table 6.5** Determination of zinc and copper in pharmaceutical preparations

Pharmaceuticals	Unit	Certified value	Found <sup>a</sup>	Recovery (%)
Unizink <sup>®</sup> 50	mg/tablet	10	9.74	97.4
Zineryt <sup>®</sup>	mg/g	68.59	65.88	96.0
Zinkit <sup>®</sup> 3	mg/tablet	3	2.93	97.8
Zinkit <sup>®</sup> 10	mg/tablet	10	9.73	97.3
Zinkit <sup>®</sup> 20	mg/tablet	20	19.58	97.9
Zincfrin <sup>®</sup>	mg/mL	2.5	2.43	97.1
Zinkorotat 20	mg/tablet	3.2	3.06	95.6
Zink-D-Longoral <sup>®</sup>	mg/tablet	6.54	6.26	95.6
Zinkoxid Salbe Law	mg/g	80	77.08	96.3
Zinkoxid Emulsion	mg/g	200	195.32	97.7
Zinkpaste Law	mg/g	160	155.37	97.1
Zinksalbe Dialon <sup>®</sup>	mg/g	80	77.44	96.8
Kupferorotat	mg/tablet	0.31	0.31	99.9

<sup>a</sup> average of three measurements

As can be seen in Table 6.5, the recoveries of the zinc and copper are lower than 100% but higher than 95%. The loss of the targeted compounds could be caused by several factors. The samples were measured after digestion process with acids, meanwhile the measurements of the standard solutions of zinc and copper for preparing the calibration curves was carried out without any digestion process included in preparation steps. The best technique of the digestion process should be further investigated because the application of the different digestion technique might produce the different results. This is still a challenge for the future research. Comparison of the measurements with the present standard method (i.e. ICP) should be carried out in order to test the accuracy and to prove the feasibility of the proposed method. In this research work, the accuracy of the proposed method was proven by measuring the concentration of the zinc and copper spiked from the certified reference solution.

PAN is a non-selective chelating agent and favorably reacts with many metal ions. The main interfering species are Cd(II), Hg(II), Ni(II)[146], Mn(II), Co(II), Fe(II), and Fe(III) [142]. Phosphates and organic matter in the form of humic acids ( $5 \text{ mg L}^{-1}$ ), citrates, tartarates, sulfide, oxalates, fluoride ( $8 \text{ mg L}^{-1}$ ) and nitrates ( $20 \text{ mg L}^{-1}$ ) are not found to impair the formation of the Zn-PAN complex [150]. These metal ions, however, are not present in the pharmaceutical samples analyzed by the proposed method and should not present a drawback to its use for this kind of determination. Therefore, the effect of those interferences was not investigated.

The main advantage of the proposed method is the flexibility 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. The analytical throughput of this method can be performed by increasing the flow rate of the sample and reducing the preconcentration time. Although the analytical throughput of this method is low, it still outclasses the FIA counterparts owing to the fact that, it uses cheap and readily available equipment; it uses microliters of the reagents and solutions per analytical cycle, full automated and very simple to assemble and operate.

#### **6.1.4 Summary**

In this research work, a procedure for the determination of zinc and copper in pharmaceutical preparations by spectrophotometric FIA method coupled with the chromatomembrane cell for extraction and pre concentration manifold in flow system is described. This procedure includes the formation of the metal-PAN complexes, liquid-liquid extraction of the



metal-PAN complexes using chloroform in the CMC, elution of the metal-PAN complexes from the CMC, and subsequent analysis of the compounds by spectrophotometer at 556 nm.

The flow system was developed to provide the combination of subsequent extraction, preconcentration and spectrophotometric determination of the samples. The FIAS software package obtainable from Perkin Elmer for computer-aided flow analysis, device control and data acquisition was used throughout. The absorbance was continuously monitored on the screen of the PC (with the aid of the software package) and automatically saved on the hard disk.

The instrumental variable, chemical variables, and flow injection variables were investigated. Higher adsorption capacity of the metal-PAN complex on PTFE caused longer elution time, which is correspond to the read time of the peak. The elution time was optimized based on the highest standard solution of zinc and copper used for providing the calibration curves. It was found that the elution time of 240 s ensured the elution of metal-PAN complexes from the CMC quantitatively.

Optimization of chemical variables indicated that the formation of zinc- and copper-PAN complexes was affected by pH and the concentration of PAN. The optimum pH for the formation of Zn-PAN and Cu-PAN complexes were 6 and 4. The use of  $8 \cdot 10^{-4}$  M PAN produced the higher analytical signal of Zn-PAN. However, the concentration of PAN was not limiting factor in the system designed for determination of copper. Variation of the PAN concentrations from  $2 \cdot 10^{-4}$  to  $10 \cdot 10^{-4}$  M produced relatively constant analytical signal of Cu-PAN.

The flow injection variables were also optimized. The preconcentration time, the sample flow rate, the eluent flow rate, and the reaction coil length affected the analytical signal of the metal-PAN complexes formed. In general, by increasing the preconcentration time, the sample flow rate, and the reaction coil length, the analytical signal of the metal-PAN complexes was also increased. In case of copper, it was found that the increase of the reaction coil length didn't increase the analytical signal of the Cu-PAN complex. In contrary, by increasing the eluent flow rate, the analytical signal was significantly decreased. This is due to the dissolution of the metal-PAN complexes by the eluting solution (chloroform). Thus, the eluent flow rate was chosen as a compromise between higher analytical signal of the metal-PAN complexes and the elution time.

The method performance was evaluated by the determination of the enrichment factor, linearity, reproducibility, accuracy, and sensitivity of the method. In the optimum conditions, the enrichment factor of 5 was achieved by introducing 0.825 mL of the sample. The end volume of the eluting solution was estimated 0.36 mL. The enrichment factor can be increased by changing either the flow rate of the sample in the fixed preconcentration time or by increasing the preconcentration time in the fixed sample flow rate. Good linearity of zinc ( $r^2 > 0.9961$ ) and copper ( $r^2 > 0.9968$ ) were observed. The relative standard deviation (<3%) indicated that the method has very good reproducibility. The accuracy of the method was indicated by recovery percentages.

Satisfactory recoveries were observed for zinc (98.8-100.3%) and copper (99.6-101.2%) from measurement of a reference standard solution which indicated a good accuracy. Applications of the method on the analysis of

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those compounds in pharmaceutical preparations were found to be in good agreement with the certified content with the percent recoveries lie between 95.6 to 99.9%.