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# Determination of lead in dialysis concentrates using flow injection hydride generation atomic absorption spectrometry

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**Abstract**

Flow injection hydride generation atomic absorption spectrometry (FI-HGAAS) was used for determination of lead in dialysis concentrates. The parameters such as acidity, concentration of oxidising and reducing agents and argon gas flow rate were investigated to reach the best peak height sensitivity. No significant background signal was observed at high salt concentrations. The detection limit, concentration giving a signal equal to three times standard deviation of the blank signal, was 0.7 ng ml$^{-1}$ for a 500 μl injection volume. Precision of the measurements at the 20 ng ml$^{-1}$ level was 3.7% R.S.D. The dialysis concentrates analysed by FI-HGAAS were found to have 10–70 ng ml$^{-1}$ of lead. The same samples were analysed by ETAAS after removing the matrix using solid phase extraction with Chelex 100. The results were in agreement with those obtained by FI-HGAAS.


*Keywords:* Lead; Hydride generation; Salt matrices; Dialysis concentrates

## 1. Introduction

Several groups have reported that dialysis patients are at risk of developing trace element imbalances [1,2]. Krachler et al. [1] used inductively coupled plasma mass spectrometry to investigate concentrations of the barium, calcium, copper, lead, magnesium, strontium and zinc in plasma and dialysis fluids of seven maintenance dialysis patients. They found that the copper concentration remained within the reference range; on the other hand, zinc concentration was lower while calcium, strontium, magnesium, barium and lead concentrations were higher than the reference range for healthy adults. However, Sampson et al. [3] reported that there were significant increase in blood lead and plasma aluminium concentrations in all patients with chronic renal failure, but they also mentioned that all blood lead concentrations were within the accepted safe exposure range, less than 380 μg l$^{-1}$.

The routine analysis of dialysis concentrates for trace elements is necessary to reduce exposure and health risk. Lead is the one of the toxic elements in dialysis concentrates. Electrothermal atomic absorption spectrometry is the most frequently used technique for determination of lead. However, the interference effects of alkali chlorides and their mechanisms are still under investigation [4,5]. Formation of volatile lead chloride at low temperatures and deposition of chlorides at cooler ends of graphite tube take place. As the temperature rises, revolatilisation and atomisation of deposited compounds result in formation of lead halides, reducing atomic signal as a result of vapour phase interferences. In addition, molecular absorption and scattering take place [6,7]. Dialysis concentrates have high salt content, causing large background signal and non-spectral interferences [8,9].

In order to stabilise the lead or to evaporate the salt matrix in ashing step, several approaches have been used employing matrix modifiers [10,11]. To remove matrix and/or preconcentrate the analyte, other techniques often used are solid phase extraction [12] and coprecipitation [13].

Inductively coupled plasma mass spectrometry (ICP-MS) is a technique of choice because of its high sensitivity and multielement capability. However, the sample with high salt content causes deposition of matrix compounds at the MS interface and reduces transport efficiency. Interference due to ionisation suppression is also possible [14]. Matrix separation or high dilution is necessary to get rid of the matrix effect. Hydride generation in situ trapping of the analyte in

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the graphite surface [15,16] and electrothermal vaporization to a plasma source [17] are the other alternative techniques to reduce salt effect on the analytical signal. The determination of the trace metal in high salt matrix has been focused on seawater samples because of importance in terms of environmental aspect. Dialysis concentrates contain larger amount of salt than the seawater, the total dissolved solid content is about 8.4–40%; the matrix, therefore, is problematic matrix for ETAAS and ICP-MS.

The generation of lead hydride is known to have difficulties, namely low yield and low stability of volatile hydrides. However, it has been demonstrated that the use of acidic oxidizing media increases the reaction rate and sensitivity [18–21]. Highest sensitivity has been reported with a relatively mild oxidant, $K_3Fe(CN)_6$ [15,22,23].

Further improvements in precision and relative freedom from interferences have been provided by combining flow injection with lead hydride generation AAS. Two of the shortcomings of lead hydride generation; low reaction rate and interferences caused by transition metals were alleviated or even eliminated [20]. Currently, FI-HGAAS for the determination of lead has become a powerful technique with its high sensitivity and freedom from interferences.This paper describes direct determination of lead in dialysis concentrates using flow injection hydride generation atomic absorption spectrometry (FI-HGAAS). The parameters affecting the analytical signal in sample matrix in different type of dialysis concentrates were studied.

## 2. Experimental

### 2.1. Reagents and samples

Deionised water (18 MΩ cm) produced by Milli-Q water system was used for the preparation of all solutions. The lead standard solutions were prepared by diluting the 1000 mg $l^{-1}$ stock solution (Fisher). Standards and sample solutions were prepared in 2.0% (w/v) $K_3Fe(CN)_6$ (Merck) and 0.1 M HCl (Riedel de Haen). $NaBH_4$ (Aldrich) was used as reducing agent at the concentration of 1% (w/v), in 0.1% (w/v) NaOH (Merck). Extra pure NaCl (Merck) was used to investigate effect of sodium chloride on the analytical signal.

Twelve dialysis concentrates were obtained from two different producers. The samples may be classified in two main groups. The first group of samples covering sample nos. 1–9 were composed of 202.7–220 g $l^{-1}$ NaCl, 0–5.5 g $l^{-1}$ KCl, 3.7–9.5 g $l^{-1}$ $CaCl_2{\cdot}2H_2O$, 3.3–7.5 g $l^{-1}$ $MgCl_2{\cdot}6H_2O$, 0–7.2 g $l^{-1}$ $CH_3COONa$ and 35 g $l^{-1}$ anhydrous dextrose. In the second group, the samples 10 and 11 contained 84 g $l^{-1}$ $NaHCO_3$ and the sample 12 was composed of 30.6 g $l^{-1}$ NaCl and 84 g $l^{-1}$ $NaHCO_3$.

### 2.2. Instrumentation and apparatus

The measurements were carried out on ATI UNICAM 939 atomic absorption spectrometer (Cambridge, UK) equipped with a 5 cm air-acetylene burner. UNICAM data coded Pb hollow cathode lamp was operated at 7.5 mA and at 283.3 nm, with a 0.5 nm spectral band pass. Deuterium background correction was used. UNICAM GF 90 electrothermal atomizer with FS 90 Plus autosampler and pyrolytic graphite coated graphite tubes were used for the ETAAS measurements.

Three channel ALITEA VS 3 midi pump was used to pump the carrier and reducing solutions. Flow injection system was constructed using Tygon peristaltic pump tubing (1.8 mm i.d.) delivering at a flow rate of 6.6 ml $min^{-1}$. The connecting tubes were made from 0.8 mm i.d. PTFE, and fittings were obtained from Cole Parmer. Rheodyne Model 5020 low-pressure injection valve with 500 μl injection loop was used for standard and sample introduction. A standard U-type gas–liquid separator of UNICAM VP 90 vapour system was used for the gas–liquid separation. Argon was used as the carrier gas to sweep out the lead hydride to atomizer. Silica tubing was supplied from Quartz Scientific Inc., Ohio, to be used as atomizer. The silica atomiser was 13 cm in length with a 10 mm i.d.; the T-connection was 8 cm in length with a 4 mm i.d. The T-tube silica atomizers were constructed in the university glass shop. The flow injection manifold used is shown in Fig. 1.

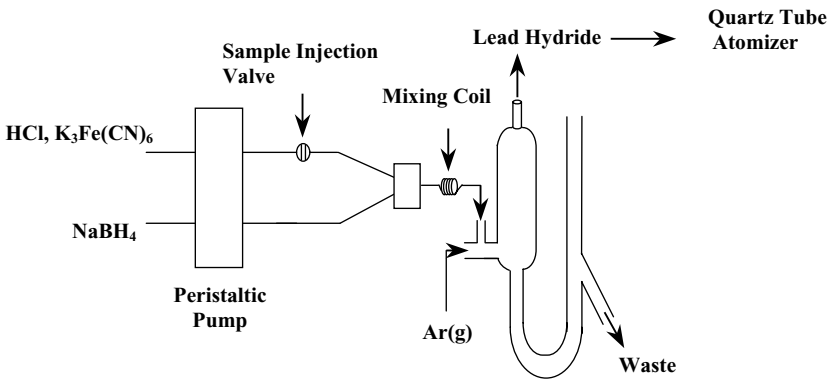


Fig. 1. Flow injection manifold for the determination of lead using HG-AAS.

### 2.3. Procedure for HG-AAS

To 5–10 ml portion of dialysis concentrate, 10 ml of 5% (w/v) $K_3Fe(CN)_6$ in 0.25 M HCl were added and the contents were diluted to 25 ml. The final concentrations of $K_3Fe(CN)_6$ and HCl in the test solutions were 2.0% (w/v) and 0.1 M, respectively. The carrier solution was 2.0% (w/v) $K_3Fe(CN)_6$ in 0.1 M HCl. Reducing solution, 1.0% (w/v) $NaBH_4$ was prepared in 0.1% (w/v) NaOH. An aliquot of 500 μl standard or sample was injected to the flowing stream. The peak height was used for quantitative determination of the standards and samples. Standard addition samples were prepared manually. A total of 12 dialysis concentrates obtained from two different producers were analysed for lead.

A certified reference material, Waste Water EU-L-1 (SCP Science, Canada) was used to validate the proposed method.

### 2.4. Procedure for comparison method

Electrothermal atomic absorption spectrometry was used as a comparison method. The samples were analysed for lead after solid phase extraction; Chelex 100 (BioRad) was used to eliminate the salt matrix. A buffer solution, 3.0 ml of 2.0 M $CH_3COONH_4$, was added to 10–20 ml of sample solution and the pH was adjusted to 6.0 by adding either 1.0 M $NH_3$, or 1.0 M $CH_3COOH$. 0.4 g of Chelex-100 was packed into a Teflon column (4 mm i.d.) and the two ends were capped with pieces of sponge. The column was fitted to the peristaltic pump tubing, washed with 5 ml of 2 M $HNO_3$, conditioned with 10 ml of pH 6.0 buffer solution and the prepared sample solutions were passed through the column with a flow rate of 0.5 ml $min^{-1}$. The column was then washed with 5 ml of buffer solution and the analyte was eluted with 10 ml of 2.0 M $HNO_3$. The necessary amount of $Mg(NO_3)_2$ solution was added to standard and sample solutions to have 50 μg of $Mg(NO_3)_2$ for each injection as matrix modifier. The analytical performance for the solid phase extraction was characterised by percent recoveries obtained from the column. The furnace temperature program used was as follows; drying step; 10 °C $s^{-1}$ ramp up to 120 °C and hold for 30 s, pyrolysis; 50 °C $s^{-1}$ ramp to 850 °C and hold for 30 s, atomisation at 1400 °C for 3 s and cleaning step was at 2400 °C for 3 s. The peak height of the signal was considered for the analysis using ETAAS.

## 3. Results and discussion

### 3.1. Optimisation studies

Optimisation of HCl concentration was carried out using 20 ng $ml^{-1}$ lead standard solutions and 0.1 M was selected as optimum concentration of HCl. However, the dialysis concentrates containing large amount of $CH_3COONa$ and $NaHCO_3$, forming buffers upon addition of HCl and affecting the acidity of samples. Therefore, the effect of HCl concentration on signal was studied both for the solution containing one component at a time and for the mixture to see their combined effect. Sample 1 was used for this purpose for the combined effect and the single component solutions were 8.6% (w/v) NaCl, 6.6% (w/v) $CH_3COONa$ and 3.4% (w/v) $NaHCO_3$. The concentrations selected correspond approximately to those obtained by diluting the original samples 2.5 times. Samples were spiked with 20 ng $ml^{-1}$ Pb. The effect of HCl concentration on peak height signal in different matrices described above is shown in Fig. 2. It was observed that sodium acetate was suppressing the signal and the highest signal was obtained at 0.06 M HCl concentration, while in the sodium chloride matrix the optimum acid concentration was found to be same with the aqueous standard. The effect of HCl concentration in dialysis concentrate has characteristics of both $CH_3COONa$ and NaCl since they are major components in matrix (Fig. 2). As a result, for the first group of samples, 0.1 M HCl concentration was used throughout the study. However, for the second group of samples, the major component was $NaHCO_3$ and 0.5 M HCl concentration was necessary to reach maximum signal. During the optimisation of HCl concentration, 3.0% (w/v) $K_3Fe(CN)_6$ and 1.0% (w/v) $NaBH_4$ were used as hydride generating medium.

In order to optimize the concentration of $K_3Fe(CN)_6$, solutions containing 0.1 M HCl (carrier) and 1.0% (w/v) $NaBH_4$ (reductant) were used; analyte concentration was kept as 20 ng $ml^{-1}$ Pb in 0.1 M HCl. The concentration of $K_3Fe(CN)_6$ was varied in the range of 0.5 and 3.0% (w/v), same value was used both for sample and carrier solutions. The signals for different $K_3Fe(CN)_6$ concentrations were corrected against blank readings since this reagent was the main source of the blank signal. An optimum value of 2.0% (w/v) for $K_3Fe(CN)_6$ was selected for further experiments. The optimum concentration for $K_3Fe(CN)_6$ was not affected from the sample matrices.

Optimisation of $NaBH_4$ was carried out using solutions prepared in 0.1 M HCl and 2.0% (w/v) for $K_3Fe(CN)_6$. A concentration of 1.0% (w/v) $NaBH_4$ was used to obtain the best peak height sensitivity. Other parameters, such as carrier gas flow rate, injection volume, pumping rate and mixing coil length were optimised sequentially and the optimum conditions are given in Table 1.

Table 1
Optimised conditions for FI-HGAAS.

| | |
|---|---|
| HCl (carrier and sample) | 0.1 M (0.5 M for $NaHCO_3$ matrix) |
| $K_3Fe(CN)_6$ (carrier and sample) | 2.0% (w/v) |
| $NaBH_4$ | 1.0% (w/v) in (0.1% (w/v) NaOH) |
| Carrier gas (Ar) | 300 ml $min^{-1}$ |
| Pumping rate | 6.6 ml $min^{-1}$ for both carrier and reductant |
| Sample volume | 500 μl |
| Mixing coil length | 55 cm |

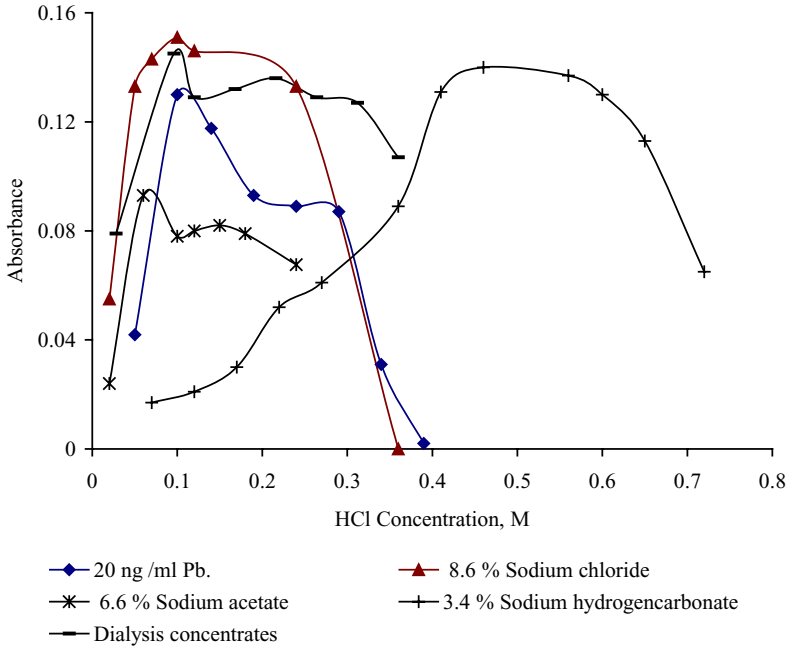


Fig. 2. Optimisation of HCl concentration in different matrices. The concentrations of salts selected approximately correspond to those obtained by diluting original matrices 2.5 times. All the salt solutions were spiked with 20 ng ml$^{-1}$ Pb.

### 3.2. Analytical figures of merit

The detection limit, $3s_b$ m$^{-1}$, and characteristic sensitivity (defined as the concentration that gives 1% absorption) obtained using 500 μl loop volume and peak height measurements were found to be 0.7 and 0.6 ng ml$^{-1}$, respectively. The calibration lines were linear between 5 and 100 ng ml$^{-1}$ aqueous lead standard solutions. The equation of the calibration line obtained by linear regression was $A = 0.0054C + 0.0115$, where $A$ is the peak height absorbance and $C$ is the concentration in ng ml$^{-1}$. The precision of the system in terms of % R.S.D. for 11 replicate measurements at 20 ng ml$^{-1}$ level was found as 3.7. The sampling frequency was 70 measurements per hour.

Typical signal obtained with sample no. 1 (2.5 times diluted and spiked with 40 ng ml$^{-1}$ Pb) is shown in Fig. 3. It should be noted that almost no background signal was observed in the signal; this is a distinct advantage of the hydride technique for such a heavy salt matrix. However, non-spectral liquid phase interferences were responsible for the variations in the slope of calibration line; and standard addition calibration method was found to be necessary for the samples.

### 3.3. Determination of lead in certified reference material

Lead was determined in a certified reference material, Waste Water EU-L-1 (SCP-Science, Canada) at the

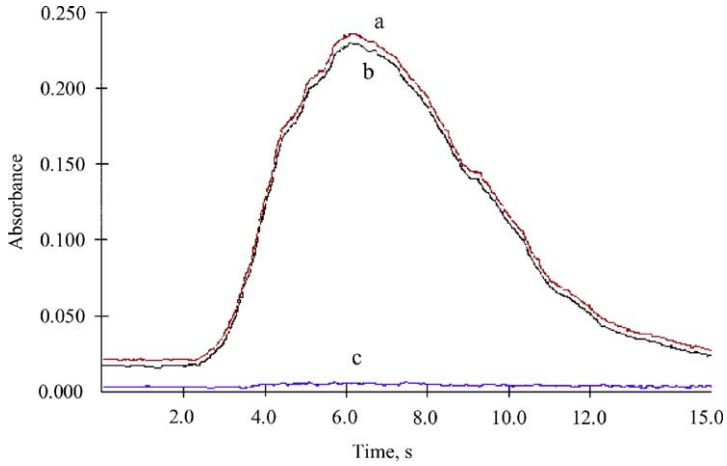


Fig. 3. Flow injection profiles for the determination of lead in dialysis concentrates using FI-HG-AAS, sample 1 spiked with 40 ng ml$^{-1}$ lead: (a) total signal, (b) corrected signal, and (c) background signal.

Table 2
Concentration of lead in dialysis concentrates obtained with FI-HG-AAS and ETAAS

| Sample no. | Pb ($ng\,ml^{-1}$), $\bar{x} \pm s$ ($n = 3$) | |
|---|---|---|
| | FI-HG-AAS | ETAAS |
| 1 | 25 ± 1 | 24 ± 1 |
| 2 | 70 ± 1 | 68 ± 3 |
| 3 | 68 ± 3 | 69 ± 3 |
| 4 | 36 ± 1 | 34 ± 2 |
| 5 | 33 ± 1 | 33 ± 3 |
| 6 | 37 ± 1 | 39 ± 3 |
| 7 | 37 ± 3 | 39 ± 1 |
| 8 | 34 ± 2 | 36 ± 1 |
| 9 | 51 ± 3 | 50 ± 1 |
| 10 | 11 ± 1 | 12 ± 1 |
| 11 | 10 ± 1 | 10 ± 1 |
| 12 | 13 ± 1 | 13 ± 1 |

optimised experimental conditions. The concentration of lead was found as $100.3 \pm 2.3\,ng\,ml^{-1}$ while the certified value was reported as $100 \pm 2\,ng\,ml^{-1}$. This reference material is not totally representative for the sample matrix. Since certified reference material for this kind of matrix is not available yet, accuracy for the sample matrix was further tested using ETAAS as a comparison method.

### 3.4. Determination of lead in the sample

Fig. 2 shows that each of the matrix component affects the signal in a different manner. In addition, the sample compositions have differences in terms of sodium acetate concentration. Therefore, each sample has its own effect on the signal. This was also observed from the ratio of slopes of external and standard addition curves, which were changing between 0.90 and 1.27 for the first group of samples and around 1.54 for the second group, bicarbonate samples. As a result, the use of standard addition method was found to be necessary. The results obtained with the proposed method were given in Table 2.

Electrothermal atomic absorption spectrometry was used as a comparison method. The samples were analysed after separation of the matrix using Chelex 100. The percent recoveries were tested with spiked samples to determine whether there was loss or contamination during the separation step. Spike recoveries were between 92.6 and 105.3% with a precision of 7.1% for triplicate analysis. The detection limit and characteristic sensitivity were 0.4 and $0.5\,ng\,ml^{-1}$, respectively, for a 20 μl injection volume.

The results obtained with FI-HGAAS and solid phase extraction ETAAS are shown in Table 2. For all the samples, FI-HGAAS results were in agreement with ETAAS results, using *t*-test at 95% confidence level.

## 4. Conclusion

Determination of lead in dialysis concentrates using FI-HGAAS technique offers distinct advantages in terms of spectral interferences. No background signal was observed with sample solutions. Therefore, direct analysis is possible without any pre-treatment. This is a distinct advantage as compared to ETAAS where high background signals would be a problem in the presence of heavy salt matrix. The technique is suitable for routine analysis since it is fast and simple. It can be performed with any standard hydride generation equipment. The low cost is another advantage as compared to ICP-MS.

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