FIA-extraction applied to the limit test for heavy metals

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Abstract:A method is presented that allows rapid determination of the total concentration of heavy metals in a sample. The method is based on FIA-extraction and photometric measurement of the metals as their dithiocarbamate complexes. The analytical parameters have been chosen such that the sensitivities for toxic elements are enhanced compared with those of less toxic heavy metals. The sampling capacity of the system is 40 samples h^{-1} and the repeatability (RSD) is 1.9% at 0.1 mg l^{-1}. Raw materials for the production of pharmaceuticals as well as analytical grade salts and household commodities have been tested.

Keywords: *Heavy metals; limit test; flow injection analysis (FIA); extraction; segmented flow; dithiocarbamate.*

Introduction

The risks of adverse health effects from the oral intake of heavy metals have been known for some time. Acute effects of large amounts of elements like arsenic and lead have been known from the seventeenth century. Gradually this knowledge has led to a reduction in cases of acute metal poisoning. However, a number of cases of severe poisoning from long-time exposure to relatively low levels of heavy metals in the environment has occurred, e.g. the Minamata and Itai-Itai events in Japan. This has led to an increasing emphasis on control of the intake of heavy metals from all kinds of sources. Thus control programmes in respect of the contents of heavy metals in commodities such as foods, beverages, water and kitchen utensils are now applied in most developed countries. For the producers this often means that the heavy metal content of raw materials of various kinds has to be controlled. The pharmaceutical industry is in a special situation in that the control of heavy metal concentrations in raw materials and products has been a routine for many years.

In some instances the control measures are aimed specifically at one element, e.g. the leaching of lead from glazed earthenware. Most frequently, however, no specific metal is analysed but rather a general "heavy metal" concentration is sought. Thus the ideal

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method should give a response of similar magnitude for all heavy metals without interference or response from other elements.

The methods now in use for this purpose are all similar to that of the European Pharmacopoeia [1]. This method utilizes the fact that most heavy metals can be precipitated as sulphides at pH 3.5. On addition of a sulphide-containing or sulphide-forming reagent to a buffered sample solution containing heavy metals, a colloidal precipitate is formed. The colour of this suspension is then compared visually with that of a standard solution, in the same buffer, containing an amount of lead corresponding to the permissible level. At the levels normally tested for, 10–40 $\mu g\ g^{-1}$ in solid materials, the colour formed is very weak and difficult to estimate. At best it is possible to decide whether a sample has a higher or lower concentration of heavy metals than the comparison standard; further quantification is not possible. Attempts to overcome this problem by measuring the absorbance of the suspensions spectrophotometrically [2] or by collecting the precipitates on a filter-paper before measurements [3] have failed to gain acceptance. Another drawback with the method is that different elements give suspensions of different colours, making visual comparison very difficult. Since a lead standard is used for comparison, the results obtained are given as "heavy metals as lead".

The reasoning in trying to improve the method used for the limit test was that it should be possible to use one of those relatively non-specific reagents, incorporating the sulphide group, commonly used for spectrophotometry after extraction. With such a reagent it was believed to be possible to include the same elements as in the original test. One of the more widely utilized reagents of this group is diethyldithiocarbamate (DDC); this reagent has been found to extract a large number of elements into carbon tetrachloride [4–6]. After a closer look at DDC it was soon found that at the wavelengths normally used there were large differences between the absorptivities of the different metal complexes [4]. However, in the UV-range all complexes showed a good response within a relatively narrow range of absorptivities. Work at these wavelengths is usually avoided because the reagent also absorbs strongly here [7]. Performing extractions at a high enough pH to avoid the co-extraction of the reagent would exclude a number of elements owing to their hydrolysis [8]. If the extractions are performed at pH 3.5, most of the excess reagent will be transferred to the organic phase. In order to be able to make use of UV absorption, this excess has to be removed prior to measurement with the associated risks for losses of some metals. Utilizing the high rate of extraction obtainable in a liquid–liquid segmented flow [9] and the precise timing of a flow system devoid of compressible gases it is possible to perform a backwashing of excess reagent practically without losses of extracted metals. This is based on the fact that the rate of backwashing of the free reagent is more rapid than the corresponding rate for a metal complex that has to be dissociated first.

Experimental

Apparatus

The manifold used is schematically shown in Fig. 1. The flow was driven with a peristaltic pump (Gilson, Minipuls 2, France) with Tygon pump tubing (Technicon, USA) for all channels. For the organic solvent, CCl_4, a displacement bottle (Tecator, Sweden) was used in order to avoid solvent attack on the pump tubing. Segmented flow was obtained with an ordinary T-piece with a bore of 0.7 mm. The two phases were separated after extraction in a membrane phase separator of the type previously
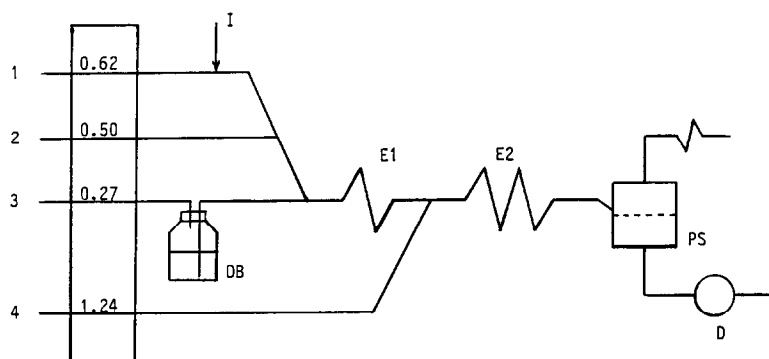
Figure 1
Extraction manifold. (1) Carrier stream, 0.1 M acetate at different pH, with and without EDTA. (2) Diethyldithiocarbamate solution, 1 g l^{-1}. (3) Aqueous stream to displacement bottle. (4) Borax solution, 0.1 M. I, loop injector, 0.45 ml; DB, displacement bottle; E1, E2, Extraction coils 1 m and 2 m $\times$ 0.5 mm, i.d., respectively; PS, phase separator; D, photometric detector, wavelength 274 nm. Flow rates are given in the figure in ml min^{-1}.

described [10]. In this case a separator was used with cavity volumes of 45 and 8 µl on the segmented and unsegmented sides, respectively. The restrictor on the aqueous outflow from the separator was adjusted so that essentially complete recovery of the organic phase was obtained, thus minimizing dispersion in this part of the system [11]. The separator and the T-pieces used in the flow system were machined from PVDF in the Institute workshop. The photometric detector used was a LKB 2151 variable wavelength detector with an optical path length of 10 mm and a cell volume of 10 µl. The signal from the detector was fed to an x/t recorder (W/W Tarkan, Switzerland).

Reagents

All reagents were of analytical grade and were used without purification. The carrier solution was varied by adjusting the pH of a 0.1 M acetate solution to 3.5 and 4.7, respectively. Performance at these two pH levels was also tested in the presence of a strong complexing agent, 0.003 and 0.002 M EDTA, respectively. Single element standard solutions at a concentration of 1 g l^{-1} were prepared for the different metal ions according to Table 1. When diluting these standards to appropriate concentrations for experiments, enough acid to prevent hydrolysis was added.

Tests on samples

Different types of samples were tested for their content of heavy metals using the extraction system. In all cases the carrier stream had a pH of 3.5 and contained 0.003 M EDTA. Samples that easily dissolve in water, such as table salt, were first dissolved in dilute acid in order to attack colloidal hydroxides that might otherwise be inert under the conditions used. The acidified samples were then diluted and a solution of buffer-masking agent was added such that the final concentration was the same as in the carrier stream.

Lump sugar and table salts, about 2.5 g, were dissolved in 10 ml of acidified water (pH 2) and then diluted to 25 ml with de-ionized water. To 5 ml of this solution, 1 ml of 1 M acetate buffer (pH 3.5) containing 0.03 M EDTA (Buffer A) was added, and the mixture diluted to 10 ml.

Table 1
Stock standard solutions

Ion	Substance	Stabilizer
Se(IV)	SeO_2	0.01 M HNO_3
Sb(III)	$K(SbO)C_4H_4O_6$	0.01 M $KNaC_4H_4O_6$
Hg(II)*	$Hg(NO_3)_2$	0.01 M HNO_3
Bi(III)	Bi	0.01 M HNO_3
Pb(II)*	$Pb(NO_3)_2$	0.01 M HNO_3
Co(II)*	$CoCl_2$	0.01 M HNO_3
Fe(III)*	$FeCl_3$	0.01 M HCl
Cr(VI)	$K_2Cr_2O_7$	0.01 M HNO_3
Cd(II)*	$CdCl_2$	0.01 M HNO_3
As(III)	AsO_2	0.01 M HCl
Zn(II)*	$ZnCl_2$	0.01 M HNO_3
Tl(I)	$TlNO_3$	0.01 M HNO_3
Cu(II)*	$CuCl_2$	0.01 M HNO_3
Ag(I)	$AgNO_3$	0.01 M HNO_3
Pd(II)	Pd	0.01 M HNO_3
Mn(II)*	$MnCl_2$	0.01 M HNO_3
Ni(II)*	$NiCl_2$	0.01 M HNO_3

*These standards were prepared from Titrisol ampoules.

Analytical grade salts were tested after dissolving 2.5 g of salt in a small volume of deionized water, adding 2.5 ml of buffer A and diluting to 25 ml.

Tap water was acidified with a small amount of nitric acid. To 5 ml of this sample, 1 ml of buffer A was added, and the mixture was diluted to 10 ml.

A group of raw materials for the production of pharmaceuticals was subjected to the heavy metal test after digestion according to the European Pharmacopoeia [1]. The procedure started with a wet ashing in sulphuric acid in silica crucibles. The temperature was slowly increased up to 250°C; thereafter the crucibles were placed in an oven and the temperature slowly raised up to 500°C. The samples were then treated at this temperature overnight. The white residue was dissolved in dilute hydrochloric acid. After neutralization with sodium acetate, 1 ml of buffer A was added and the solution diluted to 10 ml. The amount of sample taken was adjusted according to the concentration limit of heavy metals. For a limit of 10 $\mu g\ g^{-1}$ of "heavy metals as lead", 0.4 g of sample was used. For different limits, the amount of sample was adjusted accordingly. Thus, in all cases, the limit in the final solution corresponded to 400 $\mu g\ l^{-1}$ and this simplified evaluation of the results.

Results and Discussion

Initially, experiments were carried out to elucidate the influence of the different parts of the manifold on the extractions. Using single element standards and making measurements at the respective optimum wavelengths it was found that an extraction coil of 1 m × 0.5 mm, i.d., was sufficient to achieve adequate extraction. If in this step the extraction is <100% complete, the results will be affected only to a minor extent. In the second step, where excess reagent is backextracted, it is essential that virtually complete extraction is obtained. For work in the UV range, the absorbance spectra for reagent and complex will coincide and the reagent is present in at least 100-fold excess. Borate was chosen as buffer substance in the backwashing because its complexes with metal ions are

weak, thus reducing the risk for losses of metals in this step. The efficiency of backextraction was followed by measuring the absorbance in the organic phase of the proposed system, compared with the absorbance when the dithiocarbamate reagent was exchanged for de-ionized water. Using 2 m × 0.5 mm, i.d., tubing the backextraction was very efficient; the baseline absorbance was reduced to 0.04 at 274 nm, compared with a system without reagent. The residual absorption is caused by remaining dithiocarbamate reagent in the aqueous phase plus extracted metal complexes originating from the buffer solutions.

The wavelength to be used for measurement was chosen with the aid of data obtained by pumping 1 mg l^{-1} solutions of the metals as carrier and manually scanning over the range 260–330 nm. A set of signals at different wavelengths were thus obtained for each metal. From a comparison of these signals a wavelength was chosen such that high and similar sensitivities were obtained for as many elements as possible. The wavelength chosen, 274 nm, is a compromise where some consideration has also been given to the relative responses of lead, used as a standard, and other highly toxic elements such as cadmium and mercury.

The sensitivity for various elements was tested in a manifold (Fig. 1) with small variations in the composition of the carrier stream. In all cases the metal standards used were prepared to contain the same concentrations of buffer and complexing agent as the carrier stream. Each series of experiments was started by injecting a number of lead standards in the range 0.2–3 mg l^{-1}; 1 mg l^{-1} solutions of different metals were then injected in duplicate and the signal noted. If the signal was out of range, injections were repeated with more dilute standards. The first set of results, presented in Table 2, were obtained with a 0.1 M acetate buffer at pH 3.5. This is an unusual pH for an acetate buffer but it is used because it corresponds to the conditions used in the Pharmacopoeial method. If a pH of 4.7 is used instead, clear advantages are obtained; at this pH the

Table 2
Relative response for different ions

| Ion | pH 3.5 | | pH 4.7 | |
	0.1 M Acetate	0.1 M Acetate† 0.003 M EDTA	0.1 M Acetate	0.1 M Acetate 0.002 M EDTA
Se(II)	1.85	1.89	1.92	1.14
Sb(II)	1.47	1.59	1.47	1.75
Hg(II)	1.01	1.04	0.93	1.00
Bi(III)	1.00	1.10	0.97	1.11
Pb(II)	1.00	1.00	1.00	1.00
Co(II)	0.94	0.13	0.91	0.028
Fe(III)	0.81	0.0067	0.81	0.0024
Cr(VI)	0.80	0.83	0.46	0.65
Cd(II)	0.76	0.66	0.76	0.78
As(III)	0.71	0.66	0.71	0.63
Zn(II)	0.68	0.073	0.67	0.0099
Tl(I)	0.63	0.11	0.57	0.21
Cu(II)	0.51	0.47	0.50	0.49
Ag(I)	0.48	0.49	0.48	0.59
Pd(II)	0.45	0.44	0.42	0.40
Mn(II)	0.28	0.0019	0.37	0.0048
Ni(II)	0.15	0.0015	0.14	0.0040

Relative sensitivities*

*Comparisons are based on molar concentrations with the sensitivity for lead as unity.
†Conditions used for tests on real samples.

buffering capacity of the acetate is much higher, and therefore it is easier to obtain the correct pH. Furthermore, the higher pH decreases the extraction of reagent and thus simplifies the subsequent backextraction. The results obtained using a pH of 4.7 are also given in Table 2. The responses obtained in the two systems are similar for most elements. In Table 2, the responses are given as relative sensitivities, compared with that of lead. Thus the value given for a metal ion is the response obtained for a certain molar concentration of that ion in relation to the response for the same concentration of lead. Increasing the pH increases the response for manganese, whereas the response for chromium(VI) decreases. For manganese this behaviour is explained by the low extraction constant [12], whereas for chromium(VI) the explanation is probably more rapid kinetics at the lower pH. The relative sensitivities obtained are by no means uniform but vary from 0.45 to 1.85 for 15 of the most toxic heavy metal ions at pH 3.5. The stability of the relative sensitivities given in Table 2 were tested by preparing mixtures of up to five elements and testing these mixtures in the system against a pure lead standard. In all cases the experimental response corresponded, within a few percent, to that calculated from the relative sensitivities. A more direct comparison with the Pharmacopoeial method is not possible due to the subjective nature of the manual measurement. It is, however, clear from Table 2 that the FIA-method is not only more sensitive, but also provides a more uniform sensitivity over the range of elements included. Apart from the ions shown in Table 2, tin(II) and tin(IV) were tested but no response was found. This is somewhat surprising in light of the results presented by Tao *et al.* [6]. The apparent discrepancy is probably explained by a rapid backextraction coupled with tin hydrolysis.

In many cases the reason for applying a limit test for heavy metals is to assess the toxicity of the sample. In those cases it can be a drawback that ubiquitous elements such as iron and zinc give a strong response in the test, in spite of the fact that they are relatively nontoxic. In an attempt to emphasize the more toxic elements, masking agents were used to reduce the response from elements such as iron and zinc. EDTA was used as the masking agent and the concentration chosen such as not to decrease the response for the more toxic elements. The concentrations used were 0.003 M at pH 3.5, and 0.002 M at pH 4.7, and the results are given in Table 2. The depression of the response for iron, manganese, nickel and zinc equals or exceeds 100-fold at pH 3.5 whilst it is somewhat less at pH 4.7. For the other ions, the effect of adding masking agents is small. At pH 3.5 this means that at a concentration of 0.5 mg l^{-1} of lead, or corresponding amounts of other heavy metals, the presence of up to 20 mg l^{-1} of iron gives <10% increase of the response.

Calibrations were performed using single element lead standards. The responses for lead in the different systems were very similar, 1 mg l^{-1} giving a peak absorbance in the range 0.198–0.224. The calibration curve is strictly linear (correlation coefficient = 0.99997) from the detection limit to at least 1 mg l^{-1}. The repeatability was measured for 10 injections of 1 and 0.1 mg l^{-1} standards. At these two levels the repeatability, measured as the RSD of the signals obtained, was 0.8 and 1.9%, respectively. From these data the detection limit can be estimated to be 0.005 mg l^{-1}. These characteristics of the method were measured using an acetate buffer (pH 3.5) containing 0.003 M EDTA, but in this respect the slight variations in the carrier tested had little effect. The sampling rate, using 0.45-ml samples, was 40 h^{-1}.

The two kinds of table salt tested had similar concentrations of heavy metals, 2.1 and 2.6 $\mu g\ g^{-1}$, respectively, for pure sodium chloride and so-called "sodium reduced salt"

which contains 25% (m/m) of potassium chloride. The sugar, a highly refined, white sugar contained 1.2 μg g^{-1} of "heavy metals as lead". The results for cold and warm tap water showed concentrations of 0.68 and 0.88 μg g^{-1}, respectively. Thus the method is capable not only of detecting, but also of measuring the content of heavy metals in important household products.

The results from the analysis of analytical grade salts are collected in Table 3. For the salts tested it was possible to prepare solutions with a salt concentration of 10%, and thus it should be possible to detect heavy metal contents down to 5×10^{-6}%. The stated limits for analytical grade chemicals are normally of the order of magnitude 1×10^{-4}%. Thus a test for this limit could be performed with the system presented on much more dilute salt solutions than those used here.

Results for raw materials from the production of pharmaceuticals are given in Table 4 and the recorder chart is shown in Fig. 2. The largest amount of any sample taken for these tests was 0.4 g, less than what is recommended for the procedure based on visual comparison. This amount of sample was taken for materials with a test limit of 10 μg g^{-1}. For materials with higher limits, further reductions of the sample weights were made. The small amounts of sample simplified the ashing step without too much sacrifice of signal corresponding to the limit of acceptance. By proper adjustment of the amount of sample taken the concentration of heavy metals in the final solution, corresponding to the acceptance limit, can be the same for all samples tested. This concentration limit can then be expressed in terms of an absorbance limit as represented by the horizontal

Table 3
Heavy metals in analytical grade salts

Salt†	In solution (μg l^{-1})	Heavy metals* In salt (g g^{-1})	Stated limit (% m/m)
NaCl	70	7×10^{-7}	$<5 \times 10^{-4}$
Na$_2$SO$_4$	70	7×10^{-7}	$<5 \times 10^{-4}$
NaNO$_3$	92	9×10^{-7}	$<5 \times 10^{-4}$
NH$_4$Cl	54	5×10^{-7}	$<5 \times 10^{-4}$
NaF	100	5×10^{-6}	$<1.8 \times 10^{-3}$

*Measured as lead using 0.1 M acetate (pH 3.5) + 0.003 M EDTA as carrier.

†All salts were Merck Analytical Grade; analysed solutions were 0.1 g ml^{-1} except NaF which was 0.02 g ml^{-1}.

Table 4
Heavy metals* in raw materials for the production of pharmaceuticals

Material†	Concentration limit (μg g^{-1})	Amount taken g	Concentration found (μg g^{-1})
Polyvinylpyrrollidone, K90	10	0.4	4
Hydrogenated castor oil	10	0.4	1
Guar gum	20	0.2	7
Sodium starch glycolate	20	0.2	5
Ethyl cellulose, 10 cps	40	0.1	61

*Measured as lead using 0.1 M acetate (pH 3.5) + 0.003 M EDTA as carrier.

†These materials, except ethyl cellulose, were found to pass the limit test when the manual procedure was used.

Figure 2
Recorder printout from the determination of heavy
metals in pharmaceutical raw materials. Flow system
as in Fig. 1 using 0.1 M acetate (pH 3.5) with 0.003 M
EDTA as carrier. Standard concentrations are given
in $\mu g\,l^{-1}$. Samples are: B1, blank digested according
to procedure given. (1) Polyvinylpyrrollidone, K 90.
(2) Hydrogenated castor oil. (3) Ethyl cellulose, 10
cps. (4) Guar gum. (5) Sodium starch glycolate. The
dashed line represents the permissible level of heavy
metals corrected for digestion blank.
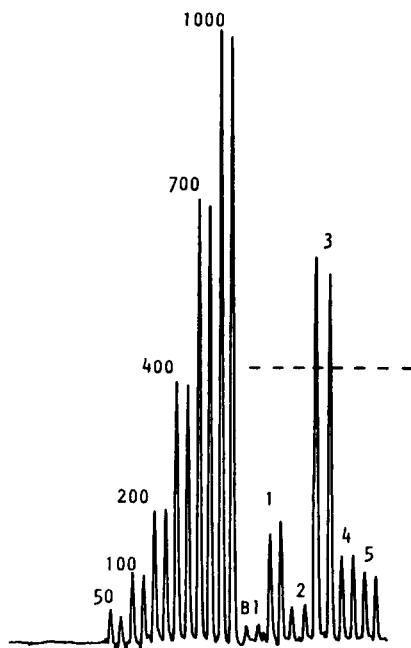
dashed line in Fig. 2. This limit is conveniently set each time the system is used by
injecting a lead standard of the appropriate concentration. Thus the decision to pass or
not to pass a sample can be easily taken.

Conclusions

Using liquid–liquid extraction in segmented flow it is possible to design a system with a
broad response to most heavy metals. The response varies between different elements
but not to a very large extent. In contrast to the standard procedure based on visual
comparison this varied response can be quantified. It is also possible to make a
quantitative estimate of the amount of heavy metals present in a sample.

The system is more sensitive than the standard procedure making it possible to use
smaller samples thus simplifying ashing procedures. Repeatability is good, 1.9% RSD at
0.1 mg l^{-1}, and the sampling rate, 40 samples h^{-1}, is adequate for most applications. By
using masking agents the response from less toxic elements such as iron and zinc can be
suppressed to emphasize more toxic elements. The system presented can be used to
screen many kinds of materials for their content of heavy metals.

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