APPLICATION OF RADIOACTIVE TRACERS TO THE DEVELOPMENT OF SEPARATION PROCEDURES FOR DETERMINATION OF TRACE ELEMENTS IN BIOLOGICAL MATERIALS

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INTRODUCTION

Humans unlike other animals are the only beings capable to modify their environment. The wish of changing their living conditions originated the wide spread of industrial activities. The chain resources with certain toxic inorganic trace elements(1).

Certainly food is the main transporting medium carrying trace elements into the human body and other than food, modern agricultural practices including fertilizers, inseticides, processing and packaging are co-responsible for the continuous changing of the food character itself. This is the way toxic elements are most usually introduced into food items(2). Monitoring toxic elements levels became then a rule because of the adverse impact of the toxic metals.

Because the diet is the main source of nutrients, it is important to study the elemental concentration of the diet(3).

During the past years some Brazilian food items as bread and milk(4), rice(5), and drinking water(6), have been analyzed for their elemental contents by nuclear methods.

In the last 3 years, we concentrated our studies in the development of radiochemical separation in order to determine elements : As. Cr. Hg. Sb and Se(7).

These elements are commonly found in biological materials. at the ppm or subppm level. At these levels, problems occur in accurately determining the concentrations of these elements due to several reasons: losses during sample treatment, contamination, reagent blank and interferences.

Several analytical procedures have been developed to determine these elements by radiochemical neutron activation analysis (RNAA). RNAA offers the advantages of high sensitivity, excelent selectivity and no reagent blank. Furthermore, the ability to add carriers during the chemical dissolution and separation enables a quantitative recovery of both elements by minimizing volatility losses and losses of microamounts into the Teflon vessels used.

The aim of this work was to develop faster and more reliable radiochemical methods than those previously utilized, such as distillation and precipitation(5). For sample dissolution, teflon bombs were employed, using conventional and microwave ovens. The main elements studied in this work were As, Cd, Cr, Cu, Hg, Mo, Sb and Se and the separation techniques used were ion-exchange, retention on inorganic exchangers, precipitation and solvent extraction. Several authors have utilized similar techniques for the analysis of biological materials with good results(8-14).

Another radiochemical procedure adapted from Chatt(15), was applied to determine Cu, Mo and Se elements in forage samples. The aim of this study was to apply a method for determining Cu content in forages used to feed a defined group of male calves and the determination of Mo content in serum of these animals.

The developed procedures were applied to the analysis of the several biological reference materials, andin four human diet samples: diet of the University Students, diet of Manaus region (North of the country) and two diets of Santa Catarina region (South of the country), and in forage samples used to feed male calves.

EXPERIMENTAL

1. Diet Preparation

1.1. Student diet

About 50 meals (lunch and dinner) served at the central restaurant in the campus of the University of São Paulo, used by students and staff . were collected. Foodstuff contents in these meals follow Brazilian basic diets for medium income groups and are composed by rice, beans, meat or derivates, greens and vegetables, fruits, sweets, bread and milk(14).

Food was ordinarily mixed for cooking preparation and were separately weighed, placed in stainless steeltrays and dried in ventilated oven at 60°C, during 12 hours or until totally dried.

The foods were pulverized and homogenized in a knife mill and conservated at 4°C.

1.2. Manaus and Santa Catarina diets

These diets were prepared from foods consumed and produced in these respective regions. The Manaus and Santa Catarina 1 diets are related to low-income groups, while Santa Catarina 2 is related to higher income groups.

The foods were obtained in the local supermarkets and fairs and prepared according to costums of each region. Thereafter, the preparation procedure of the diets was as previously described.

2. Irradiation and dissolution of the reference materials and samples

About 200 mg of reference materials and samples were weighed in clean quartz ampoules. A multielemental standard containing elements As, Hg, Sb, Cu, Cd, Cr and Se was prepared by mixing appropriate aliquots of solutions of these elements made from spectroscopically pure reagents. Aliquots of the multielemental solutions were pipetted into quartz ampoules.

Samples together with standards were irradiated in the IEA-R1 Research Reactor, for 8 hours, under a thermal neutron flux of 10^{13} $n.cm^{-2}.s^{-1}$. After irradiation, samples and standards were processed in the same way.

3. Counting Systems

Gamma ray measurement of irradiated samples and standards was carried out by using high resolution Ge Hiperpure detectors and associated electronics.

Spectrum analysis was performed by means of the Vispect2 software, developed by Dr. D. Piccot, from Saclay, France.

4. Separation Procedures

PROCEDURE I

4.1. Determination of As, Hg, Sb and Se by using inorganic exchanger TDO.

4.1.1. Radiochemical Separation

Irradiated samples and standards were transferred to teflon bombs and 100 ug carriers of each element were added. After addition of 4 ml of HNO_3, the teflon bombs were taken to a conventional oven at 130°C, for 15 to 20 hours, depending on the reference material.

Dissolved samples were cooled and 10 drops of H_2O_2 and distilled water were added.The solution was passed through a TDO column, previously treated(7). The column was washed twice with 15 ml of 1M HNO_3, allowed to dry, and the TDO transferred to a plastic counting vial for the measurement of ^{76}As, ^{75}Se and $^{122-124}Sb$ on gamma ray spectrometer.

The effluent of the column was adjusted to pH 1 with NH_4OH and Hg was extracted with 20 ml of 0.005M $Ni(DDC)_2$ in chloroform.The aqueous phase was discarded and the organic phase was measured for $^{197-203}Hg$.

PROCEDURE II

4.2. Determination of As, Hg, Sb and Se by using anionic resin (DOWEX 2X8) and inorganic exchanger TDO (tin dioxide)

4.2.1 Radiochemical Separation

After addition of 3 ml HNO_3 , 1 ml H_2SO_4 and carriers solutions, teflon bombs were taken to a conventional oven at 130°C, for 1 to 4 hours, depending on the material analysed.

Dissolved samples were cooled and 3 ml of a 10% urea solution, 5 ml HCl and 0.2g $NaBrO_3$ were added. Solutions were slowly heated and a clear yellow colour was obtained. After cooling, solutions were passed through the Dowex 2X8 anionic resin column,previosly treated(7), to retain Hg and Sb on the column. The column was washed with 20 ml 6M HCl and transferred to counting vials.

Equal volume of H_2O was added to the effluent of the resin, and this solution was passed through a TDO column, that was washed with 15 ml 3M HCl and transferred to a counting vial for measurement of ^{76}As and ^{75}Se.

PROCEDURE III

4.3. Determination of Cd, Cr, Cu and Se by using inorganic exchanger HMD and a solvent extraction system using diethylditiocarbamates compounds.

4.3.1. Radiochemical Separation

Samples and standards were dissolved in Parr acid digestion bombs in microwave oven at 360 W for 2 min.,after addition of Cr, Cu and Se carriers and 4 ml of HNO_3.

Two mililiters of $HClO_4$ and 5 drops of HF were added and the solution was slowly evaporated to a final volume of about 0.5 ml. This solution was diluted with 15 ml 1M HNO_3 and passed through a preconditioned HMD column (7).The columns were washed twice with 15 ml of 1M HNO_3 each time and transferred to polyethylene counting vials to determine Cr and Se.

The pH of the eluated fractions containing Cu and Cd was adjusted to 1.5 with NH_4OH, and 100 mg of Zn holdback carrier was added. Copper was extracted with 25 ml of 0.003M $Bi(DDC)_3$in chloroform(7), and cadmium was extracted from the remained aqueous solution with 25 ml of 0.005M of $Zn(DDC)_2$ in chloroform.

The organic fraction containing ^{64}Cu were counted immediately after separation while that the organic fractions containing Cd were allowed to stand for 24h to establish the equilibrium between $^{115}Cd-^{115m}In$.

PROCEDURE IV

4.4. Determination of Cu, Mo and Se by precipitation in sulphide medium

4.4.1. Irradiation and dissolution of the samples

About 200 mg of the sample was weighed in clean polyethylene bags. A multielemental standard containing the elements Cu, Mo and Se was pipetted into filter paper Whatman n. 40 (area about 1 cm^2)

After dissolution samples and standards were evaporated until almost dried. Drops of HCl and 40 ml of water were added and the pH was adjusted to 2. Solutions were gently heated until 60°C and then 6 ml of 2.5% thyoacetamide solution was added. Solutions were set aside for about 50 minutes at room temperature and after this, heated until reaching boiling point. These solutions were cooled and precipitates were filtered through Whatman n. 41 filter paper, dried and counted.

RESULTS

1. Chemical Yield

Table 1 shows the results of chemical yields obtained for four developed procedures describe above.

2. Quality Control

The developed procedures were applied to biological reference materials analysis. The results are presented in Tables 2 to 5.

3. Human Diet Analysis

Procedures I, II and III were applied to analysis of Students, Manaus , Santa Catarina I and II diets. The results obtained for the determination of As, Cd, Cu, Cr, Hg, Sb and Se concentration in these diets are presented in Table 6.

4. Forage Analysis

Table 7 shows the results of Cu, Mo and Se concentration in forage samples by procedure IV.

DISCUSSION AND CONCLUSIONS

The results obtained using the radiochemical separation schemes presented in this work (Tables 2 to 5) are, in general, in good agreement with literature and reference concentration values.

The separation methods outline in this work present a good alternative for the isolation and subsequent determination of As, Cd, Cr, Cu, Hg, Sb and Se in foodstuffs and diets. The procedures have proven to be fast and give good results. Although in some cases the concentration values determined by these methods differ from the certified ones, as the Cr case, they are within the error indicated by the supplier of the reference material concerned.

The results obtained for determination of As, Hg and Se concentration by procedures I and II (Table V) in Brazilian diets shown:

1. Good agreement between results for As, Hg and Se concentration in Manaus diet.
2. For Santa Catarina 1 diet the results did not agree.
3. For Santa Catarina 2 diet the results agreed for As and Se, but not for Hg and Sb.

The results obtained by procedure I for Sb were, in all cases, higher than those obtained by procedure II.

Comparing the results obtained for Cr and Se in Brazilian diets with diets of different countries(15) (Table 8), it could be seen that the results obtained for Se were in agreement with other results, but the results for Cr were higher than those obtained in other countries. It could be that the diets were contaminated during preparation step.

For the other elements, literature values of concentration in diets were not found for comparison. However, Se content in student diet, shows a very low concentration level.

The diets, supplied by Food and Experimental Nutrition Department of USP, were prepared for other studies and purposes, such as bioavaliability of elements Zn, Se and Cu.

Once in Brazil there are no table information on major, minor and trace element contents for diets of different regions, we intend to obtain these data and to compare with data from other countries.

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Table 1

Chemical yield results obtained for
each procedure, in %

Element	Procedure			
	I	II	III	IV
As	96+-1	99+-5	-	-
Hg	97+-1	96+-5	-	-
Sb	82+-1	100+-13	-	-
Se	-	85+-11	99+-1	95+-2
Cu	-	-	98+-2	95+-2
Cd	-	-	95+-4	-
Cr	-	-	97+-4	-
Mo	-	-	-	82+-6

- not determined

Table 2

Results of the determination of As, Hg, Sb and Se in biological materials using TDO inorganic exchanger

(PROCEDURE I)

Reference material	This work (ug/g dry weight)	RSD (%)	Relative error (%)	Literature values(16)
Bowen's Kale	As: 0.135+-0.019	14.1	3.1	0.131+-0.045
	Hg: 0.192+-0.014	7.3	12.3	0.171+-0.027
	Sb: 0.078+-0.017	21.8	13.9	0.069+-0.014
	Se: 0.127+-0.005	3.9	5.2	0.134+-0.020
Copepod *IAEA-MA-A-1*	As: 7.3+-0.7	9.6	8.9	6.7+-0.6
	Hg: --	--	--	0.28+-0.01
	Sb: --	--	--	0.07+-0.03
	Se: 2.4+-0.2	8.3	20.0	3.0+-0.2
Bovine Liver *NBS-SRM-1577a*	As: 0.056+-0.004	7.1	19.1	0.047+-0.006
	Hg: nd	--	--	0.004+-0.002
	Sb: nd	--	--	0.003
	Se: 0.61+-0.05	8.2	14.1	0.71+-0.07
Fish-Flesh *IAEA-MA-A-2*	As: 2.61+-0.12	4.6	0.4	2.6+-0.1
	Hg: 0.51+-0.05	9.8	8.5	0.47+-0.02
	Sb: 0.014+-0,002	14.3		0.005+-0.001
	Se: 1.18+-0.09	7.6	30.6	1.7+-0.3
Mixed Human Diet *IAEA-H-9*	As: 0.072+-0.008	11.1	18.2	0.088+-0.033
	Hg: --	--	--	0.0048+-0.0014
	Sb: 0.016+-0.004	25.0	33.3	0.012+-0.008
	Se: 0.094+-0.019	20.2	14.5	0.11+-0.01
Rice Flour NBS-SRM-1568	As: 0.372+-0.020	0.0	9.3	0.41+-0.05
	Hg:0.0067+-0.0003	4.5	11.7	0.0060+-0.0007
	Sb: 0.016+-0.002	12.5	26.0	0.0127+-0.0007
	Se: 0.32+-0.09	28.1	20.0	0.40+-0.10

-- - not determined
n.d. - not detected

Table 3

Results of the determination of As, Hg, Sb and Se in biological reference material using TDO inorganic exchanger and Dowex 2-X8 anionic resin

(PROCEDURE II)

Reference material	This work (ug/g dry weight)	RSD (%)	Relative error (%)	Literature values(16)
Bowen's Kale	As: 0.128+-0.014	10.9	2.3	0.131+-0.045
	Hg: 0.136+-0.022	16.2	20.5	0.171+-0.027
	Sb: 0.068+-0.006	8.8	0.7	0.069+-0.014
	Se: 0.12+-0.02	16.7	10.4	0.134+-0.020
Rice Flour NBS-SRM-1568	As: 0.49+-0.07	14.3	19.5	0.41+-0.05
	Hg: 0.008+-0.001	12.5	33.3	0.0060+-0.0007
	Sb: 0.007+-0.003	42.9	7.9	0.0076
	Se: 0.35+-0.01	2.9	12.5	0.40+-0.10
Citrus Leaves NBS-SRM-1572	As: 3.17+-0.54	17.0	2.3	3.10+-0.30
	Hg: 0.084+-0.004	4.8	5.0	0.08+-0.02
	Sb: 0.036+-0.006	16.7	10.0	0.04

Table 4

Results for Cd, Cr, Cu and Se determination in reference materials by using inorganic exchanger HMD

(PROCEDURE III)

Reference material	This work (ug/g dry weight)	RSD (%)	Relative error (%)	Literature values(16)
Mussel Tissue *(IAEA-MA-M-2)*	Cu: 9.0+-0.5 (4)	5.6	6.3	9.6+-0.2
	Cd: 0.34+-0.02 (4)	7.2	0	0.34+-0.02
	Se: 1.64+-0.08 (4)	4.8	1.2	1.66+-0.04
	Cr: 1.2+-0.2 (4)	16.7	50.0	0.80+-0.08
Bovine Liver *(NBS-SRM-1577A)*	Cu: 157+-5 (5)	3.5	0.6	158+-7
	Cd: 0.45+-0.02 (5)	5.5	2.3	0.44+-0.06
	Se: 0.80+-0.03 (5)	3.3	12.7	0.71+-0.07
	Cr: -	-	-	-
Fish Flesh *(IAEA-MA-A-2)*	Cu: 3.7+-0.2 (6)	5.6	7.5	4.0+-0.1
	Cd: 0.067+-0.004(4)	5.3	1.5	0.066+-0.004
	Se: 1.4+-0.2 (6)	10.6	17.6	1.7+-0.3
	Cr: 1.8+-0.3 (5)	16.7	38.5	1.3+-0.1
Citrus Leaves *(NBS-SRM-1572)*	Cu: 16.3+-2.3 (6)	14.1	1.2	16.5+-1.0
	Cd: 0.036+-0.002(5)	5.6	20.0	0.03+-0.01
	Se: 0.036+-0.006(4)	16.7	-	0.025
	Cr: 1.0+-0.2 (5)	20.0	25.0	0.8+-0.2
Total Diet *(NBS-SRM-1548)*	Cu: 2.7+-0.2 (4)	7.4	3.8	2.6+-0.3
	Cd: 0.029+-0.008(4)	17.2	3.6	0.028+-0.004
	Se: 0.26+-0.03 (4)	11.5	6.1	0.245+-0.005
	Cr: 0.5+-0.2 (4)	40.0	-	-

( ) - number of determinations

Table 5

Results of the determination of Cu, Mo and Se in biological reference materials using precipitation with thyoacetamide

(PROCEDURE IV)

Reference material	This work* (ug/g dry weight)	RSD (%)	Relative error (%)	Literature values(16)
Rice Flour (*NIES-CRM-10C*)	Cu: 4.2+-0.4	9.5	2.4	4.1+-0.3
	Mo: 1.6+-0.2	12.5	0	1.6+-0.1
	Se: 0.13+-0.05	38.5	--	0.07
Rye Grass (*BCR-CRM-281*)	Cu: 9.6+-1.2	12.5	0.5	9.65+-0.38
	Mo: 0.85+-0.12	14.1	1.2	0.84+-0.06
	Se: 0.24+-0.07	29.2	--	0.028+-0.004
Pig Kidney (*BCR-CRM-186*)	Cu: --	--	--	31.9+-0.4
	Mo: 3.3+-0.4	12.1	--	--
	Se: 10.4+-0.4	3.8	1.0	10.3+-0.5

* - mean of 4 determinations

Table 6

Results of the determination of trace elements in diets by different radiochemical procedures

Diet	Procedure I		Procedure II		Procedure III	
	This work* (ng/g dry weight)	RSD (%)	This work* (ng/g dry weight)	RSD (%)	This work** (ug/g dry weight)	RSD (%)
Students	As: 42.4+-0.9	2.1	As: -	-	Cd: < 0.030	-
	Hg: 19+-3	15.8	Hg: -	-	Cr: 0.49+-0.06	12.2
	Sb: 10.6+-0.2	1.9	Sb: -	-	Cu: 3.3+-0.2	6.1
	Se: 72+-5	6.9	Se: -	-	Se: 0.07+-0.01	14.3
Manaus	As: 37.2+-0.5	1.3	As: 34+-2	5.9	Cd: < 0.073	-
	Hg: 87+-9	10.3	Hg: 85+-5	5.9	Cr: 0.97+-0.05	5.2
	Sb: 7.4+-0.1	1.4	Sb: 2.3+-0.4	17.4	Cu: 2.42+-0.02	0.8
	Se: 210+-16	7.6	Se: 228+-13	5.7	Se: 0.208+-0.018	8.7
Santa Catarina 1	As: 138+-5	3.6	As: 302+-27	8.9	Cd: 0.021+-0.004	19.0
	Hg: 19+-3	15.8	Hg: 6.9+-0.5	7.2	Cr: 0.35+-0.04	11.4
	Sb: 9.0+-0.5	5.6	Sb: 3.3+-0.3	9.1	Cu: 1.87+-0.05	2.7
	Se: 141+-19	13.5	Se: 151+-13	8.6	Se: 0.147+-0.011	7.5
Santa Catarina 2	As: 259+-17	6.6	As: 279+-11	3.9	Cd: 0.024+-0.005	20.8
	Hg: 22+-3	13.6	Hg: 7.2+-0.3	4.2	Cr: 0.68+-0.01	1.5
	Sb: 10.7+-0.9	8.4	Sb: 2.7+-0.3	11.1	Cu: 2.1+-0.1	4.8
	Se: 241+-12	5.0	Se: 201+-11	5.5	Se: 0.246+-0.010	4.1

* - mean of 2 determinations
** - mean of 4 determinations
\- - not determined

Table 7

Results obtained for Cu in forage samples by using precipitation with thyoacetamide

Sample collected in	This work (ug/g dry weight)	RSD (%)
March/89	4.9+-0.4	4.9
April/89	10.0+-0.7	6.7
June/89	5.55+-0.07	1.3
August/89	5.8+-2	36.6
September/89	5.7+-0.2	3.8

Table 8

Comparison of Cr and Se concentrations in freeze-dried diets consumed in Brazil and other countries(17)

Element	Cr (ug/g)	Se (ug/g)
Brazil*	0.35 - 0.97	0.07 - 0.25
USA	0.07 +- 0.01	0.26 +- 0.01
Norway	0.13 +- 0.09	0.13 +- 0.07
Germany	0.044	0.042
Turkey	0.13 +- 0.05	0.11 +- 0.04

* - Range of results of diets analyzed in this work

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