A METHOD FOR THE DETERMINATION OF Sr^{90} - Y^{90} BY USING EDTA AND ION-EXCHANGERS-APPLICATION TO THE DETERMINATION OF THOSE RADIOISOTOPES IN MILK.

CÊLIA MACHADO SILVA and FAUSTO W. LIMA

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INSTITUTO DE ENERGIA ATÔMICA
Caixa Postal 11049 (Pinheiros)
CIDADE UNIVERSITÁRIA "ARMANDO DE SALLES OLIVEIRA"
SÃO PAULO — BRASIL

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Célia Machado Silva[**] and Fausto W. Lima

RADIOCHEMISTRY DIVISION
INSTITUTO DE ENERGIA ATÔMICA
São Paulo - Brasil

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[**] On leave of absence from the Departamento de Produção Animal, Secretaria da Agricultura, São Paulo, Brazil. On fellowship of the Comissão Nacional de Energia Nuclear.

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Célia Machado Silva and Fausto W. Lima
Radiochemistry Division - Instituto de Energia Atômica
São Paulo - Brasil

SUMÁRIO

Descreve-se o método desenvolvido para a determinação de Sr^{90}-Y^{90} por meio de ácido etilenodiaminotetraacético (EDTA) e resinas trocadoras de ions. O método foi aplicado á determinação da concentração daqueles radioisótopos em leite, evitando-se, dessa forma, a eliminação das proteinas por precipitação ácida ou por evaporação do leite e queima do resíduo.

Foram feitas análises de amostras de leite procedentes de vários lugares no Estado de São Paulo, Brasil, apresentando-se os resultados correspondentes. Os valores encontrados estão muito abaixo da concentração máxima permissível.

RESUMÉ

On decrit une méthode pour déterminer Sr^{90}-Y^{90} avec l'acide etilenediaminetetraacetique (EDTA) et les resines echangeurs d'ions. On a utilisé cette methode pour la determination de la concentration du Sr^{90} - Y^{90} dans le lait. Ainsin on évite l'elimination des proteines par precipitation acide ou même par la evaporation du lait et brûlement du résidu.

On a analysé des échantillons de lait provenants de differentes parties de l'État de S.Paulo, Brasil et les resultats sont rapportés. Les valeurs trouvés sont inferieurs à la concentration maxime permissible.

SUMMARY

A method in which the complex of ethylenediaminetetraacetic acid (EDTA), with yttrium, used in conjunction with ion-exchangers for determination of $Sr^{90} - Y^{90}$ is described. The method was applied to the determination of concentration of $Sr^{90} - Y^{90}$ in milk, avoiding, in this way, protein elimination by acid precipitation or by evaporation of milk and ashing the residue.

Analysis of samples of milk from various places in the State of S.Paulo, Brasil, were carried out and the results are reported. Values found are much lower than maximum permissible concentration.

INTRODUCTION

Determination of Sr^{90} in milk is usually carried out by lengthy procedures consisting of an initial protein separation, the protein being usually eliminated by acid precipitation, or by evaporation of milk and ashing of the residue. These methods are effective but are space and time consuming and search for simpler procedures is justifiable. Porter et al(1) developed a procedure in which yttrium citrate carrier is added to the milk sample and a volume of about one liter of milk is passed through a cationic ion-exchanger and next through an anionic exchanger. Yttrium is retained in the anion exchanger, eluted, precipitated as oxalate, and counted. The total yield of the procedure, for the recovery of yttrium, is reported as being of 55%.

The final counting of the separated Y^{90}, in low-background counting systems, is usually of the order of 2 to 6 counts per minute for one liter samples of milk. Procedures that would give higher recovery for yttrium are always desirable in order to give samples with higher activities for the final counting determination.

The method described in this paper is based on the fact that yttrium-EDTA complex is much more stable than the yttrium-citrate complex. Citrate-ions and proteins are both major components of milk and yttrium would probably exist as the anionic complexes of those milk constituents. The retention of milk by the anionic ion-exchanger, on the process developed by Porter et al(1), is due to the existence of those complexes at the normal milk pH of 6.6 to 7.0, in accordance with the mentioned authors.

If yttrium-EDTA complex is added as carrier to the milk samples, a higher recovery than 55% will be obtained. For samples with very low content of Sr^{90}-Y^{90}, a higher recovery of Y^{90} will allow the determination of Sr^{90} in milk with a higher precision.

In order to verify the method, samples of milk were purposely contaminated with Y^{90} and/or Sr^{90}, at various activity levels, and the separation procedure described by Porter et al(1), as well as the one described in this paper, were applied. A Y^{90} recovery of 55% was observed for the first method, in accordance with the value reported by Porter, and of 85% for the second.

Biological trace results are sometimes open to doubt because of possible differences in chemical form between radionuclides existing in the cow's milk and those purposely added to it afterwards. In order to decide if the tracer experiments mentioned above were valid, samples of milk were analysed by the procedure described in this paper, as well as by standard ashing procedures(2). From Tables I and II it is seen that results are in agreement, within experimental errors. Precision can be evaluated from the data presented in Table III in which duplicate and triplicate set of analysis, for various samples, are recorded.

TABLE -I-

Results of analysis of milk samples to which known amounts of Y^{90} were added.

Sample*	Activity added (cpm/ml)	Activity recovered (cpm/ml)	% Recovered
1	8671	7346	84.6
2	12055	10225	84.8
3	33500	28358	84.6
4	49480	25368	51.3

*Samples 1, 2 and 3 processed in accordance with method described in this work; sample 4, method described in ref. (1).

TABLE -II-

Results of analysis of samples of milk for Sr^{90}.

Sample	Type of milk	Present work	Ashing*
1	Powder, whole	6.1 pc/100g	6.1 pc/100g
2	Powder, skim	3.5 pc/100g	3.2 pc/100g
3	Fresh, whole	9.3 pc/l	9.6 pc/l

*Process described in ref. (2).

TABLE -III-

Results to evaluate precision of analysis carried out by the method proposed in this paper.

Sample	Sr^{90} pc/1
1	8.3 ± 0.1 8.2 ± 0.1
2	9.3 ± 0.6 10.1 ± 0.6
3	6.6 ± 0.9 7.9 ± 0.9
4	10.9 ± 0.1 10.8 ± 0.1
5*	6.1 ± 0.4 6.3 ± 0.4 5.6 ± 0.4

* Powder milk, pc/100g

METHOD

Materials and Reagents

Glass column, with syphon, 5 cm diameter and 25 cm length (cationic exchanger).

Glass column, with syphon, 3 cm diameter and 18 cm length (anionic exchanger). Carrier solution of Y-EDTA. Dissolve 5.75 g of EDTA in concentrated ammonium-hydroxide. Add 15 ml from a solution of yttrium nitrate with concentration of 10 mg of yttrium per ml. Add ammonium hydroxide to adjust pH to 7 (universal indicator paper) and make to volume in a 50 ml volumetric flask.

Cation ion-exchanger, Amberlite IR-120, 30 to 50 meshs.

Anionic ion-exchanger, Amberlite IR-401, 30 to 50 meshs.

Prepare the 5 cm diameter column with 140 ml of cation exchanger. Put the exchanger in the sodium form by passing 1,000 ml of a 4 M sodium chloride-solution at 5 ml per minute. Wash the excess of sodium chloride with deionized water. The exchanger regenerated, after each analysis, with 500 ml of 4 M sodium chloride solution.

Prepare the 3 cm diameter column with 30 ml of anion exchanger. Put the exchanger in the chloride form by passing 400 ml of a 2 M hydrochloric acid solution. Wash with deionized water until no more acid indication on the universal indicator paper is shown. After each analysis regenerate the exchanger with 200 ml of a 2 M hydrochloric acid solution.

METHOD

To a one liter sample of milk 3 ml of formaldehyde at 40% and 2 ml of carrier solution of Y-EDTA, are added. The sample is stored under refrigeration at about 10ºC, for 15 days, in order that secular equilibrium Sr^{90} - Y^{90} may practically be attained.

The sample is transferred to a separatory funnel and passed through the cationic and next the anionic exchangers columns at about 5 ml per minute. The flow is stopped when milk surface is at about 2 mm of the cationic exchanger. 200 ml of deionized water is next passed through the columns in order to remove the milk from the resin interstices. Next, warm water is passed until the resin is completely washed out of milk and protein.

The anionic exchanger column is then eluted with 250 ml of 2 M hydrochloride acid solution at the rate of 1 ml per minute. The first portion of the eluate is disposed off. When the pH of the eluate reaches a value of 4 the next 100 ml of eluted fraction are collected. Thymol blue indicator is added, next concentrated ammonium hydroxide solution, up to yellow-rose colour of indicator, followed by 10 ml of a one molar solution of oxalic acid. The solution is stirred for two minutes. Ammonium hydroxide solution is again added until yellow- rose colour of the indicator and the precipitate is left to settle in a refrigerator for about one hour.

The precipitate is centrifuged, washed with 10 ml of hot water and centrifuged again. The precipitate is dissolved with 1 ml of hydrochloric-acid 6 M and 15 ml of hot water. If any insoluble material remains it is separated by centrifuging.

In order to precipitate the yttrium oxalate the solution is first treated with concentrated ammonium hydroxide up to yellow-rose colour of thymol blue indicator. 10 ml of oxalic acid one molar is added and the solution is stirred for about two minutes and a new portion of ammonium hydroxide is added until yellow-rose colour of the indicator. The precipitate is left standing for about one hour and then centrifuged. The precipitate is transferred, with hot water, to a chimney type sintered glass filter and mounted for counting.

The yttrium oxalate sample is counted in a low background counting system and a decay line is drawn to check purity by half-life determination. Zero time for the separation of Y^{90} and Sr^{90} is taken as half the time for the one liter sample to percolate through the ion exchangers columns.

RESULTS AND DISCUSSION

Table IV summarizes the results obtained for samples of milk from various places in the State of São Paulo, Brazil.

Amounts of Sr^{90} for milk from places 3, 4 and 5 are lower than for the ones from places 1 and 2. Milk from 3, 4 and 5 are from cows living, part of the time, in stables; cows from places 1 and 2 are kept in open field most of the time, although they have their food suplemented by an ammount of sugar beet cane and bean straw, when green pasture becomes scanty. Animals from places 3, 4 and 5 are fed with corn and cottonseed cakes, rice, and, partly, natural green pasture(3).

It has been shown by Tukey et al (4), Russel et al(5)

TABLE -IV-

Sr.^{90} in milk samples from various places in the State of São Paulo, Brazil
(August 1962 to February 1963) (picocuries/liter).

Place	August		September		October		November		December		January		February	
	Day	pc/l	Day	pc/l	Day	pc/l	Day	pc/l	Day	pc/l	Day	pc/l	Day	pc/l
1	3	9.5	4	9.3	9	9.8	16	9.6						
	6	6.9	20	9.3	9	9.7								
	10	9.5	20	10.1	17	10.9								
	14	8.7	28	6.6	17	10.8								
	24	9.8	28	7.9										
	27	7.4												
	31	8.3												
	31	8.2												
2							27	9.6	17	10.0	8	10.0	5	10.9
									26	13.4	15	9.5		
											23	9.7		
											31	11.4		
3							27	2.6	17	3.4	8	2.7	1	2.1
									26	2.7	15	2.3	5	2.4
											23	1.5	6	2.4
4							27	6.6	17	5.1	8	2.8		
									26	3.4	15	3.8		
											23	4.3		
5							28	2.9	17	3.3	8	2.9	5	1.8
									26	4.0	15	2.4		
											23	1.9		
											31	1.9		

Larson et al(6), Nishita et al(7) that Sr^{90} will not move within the plant. The contamination is a consequence of a direct absorption of strontium deposited on the leaves and other exposed parts.

Corn and cottonseed cakes as well as rice rations are prepared from parts of the plants which are protected from direct exposure to fallout particles. This would give a lower concentration of Sr^{90} in seeds and in cotton and corn cakes made up of them, such as the ones given to cows kept in stables (places 3, 4 and 5).

Cows from places 1 and 2 are fed, mainly, by green pasture, and also by sugar beet cane and bean straw. It is thus seen that their feeding habits are such that a prominent part of the food is composed of external or exposed parts of the plant. This might be the cause for a higher amount of Sr^{90} in their milk.

Maximum permissible concentration of Sr^{90} in milk has been reported as 80 picocuries per liter, Campbell (9), quoted in Tentoni and Morisi(8). Concentration of that radioisotope in milk from places reported in Table IV are much lower than that value.

Milk samples listed on Table III have also been analysed for calcium content. The concentration of calcium is the same, within experimental errors, for milk from places 1, 2, 3, 4 and 5, meaning 1.3 grams per liter of milk. No correlation seems to exist for the calcium content and Sr^{90} in the samples mentioned. The same lack of correlation may also be noticed in the data published by Campbell et al$^{(10)}$, for whose milk samples Sr^{90} varied from 1.0 to 37.3 picocuries per liter and calcium from 1.01 to 1.33 grams per liter. It seems that the concentration of calcium is so much higher as compared to the very small concentration of the alkaline earth radioisotope Sr^{90}, that the small variation of calcium concentration found in milk will not affect the concentration of Sr^{90}.

Concerning the chemical processing of samples by using ion-exchangers, the method is much more simpler than the ashing procedures and can be carried out in shorter periods of time.

The higher stability constant of yttrium - EDTA , as compared to the complex yttrium-citric acid, makes the use of that complexing agent preferable to citric acid, as shown by the higher yield of 85%, as presented in this paper, compared to the yield of 55% obtained by using citric acid.

Stability of calcium and strontium ions with EDTA at the pH values at which the milk samples are percolated through the ion-exchangers, i.e., at pH values of about 6.5 , is not high. At this pH value yttrium complexes very well with EDTA. In this way, by working with the milk samples at the pH conditions described, the formation of the anionic species Y-EDTA will be enhanced and the corresponding ones for the alkaline earths, Ca-EDTA and Sr-EDTA, will be diminished. Consequently, calcium and strontium will be retained efficiently by the cationic exchanger while the anionic Y-EDTA complex will pass through and be retained by the anionic exchanger.

TABLE -V-

Stability constants for citric acid and EDTA complexes, Chaberek and Marteel(11).

	log K
Ca Cit^{-}	3.22
Sr Cit^{-}	2.7
Y Cit	3.6
Ca EDTA^{--}	10.59
Sr EDTA^{--}	8.63
Y EDTA^{-}	18.09

From Table V it is seen that log K values for Y-citric acid is 3.6, (while for Y-EDTA it is equal to 18.09); for Ca-citric acid it is equal to 3.22 and for Sr-citric acid,2.7. These figures show that the M-citric acid complex constants are lower than the M-EDTA constants and, consequently , the citric acid complexes would be more easily broken by the cationic exchanger than the EDTA complexes, specially in the range of pH at which milk is passed through the exchangers . This would result in a lower amount of yttrium absorbed , as negative complex, into the anionic exchanger as compared to the amount of yttrium complexed with EDTA for which the stability constant is very high. Moreover, the stability constants for Y-citric acid and Sr-citric acid are more closer to each other (3.6 and 2.7, with a quotient equal to 1.33) than the ones for Y-EDTA and Sr-EDTA (18.09 and 8.63, with a quotient equal to 2.1). This would result in a much larger possibility of yttrium being contaminated by strontium in the anionic exchanger, when using citric acid, as complexing agent, than when using EDTA.

One could not expect that in a complex solution such as milk, considerations based purely on stability constants of yttrium and strontium with citric acid or EDTA would permit to foresee theoretically the exact behaviour of the complexes and corresponding ions. However, the basic facts, as considered above, prevail as shown by the experiments.

As conclusion it can be said that the method presented in this paper avoids the cumbersome elimination of proteins by acid treatment or by concentrating and ashing the sample. The higher yield for Y^{90} recovery, when using EDTA, as described, makes this complexing agent preferable to citric acid. Also, radiochemical purity of separated Y^{90} is better when using EDTA. A complete chemical processing by the method presently described takes only about eight hours with various samples being processed at same time by one single analyst.

Contamination of the separated Y^{90} by other radioisotopes of rare earths, from fissiom products, was not observed . Rare earth elements, and of this only the long half-lives radioisotopes should be considered, are not absorbed from the gut and also are very poorly taken up by plants(12). Determination of the half-life of the separated Y^{90}, from milk, showed that contamination by other fission product radioisotopes did not occur.

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