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WHOLE BLOOD SOLUBILIZATION AND DISCOLORATION BEFORE LSC OF YTRIUM SAMPLES

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ABSTRACT

Liquid scintillation counting of whole blood and animal tissues samples could be severely impaired owing to quenching by their compounds. The objective of this previous study is preparing one protocol of ^{90}Y measurement to apply in biodistribution and dosimetry studies of radiopharmaceuticals labeled with this isotope and other beta emitters in *in vivo* and *ex vivo* samples. The first parameters considered to choose a method were: the largest blood sample per collection (80µl-90µl), attending the collection limit of less than 7.5% of total circulating blood volume for *in vivo* samples. Other parameters were the use of EDTA and Cyclohexydine as solubilization and lytic agents, HNO_3 and H_2O_2 as mineralizing agents and NH_4OH as neutralization agent. One samples batch was tested in a water bath under the lower temperature to prevent the volume lose of the ionic phase. Other samples batch was mineralized over a hot-plate at 120^0C following the currently largest sample amounts processing procedure in our laboratory by using HNO_3 and H_2O_2. Results show the contribution of the blood fragments as quenching in the region A (<500keV) only for the first batch samples. The mineralized samples did not present significant quenching in any region of the spectrum showing lowest interference due the use of HNO_3 and/or NH_4OH in the hot-plate digestion. As expected, the measurements in the three spectral regions show the proteins and colored fragments were completely removed by the hot-plate digestion. The rate between efficiency and ^{90}Sr-^{90}Y concentration had not significant differences in the range between 120Bq and 1200Bq.

1. INTRODUCTION

Liquid scintillation counting of whole blood and animal tissues samples could be severely impaired owing to quenching by their compounds. Samples from small animals such rats and mice usually presents problems due to the limited volume of each collection. Suggested sample preparation methods include solubilization and sample combustion, although the chemicals used in the pretreatment could, in the most part of the procedures, increase the quenching effects.

The objective of this previous study is preparing one protocol of ^{90}Y measurement to apply in biodistribution and dosimetry studies of radiopharmaceuticals labeled with this isotope and other beta emitters in *in vivo* and *ex vivo* samples.

IPEN radiopharmaceuticals research and quality control have been used only mice and rats.

Muride sample blood collection is based on type of experiment and total circulating volume in the laboratory animals. Currently ATLA associations recommended to small rodents two sites for venepuncture to repeated sample collection to mouse:

submandibular vein and retrobulbar plexus and one site to rat; lateral tarsal vein. The collection amount is limited to 7.5% of total circulating blood volume for *in vivo* samples. The largest blood samples collection in 24h are 80µl for mice and 150µl for rats [1].

Additionally, the high activities present in these small volumes, in the order of 200kBq, demand fractionating of the most active samples to attempt LSC analyzer equipment operation range.

Once the methods suggested by the equipment and chemical suppliers need patented products and are very specific to lower energy Beta emitters like ^{3}H and ^{14}C, ó*in-house*ô traditional digestion methods seemed more economic. [2, 3]

Consequently, several procedures of whole blood preparation were examined on the basis of the blood sample amounts, time expended on the samples discoloration, quenching influence on the counting efficiency. [2, 3, 4]

2. METODOLOGY

The study was developed on basis of the planned runs described in the Table 1. The parameters were compared with no treated blood samples and water samples.

Mouse blood was diluted to 1:10 in EDTA 1% and preserved under 5°C in amber glass bottles. Triplicate aliquots, corresponding to original blood volumes (20µl, 40µl, 50 µl and 80 µl) added with 200µl of Cyclohexidrine (10%) as described in Table 1, were taken to test each condition of solubilization and discoloration treatment.

One first samples batch was tested in a water bath (WB) under the lower temperature recommended in the literature (40°C) to prevent the volume lose of the ionic phase mixed in the total volume of the counting vial.

The second samples batch was mineralized in a 5 ml beaker over a hot-plate (HP) at 120°C following ó*in-house*ô largest sample amounts, dropping concentrated HNO_3 and H_2O_2 (30%) until discoloring. After dryness, the residual fraction was transferred to vials with distillated water.

The third batch was a group of no treated blood samples.

Nitric Acid and Hydrogen Peroxide used in biological sample mineralization are seeing as quenching agents due their oxidation capacity, thus, after reduced the samples until the dryness, the neutralization with Ammonium Hydroxide (2.5%) were also tested.

Measurements are performed in a Tri-Carb Liquid Scintillation Analyzer 2100TR Packard in three energy spectral regions (A: 0-2000keV, B: 0-750keV and C: 750-1150keV), during 20 min, considering the average beta energy of ^{90}Sr and ^{90}Y. Data was registered in the Spectral Index Sample (SIS), Transformed Spectral Index (tSIE) and 2s% modes to allow comparative studies about equipment and sample performances. [5, 6]

Table 1. Blood samples treatment runs to Yttrium measurement.

Treatment	Sample	Blood (µl)	^{90}Y-^{90}Sr Standart (Bq)	Cyclohexidrine (200µl)	EDTA (qsp)	H_2O_2	HNO_3 (qsp)	Water (ml)	NH_4OH (10%) (ml)
(HP) Hot-plate (130ºC/10min)	1	N	N	Y	Y	N	N	2	N
	2	N	N	Y	Y	Y	Y	2	N
	3	20	200	Y	Y	Y	Y	1	N
	4	20	200	Y	Y	Y	Y	0.5	0.5
	5	20	200	Y	Y	Y	Y	N	1
(WB) Water bath (40ºC/1-4h)	6	N	200	N	Y	Y	N	2	N
	7	30	200	Y	Y	Y	N	N	N
	8	40	200	Y	Y	Y	N	N	N
	9	50	200	Y	Y	Y	N	N	N
	10	80	200	Y	Y	Y	N	N	N
	11	N	N	Y	Y	Y	N	N	N
(NO TT) Direct Measurement	12	20	100	Y	Y	Y	N	N	N
	13	20	200	Y	Y	Y	N	N	N
	14	20	400	Y	Y	Y	N	N	N
	15	20	600	Y	Y	Y	N	N	N
	16	20	800	Y	Y	Y	N	N	N
	17	20	1000	Y	Y	Y	N	N	N

Y Yes N NO
Time 20min LSC Cocktail 18ml Total volume 20ml
Region A: 0-2000 Region B: 750-1150 Region C: 0-7500 QIP=tSIE

The quenching influence was analyzed considering the effects over the counting efficiency, spectral changes in position and shape, chemiluminescence contribution and sample/cocktail (Optiphase Hisafe 3 Perking Elmer) compatibility and stability.

Linearity of results in function of activity was performed only using the same proportion between the analites (1:1), since the samples were spiked with a ^{90}Sr-^{90}Y standard in radioactive equilibrium supplied by Instituto de Radioproteção (IRD).

3. RESULTS AND DISCUSSION

Table 2 shows the average LSC results from three measurements obtained with for each experimental condition summarized in the Table 1 planning.

Table 2. LSC data from blood samples treatment runs to Yttrium measurement.

Sample	CPMA	A: 2%s	CPMB	B2%s	CPMC	C:2%s	SIS	tSIE
1	188	3	2	29	180	3	164	371
2	158	3	3	25	148	4	225	362
3	15345	0	335	2	15005	0	649	371
4	15345	0	330	2	15010	0	650	370
5	15303	0	293	3	15005	0	635	365
6	15483	0	330	2	15148	0	649	367
7	15483	0	330	2	15148	0	649	367
8	15253	0	314	3	14926	0	647	366
9	15253	0	314	3	14926	0	647	366
10	15162	0	293	4	14864	0	635	364
11	74	5	2	29	66	6	440	381
12	14823	0	172	3	14648	0	598	343
13	15083	0	278	3	14800	0	636	362
14	14753	0	495	2	14254	0	684	395
15	15215	0	670	2	14541	0	720	419
16	14753	0	495	2	14254	0	684	395
17	15215	0	670	2	14541	0	720	419

The plotted results presented below were interpreted on basis of spectrum Region B.

The relative quenching influence on counting efficiency due ^{90}Sr-^{90}Y activity addition and treatment method presented in Figures 1-3 was interpreted considering water sample without blood addition measurement as 100%.

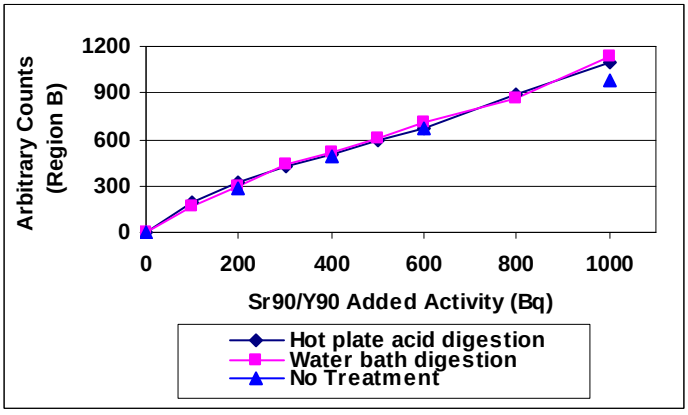

Figure 1. Quenching influence on counting efficiency due added activity and sample treatment method.

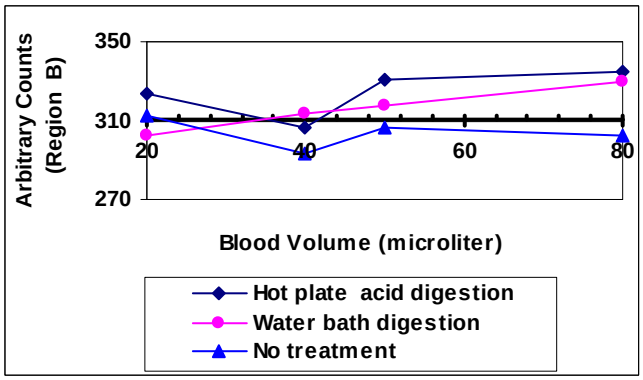

Figure 2. Quenching influence on relative counting efficiency due sample blood volume.

Correlation quenching curves growing using SIS and tSIS modes are presented in the Figure 3.

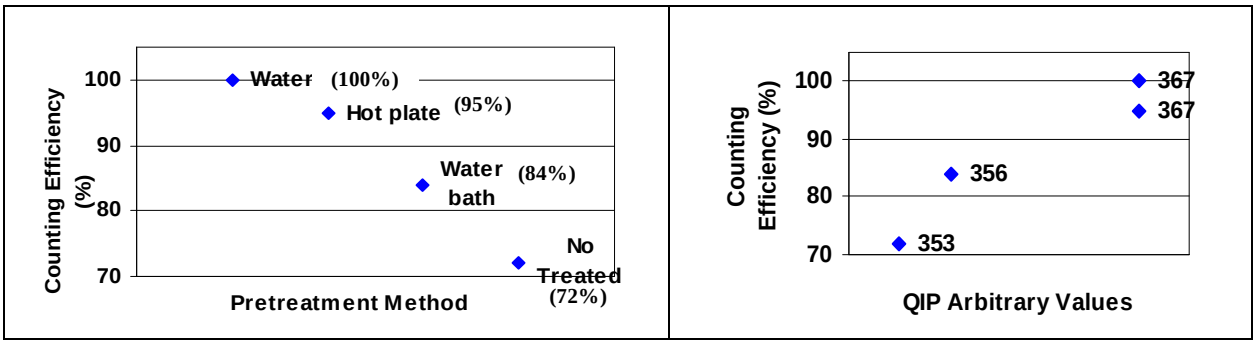

Figure 3. Sample treatment method and quenching influence on relative counting efficiency.

The average rate SIS/tSIE was (1.76±0.02) except to not spiked blood samples, (1.21±0.07) for HP, (1.15±0.12) for WB and (0.55±0.18) for NO TT since low count number causes statistical malfunctions.

The time to discoloring blood samples in water bath was proportional to total volume. A 80µl sample wasted over 4 h in the water bath until foaming and color disappearance.

Calculated correlation between the three spectrum regions (A, B and C) showed average rates of (0.017±0.041) to (B/A) and (0.018±0.041) to (B/C) shown that no significant color quench effect in spectra dislocation.

4. CONCLUSIONS

No treated samples presented quenching interference not only related with the colorless but probably with the not digested blood proteins.

Results show the contribution of the blood fragments as quenching in the region A (<500keV) only for the first batch samples (WB) without significant interference in the region B. The mineralized samples batch (HP) did not present significant quenching in any region of the spectrum showing lowest interference due the use of little amounts of HNO_3 and/or NH_4OH in the hot-plate digestion.

As expected, the measurements in the three spectral regions show the proteins and colored fragments were completely removed by the hot-plate digestion. Additionally, the rate between efficiency and ^{90}Sr-^{90}Y concentration had not significant differences in the range between 120Bq and 1200Bq.

Considering recovery and promptness, the hot-plate digestion was the best option between the three methods presented in this work and will be validate to upcoming studies.

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