2007 International Nuclear Atlantic Conference - INAC 2007
Santos, SP, Brazil, September 30 to October 5, 2007
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 978-85-99141-02-1

SYNTHESIS AND CHARACTERIZATION OF PHOSPHATE GLASS MICROSPHERES FOR RADIOTHERAPY APPLICATIONS

Frank F. Sene[1], José R. Martinelli[1], and Emico Okuno[2]

[1] Instituto de Pesquisas Energéticas e Nucleares (IPEN / CNEN - SP)
Av. Professor Lineu Prestes 2242
05508-000 São Paulo, SP
ffsene@ipen.br
jroberto@ipen.br

[2] Instituto de Física da Universidade de São Paulo
Rua do Matão, trav. R 187
05508-900 São Paulo, SP
emico.okuno@dfn.if.usp.br

ABSTRACT

Glass microspheres containing radioisotopes ^{90}Y or ^{32}P can be used for cancer radiotherapy. ^{90}Y and ^{32}P emit β^- particles with short penetration range of about 2.5 mm in living tissue with the half-life of respectively 2.7 days and 14.3 days. Glass microspheres containing such radioisotopes with suitable activity can be easily introduced in specific human organs as liver, stomach, pancreas, and uterus to kill cancer cells, reducing significantly damages to other health tissues allowing a major therapeutic effect. The use of Y_2O_3-Al_2O_3-SiO_2 glass microspheres for cancer therapy is an efficient alternative compared to conventional chemotherapy treatments. However, the activity of the glass microspheres with ^{90}Y is relatively low, due to the limitation of content of Y_2O_3 to only 17 mol %, and short half-life, in a way that ^{90}Y may decay substantially even before the treatment. A potential alternative is to produce microspheres with high content of ^{31}P that could be transmuted to ^{32}P with higher activity and longer half-life than ^{90}Y. In the present work phosphate glass microspheres were produced with different compositions and particle size distribution in the range of 20 μm to 30 μm by using two different thermal processes to spherodize glass particles originally with irregular shapes. Samples were characterized by Differential Thermal Analysis, X-rays diffraction, and Energy Dispersive X-rays Fluorescence Spectroscopy. The glass dissolution rate in water was determined at 90°C, and in Simulated Body Fluid (SBF) at 37°C. Glasses with dissolution rates close to 10^{-5} g/(cm^2.d) were obtained, which make them suitable for the present application. Scanning Electron Microscopy was used to evaluate the shape of the microspheres before and after the dissolution tests. The cytotoxicity tests showed that these microspheres can be used for biological applications.

1 - Introduction

Radiotherapy is an alternative way for cancer treatment [1]. Brachytherapy is a technique that introduces the radioactive source directly in the human body, permanently or temporally [2]. A promising brachytherapy technique uses glass microspheres to carry the radioactive source. The spherical shape and the chemical resistance of the glass particles make them a promising material for liver cancer treatment because it reduces the damage of

ISBN: 978-85-99141-02-1
healthy tissues caused by sharp edges in the glass particles, avoiding unnecessary hemorrhage [3].

Several types of cancers are difficult to be treated, and liver cancer is one of them. Generally it is terminal, and after being diagnosed the patient's expected life time is only few months. Surgery is an option seldom used due to the high probability of causing metastases. Chemotherapy might offer a temporary release however it is often suspended before killing all the malign cells and consequently aggravating the clinic condition of the patient. On the other hand, radiotherapy treatments that use external radiation sources such as teletherapy, do excessive harm to the neighboring healthy tissues. The dose distribution applied in multiple plans and steps aims to minimize the side effects related to the irradiation; however, it is still not enough to precisely restrict the irradiated area to the cancer tissues without affecting a large area of healthy tissues. Usually this treatment requires an average of 10 irradiations in a period of 30 days with doses of approximately 2,500 rads. On the other hand, if the radiation source is localized as in the brachytherapy, doses of up to 15,000 rads can be used in a single step, being enough to kill cancer cells without damaging significantly the healthy neighboring tissues [3-5]. The use of β^- particles is an additional advantage over the γ rays, because the short penetration range of these particles allows an effective energy absorption in the restricted area of the tumor to be treated. Glass microspheres of $17Y_2O_3$-$19Al_2O_3$-$64SiO_2$(mol%) with sizes in the range of 20μm to 40μm have been reported to be used in cancer brachytherapy treatments [3-5]. The isotope ^{89}Y is transmuted to ^{90}Y by neutron activation resulting in a β^- emitter with half-life of 64.1h [6]. Other elements that make part of the glass structure, such as ^{27}Al, and ^{30}Si, are also activated becoming β^- emitters; however the half-life is only 2.25min and 2.62h, respectively [6]. These microspheres are chemically resistant to body fluids and are non-cytotoxic [7]. After the neutron activation, these microspheres are injected in the liver through the hepatic artery, allowing a relative high radiation dose restricted to the cancer tissue area [3-5]. These glasses were submitted to clinical testes for treatment of cancer in liver and kidney, as well for synovectomy [7-15]. Similarly to the isotope ^{89}Y, the isotope ^{31}P can be transmuted to ^{32}P which is β^- emitter with a half-life of 14.3 days and can be more effective to cancer treatment when compared to ^{90}Y. In a previous work [16] P^+ ions were implanted in Y_2O_3-Al_2O_3-SiO_2 glasses showing promising results. However, phosphorus can be a problem in some types of glasses because it usually plays an important role in the nucleation of crystalline phases, as observed for some silicate glasses.

The specific activities for the radionuclides ^{90}Y and ^{32}P are 92.5 GBq/g and 66.6 GBq/g, respectively. The cross sections for neutron absorption reaction, σ_n, for ^{89}Y and ^{31}P are $1.28\times10^{-24}cm^2$ and $0.17\times10^{-24}cm^2$, respectively, making necessary longer radiation times for glass microspheres containing phosphorus when compared to yttrium. However, the amount of phosphorus in the glass microspheres is usually higher when compared to yttrium in the similar microspheres. This fact compensates the lower cross section of P and leads to microspheres with activity suitable for radiotherapic treatments.

Phosphate glasses are relatively easy to be obtained at low temperatures (900 - 1200°C) and have low work temperature. In the present work glass microspheres containing phosphorous were produced by using two different thermal processes described in the next section.

2 - Experimental Procedures

Glasses based on the composition showed in Tables I were prepared by mixing analytical grade $(NH_4)_2HPO_4$, Al_2O_3, MgO, and SiO_2, melting in an electric furnace in the temperature range of 1250°C - 1550°C for 2h, stirring each 30 min with a high purity silica

ISBN: 978-85-99141-02-1
rod to achieve a good homogeneity and fining. This material was ground in stainless steal ball milling and sieved to separate particles in the size range of 20 - 50μm. A 1x1x10mm^3 piece was used for leaching tests in water and Simulated Body Fluid (SBF). The amount of SiO_2 and Al_2O_3 were varied to evaluate the effects on the chemical durability in the final glass composition.

Table I – Glass composition (wt %).

Glass type	P_2O_5	Al_2O_3	SiO_2	MgO
MVP1	60	15	15	10
MVP2	60	10	15	15
MVP3	60	10	10	20
MVP5	60	10	5	25
MVP6	40	5	5	30
MVP8a	45	5	5	45
MVP8b	43	4	10	43
MVP8c	42	4	12	42
MVP8d	41	3	15	41
MVP9a	42	10	4	44
MVP9b	42	12	4	42
MVP9c	41	15	3	41

Glass particles with irregular shapes were transformed to microspheres by using two different processes. In the first process glass microspheres with particle size distribution in the range of 20 - 150μm were obtained by re-melting the irregular particles in a hot flame produced by a torch burning a mixture of oxygen and Petrol Liquefied Gas. The microspheres are collected in a metal cylinder. Although this process, known as "spheronization by flame", has been previously reported [17], the experimental parameters must be determined for each type of glass. The second process consists of introducing glass particles with irregular shapes on the top of a vertical tubular furnace, and let them falling down. This process is named "spheronization by gravitational fall in a tubular furnace".

Samples were characterized by X-rays diffraction (XRD), Differential Thermal Analysis (DTA), and Energy Dispersive X-rays Fluorescence Spectroscopy (EDS) coupled to a Scanning Electron Microscope or by using an EDX (Shimadzu model 720). Scanning electron microscopy was used to observe glass samples before and after being exposed to a Simulated Body Fluid (SBF).

To check the chemical resistance of theses glasses, samples were immersed in a *Pyrex* container with 100 ml of SBF at 37°C during 28 days, or in distilled water at 90^{o}C. The sample's weight and the pH of the aqueous solution were monitored after 1, 3, 7, 14, and 28 days.

Glass samples were tested "in vitro" to evaluate the toxic effects on cells by using a diluted extract of the material in contact with cells of Chinese Hamster Ovarian (CHO), American Type Culture Collection (ATCC) (IS0 10993-5, 1992)[10-11].

3 - Results and discussion

The XRD patterns for microspheres produced with compositions MVP3, MVP8c, and MVP9c do not show any evidence of crystalline phases after the spheronization process. Usually the nucleation of crystalline phases is undesirable because it can cause mechanical stresses and jeopardize the microspheres performance by creating cracks and other defects.

According to the DTA curves, no exothermic peaks could be assigned to a crystallization process. We can then infer that these glasses are very resistant to crystallization.

ISBN: 978-85-99141-02-1

Table II presents the amount of constituent oxides in the MVP3 and MVP9c glasses as a function of the immersion time in SBF at 37°C, determined by EDS. It is noticed that there is not a large variation among these values; therefore, there was not a selective leaching of the elements. These results complement the ones obtained to determine the chemical durability by weight loss. These results can be useful to estimate the activity of microspheres and the absorbed dose. Table III presents the dissolution rate for glasses immersed in SBF at 37°C.

Table II – Surface glass composition after immersion in SBF at 37°C as a function of immersion time: a) MVP3 and b)MVP9c.

a)	**wt %**				**b)**	**wt %**			
t (day)	**P_2O_5**	**SiO_2**	**Al_2O_3**	**MgO**	**t (day)**	**P_2O_5**	**SiO_2**	**Al_2O_3**	**MgO**
0	62.70	9.70	10.08	17.55	**0**	38.12	4.22	17.21	40.39
7	62.97	9.91	10.12	17.70	**7**	38.36	4.28	17.32	40.42
14	63.32	9.92	10.20	17.12	**14**	38.76	4.31	17.41	40.62
21	62.54	9.72	10.04	17.24	**21**	38.14	4.17	17.12	40.36
28	62.52	9.69	10.02	17.18	**28**	38.50	4.29	17.28	40.72

Table III –Dissolution rate (Dr) as a function of immersing time in SBF at 37°C.

	Dr ($g/cm^2.d$)				
t(day)	MVP3	MVP8c	MVP8d	MVP9b	MVP9c
1	$1.2x10^{-7}$	$8.2x10^{-7}$	$9.7x10^{-7}$	$4.6x10^{-7}$	$6.3x10^{-7}$
3	$9.1x10^{-6}$	$8.1x10^{-7}$	$9.2x10^{-7}$	$4.5x10^{-7}$	$6.2x10^{-7}$
7	$8.7x10^{-6}$	$7.8x10^{-7}$	$9.1x10^{-7}$	$4.5x10^{-7}$	$6.1x10^{-7}$
14	$8.6x10^{-6}$	$7.8x10^{-7}$	$8.8x10^{-7}$	$4.4x10^{-7}$	$6.1x10^{-7}$
28	$8.2x10^{-6}$	$7.6x10^{-7}$	$8.9x10^{-7}$	$4.2x10^{-7}$	$5.9x10^{-7}$

All samples show a decreasing dissolution rate as a function of time which is related to the decrease of mass transfer from the glass surface to the medium. This fact might be related to the precipitation of secondary phases on the glass surface or to the saturation of the leaching solution with the corroded elements. In any case, these glasses have a higher chemical durability than commercial window glasses (DR= 10^{-5} g/cm^2 d). It is noticed that the MVP9b composition has the highest corrosion resistance either in water or in SBF.

Figure 1a shows the micrograph of glass particles with irregular shapes used to prepare glass microspheres. Figures 1b, 1c, and 1d show the micrographs of microspheres with composition MVP3 (different magnifications are shown in the figures). Figures 2a and 2b show the micrographs of microspheres with composition MVP9c. In all cases the shapes are regular and very close to spheres. Therefore the procedure to obtain this material is appropriated. These micrographs will be used to determine the average particle size after processing the images.

ISBN: 978-85-99141-02-1

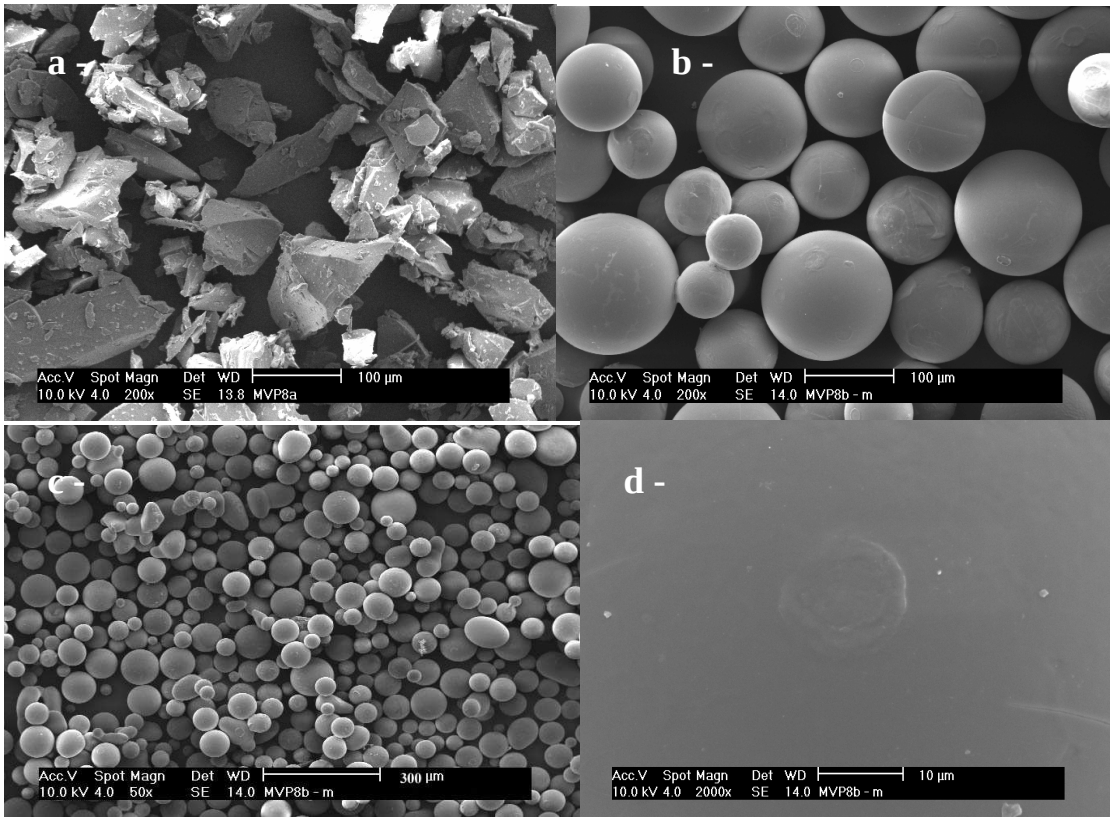

Figure 1 – Scanning Electron Micrographs of: a) glass powders used to obtain microspheres , b), c) and d) microspheres with composition MVP3 (different magnifications).

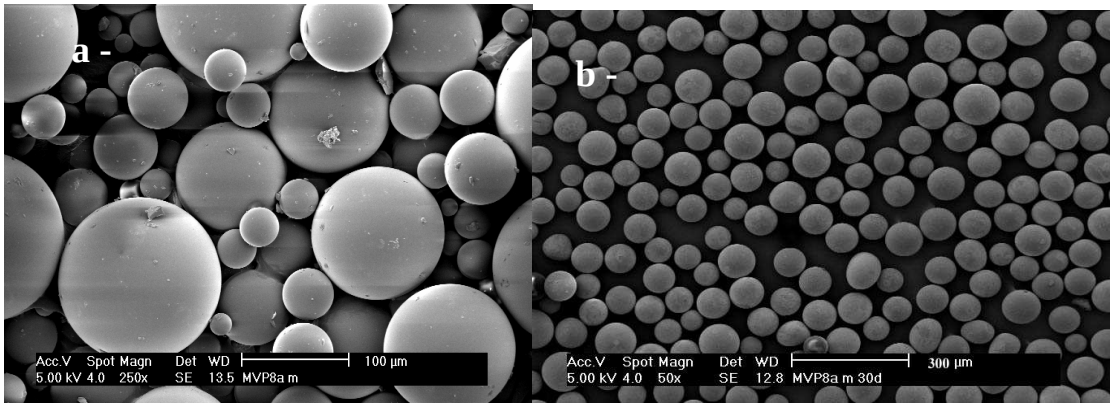

Figure 2 – Scanning Electron Micrographs of microspheres with composition MVP9c (different magnification).

Figures 3a and 3b show the scanning electron micrographs of microspheres with composition MVP3 and MVP9c after withdrawing from a bath containing SBF during 21 days at 37^{o}C, and dried afterwards. Figures 3c and 3d show the same micrographs but with higher magnification. Considering the low corrosion attack of these materials by the SBF, it is assumed that MVP9c microspheres are promising materials for the application of the present work. On the other hand, MVP3 microspheres present some undesirable features. These results are in agreement with the chemical durability tests either in SBF or in distilled water.

ISBN: 978-85-99141-02-1

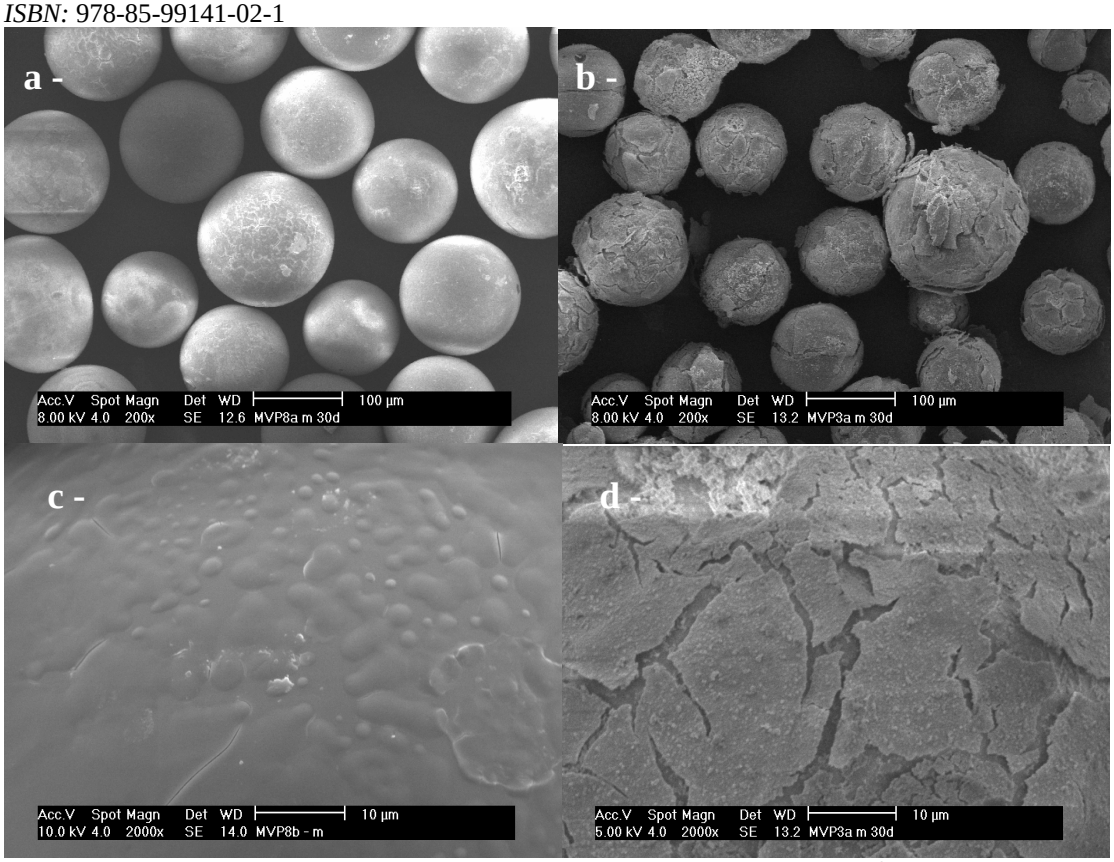

Figure 3 – Microspheres after withdrawing from a bath containing SBF at 37 ^{o}C during 21 days– a) and c) MVP9b; b) and d) MVP3.

Based on the cytotoxicity tests microspheres with composition MVP9a, MVP9b, and MVP9c are suitable for the application aimed at this work, however the MVP3 and MVP8c microspheres are inadequate because they can initiate an inflammatory process and, consequently, the cellular tissue necrosis. The inadequacy of MVP3 might be related to the high dissolution of glass elements, since this material presents a lower chemical durability compared to the MVP9b and MVP9c. The MVP8c glass has a relatively high chemical durability; however the low cellular viability is due to the dissolution of silicon and phosphorus, which, even in low concentrations, can affect the cellular viability. This problem was solved by changing the composition to the MVP9, with higher amounts of Al_2O_3 and consequently the decrease of the dissolution rates of all glass constituents.

Figure 4 presents the results of the cytotoxicity tests performed by the method of neutral red incorporation.

4 – Conclusions

Glass microspheres with different compositions and resistant to crystallization were obtained with size and shapes appropriated to brachytherapy applications. The composition named MVP9 presents the best chemical durability and cellular viability. The glass composition named MVP9b was considered the most promising material to produce microspheres because it has the best chemical durability and resistance to crystallization after annealing to eliminate the residual stresses.

The loss of phosphorus during the glass processing does not affect the aim of the present work because the remaining amount is enough to achieve the required activity for radiotherapy applications.

ISBN: 978-85-99141-02-1

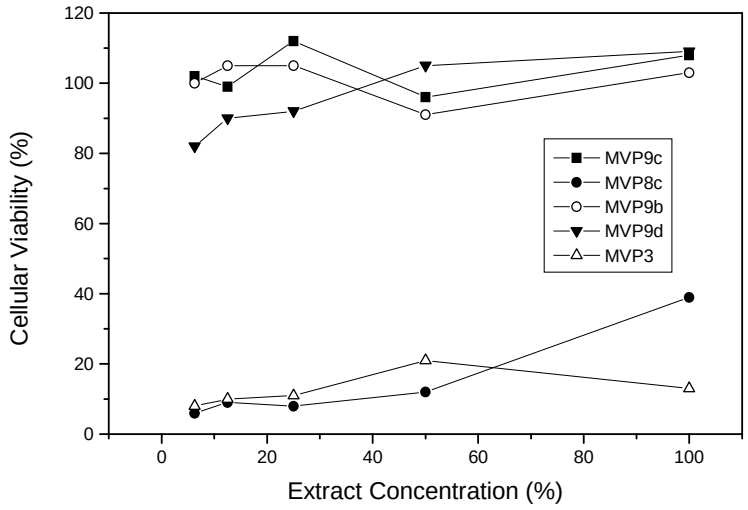

Figure 4 – Cellular viability of glass samples as a function of the extract concentration

References

[1] G.J. Ehrhardt, D.E. Day, Nucl. Med. Bio. 14 (1987) 233.
[2] Stribley K.V. et al: Internal radiotherapy for hepatic metastases II: The blood supply to hepatic metastases, J. Surg. Res. 34, (1983) 25.
[3] G. J. Ehrhardt, D. E. Day, Nucl. Med. Biol. 14 (1987) 233.
[4] M. J. Hyatt, D. E. Day, J .Am. Ceram. Soc. 70 (1987) 283.
[5] E. M. Erbe, D. E. Day, J. Biomed. Materials Res. 27 (1993) 1301.
[6] R. C. Weast, D. R. Lide, M. J. Astle, W. H. Beyer, CRC Handbook of Chemistry and Physics, in: CRC, Boca Raton FL, B-28 (1989) 231.
[7] R.V. P. Mantravadi, D. G. Spignos, W. S. Tan, E. L. Felix, Radiology 142 (1982) 783.
[8] M. J. Herba, F. F. Illescas, M. P.Thirlwell, L. Rosenthall, Smith, Radiology 169 (1988) 311.
[9] M. J. Herba, F. F. Illescas, M. P.Smith, Cancer 61 (1983) 1336.
[10] S. Houle. T. K. Yip, F. A. Shepherd, L . E. Rotstein, K. W. Sniderman, E. Theis, R. H. Cawthorn, K., Radiology 172 (1989) 857.
[11] J. H. Anderson, J. A. Goldberg, R. G. Bessent, D. J. Kerr, J .H. Mckllop, I. Stewart, T. G. Cooks, C. S. Meardle, Radiotherapy Oncol, 25 (1992) 137.
[12] M. A. Burton, B. N. Gray, C. Jones, A. Coletti, Nucl. Med. Biol. 16 (1992) 495.
[13] F. A. Shepherd, L .E. Lotstein, S. Houle, T. K. Yip, K. Paul, K. W. Sniderman, Cancer 70 (1992) 2250.
[14] D. E. Day, T. E. Day, An Introduction to Bioceramics, in: L. L. Hench, J. Wilson (Eds.), World Sconce, Singapore, 1993, p.305.
[15] J. C. Andrews, S. C. Walker, R. J. Ackermann, L. A. Cotton, W. D. Newsmonger, B. Shapiro, J. Nucl. Med. 35 (1994) 1637.
[16] M. Kawashita, F. Miyaji, T. Kokubo, G. Takaoka, I. Yamada, Y. Suzuki, M. Ionue, J. Non-Cryst. Solids, 255 (1999) 140.
[17] Huang W. et al: The Preparation and application of the micro-spheres containing Yttrium-90, J. Shanghai Inst. of Bldg. Mat. 4 (1992) 347.

2007 International Nuclear Atlantic Conference - INAC 2007
Santos, SP, Brazil, September 30 to October 5, 2007
ASSOCIAÇÃO BRASILEIRA DE ENERGIA NUCLEAR - ABEN
ISBN: 978-85-99141-02-1

Acknowledgements

The authors acknowledge CNPq for granting a pos-doc scholarship and for the financial support (Project # 471712/2004-3), and Fapesp (Project # 05/53241-9). The authors are indebt with Dr. Sizue Rogero for the cytotoxicity tests, Mr. Reinaldo Aparecido da Costa (IPEN-CCN) for the DTA analyses, and Ms. Sandra M. Cunha (CCTM-IPEN) for the FTIR analyses.