Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Nuclear Instruments and Methods in Physics Research B 236 (2005) 521–525
NIM B
Beam Interactions with Materials & Atoms
www.elsevier.com/locate/nimb

Silicone crosslinked by ionizing radiation as potential polymeric matrix for drug delivery

Sizue O. Rogero [a,*], José S. Sousa [a], Dante Alário Jr. [b], Lilian Lopérgolo [b], Ademar B. Lugão [a]

[a] *Chemistry and Environmental Centre, Nuclear Energy Research Institute, IPEN, Avenida Prof Lineu Prestes, 2242, Cep 05508-900, Cidade Universitária, São Paulo, SP, Brasil*
[b] *Biolab Sanus Farmacêutica Ltda, Brasil*

Available online 23 May 2005

Abstract

This work describes the use of a catalysis-free system for crosslinking of silicone. Biomedical Grade silicone was crosslinked by ionizing radiation and the physico-chemical and biocompatibility properties of the resulting material were evaluated. High gel content (>90%) was obtained at the irradiation dose of 25 kGy, as indicated by gel fraction measurements. Swelling measurements showed a trend towards stabilization of crosslinking at 75 kGy. DMTA measurements showed that crystallization was impaired by the crosslinking reaction. The in vitro cytotoxicity data showed that radiation-induced crosslinking and degradation did not promote any toxicity in irradiated silicone.

Keywords: Silicone; DDS; Radiation-crosslinked silicone; Charlesby–Rosiak

1. Introduction

Silicone is an important biomaterial used in prosthetic devices as implant mostly for breast enlargement, for tissue augmentation and for treatment of hydrocephalus [1].

Within the field of polymeric biomaterials, great attention has been given to the study of polymeric matrixes for drug delivery. Silicone is a good candidate for use in monolithic devices where the active agent is dispersed in a polymer matrix and its release is controlled by diffusion from the matrix [2]. Silicone presents interesting properties and biocompatibility. Its use as matrix in drug delivery systems, however, requires further investigation, especially because of the presence of olygomers

* Corresponding author. Tel.: +55 11 3816 9341; fax: +55 11 3816 9325.
E-mail address: sorogero@ipen.br (S.O. Rogero).

0168-583X/$ - see front matter
doi:10.1016/j.nimb.2005.04.031

and catalysis residues during conventional silicone crosslinking, which are cause for concern.

Silicone is an important elastomer, but its radiation chemistry has received less attention than other commercial polymers from polymer scientists because its chemical curing system is already an established art. As a consequence, studies of irradiation of silicone have focused on its radioresistance connected with sterilization or its use in nuclear facilities [3–5].

In this work, crosslinking of medical grade silicone by ionizing radiation was studied. Some properties of the radiation-crosslinked matrix such as gel fraction and swelling behavior were evaluated, and its cytotoxicity was tested in vitro by the neutral red uptake assay.

2. Experimental

Plastic syringes filled with Down Corning Q7 4735 biomedical grade silicone were irradiated with a ^{60}Co source at doses of 5, 10, 25, 50 and 75 kGy. Crosslinked silicone cylinder rods were cut into slices with different mass and dimensions according to the type of test to be performed.

2.1. Dynamic mechanical thermo-analysis (DMTA)

Stress–strain oscillation measurements were performed with a dynamic mechanical thermoanalyzer NETZCH mod. DMA 242 in the linear viscoelastic region, using a three-bending point deformation mode at a frequency of 2 Hz. Test samples about 3.0 mm thick were cooled to −150 °C and heated to 80 °C under a strain-controlled sinusoidal tensile loading, at a heating rate of 5 °C/min. The maximum dynamic force used was 1 N with amplitude of 30 μm. The viscoelastic properties of storage modulus (E') and mechanical loss factor (E''), i.e. damping ($\tan d = E''/E'$), were recorded as a function of temperature.

2.2. Swelling assay

Samples were immersed into analytical grade toluene and the swollen samples were blotted and weighed at regular intervals until swelling equilibrium was reached at 23 °C. Degree of swelling was calculated as:

$$\%\ \text{Swelling} = \frac{\text{Wt. of swollen sample} - \text{wt. of initial sample}}{\text{Wt. of initial sample}} \times 100. \qquad (1)$$

2.3. Gel fraction assay

Samples were weighed and submitted to sol fraction extraction in a Soxhlet system with analytical grade toluene for 40 h. After this period the samples were dried until constant weight and their mass determined. Gel fraction was calculated as:

$$\%\ \text{Gel fraction} = \frac{\text{Wt. of dried gel after extraction}}{\text{Wt. of initial sample}} \times 100. \qquad (2)$$

2.4. Cytotoxicity assay

The cytotoxicity test was carried out using as target cell line NCTC clone 929 from American Type Culture Collection (ATCC), according to the International Standardization Organization [6] and the methodology described in a previous paper [7]. Cells were cultured for 24 h in the presence of diluted extracts of each irradiated silicone sample. The cytotoxic effect was evaluated by measuring cell viability in the neutral red uptake assay, which is based on the ability of living and undamaged cells to take up the neutral red compound. PVC pellets were used as negative control and a 0.02% phenol solution was used as positive control, following the International Standard Organization (ISO) 10993-5 [6].

3. Results and discussion

The gel fraction results of silicone irradiated at different doses of gamma radiation are presented in Table 1. From Table 1 it is possible to observe

Table 1
Gel fraction assay results of irradiated silicone in different doses

Radiation dose (kGy)	Gel fraction
2.5	Not measured
5	0.5442
10	0.8098
15	0.8434
25	0.9125
50	0.9421
75	0.9500

that silicone is an easily crosslinkable polymer. At 5 kGy of total dose the gel fraction was already 54%, and a high gel content of over 90% was achieved at 25 kGy. The rate of increase in gel fraction could be said to slow down considerably at higher doses (cf. gel fractions at 50 and 75 kGy), but it should be noted that there is a clear trend towards stabilization at 95% of gel fraction.

It was not possible to measure the gel content at 2.5 kGy due to the formation of a paste-like material composed mainly of dispersed micro-gels and with no mechanical properties. In order to obtain a more precise evaluation of the balance between crosslinking and degradation reactions, the Charlesby–Pinner and Charlesby–Rosiak equations [8] were applied to the data.

From Fig. 1 it was possible to draw the kinetics of radiation-induced degradation and crosslinking of medical grade silicone. The gelling dose was found to be 2.57 kGy according to Charlesby–Pinner's equation and 2.78 kGy according to Charlesby–Rosiak's equation. The ratio of degradation over crosslinking (p_0/q_0) was found to be 0.19 according to Charlesby–Pinner's equation and 0.22 according to Charlesby–Rosiak's equation.

The amount of gel does not inform us about network features at higher radiation doses (i.e. >5 kGy), therefore swelling experiments were undertaken to provide some useful insight into such features.

Fig. 2 shows the degree of swelling in radiation-crosslinked silicone samples as a function of time and dose. The sample at 5 kGy broke apart during the assay but it did not dissolve in spite of that the hydrogel content has a low degree of crosslinking. Samples crosslinked at 10 kGy showed an unusual behavior, i.e. a reduction in swelling after 30 h. This can be explained by the extraction of the sol fraction and consequent mass reduction in the irradiated sample. The same phenomenon could be observed to a lesser extent in samples irradiated at 15 kGy and only very slightly at 25 kGy and over, as the gel content approached 100%.

Fig. 2 shows that, upon irradiation at 75 kGy, there was only a slight reduction in swelling as compared with the 50 kGy dose. It could be reasoned that, as radiation increases, the rigidity of the resulting network is making it more and more difficult for the crosslinking reaction to occur.

DTMA was performed to evaluate the thermo-mechanical characteristics of the network. Two groups of curves were easily distinguished. The first comprises weakly crosslinked samples, i.e.

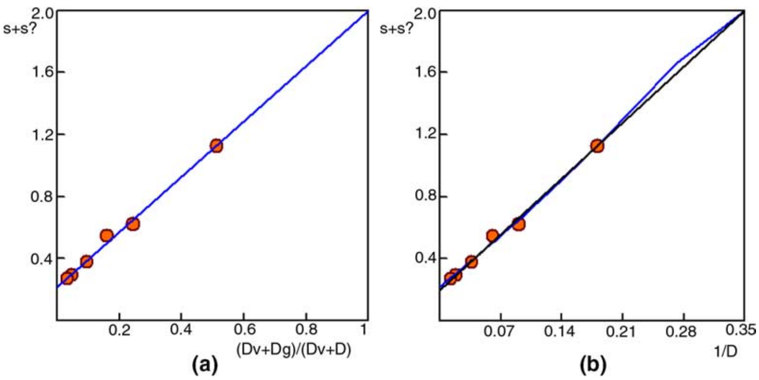

Fig. 1. Sol/gel data plotted according to (a) Charlesby–Rosiak equation and (b) Charlesby–Pinner equation.

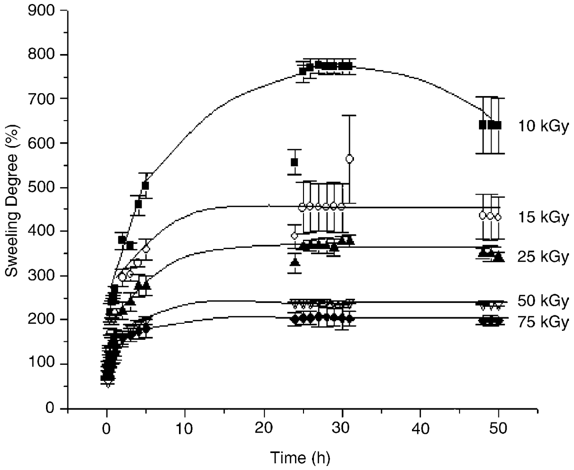

Fig. 2. Swelling assay curves of silicone irradiated under 10, 15, 25, 50 and 75 kGy doses.

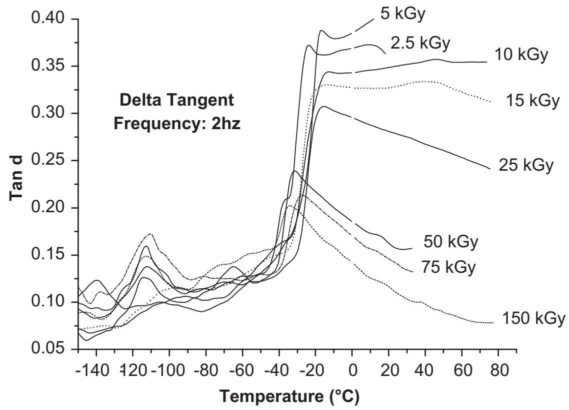

Fig. 3. TDMA of silicone irradiated under different doses.

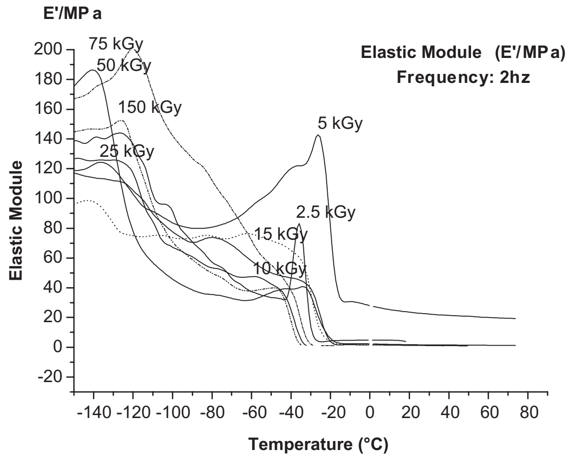

Fig. 4. TDMA of silicone irradiated under different doses.

samples exposed to 2.5–15 kGy, and the second comprises strongly crosslinked samples, i.e. those exposed to 50–150 kGy. Samples irradiated at 25 kGy presented an intermediate behavior (Fig. 3).

The glass transition temperature of silicone is known to be about −120 °C and its melting point about −40 °C. Fig. 3 shows that weakly crosslinked samples have small amplitude peaks for glass transitions temperatures and large ones for melting points. It is evident, from Fig. 4, that crystallization was reduced due to crosslinking while the amorphous region increased. This decrease in crystallinity agrees very well with a previous work by Stevenson et al. [3]. It is related to the increase in crosslinking density as measured by the degree of swelling. One might conclude that the higher the crosslinking density the lower the mobility of silicone segments, with consequent impairment of crystallization.

Because irradiation promotes mainly crosslinking but in minor level some degradation of the silicone molecules, a test for cytotoxicity was included in order to evaluate if such structural modifications jeopardize the biocompatibility of medical grade silicone.

In the cytotoxicity assay, negative and positive controls are used to demonstrate the cellular response in the presence or absence of toxicity. Our positive control showed a toxic effect with a cytotoxicity index ($IC_{50\%}$) of about 24. This means that the extract of the material used as positive control at a concentration of 24% killed or injured half (50%) the cell population in the assay. Silicone samples irradiated at 50 kGy presented the same cytotoxic behavior of the negative control, i.e. no cytotoxicity (Fig. 5).

Therefore, radiation-crosslinked silicone showed adequate properties as a polymeric matrix for drug delivery, with no in vitro toxicity and thus preserved biocompatibility, indicating that it is an excellent candidate for use in drug delivery systems. The study of biocompatibility has to be continued with

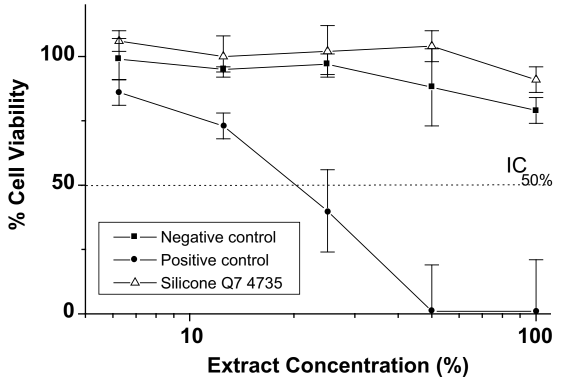

Fig. 5. Cellular viability curves in the cytotoxicity assay by Neutral Red Uptake method of silicone irradiated in a dose of 50 kGy.

in vivo tests using animal models and with subsequent pre-clinical tests.

4. Conclusions

The gelling dose was found to be 2.57 kGy according to Charlesby–Pinner's equation and 2.78 kGy according to Charlesby–Rosiak's equation. The p_0/q_0 ratio, which expresses the ratio of degradation over crosslinking, was found to be 0.19 according to Charlesby–Pinner's equation and 0.22 according to Charlesby–Rosiak's equation. Twenty five kGy was shown to be the minimum dose to promote a crosslinking level suitable as polymeric matrix for drug delivery system. Further experiments are presently under way in our laboratory to study active compound incorporation and in vitro drug release kinetics with irradiation-crosslinked silicone as matrix.

Acknowledgments

The authors wish to thank Embrarad for sample irradiation; Dow Corning for sample supply; Biolab-Sanus Farmacêutica Ltda for financial support and CNPq for granting of student fellowship.

References

[1] G.H. Gifford Jr., E.W. Merrill, M.S. Morgan, In vivo tissue reactivity of radiation-cured silicone rubber implants, J. Biomed. Mater. Res. 10 (1876) 857.
[2] B.D. Ratner, A.S. Hoffman, F.J. Schoen, J.E. Lemons, Biomaterials Science. An Introduction to Materials in Medicine, Academic Press, USA, 1996.
[3] I. Stevenson, L. David, C. Gauthier, L. Arambourg, J. Davenas, G. Vigier, Influence of SiO_2 fillers on the irradiation ageing of silicone rubbers, Polymer 42 (2004) 9287.
[4] A.A. Basfar, Hardness measurements of silicone rubber and polyurethane rubber cured by ionizing radiation, Radiat. Phys. Chem. 50 (6) (1997) 607.
[5] J. Li, M. Zhai, M. Yi, H. Gao, H. Ha, Radiation grafting of thermo-sensitive poly(NIPAAm) onto silicone rubber, Radiat. Phys. Chem. 55 (1999) 173.
[6] International Standard Organization: Biological Evaluation of Medical Devices—Part 5: Tests for Cytotoxicity: in vitro methods. ISO 10993-5, 1992.
[7] S.O. Rogero, S.M. Malmonge, A.B. Lugão, T.I. Ikeda, L. Miyamaru, A.S. Cruz, Artif. Organs 27 (5) (2003) 424.
[8] J. Olejniczak, J.M. Rosiak, A. Charlesby, Gel/dose curves for polymers undergoing simultaneous crosslinking and degradation, Radiat. Phys. Chem. 37 (1991) 499.