Pergamon

Radiat. Phys. Chem. Vol. 52, Nos 1–6, pp. 301–306, 1998

Printed in Great Britain
PII: S0969-806X(98)00157-1 0969-806X/98 $19.00 + 0.00

BIOMEDICAL APPLICATIONS OF RADIATION TECHNOLOGY IN MEXICO.

Ma. Esther Martínez-Pardo[a], R. Vera-Graziano[b] and L. E. Ramos-Durón[c]

[a] INSTITUTO NACIONAL DE INVESTIGACIONES NUCLEARES (ININ), Apdo. Postal 18-1027, Col. Escandón, 11801 México, D.F. [b]INSTITUTO DE INVESTIGACIONES EN MATERIALES (IIM/UNAM), Apdo. Postal 70-360, Coyoacán, 04510 México, D.F., [c] HOSPITAL CENTRAL SUR DE ALTA ESPECIALIDAD (HCSAE), Periférico Sur 4091, Col. Fuentes del Pedregal, 14140 México, D.F.

ABSTRACT

Mexican Health Institutions continuously require suitable medical grade prosthetic materials for reconstructive and plastic surgery. In particular, the requirements of polydimethylsiloxane, PDMS, for soft tissue replacements are rapidly growing. In addition to molecular weight, the properties of PDMS in biomedicine strongly depend on its purity, formulation and processing. High energy radiation has been used for both the synthesis of highly pure PDMS, free of catalyst and chemicals, and for sterilization of biomedical products. Here, are discussed the gamma radiation polymerization of different siloxane precursors to obtain PDMS with specific functionality and molecular structure as well as the radiation sterilization of amniotic membranes used as wound dressing.

KEYWORDS

Polydimethylsiloxanes: radiation induced polymerization: amniotic membrane: radiosterilization.

INTRODUCTION

Tolerance of polysiloxanes by the human body mainly depends on chemical structure, purity and sterilization. Pure and sterile polydimethylsiloxanes, PDMS, can be obtained by gamma radiation, because no chemicals (catalysts, initiators and others) are needed for the polymerization of precursors. These chemicals may produce serious rejection problems after the polymer material is implanted in the organism. By selecting the proper radiation dose and precursor functionality, both, the crosslinking density and the type of network are controlled. Thus, a wide range of physical properties are offered by PDMS: microgels, macrogels and elastomers. γ radiation has been used for making PDMS networks of specific functionality with different crosslinking density, and for the sterilization of amniotic membranes.

Open wounds caused by burns or ulceration are site of infections; the lost of the protective layer skin can originate the lost of fluids, protein, thermal-regulation, etc. The resulting metabolic de-rearrangements can prove fatal. The use of amniotic membranes, AM, has several advantages: diminution of evaporation, electrolytes, protein, calories, extrinsic contamination, inflammation, pain intensity and so on. However, the AM easily pick-up bacteria and fungi from the birth channel (Phillips. 1994). Conventional sterilization process of AM with antibiotic solutions, does not guarantee sterility. Radiosterilization of AM is known, but in Mexico the process was not well

known. We did a study to determine the minimum radiation dose to sterilize MA inoculated with five bacteria and a fungi.

A) POLYDIMETHYLSILOXANES (PDMS)

Experimental

The siloxane precursors (from Huls-Petrarch Sys.) were irradiated in vacuum sealed ampoules. Some precursors were mixed with D_4V_4 to enhance crosslinking. The reacting systems and irradiation conditions are shown in Tables I and II. Two separate sets of samples were dehydrated before irradiation with metallic sodium and calcium hydride respectively. Two AECL ^{60}Co radiation sources were used (Gammacell 220 and JS-6500), after the dose and dose rate were determined (Fricke solution for the Gammacell 220 and routine dosimetry with Red Perspex Dosimeter, type 4034 CG, Harwell Lab., for the JS-6500).

Sol and gel fractions were obtained by reflux extraction with toluene, at 388 K, for 24 h., according to ASTM procedure D-2765. The crosslinking densities of PDMS gel fractions were determined by both absorbency (swelling) and heat capacity (Sperling. 1992, Vera-Graziano *et al.*. 1995). Polymer structures were determined by infrared spectroscopy (Nicolet MX-5) and the molecular weight by gel permeation chromatography (Waters 150C). Thermal stability was measured by TGA (TA Instruments Co.). The densities were measured by the density-gradient-column (ASTM procedure D-1505) using mixtures of isopropanol-water (0.79 to 1.00 g/cc) and isopropanol-ethyleneglycol (0.79 to 1.11 g/cc). Contact angles of PDMS films were determined by goniometry (Ramé-Hart, Inc.).

Results and Discussion

γ-radiation. The most important chemical reactions induced by γ-radiation were polymerization and crosslinking. Activation doses, gel percent and density are shown in Table III. Ring opening polymerization of D_4, was not observed, even though the dose of 1,000 kGy, while the vinyl group of D_4V_4 only required 87.5 kGy at 22 °C and 17.5 kGy 65 °C. The activation dose for the methyl group in PDMS is about 68 kGy at 22°C and decreases more than three fold at 65°C. The partial substitution of methyl by hydrogen yield a similar situation.

D_4 reacted with the D_4V_4, forming a gel, at a dose of 393 kGy at room temperature (r.t.), for the 1/50 molar proportion. As before, the required dose was substantially reduced with temperature. Surprisingly, the dehydrated systems required higher activation doses probably because of the impurities included in the dehydration process: at r.t. all dehydrated mixtures with linear chain precursors reacted at 87.5 kGy while the non-hydrated samples required 81 kGy. It was observed that the activation dose for polymerization decreased with the molecular weight (MW) of the trimethylsiloxy and hydroxy terminated precursors. The vinyl terminated linear precursors are so reactive that no MW effect was observed. Activation dose increased in the following order of

TABLE I. Physical properties of polysiloxane precursos.

PRECURSOR	I.D.	MOLECULAR WEIGHT (daltons)	VISCOSITY cps
OCTAMETHYLCYCLOTE-TRASILOXANE	D_4	296	
1,3,5,7,TETRAVINYLTE-TRAMETHYL-CYCLOTETRASILOXANE	D_4V_4	344	
HEXAMETHYLDI-SILOXANE	HMDS	162	0.65
POLYDIMETHYLSILO-XANES,	PDMS(1)	17,250	500
	PDSM(2)	49,350	5,000
TRIMETHYLSILOXY-TERMINATED	PDMS(3)	62,700	10,000
POLYDIMETHYL-SILOXANES,	PDMS-OH(1)	26,004	1,000
		58,004	5,000
HYDROXYDIMETHYL-SILOXY-TERMINATED	PDMS-OH(2)		
POLYDIMETHYL-SILOXANES,	PDMS-V(1)	26,034	1,000
		49,384	5,000
VINYLDIMETHYLSILOXY-TERMINATED	PDMS-V(2)		
POLYDIMETHYL-SILOXANES,	PDMS-V_2	26,068	10,000
DIVINYLMETHYLSILOXY-TERMINATED			
DIMETHYLSILOXANE VINYL	PDMS-V\		
,	V-PDMS		
TERMINATED\METHYLVINY L-SILOXANE COPOLYMER			
DIMETHYLSILOXANE	PDMS\		
\METHYL-HYDROSILOXANE	H-PDMS		
COPOLYMER			

functionality: vinyl, methyl and hydroxy: For samples of same MW (5,000) irradiated at 338 K, the dose was 22, 33 and 61 kGy. Concerning gel content, the dose for non-dehydrated precursors was about the same independently of functionality but the gel percent indeed increased with the MW of the precursor (Guzmán. 1995).

TABLE II. REACTING SYSTEMS

I.D.	MOLAR PROPORTIONS
D_4V_4/D_4	1/50
D_4V_4/D_4	1/100
D_4V_4/D_4	1/200
D_4V_4/HMDS	1/1
D_4V_4/PDMS(X)	1/1
D_4V_4/PDMS-OH(X)	1/1
D_4V_4/PDMS-V(X)	1/1
D_4V_4/PDMS-V_2	1/1

X REFERS TO THE MOLECULAR WEIGHT OF THE PDMS'S SHOWN IN TABLE I

Infrared spectroscopy. The intensity of the D_4 precursor band (1263 cm^{-1}) corresponding to the CH stretch vibrations of methyl groups on Si decreased with polymerization. The 1263 cm^{-1}, band is always accompanied by one or more equally intense bands, from the 763 to 847 cm^{-1}. This region gives useful information about the structure of the molecule (Vera-Graziano *et al.*.1995) The two

TABLE III. Average activation doses for chemical reaction, gel percent and density of reacting systems.

ID.	DEHYDRATED 295 K	DEHYDRATED 338 K	NON-DEHYDRATED 295 K	NON-DEHYDRATED GEL CONTENT %	295 K DENSITY g/cc
D_4 OCTAMETHYL		non up to 1,000		NON	
PDMS(2) TRIMETHYL SILOXI		17.5	80.4	73.40	1.15454
PDMS-/VYNYL-PDMS COPOLYIMER		17.5	67.5	32.60	0.9078
PDMS/HYDROSILOXANE-PDMS COPOLYMER		357.8* 398.6	317.5* 527.9	58.70	0.9597
D_4V_4		17.5	86	18.46	0.9280
	REACTING SYSTEMS:				
D_4V_4[1]/D_4[50] a	393.0*	94.0*	123.8*	3.85	0.9889
D_4V_4[1]/D_4[100] b	550**	151.7	123.8*		
D_4V_4[1]/D_4[200] c	550**	220.1	123.8		
D_4V_4/HMDS P.M. (162)	87.5	17.5	81.0	53.96	1.0329
D_4V_4/PDMS (1) (17.250)	87.5	52.8	81.0	44.69	0.9578
D_4V_4/PDMS (2) (49.350)	87.5*	33.1	81.0	60.90	0.9630
	108.6 COMP.				
D_4V_4/PDMS (3) (62.700)	87.5	22.0	81.0	65.27	0.9623
D_4V_4/PDMS-OH (1) (26.004)	87.5	(52.8) 73.5	81.0	37.06	0.9578
D_4V_4/PDMS-OH(2) (58.004)	87.5	60.8	81.0	79.12	0.9436
D_4V_4/PDMS-V (1) (26.034)	87.5	17.5	81.0	87.98	0.9659
D_4V_4/PDMS-V (2) (49.384)	87.5	17.5	81* 102.5	89.69	0.9710
D_4V_4/PDMS-V_2 (26.068)	87.5*	17.5	81* 102.5	87.73	0.9635
	108.6				

* a gel was formed ** no polymerization was observed

methyl groups of the cyclic precursor absorb differently than those of PDMS. Also the 1076 cm^{-1} band, arising from an asymmetric Si-O-Si stretch vibration in the cyclic precursor, splits into two overlapping components in progressively longer chain compounds, with points of maximum absorption at 1020 cm^{-1} and 1087 cm^{-1} (Kendrick *et al.*. 1989). Also the double bond bands decreased as gel content increased.

Thermal Stability. The thermal stability of the crosslinked PDMS was much higher than that of the non-crosslinked polymer. TGA showed that gel fractions started loosing weight above 425°C while for the non-crosslinked material started volatilization at 100°C. It was also observed that thermal stability increased with crosslinking density.

Molecular Weight and Crosslinking Density. The MW of both sol fractions and linear PDMS were analyzed by GPC. The sol fractions had average molecular weights, Mw, from 1,200 to 14,000, in contrast the Mw of soluble PDMS was up to 430,000, corresponding to about 5850 repeating units. Crosslinking densities of gel fractions increased with both D_4V_4 concentration and dose. The results obtained by heat capacity measurements are in agreement with those obtained by absorbency (Vera-Graziano *et al.*.1995).

Wetting behaviour. PDMS films, supported on glass plates showed contact angles from 66 to 100 degrees. The contact angle increased with the MW of the polymer indicating that the high MW polysiloxanes can also be used as coating materials for soft tissues, including blood. Preparation of PDMS films is reported elsewhere (Rivera *et al.*. 1997).

B) AMNIOTIC MEMBRANES (AM)

Experimental

Membranes from placenta following childbirth were collected at HCSAE, taking into account some criteria: mother without any infectious process (negative HIV), prolonged drug therapy, age, completed gestation, aseptic recuperation technique less than 72 h with aseptic procedure. The AM were cut into small pieces, washed with water and put in a culture solution for bacteria and fungi only for negative control. Then they were kept in special culture tubes containing a 9% sterile saline solution at 4 °C. The bacteria and fungi used to inoculate the AM were: Enterobacter sp, Escherichia coli, Staphylococcus aureus, Streptococcus beta hemolytic, Pseudomonad sp and Candida albicans. The AM were gamma irradiated at doses from 5 to 30 kGy.

Results and discussion

Histopathology analysis did not reveal any structural changes after irradiation. It is important to emphasize that the membranes kept their structural stability at the maximum dose, so a waste tissue has now an added value (De la Tejera. 1995, Martínez-Pardo *et al.*. 1995). The IAEA recommends

to freeze-dry and support the membranes, obtained by Caesarean section, in sterile cotton and packaged, followed by irradiation at 25 kGy. We did it the same, but supporting the AM on local polyester or nylon fabric to facilitate handling. These AM had a successful clinical use. Other use of AM is as a cover of silicone implants. HCSAE did an investigation to determine the biological behaviour and tolerance of silicone macro implants covered with AM (from rats and from human being). A covered implant (homograft, from rats) produces a minimum of inflammatory reaction, granulomatous, fibrosis, neovascularity, capsule sheath and rejection. The silicone covered with AM (homograft) has better biological behaviour than the uncovered silicone graft or than the allograft covered silicone (Lizárraga. 1996). Our results were the base to develop a co-operation program among ININ, HCSAE and UNAM, with technical assistance of the IAEA to promote radiation sterilization of tissues and other alloplastic materials, as well as to establish a tissue bank in Mexico.

REFERENCES

G. O. Phillips (1994) "Radiation Sterilisation of Tissue Allografts for Transplant Surgery"

Sperling L. H. (1992) *Introduction to Physical Polymer Science,* 2nd edn., Wiley-Interscience, New York.

Vera-Graziano R., Hernández-Sánchez F. and Cauich-Rodríguez J. V. (1995) Study of Crosslinking Density in Polydimethylsiloxane Networks by DSC. *Journal of Applied Polymer Science,* **55,** 1317.

Guzmán Gutiérrez P. (1995) Bachelor Thesis, Universidad Autónoma del Edo. de México, December. "Obtención de polisiloxanos de alta pureza por copolimerización mediante irradiación gamma".

Kendrick, T. C., Parbhoo B. and White J. W. (1989) Part 2, pp 1289, in *The Chemistry of Organic Silicon Compounds,* S. Patai and Z. Rapoport De., John Wiley & Sons, New York, N.Y.

Rivera F. and Vera R. (1997) "Sintesis y caracterización de polisiloxanos" IIM/UNAM informe técnico IIM-01/97.

De la Tejera García, M. F. (1995) "Esterilización de membranas amnióticas (como apósitos biológicos) mediante radiación gamma", tesis de posgrado, Facultad de Medicina, UNAM.

Martínez Pardo M. E., Breña V., M., Ramos Durón L. E. y De la Tejera G., M. F. (1995) "Determinación de la dosis mínima de radioesterilización de membranas amnióticas para su uso como apósitos biológicos", ININ informe técnico FQ-95-12.

Lizárraga R, R. L. (1996) " El uso de las membranas amnióticas en la tolerancia de implantes de silicón", tesis de posgrado, Fac. de Medicina, UNAM.