3 Results and Discussion

3.1 Solubility and miscibility

The solubility of different PLGA polymers in some solvents frequently used for the preparation of in situ forming implants was proved (Table 3.1). After 48 hours agitation, RG 752 and R 202H showed to be soluble in all the solvents tested, exhibiting a single clear phase while the other three polymers (RG 502H, RG 503H and RG 504) swelled after being shaken with benzyl alcohol. RG 503H and RG 504 also swelled with benzyl benzoate. Additionally RG 503H was not soluble in PEG 400 and RG 504 not soluble in ethyl acetate. Partial miscibility was found for RG 504 in 2-pyrrolidone (Table 3.2). The polymer selected for the next studies was RG 752.

Table 3.1 Solvents used for in situ implants formation.

Solvent	Melting Point	Boiling Point	Solubility water	Density 20°C
N-methyl-2-pyrrolidone	-24.0°C	202.0°C	1000 g/l	1.03 g/cm^3
2 -pyrrolidone	24.0 - 26.0°C	245.0°C	1000 g/l	1.11 g/cm^3
Polyethylene glycol 400	4.0 - 8.0°C	>250 °C	1000 g/l	1.13 g/cm^3
Ethyl acetate	-83.0°C	77.0 °C	85.3 g/l	0.90 g/cm^3
Benzyl alcohol	-15.3°C	205.0 °C	40.0 g/l	1.05 g/cm^3
Benzyl benzoate	21.0°C	324.0°C	insoluble	1.12 g/cm^3
Triacetin	-78.0°C	258.0°C	64.0 g/l	1.16 g/cm^3
Dimethyl sulfoxide	18.4°C	189.0°C	1000 g/l	1.10 g/cm^3

Data obtained from http://chemdat.merck.de/mda/int_en/catalog/

Table 3.2 Solubility of different PLGA polymers in different solvents.

	Polymer				
Solvent	RG 752	RG 502H	RG 503H	RG 504	R 202H
2-pyrrolidone	+	+	+	+/-	+
N-methyl-2-pyrrolidone	+	+	+	+	+
Polyethylene glycol 400	+	+	-	+	+
Ethyl acetate	+	+	+	-	+
Benzyl alcohol	+	Sw	Sw	Sw	+
Benzyl benzoate	+	+	Sw	Sw	+
Triacetin	+	+	+	+	+

+ soluble
+/- partially soluble
- insoluble
Sw swelling

For the preparation of O/W-ISM systems, the suitable solvent had to be immiscible with water and able to dissolve the polymer, as it was the case of benzyl benzoate or other low water soluble solvents like benzyl alcohol and triacetin (Table 3.1 and 3.2). O/O-ISM systems required an organic solvent immiscible with the oil but able to dissolve the polymer. Some commonly used oils for parenteral products are listed in table 3.3. Peanut oil and sesame oil are slightly miscible or immiscible with the three solvents tested. Since corn oil and miglyol were miscible with NMP, sesame oil and peanut oil were selected for the study. Additionally sesame oil was reported as being more stable, knowing the possible oxidative changes of fixed oils, due to the presence of unsaturated fatty acids [102].

Table 3.3 Properties of commonly used oils in parenteral products.

Oil	Viscosity	Density	Constitution			
Peanut oil	41 cP	0.910 - 0.915 g/ml	C16 C18 C18:1 C18:2	8.3% 3.1% 56.0% 26.0%	C20 C22 C24	2.4% 3.1% 1.1%
Miglyol 812	28 - 32 cP	0.940 - 0.960 g/ml	C6 C8 C14	0.5% 58.0% 1.4%	C10 C12 C16:1	40.0% 1.0% 1.5%
Corn oil	39 cP	0.916- 0.921 g/ml	C16 C18:1	10.2% 49.6%	C18 C18:2	3.0% 47.8%
Sesame oil	57 cP	0.916 - 0.920 g/ml	C16 C18:1 C18:2	9.1% 45.4% 40.4%	C18 C20	4.3% 0.8%
Soybean oil	50cP	0.930 g/ml	C12 C14 C16 C18 C20	0.2% 0.1% 9.8% 2.4% 0.9%	C16:1 C18:1 C18:2 C18:3 C24	0.4% 28.9% 50.7% 6.5% 1.1%

Data from reference [103]

Table 3.4 Solvent/oil miscibility for O/O-ISM systems.

Oil	Solvent		
	N-methyl-2-pyrrolidone	2-pyrrolidone	Polyethylene glycol 400
Peanut oil	+	-	-
Corn oil	+++	-	-
Miglyol	+++	-	-
Sesame oil	+	-	-

+++	miscible, one phase that stay after rotation of the vial
++	partial miscible, one phase that forms lamps after rotation of the vial
+	slightly miscible -two phases, one bigger than the other
-	inmiscible - two phases of equal height

3.2 Rate of dissolution

A rapid dissolution of PLGA in the biocompatible organic solvent during reconstitution is essential for the potential use of solid PLGA for the formation of in situ systems. The rate of dissolution of solids can be described by the Noyes-Whitney equation:

$$dm/dt = kA(C_s-C),$$

Where:
m = mass of solute that passed into solution in time t,
dm/dt = mass of solute going in solution per unit time,
A = surface area of the undissolved solid in contact with the solvent,
C_s = concentration of solute required to saturate the solvent at the experimental temperature,
C = solute concentration at time t and
k = dissolution rate constant, it has the dimensions of length^{-2} time^{-1} :

$$k = D/ Vh$$

Where:
D = diffusion coefficient of the solute in the dissolution medium (or solvent),
V = volume of the dissolution medium and
h = thickness of the boundary layer [104-106].

From the terms present in the previous equation is possible to deduce some of the factors that could have influence on the dissolution rate of solids in liquids and therefore in the dissolution rate of solid PLGA particles in organic solvents (Table 3.5).

Table 3.5 Factors affecting dissolution rate of solids in liquids.

Noyes-Whitney equation terms	Affected by	Comments
A, surface area of undissolved solid	Size of solid particles	Particle size
	Dispersibility of powdered solid in solvent	Wetting agent
	Porosity of solid particles	Large enough pores
Cs, Solubility of solid in solvent	Temperature	exothermic - endothermic
	Nature of solvent	Solubility parameters, cosolvents, pH
	Molecular structure of solute	different polymers, drugs
	Crystalline form of solid	polymorphism
	Presence of other compounds	complex formation, solubilizing agents
C, concentration of solute in solution at time t	Solvent volume	different concentrations
	Any process that removes dissolved solute from the solvent	partition into oil
k, dissolution rate constant	Thickness of boundary layer	mixing rate, shape and size of container, volume of solvent, viscosity of solvent
	Diffusion coefficient of solute in the solvent	viscosity solvent, particle size of diffusing molecules

Adapted from [105].

The rate of polymer dissolution thus depends on the surface area (particle size) of the polymer. The particle size of PLGA (as received from the supplier) was > 500 µm, The particle size could be reduced by cryogenic milling to 50 µm. As

expected, the dissolution time (as represented by the number of mixing cycles) decreased with decreasing particle size (increasing surface area) from 45 cycles for the size fraction larger 160 µm to 20 cycles for the 50 – 100 µm fraction (Figure 3.1).

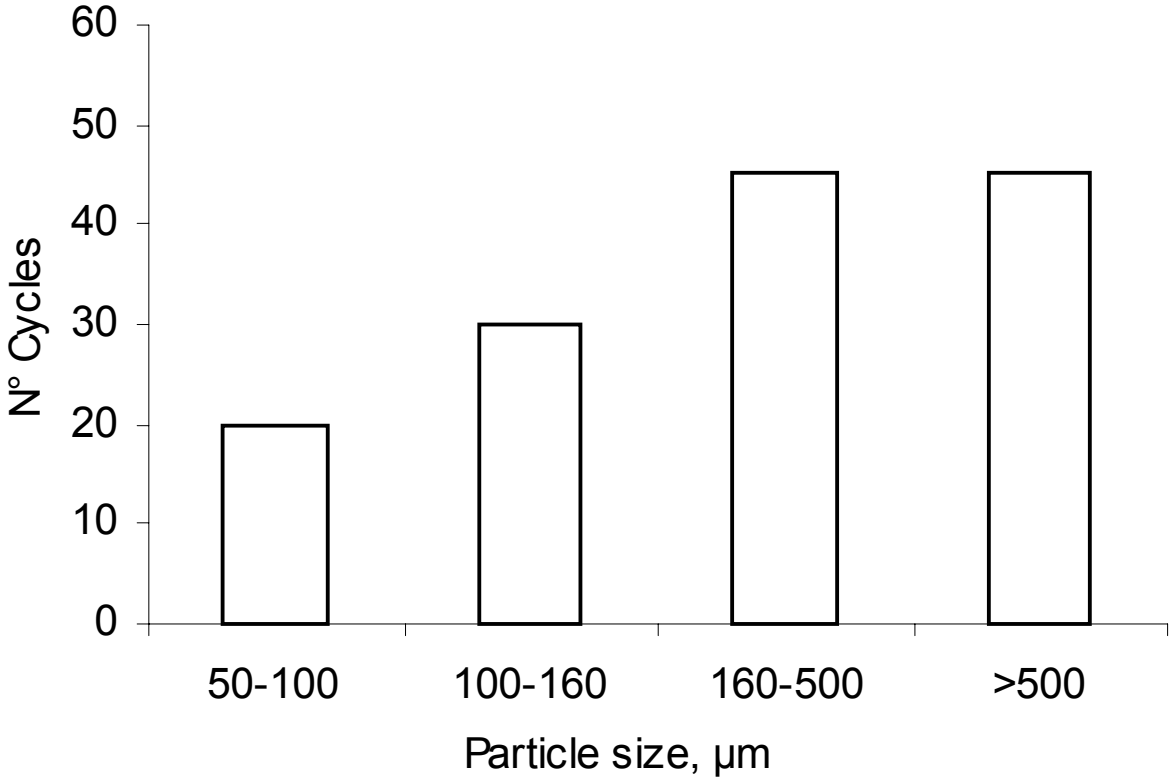


Figure 3.1 Effect of the particle size on the dissolution rate of 30% w/w R 202H in 2-pyrrolidone (0.5 cycles/s)

A key factor for the polymer dissolution process is the selection of the organic solvent, which will affect the rate of dissolution primarily through the solubility of

the polymer and the viscosity of the resulting polymer solution [107]. Since PLGAs are water-insoluble, various organic biocompatible solvents, which have been in used in parenteral formulations, were evaluated for their ability to rapidly dissolve PLGA. The time required for complete dissolution of PLGA RG 752 powder (as received from the supplier) was in the following order: ethyl acetate < NMP < benzyl alcohol < triacetin < benzyl benzoate < 2-pyrrolidone < PEG 400 (Table 3.6).

Table 3.6 Dissolution time of PLGA RG752 in different solvents and their corresponding solvent/ PLGA solution viscosities.

Solvent	Dissolution time, min			Viscosity, mPa s				Hildebrand Solubility parameter, MPa $^{1/2}$ [a]
	Polymer concentration			Polymer concentration				
	10%	25%	40%	0%	10%	25%	40%	
Ethyl acetate	1.1	1.8	10.2	0.5	-	-	-	18.6
NMP	3.0	10.7	33.0	1.7	-	48.9	-	23.1
Benzyl alcohol	18.0	33.2	51.0	3.7	-	113.1	-	24.8
Triacetin	-	84.4	-	17.0	-	761.9	-	23.7
Benzyl benzoate	-	132.4	-	6.8	-	419.6	-	-
2 -pyrrolidone	-	178.5	-	12.2	-	292.8	-	30.1
PEG 400	1355.0	1860.0	*	90.0	-	1243.0	*	-

* not completely dissolved

- not determined (viscosity) / not found (solubility parameters)

[a] From [108]

The rank order of the solvents was the same at all three concentrations tested (10%, 25% and 40% w/v). The viscosity of the biocompatible solvent ("dissolution medium") increased significantly during the dissolution of the polymer. The time for dissolution correlated with the viscosity of the pure solvents/resulting polymer solutions and the solubility parameter of the solvent (Table 3.6). Solvents with a lower viscosity, such as ethyl acetate, NMP or benzyl alcohol dissolved the polymer in between 1 and 50 min at all polymer concentrations tested, while the most viscous solvent PEG 400 required almost

one day at a PLGA concentration of only 10% w/v. The dissolution time increased with increasing polymer concentration because of an increased solution viscosity.

The solvent quality for a solute is generally reflected by the solubility of the solute in the solvent. However, the solubility of polymers is difficult to determine experimentally because of their high solution viscosity. The solvent quality for polymers has been reflected empirically by the solubility parameter [108, 109]. Shivley et. al determined the solubility parameter of PLGA as being 20.05 $MPa^{1/2}$ and Lambert and Peck reported the best solubility of PLGA in solvents with solubility parameters between 18.41–22.50 $MPa^{1/2}$ [43, 45]. Ethyl acetate (18.6 $MPa^{1/2}$), NMP (23.1 $MPa^{1/2}$), triacetin (23.7 $MPa^{1/2}$) and benzyl alcohol (24.8 $MPa^{1/2}$) had solubility parameters within or close to this range, thus resulting in rapid dissolution times. In contrast, 2-pyrrolidone (30.1 $MPa^{1/2}$) had a solubility parameter outside this solubility parameter range and longer dissolution times (Table 3.6).

An ideal organic solvent for rapid dissolution of PLGA should thus have a solubility parameter close to the one of the polymer and result in a low solution viscosity. With regard to injectability, solvents resulting in a lower polymer solution viscosity at the same polymer concentration are more desirable because of an easier and less painful injection.

3.3 In situ forming implants (ISI)

Next, the dissolution time of PLGA was investigated within a possible application device to be used with patients, a two syringe system with a connector. PLGA was filled in one syringe and the solvent in the second syringe. The two syringes were connected via a connector; the solvent was then pushed in the polymer-containing syringe followed by pushing forward and backward the plungers of the two syringes in order to dissolve the polymer (Figure 3.2).

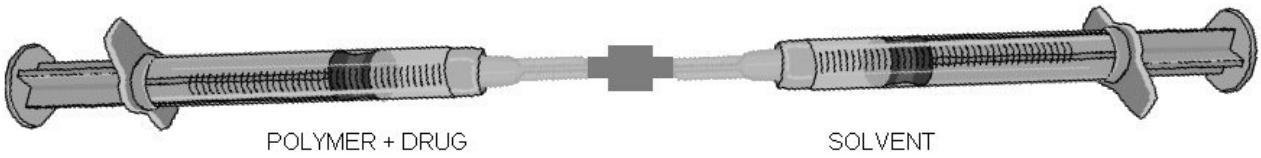


Figure 3.2 Device for the preparation of in situ forming systems.

3.3.1 Factors affecting the dissolution rate

The rank order in dissolution time was the same as with the one obtained with the shaker method (Table 3.6) except for 2-pyrrolidone, which dissolved the polymer slightly faster than triacetin (Figure 3.3A). The dissolution times with the two syringe system were much shorter (less than one minute) than the dissolution times obtained with the shaker method, because the shear stress at the PLGA particle/solvent interface was much higher with the syringe system than within a shaken vial. The short reconstitution times of less than one minute are in a time frame acceptable for the clinical end user, who prepares the injectable in situ system.

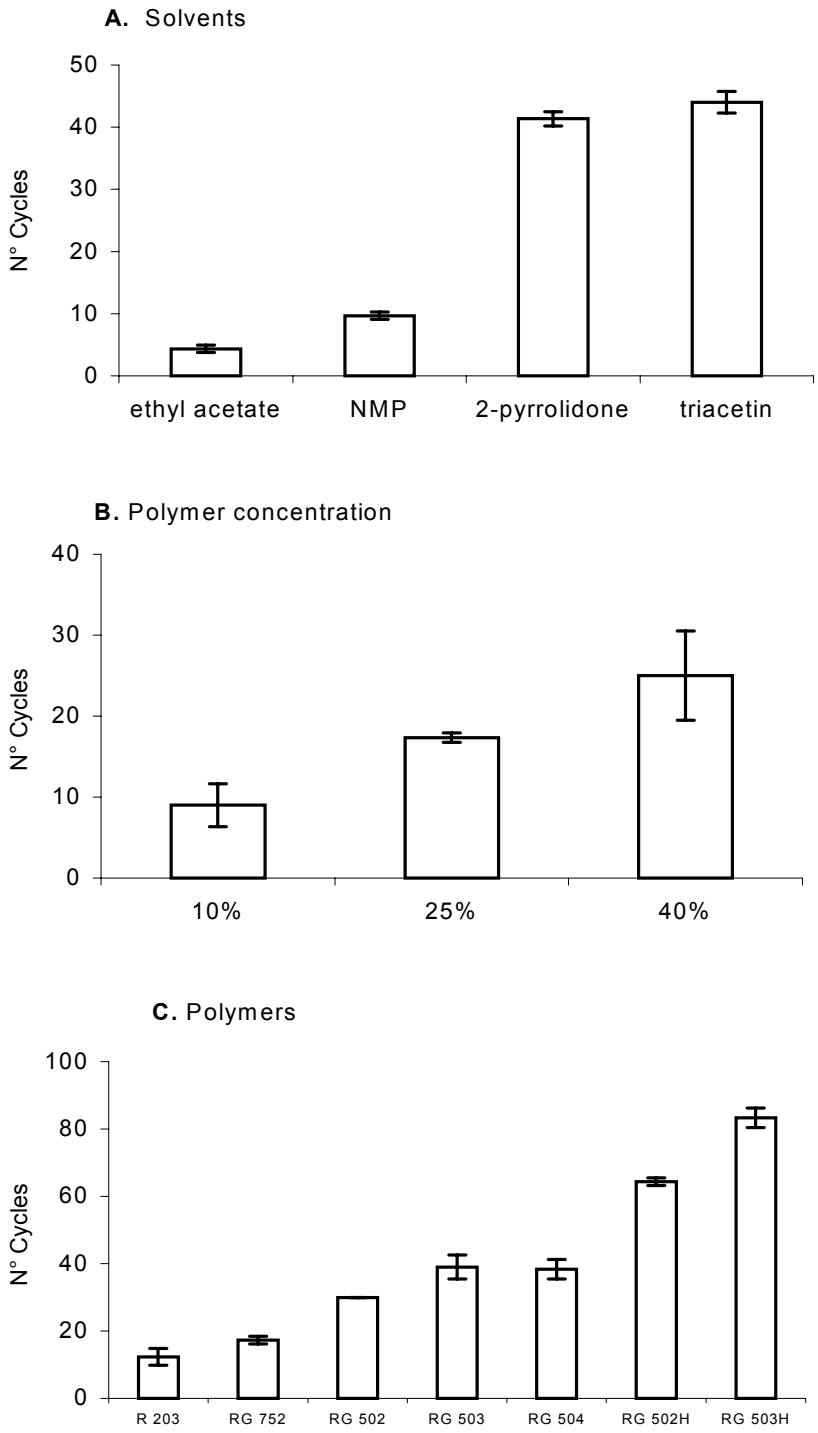


Figure 3.3 Effect of formulation parameters on the dissolution time (number of cycles) of (A) 10% w/w R 203 in different organic solvents; (B) different RG 752 concentrations in NMP; (C) type of polymer in NMP (25% w/w). (1 ml syringe size, mixing rate of 0.5 cycles/s, polymer as received, n=3).

The drug release from in situ forming systems usually decreases with increasing polymer solution concentration because of the formation of a denser polymer matrix after precipitation of the polymer in situ in the body [40, 75]. The required number of cycles to dissolve the polymer increased with increasing polymer concentration. However, the dissolution process was still very rapid, even a solution with a high polymer content of 40% formed after only approx. 20 cycles (40 sec) (Figure 3.3B).

The possible drug release time periods from in situ systems also depend on polymer properties such as the molecular weight, lactide/glycolide ratio and end group functionality of PLGAs, which control the degradation process of the PLGAs. The dissolution time of various PLGA polymers in NMP increased with increasing molecular weight because of an increased solution viscosity (RG 502H vs. RG 503H) (Figure 3.3C). The uncapped PLGAs (free carboxylic end groups, marked H) RG 502H and 503H are more hydrophilic than the end-capped PLGAs RG 502 and 503 and required more cycles to be dissolved. Polymers with a lower glycolide content (RG 752 vs. RG 502) or pure D,L-lactide (R 203) also resulted in faster dissolution times because of the less hydrophilic character of the polymer and also due to the lower viscosity of their polymeric solutions (Figure 3.4)

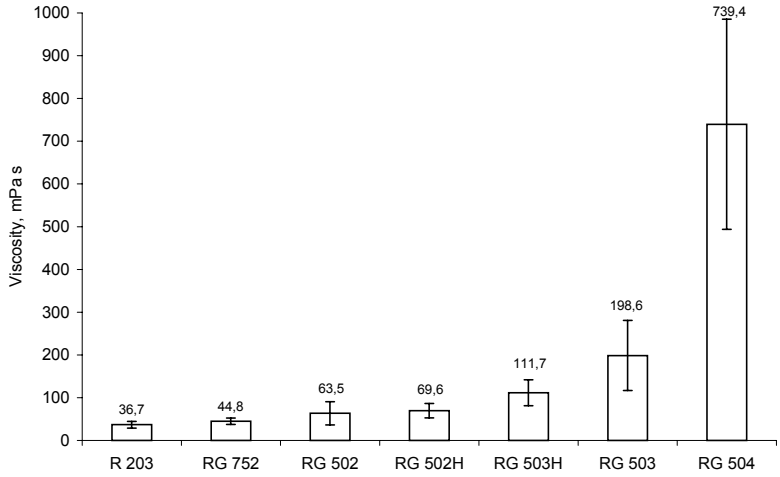


Figure 3.4 Viscosity of solutions prepared with different polymers at 20% w/w in NMP.

The mixing rate (number of cycles per second) did not affect the required number of cycles to dissolve the polymer (Figure 3.5A). This indicated a robust reconstitution process independent of variable mixing speeds applied by the end user. The dissolution time slightly increased with increasing syringe size at the same polymer solution volume because of a less intensive mixing with the larger syringes (Figure 3.5B).

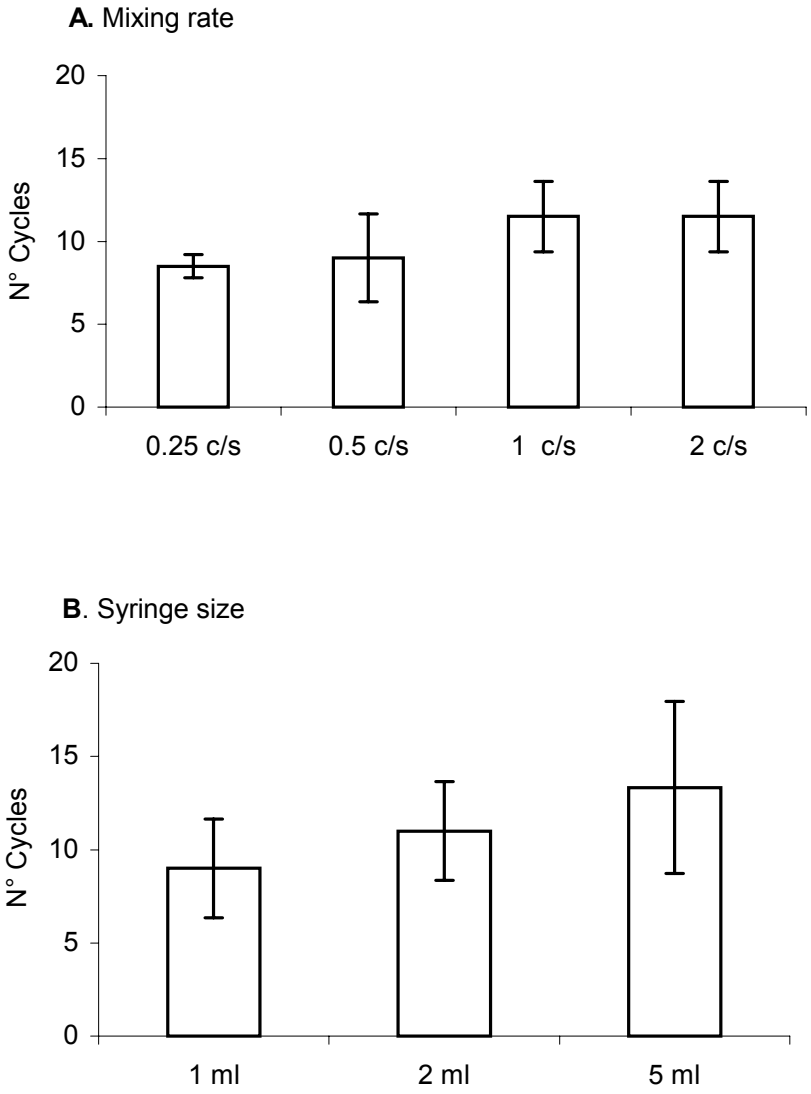


Figure 3.5 Effect of (A) mixing rate (1ml syringe size) and (B) syringe size (mixing rate of 0.5 cycles/s) on the dissolution time of RG 752 in NMP (10% w/w, polymer as received, n=3).

3.4 Freeze-drying

Powder filling of the PLGA particles into vial or syringes could be problematic because of the small amount of PLGA (approx. 30-100 mg) required per dose and because of the poor flow of small particles and static charge problems. Liquid filling is an interesting alternative, which could result in a better dosing accuracy and eliminate powder flow properties. For example, the polymer particles could be filled as an aqueous suspensions followed by lyophilization. Potential polymer degradation by hydrolysis (although the contact time with water would be short) and sedimentation problems during filling have to be addressed in this case. Alternatively, the polymer could be dissolved in an organic solvent followed by filling of the polymer solution into a syringe (vial) followed by lyophilization of the polymer solution, resulting in a highly porous cake (sponge).

An additional advantage of the lyophilization process is the easy incorporation of drugs into the in situ system. Drugs could be dissolved/dispersed in the polymer solution prior to freeze-drying thus assuring a homogeneous distribution of the drug within the reconstituted polymer solution and eliminating the problems seen with filling of small amounts of drug.

3.4.1 Solvent selection

Solvents for freeze-drying had to be identified, which dissolve PLGA and which can be frozen and be removed during the lyophilization process. The solvent should therefore possess a melting point, which allows freezing in the temperature range used in lyophilization (Tm > -70°C) and, ideally, a low toxicity. From many solvents commonly used to lyophilize, 1,4-dioxane and acetic acid fulfilled these criteria and were evaluated (Table 3.7).

Acetic acid and 1,4-dioxane are solvents for PLGA and can also be freeze-dried because of a high enough melting point and vapor pressure to allow removal by sublimation. Moreover, they belong to class 3 (acetic acid) and class 2 (1,4-dioxane) in accordance with the International Conference on Harmonization (ICH) guidelines. Class 3 solvents include no solvent known as a human health hazard at levels normally accepted in pharmaceuticals and class 2 solvents

should be limited. While class 1 solvents should not be employed in the manufacture of drug substances, excipients and drug products because of their unacceptable toxicity or their deleterious environmental effect [110].

Table 3.7 Solvents used for freeze- drying.

Solvent	Melting point °C (a)	Boiling point °C (a)	Vapor pressure hPa (20°C) (b)	Class (c)
1-butanol	-89,5	118.0	6,7	3
1,1,2-trichloroethene	-86,4	86.9	77,0	2
1,4-dioxane	**12,0**	**101.1**	**41,0**	**2**
1-propanol	-127,0	97.2	19,0	3
2-pyrrolidone	24,0	245,0	0,0	--
2-butanol	-114,0	99.5	16,5	3
2-methyl-1-propanol	-108,0	108,0	10,6	3
2-propanol	-89,5	82.4	43,0	3
3-methyl-1-butanol	-117,0	130.5	3,1	3
acetic acid	**10,0**	**118,0**	**15,0**	**3**
acetone	-95,4	56.5	233,0	2
acetonitrile	-45,7	81.6	97,0	2
acetylene dichloride	-95,0	60,0	475,0	2
benzaldehyde	-26,0	179,0	1,3	2
butyl acetate	-77,0	126,0	13,0	3
chlorobenzene	-45,0	132,0	12,0	2
chloroform	-63,0	61,0	213,0	2
cumene	-96,0	153,0	5,3	3
cyclohexane	6,0	80.7	103,0	1
dimethyl sulfoxide	-18,5	189,0	0,6	3
ethanol	-114,5	78.5	59,0	3
ethyl acetate	-83,0	77,0	97,0	2
ethyl ether	-116,3	34.6	587,0	2
ethyl formate	-81,0	54,0	256,0	3
Ethyl lactate	-25,0	154,0	1,6	3
formamide	2,0	210.5	0.08	2
formic acid	8 decomp.	100.5	42,0	3
heptane	-90,6	98.4	48,0	3
isobutyl acetate	-99,0	118,0	17,0	3
isopropyl acetate	-73,0	89,0	61,0	3
methyl acetate	-98,0	56.9	217,0	3
methylene chloride	-95,0	39.7	475,0	3
methylethyl ketone	-86,0	79.6	105,0	3
methylisobutyl ketone	-84,0	116-118	20,2	3
N-methyl-2-pyrrolidone	-24,0	202,0	0,3	--
pentane	-129,7	36.1	573,0	3
propyl acetate	-95,0	101.6	33,0	3
pyridine	-42,0	115.3	20,0	2
tert-butyl methyl ether	-108,6	55.3	268,0	3
tetrahydrofuran	-108,5	66,0	173,0	3
tetralin	-36,0	207.2	0,4	2
xylene	>-34	137-140	10,0	2

(a) Values from *The Merck Index* , 12th ed.; Budavari,S.,Ed,Merck: Whitehouse Station,NJ,1996

(b) Values from the web site: www.merck.de

(c) Values from Department of Health and Human services. Food and Drug Administration. International Conference on Harmonization;Draft Guideline on Impurities: Residual Solvents. 1997.

-- Not included on the list

3.4.2 Process conditions

The freeze-drying process consists of three stages: prefreezing, primary drying and secondary drying. During the freezing process the solution is transformed into a solid, usually water and solutes crystallize or, if amorphous, transformed to a rigid glass when the system is cooled below the glass transition temperature (Tg) of the amorphous phase. In practice, ice does not form at the thermodynamic or equilibrium freezing point, but normally nucleates and crystallizes only after supercooling to about 10-15°C below the equilibrium freezing point [79].

The freezing cycle of the lyophilization process was simulated by DSC, whereby the freezing point of the solvent was observed upon cooling of the PLGA solution. The freezing point of acetic acid decreased with increasing PLGA solution concentration from –2 to –17°C due to a stronger binding of the solvent and a higher solution viscosity retarding crystallization of the solvent (Figure 3.6A). The heat of fusion also decreased with increasing polymer content. The freezing point depression also depends on the type of polymer (e.g. lactide/glycolide ratio, capped vs. free carboxyl groups) and probably reflects different solvent-polymer interactions (Figure 3.6B).

The samples were frozen at –70°C in a freezer, which is a temperature well below the freezing point of the different formulations.

The freezing speed has influence in the structure of the final product, but this parameter will be discussed in 3.6.1

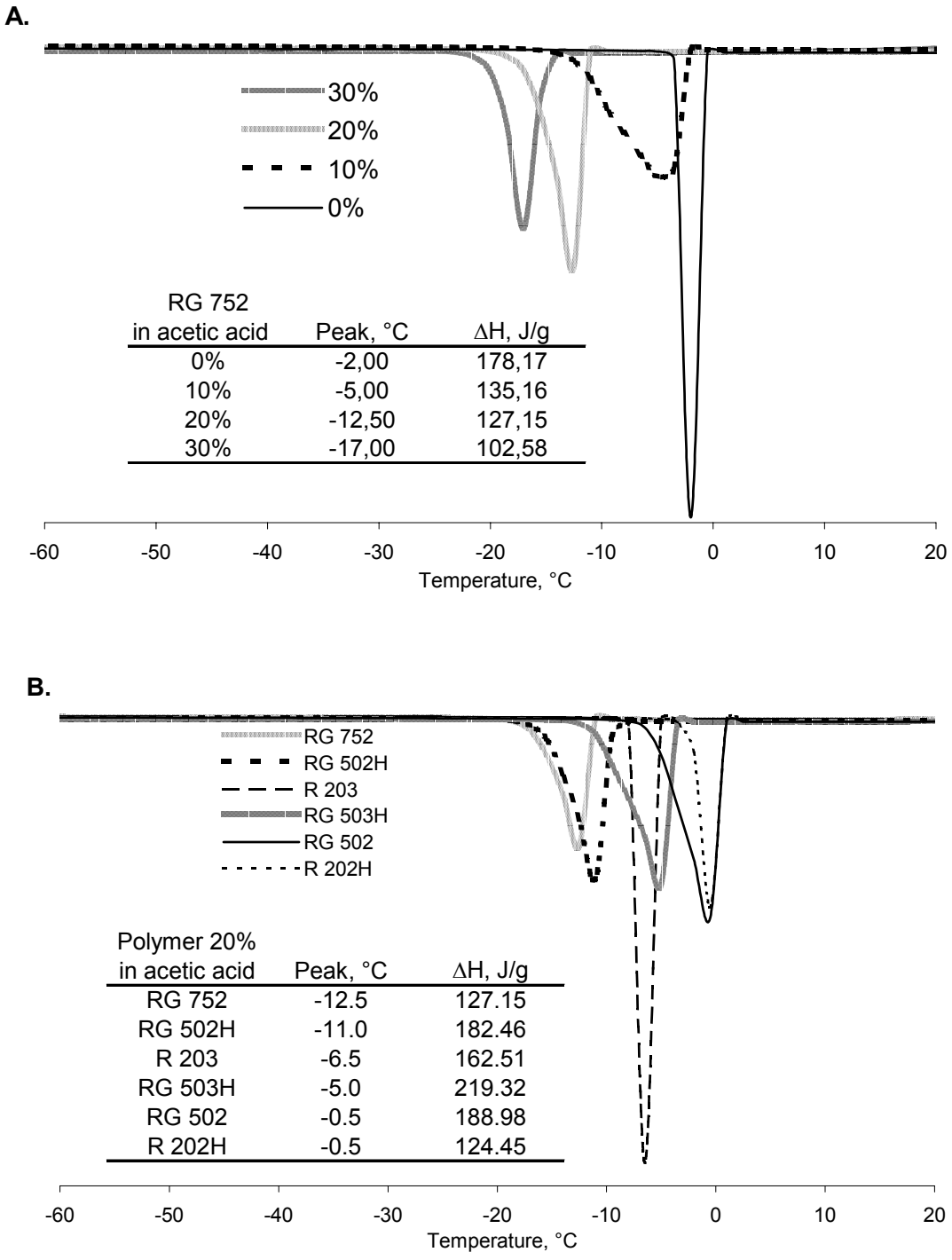


Figure 3.6 DSC thermograms of PLGA solutions: (A) RG 752 at different polymer concentrations in acetic acid; (B) different types of PLGA at 20% w/w in acetic acid.

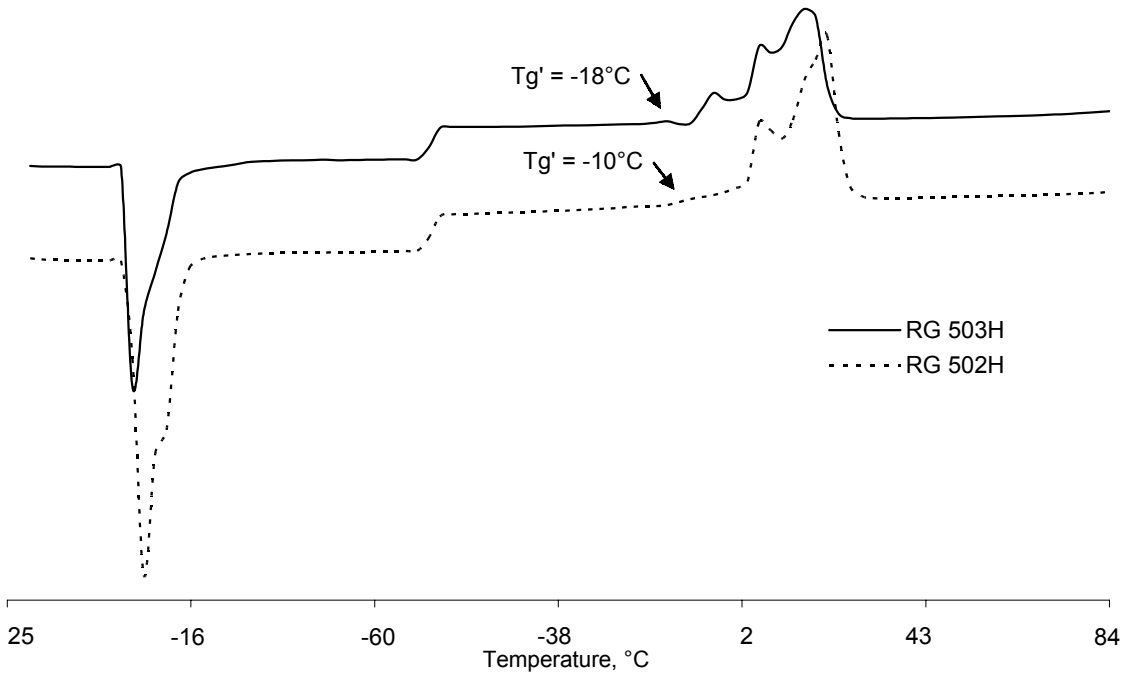


Figure 3.7 DSC thermogram of a solution of RG 503H and RG 502H with 10% lidocaine base in 1,4-dioxane (first run: 25°C to –70°C and second run: –70°C to 85°C).

After prefreezing the product, the appropriate conditions of temperature and pressure at which the solvent can be removed from the frozen product should be established. A balance between the temperature that maintains the frozen integrity of the product and the temperature that maximizes the vapor pressure of the product is the key for an optimum drying. No phase diagrams pressure vs. temperature were available, neither for acetic acid nor for 1,4-dioxane.

Therefore, the temperature (-40°C) and pressure (0.040 mbar) selected for the primary drying were as low as possible to ensure a process well below Tg' (glass transition temperature in the frozen state) of the different formulations and to allow a high sublimation rate (Figure 3.7). In the last stage of the process, the secondary drying, the objective is to reduce the residual moisture content to a level optimal for stability, which is usually less than 1%. Higher vacuum and a temperature increase above 0°C but below Tg of the product allow the desired water desorption. The conditions selected were 10°C and 0.040 mbar (Figure 3.8) [78, 79, 81].

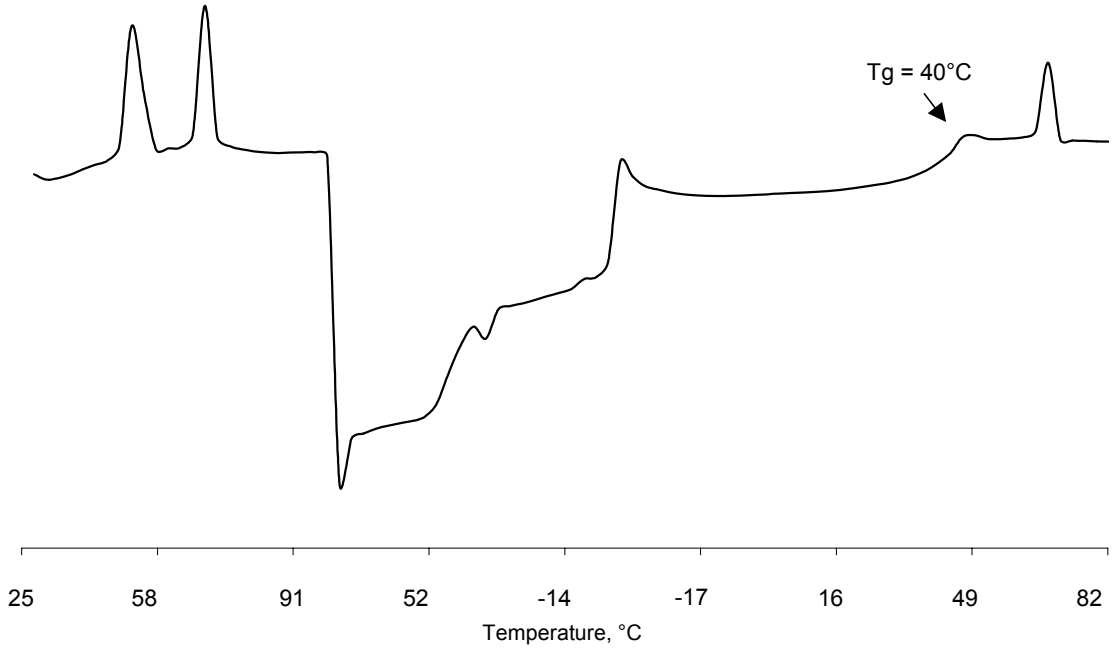


Figure 3.8 DSC thermogram of a physical mixture of RG 503H and 10% lidocaine base (first run: 25°C to –100°C, second run: 100°C to -40°C and third run: -40°C to 85°C).

3.4.3 Sponges dissolution rate

Porous, sponge-like matrices were obtained after lyophilization (Figure 3.9).

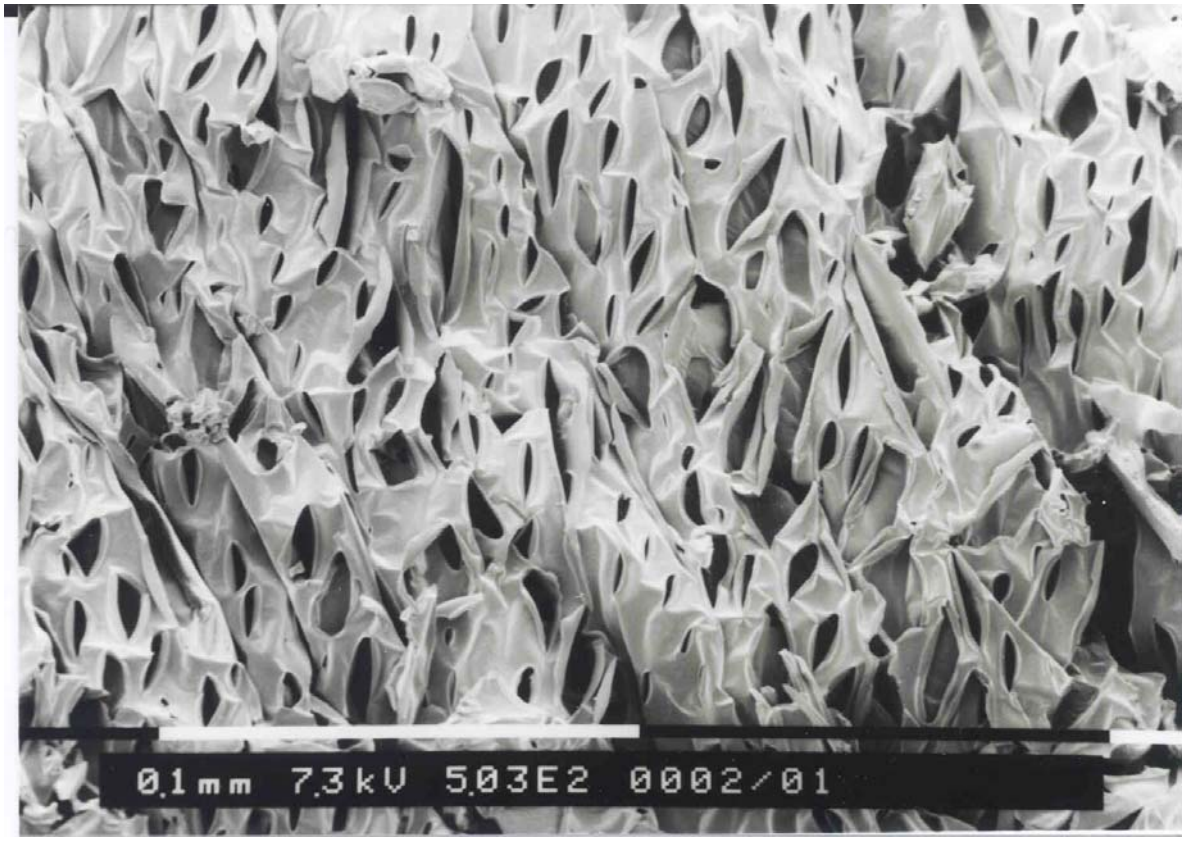


Figure 3.9 SEM picture of an R 202H sponge.

The PLGA sponges dissolved rapidly in different organic solvents and much faster than the received polymer powder because of the high internal surface area and rapid penetration of the solvent into the pores (the same number of cycles was obtained for each case n=3) (Figure 3.10)

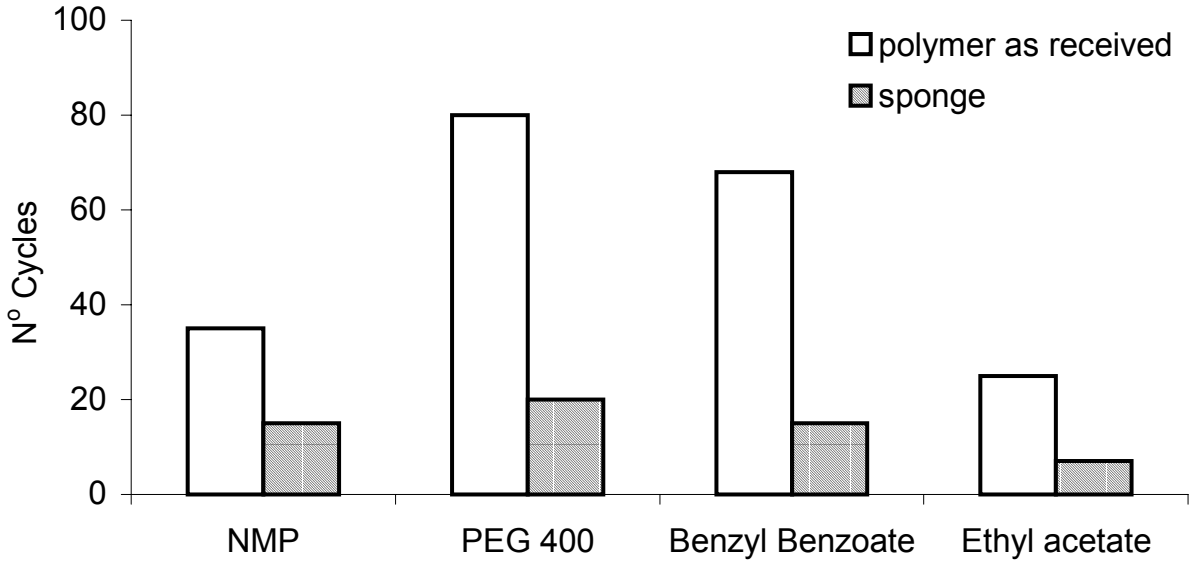


Figure 3.10 Dissolution time of freeze-dried PLGA-sponge in comparison with polymer (RG 752 as received from supplier) in different solvents (25% w/w based on solvent) for in situ implants preparation

3.5 In situ forming microparticles (ISM)

In contrast to in situ implants, which form after injection of PLGA solutions, in situ microparticles (ISM) are based on an emulsion of an internal drug-containing PLGA solution and an external water- (O/W-ISM) or oil- (O/O-ISM) phase [111, 112]. ISM-emulsion are generally prepared with the two syringe system, whereby the polymer and external phase are kept in separate syringes and are then mixed through the connector.

3.5.1 ISM dissolution rate

With ISM, the reconstitution process thus has to result in the dissolution of the polymer and the formation of the emulsion prior to injection. Surprisingly, both the polymer dissolution and emulsion formation were possible in less than a minute for both O/O- and O/W-ISM, even at a high polymer content of 30% (Figure 3.11 and Table 3.8).

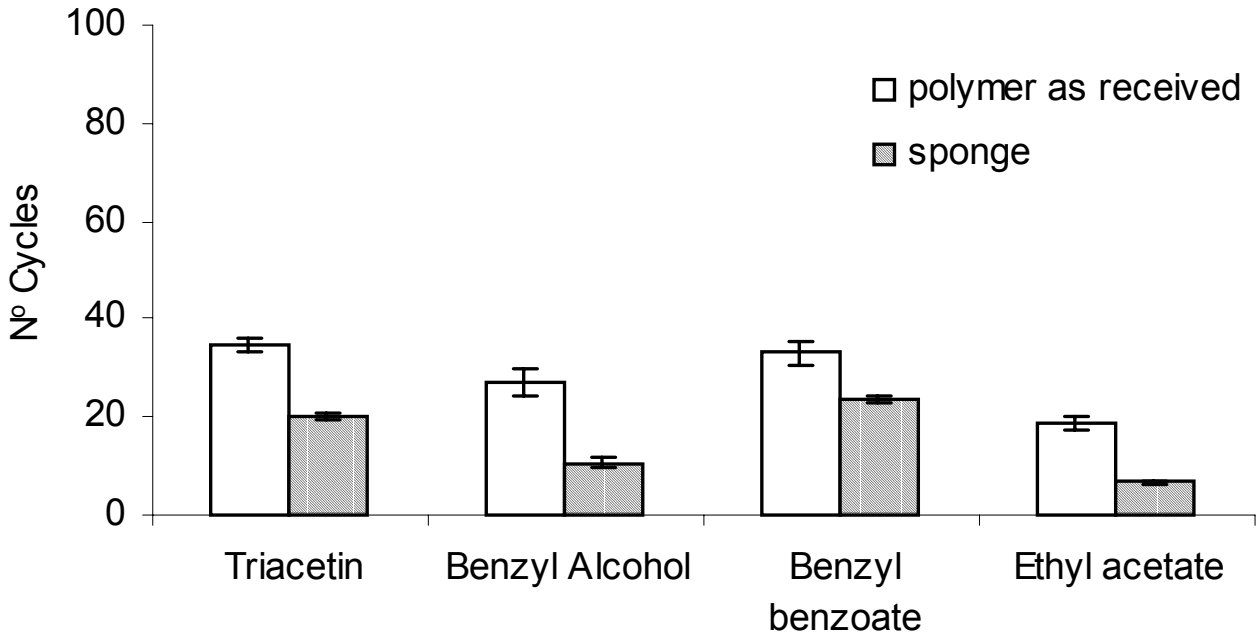


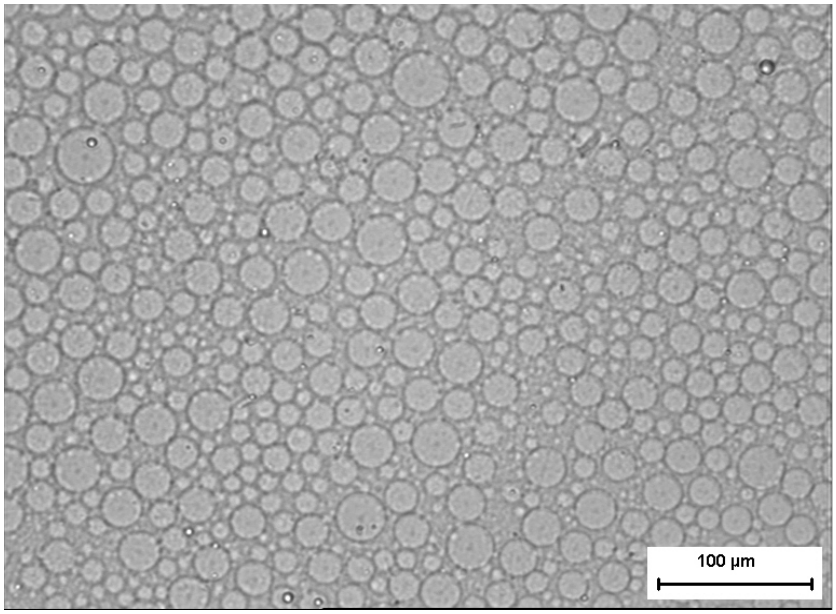
Figure 3.11 Dissolution time of freeze-dried PLGA-sponge in comparison with polymer (RG 752 as received from supplier) in different solvents (25% w/w based on solvent) for O/W-ISM (1 ml syringe size and 0.5 cycles/s).

Table 3.8 Rate of dissolution of RG 503H sponges lyophilized with 40% lidocaine base in O/O-ISM and O/W-ISM systems.

Solvent O/O-ISM	**Polymer concentration (w/v)**	**No. Cycles**
2-Pyrrolidone	30%	100
DMSO	30%	15
NMP	30%	15
O/W-ISM		
Triacetin	10%	30
Benzyl alcohol	20%	30
Ethyl acetate	30%	30

In general, the dissolution was faster with O/O- than with O/W-ISM systems, probably because of the higher shear force exerted by the more viscous external oil phase when compared to the less viscous external aqueous phase. Only droplets and no solid polymer were visible on photographs of the emulsions after 25 cycles (Figure 3.12).

A. O/O-system



B. O/W-system

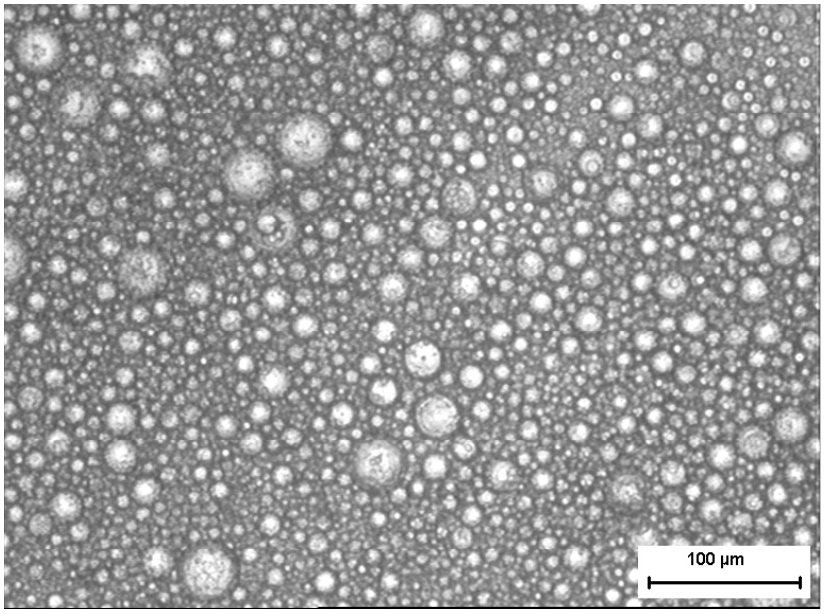


Figure 3.12 Pictures of ISM-emulsions after 25 mixing cycles (0.5 cycles/s), prepared with RG 752 10% w/w based on solvent: (A) O/O-system with 2-pyrrolidone as solvent and (B) O/W-system with ethyl acetate as solvent.

The droplet size was bigger with the O/O-ISM, because of the higher polymer solution viscosity in 2-pyrrolidone (O/O) than in ethyl acetate (O/W). The droplet size decreased with the number of increasing mixing cycles (Figure 3.13). The size of the droplets only slightly increased from 8.1 µm to 12.9 µm during a 2 h storage at a 10% polymer concentration and from 10.2 µm to 12.6 µm for 25% polymer concentration (Figure 3.14). The reconstituted emulsion, which would normally be injected immediately after preparation, thus could be used within a 2 h time frame after preparation.

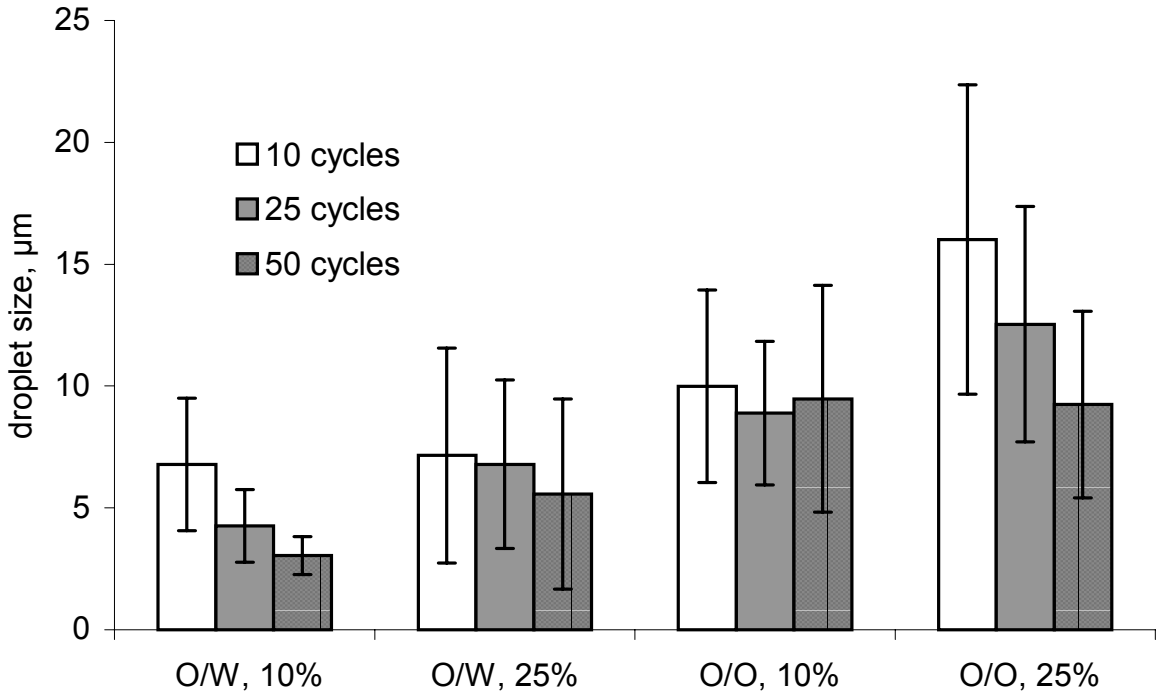


Figure 3.13 Effect of the number of mixing cycles on the droplet size of O/W- and O/O-ISM systems at two different polymer concentrations (w/w based on solvent) (polymer: PLGA RG 752, mixing rate:0.5 cycles/s).

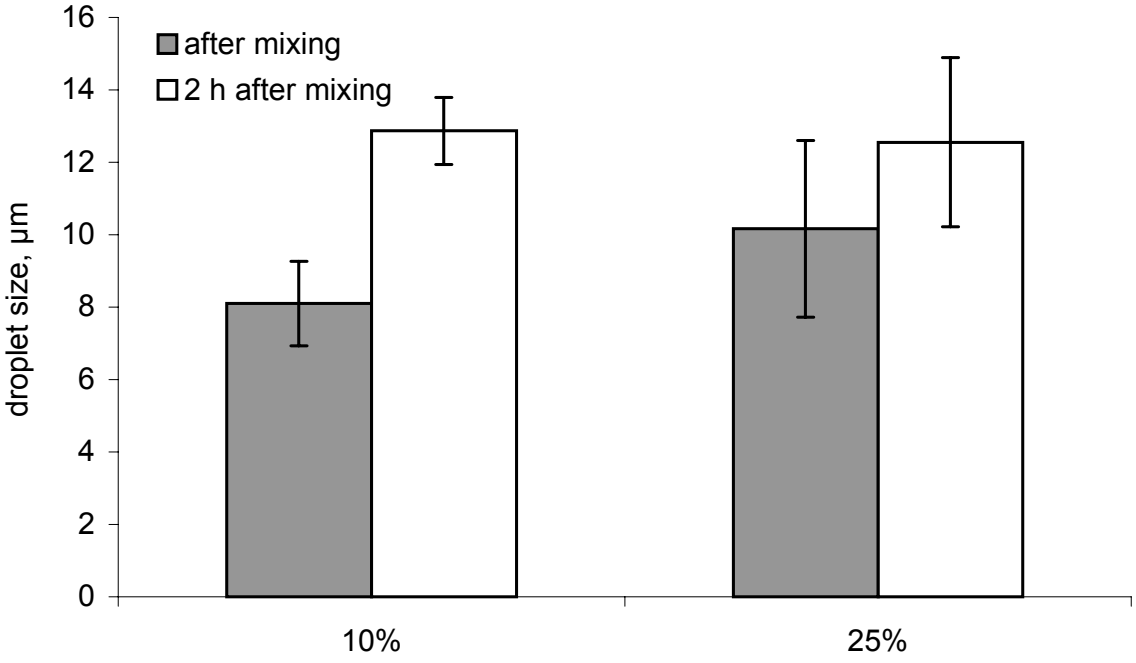


Figure 3.14 Stability of O/O-ISM prepared with RG 752 as received and NMP as solvent at two different concentrations, 10% and 25% w/w based on solvent (mixing rate:0.5 cycles/s).

3.5.2 Effect of the mixing method

Various possibilities exist to prepare the ISM-emulsions. Three different methods were compared (see section 2.2.6). In method A, the polymer was placed in one syringe and the organic solvent with the external phase (oil or water) in the second syringe without mixing (two phases); both syringes were then connected and the contents mixed to dissolve the polymer and form the ISM-emulsion. In method B, an emulsion of the organic solvent with the external phase was formed and was then pushed into the polymer-containing second syringe followed by mixing. In method C, the polymer was dispersed in the oil or

water and then added to the solvent-containing second syringe followed by mixing. Different ratios of solvent: water or oil (1:1, 1:2, 1:3 and 1:4) and different polymer particle sizes were tested with method C. O/W-systems dissolved fast, independent of the polymer particle size or the method used. However, an increase in the amount of external water phase resulted in gel formation during mixing due to polymer precipitation prior to emulsion formation because of rapid diffusion of the organic solvent into in the external phase. The dissolution time of PLGA in O/O-ISM systems decreased with the particle size decrease and improved with method C, PLGA dispersion in the external oil phase avoids agglomeration and allows a better dissolution when in contact with the solvent. Nevertheless, increase in the phase ratio above 1:2 did not improve the dissolution rate (Table 3.9).

Table 3.9 Dissolution time of RG 503H in ISM-systems prepared by different mixing methods: O/W-systems, 20% w/v polymer in benzyl alcohol and O/O-systems, 20% w/v polymer in DMSO.

	Method A	**Method B**		**Method C**		
polymer	**1:1**	**1:1**	**1:1**	**1:2**	**1:3**	**1:4**
O/W-ISM						
As received	30	30	25	25 G	25 G	25G
< 160 µm	25	30	28	15 G	20 G	15
Lyophilized	30	-	-	-	-	-
O/O-ISM						
As received	*	*	*	*	*	*
< 160 µm	90	90	40	10	10	10
< 50 µm	30	35	15	10	10	10
Lyophilized	15	-	-	-	-	-

G- gel formed
* polymer lump formed
'- not determined

3.6 Biodegradable implants

Sponge-like implants were prepared by lyophilization of PLGA solutions. The polymer and drug were dissolved in a solvent. This solution was filled in a mold, frozen and then lyophilized to remove the solvent. The advantages of this implant preparation technique compared to other classical techniques (e.g., melt compression, extrusion) are the lack of elevated temperature and a good drug distribution and hence content uniformity throughout the PLGA matrix.

3.6.1 Physical properties of the implants

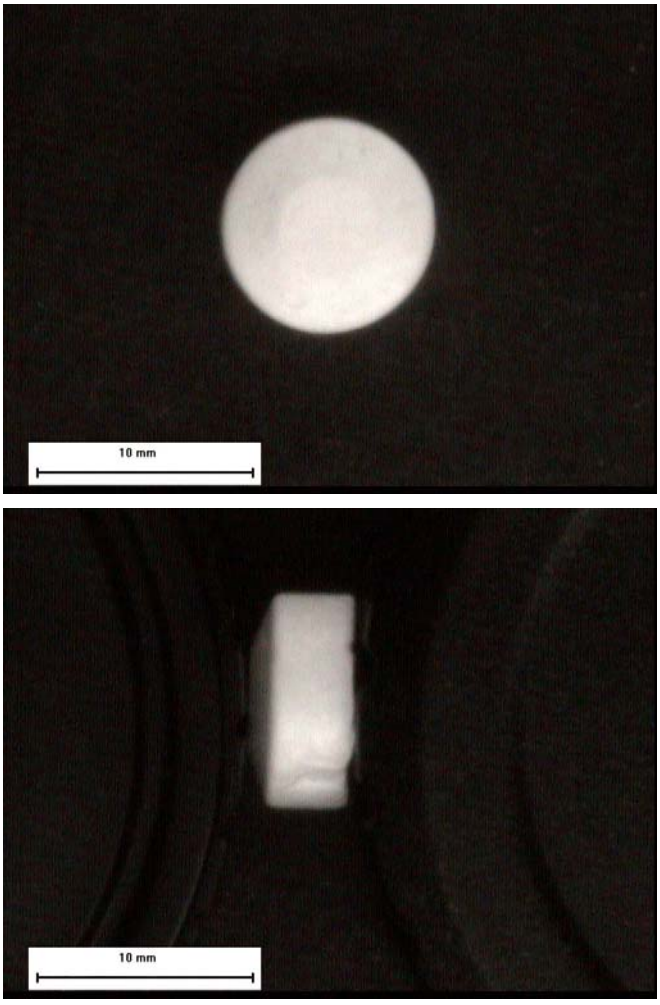


Figure 3.15 Macroscopically pictures of implants prepared by lyophilization of 20% RG 503H in acetic acid.

Morphology

Porous, sponge-like implants were obtained. 1,4-dioxane resulted in implants with regular-shaped pores and a continuous PLGA network, while acetic acid resulted in a more irregular structure, like leaflet or platelet structure. The pore structure depends on the geometry of frozen solvent crystals before sublimation and the polymer phase separation during the freezing process (Figure 3.16A-D) [113]. Foda et. al founded that sheet-like structures are produced by partial collapse of pores during freeze-drying, since unorganized ice crystal growth may induce irregular sublimation with recrystallization of solvent vapor and secondary sublimation in the same areas during the lyophilization process. While a continuous open channel structure is obtained after a regular ice crystal growth leading to a more regular sublimation between the surface and the interior of the sponge without recrystallization [114, 115].

The freezing rate of the polymeric solutions strongly affected the morphology and pore structure/size of lyophilized cakes. Implants prepared by a rapid freezing of the PLGA solution have a visually denser structure with smaller pores when compared to larger pores obtained with slower freezing rates (Figure 3.16C and E). A higher degree of supercooling and a low temperature induce a high nucleation rate and a low crystal growth rate, leading to the formation of a large number of small crystals. On the other hand, a relatively low degree of supercooling at a higher temperature, produce a small number of large crystals, due to the low nucleation rate and the high crystal growth rate [89, 115, 116].

A. Acetic acid – cross section

B. Acetic acid – surface

C. 1,4-dioxane – cross section

D. 1,4-dioxane – surface

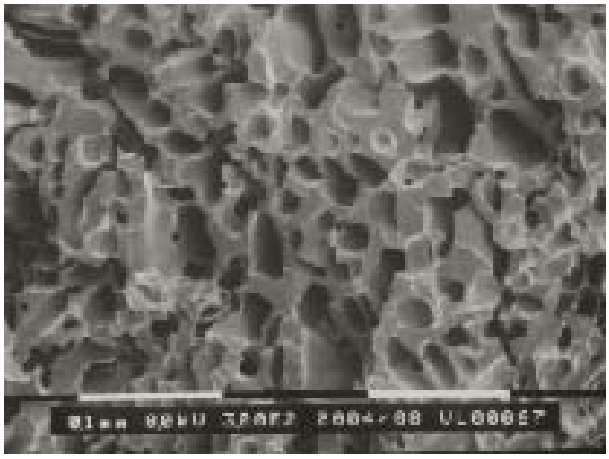
E. 1,4-dioxane – cross section fast freezing

Figure 3.16 SEM pictures of sponges prepared with (A, B) 10% RG 503H in acetic acid at slow freezing rate; (C, D) 10% RG 503H in 1,4-dioxane and at slow freezing rate; (E) 10% RG 503H in 1,4-dioxane at fast freezing rate.

As expected, at higher polymer concentrations more dense matrices were obtained (Figure 3.17). The polymer type affected the matrix structure, since more rigid structures with well interconnected pores were obtained at high molecular weight polymers (Figure 3.18B, D and F) or pure D,L-lactide polymer, as R 202H (Figure 3.18A). Low molecular weight polymers (RG 752 or RG 502H) presented superimposed porous layers (Figure 3.18C and E).

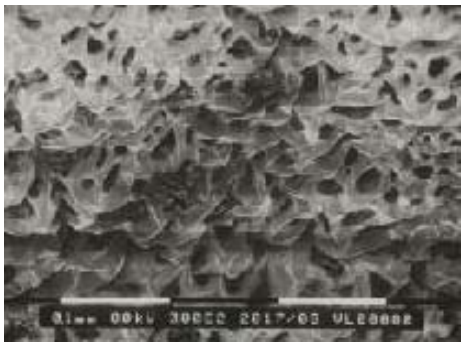
A. 5%

B. 20%

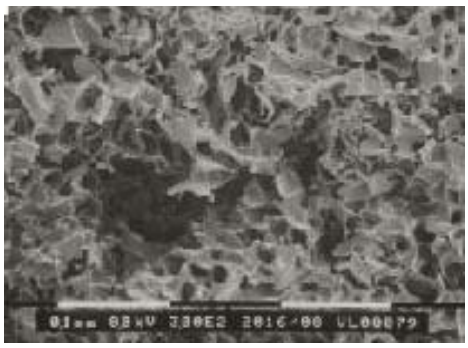


C. 30%

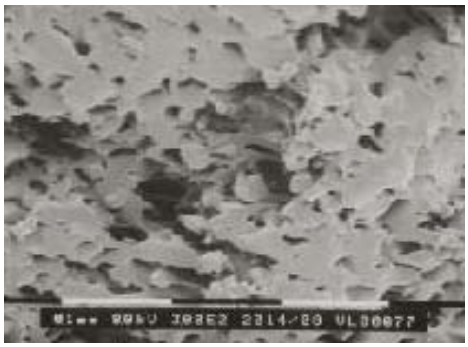
Figure 3.17 SEM pictures of sponges prepared with RG 503H in 1,4-dioxane at different concentrations and slow freezing rate, (A) 5%, (B) 20% and (C) 30%.



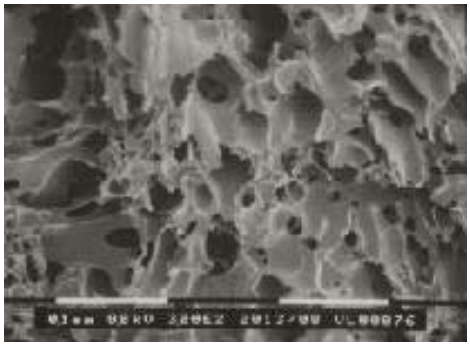
A. R 202H



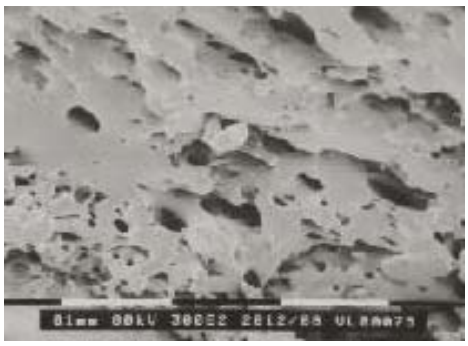
B. R 203



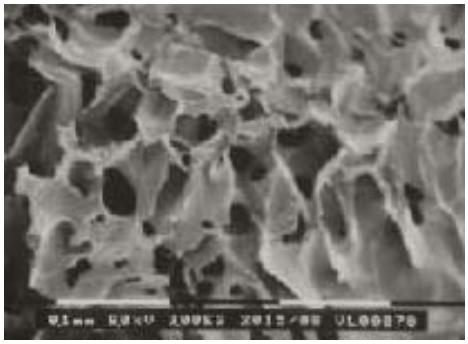
C. RG 502H



D. RG 503



E. RG 752



F. RG 755

Figure 3.18 PLGA sponges prepared with 10% drug loading (lidocaine base) in 10% 1,4-dioxane solutions of different polymers (A) R 202H, (B) R 203, (C) RG 502H, (D) RG 503, (E) RG 752 and (F) RG 755.

Glass transition temperature (Tg) and residual solvent

After freeze-drying, the glass transition temperature of the sponges decreased as a consequence of the residual solvent, that act as a plasticizer for the polymer [117]. Sponges prepared with 1,4-dioxane presented less residual solvent but a more significantly decline of Tg, suggesting a stronger plasticizer effect of 1,4-dioxane in comparison with acetic acid that did not show a significantly decrease (Table 3.10). Explained by the higher affinity of the polymer with 1,4-dioxane, a non-polar solvent (37,2% polarity), compared with the higher polar acetic acid (73,4% polarity). The freezing rate has no influence neither in the Tg nor in the residual solvent of the sponges, while the presence of lidocaine base decreases the Tg values of the sponges, as explained in 3.6.2 (Table 3.10).

Table 3.10 Glass transition temperature (Tg) and % of residual solvent of sponges prepared with 30% of PLGA RG 503H in two different solvents and 10% lidocaine base.

Polymer	Freezing rate	Solvent	Drug	Tg, °C (a)	Res. Solv., % (a)
RG 503H	slow	acetic acid	no drug	46.1(49.9)	1.4 (0.5)
RG 503H	slow	acetic acid	lidocaine base	38.5	2.5
RG 503H	slow	1,4-dioxane	lidocaine base	29.3	1.6
RG 503H	slow	1,4-dioxane	no drug	34.8	---
RG 502H	slow	acetic acid	no drug	47.3 (46.7)	1.5 (0.6)
RG 502H	fast	acetic acid	no drug	48.5	1.4

(a) Value on parenthesis corresponds to the polymer as received.

'--- not measured

The Tg value for the different polymers tested decreased between 11°C to 17°C after lyophilization in the presence of lidocaine base (Table 3.11), but without influence of the type of polymer used. Sponges prepared from PLGAs with free carboxylic end groups (H-grades) had a slight increase in the residual solvent content than sponges prepared from the end-capped polymers. The solvents thus had a few more affinity for the more hydrophilic H-grade polymers (Table 3.11)

Table 3.11 Glass transition temperature (Tg) and % of residual solvent of sponges prepared with 20% of different PLGA polymers in acetic acid and 10% lidocaine base.

Polymer	Freezing rate	Tg °C (a)	Residual solvent (a)
R 202H	slow	38.7 (50.7)	1.7%
R 203	slow	35.5 (52.9)	1.0% (0.5%)
RG 502H	slow	32.5 (46.7)	1.7% (0.6%)
RG 503	slow	38.7 (50.2)	1.4% (0.4%)
RG 504H	slow	38.8 (50.1)	1.9%

(a) Value on parenthesis corresponds to the polymer as received.

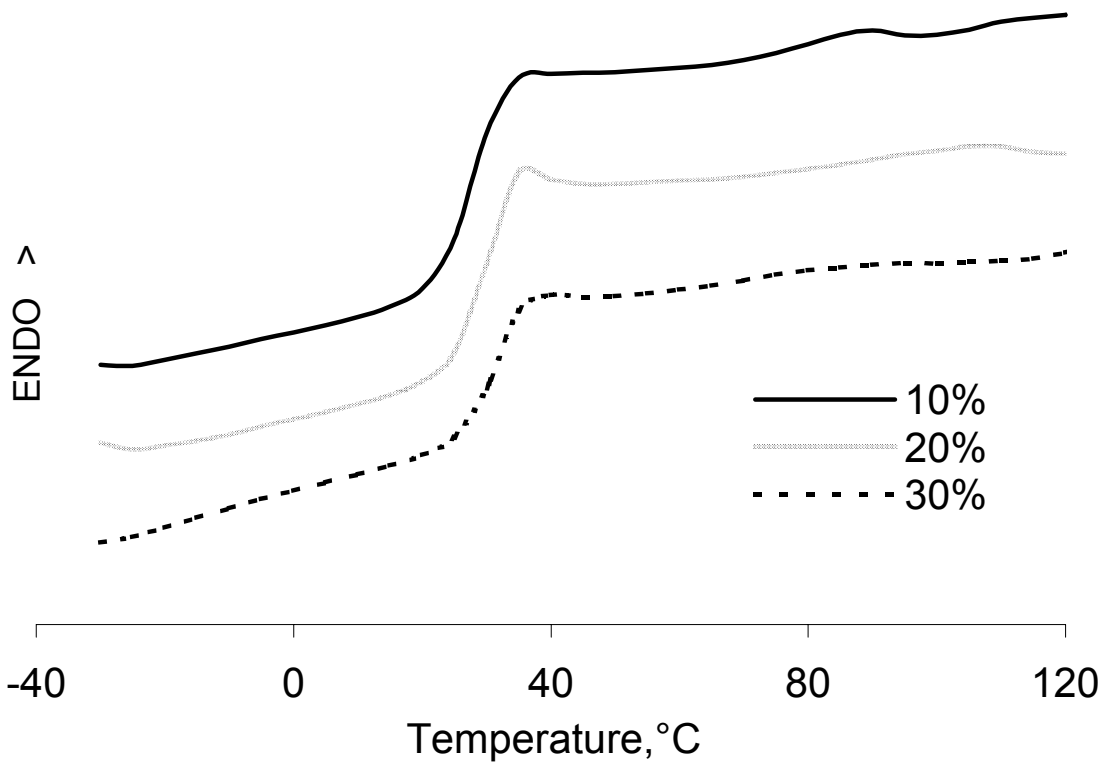


Figure 3.19 Glass transition temperature (Tg) of sponges prepared at different RG 503H concentrations in 1,4-dioxane without drug.

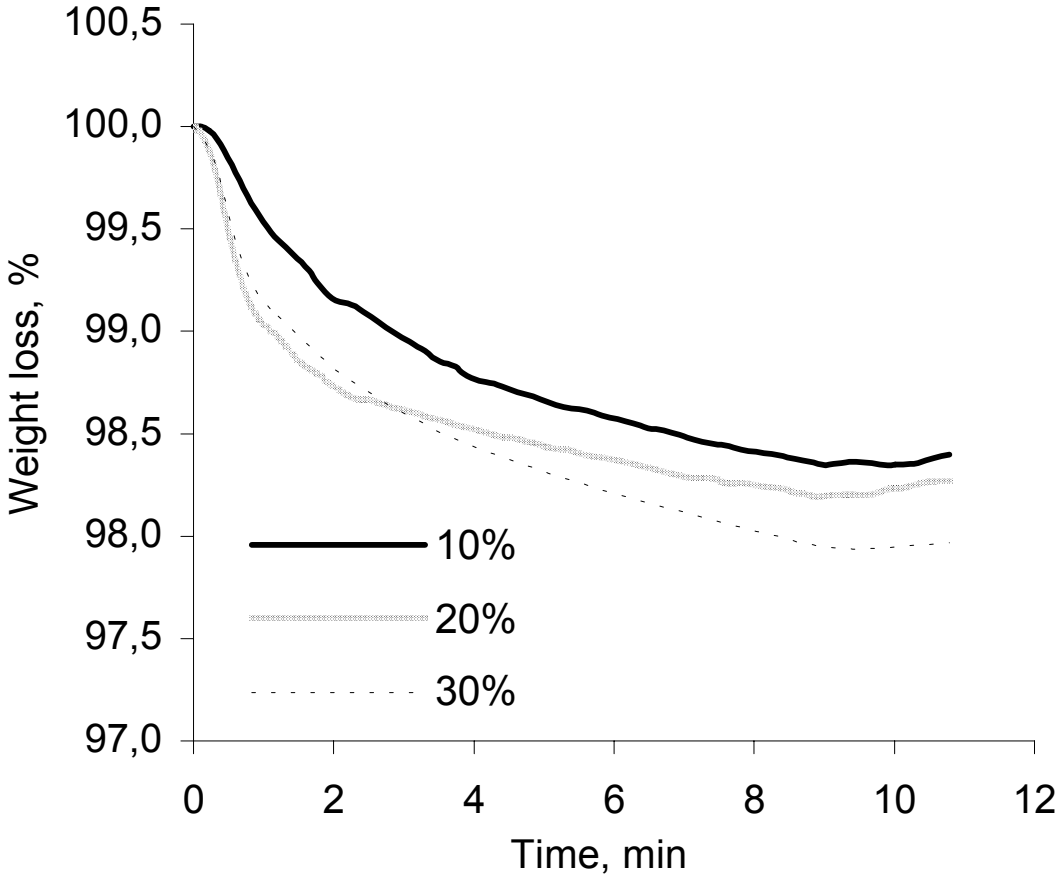


Figure 3.20 Percentage of residual solvent in sponges prepared with RG 503H at different concentrations in acetic acid and 10% lidocaine base.

The glass transition temperature, as well as the content of residual solvent were not influenced by the polymer concentrations used (Figure 3.19 and 3.20)

Mechanical properties

According to the application intended for the polymeric implants, the mechanical properties of the sponges must be sufficient so that it does not collapse during manipulation, insertion or use. Since no specific use for the PLGA sponges is here defined, the strength and % of recovery of the implants was assessed after variation of some process and formulation parameters, in order to find the versatility of the device.

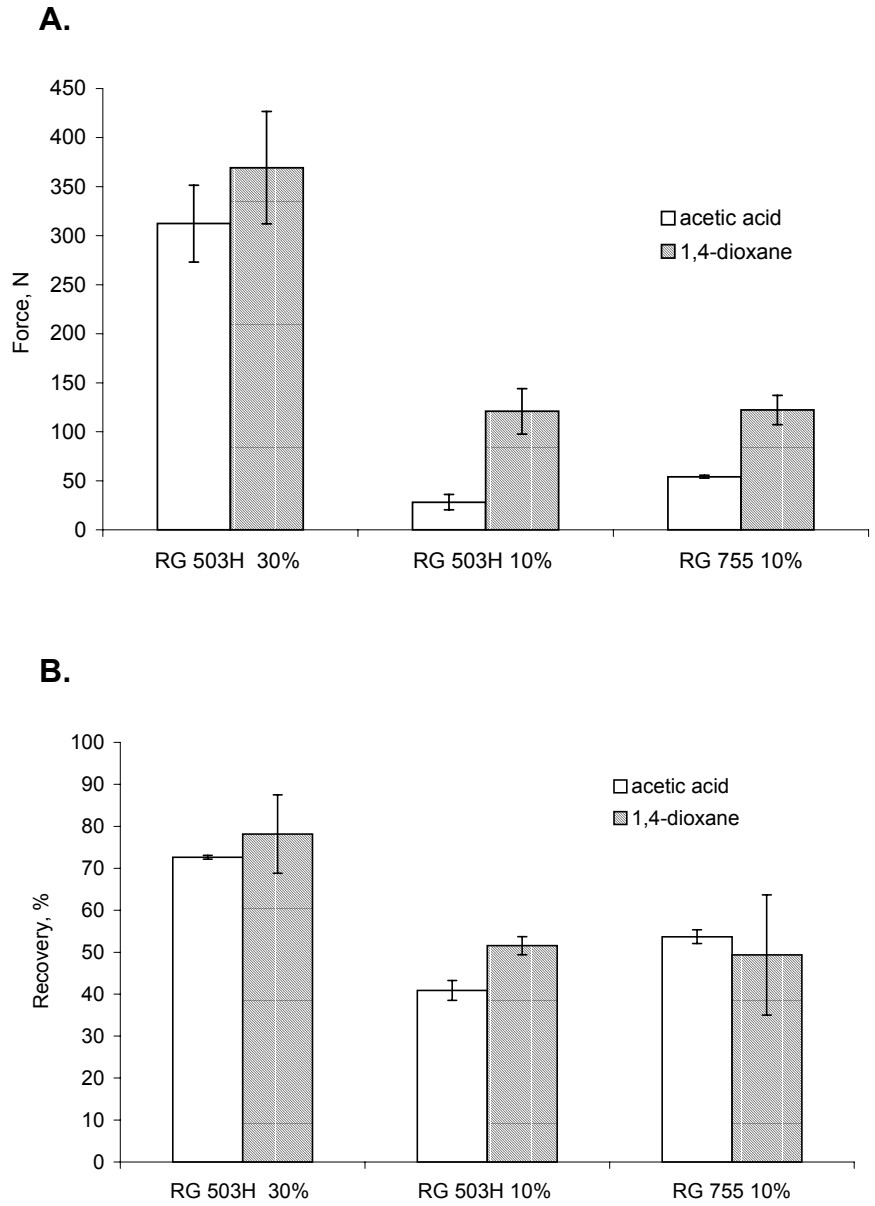


Figure 3.21 (A) Compressive strength (Force) and (B) % of recovery of sponges prepared with two different polymers at 10% and 30% concentration in two different solvents.

Implants prepared with 1,4-dioxane had a higher mechanical strength than implants prepared with acetic acid. A non well defined and irregular structure will be less resistant, as was the case of acetic acid sponges (Figure 3.21A) [118]. However the differences in the percentage of recovery were not marked (Figure 3.21B).

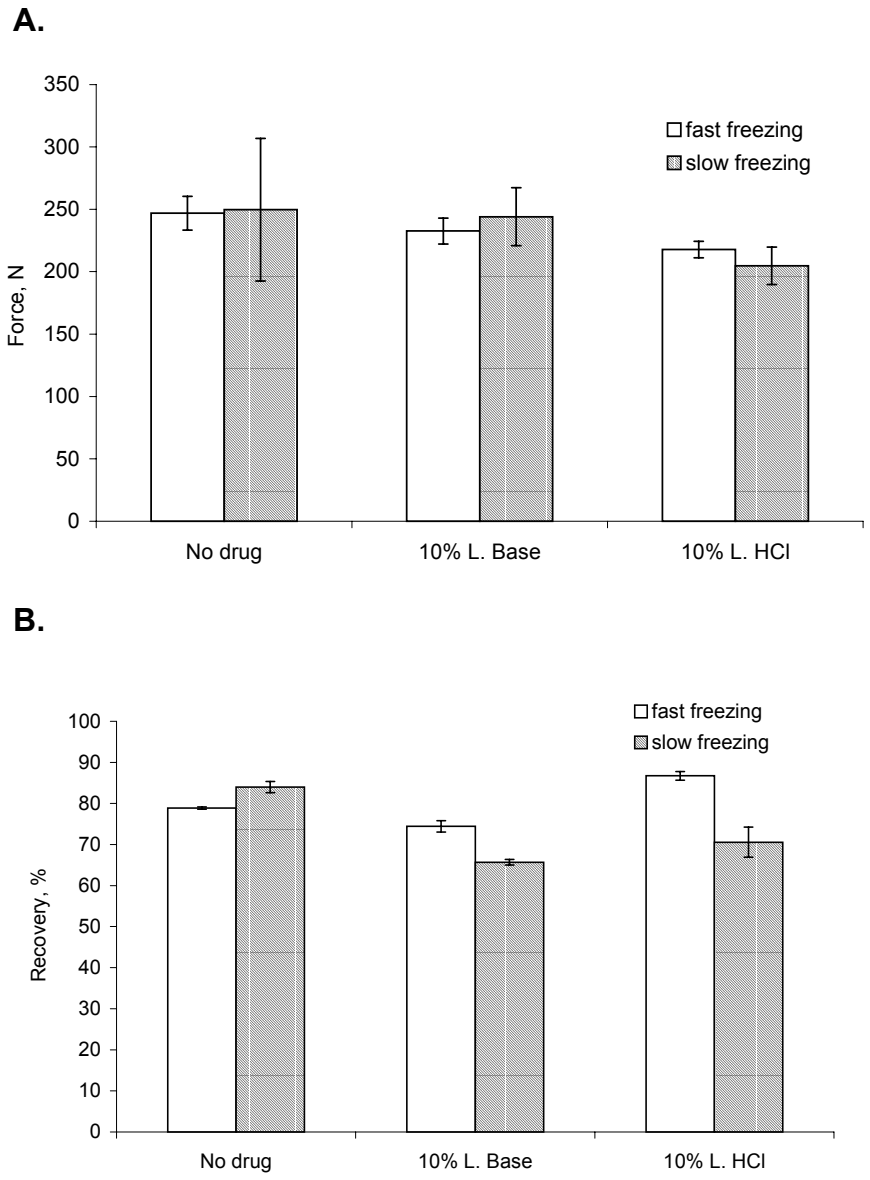


Figure 3.22 (A) Compressive strength (Force) and (B) % of recovery of sponges prepared with RG 502H 30% in acetic acid, at two different freezing rates.

Surprisingly, no difference in the mechanical properties of sponges prepared by different freezing rates was found (Figure 3.22). Since more porous sponges present weaker mechanical strength, expected was a higher resistance in sponges freezed slowly (Figure 3.16A and B) [119]. However, this suggests that the difference in the freezing rate will alter the pore size but not the porosity.

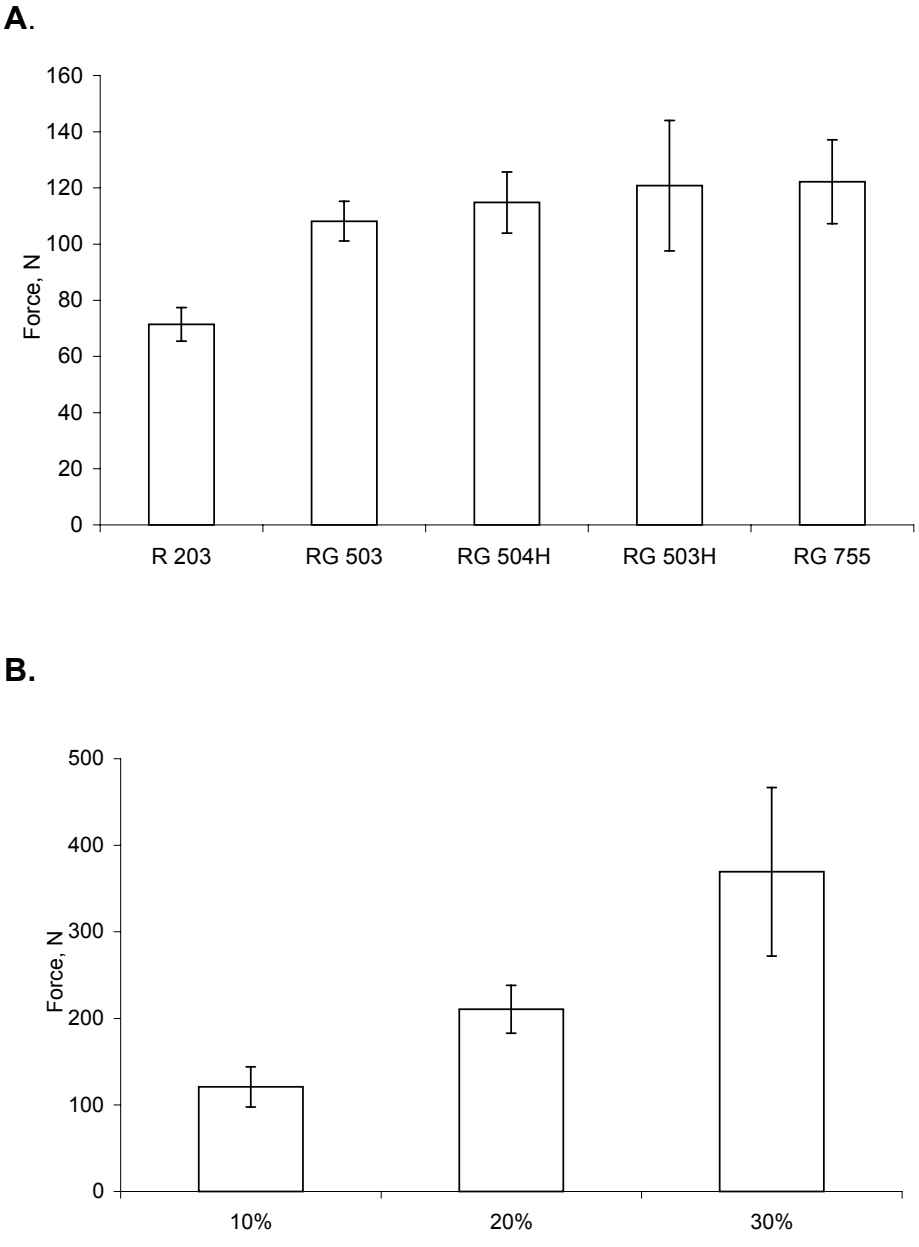


Figure 3.23 Compressive strength (Force) for sponges prepared at (A) 10% of different PLGA polymers in 1,4-dioxane and 10% lidocaine base and (B) three different concentration of RG 503H in 1,4-dioxane.

The mechanical properties were influenced mainly by the type and concentration of the polymer used (Figure 3.23 and 3.24). Pure D,L-lactide sponges (R203) were less resistant than those prepared with the copolymers, this is in agreement with the technical information that reports lower tensile strength for D,L-PLA (27.6-41.4 MPa) in comparison with PLGA (41.4-55.2 MPa) [14]. Furthermore, the strength was directly related to the polymer concentration, since denser matrices possess higher resistance to compression (Figure 3.23 and 3.17).

No effect of the drug state or loading was observed (Figure 3.22 and 3.24).

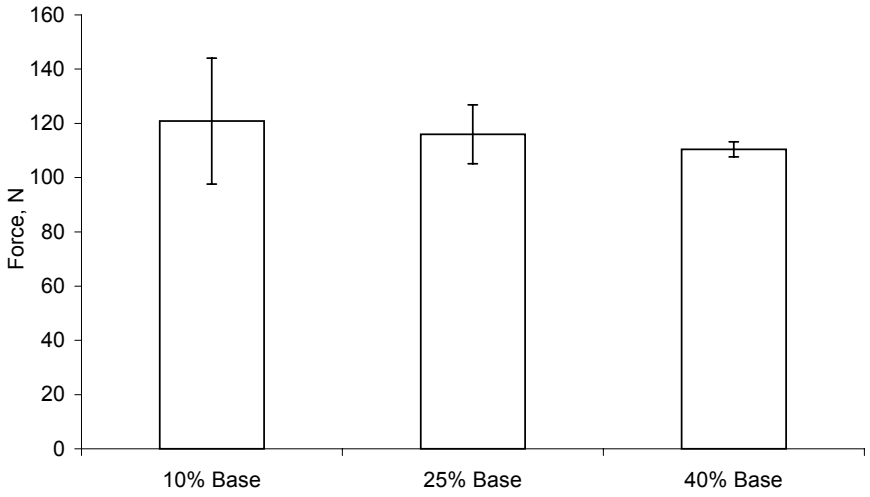


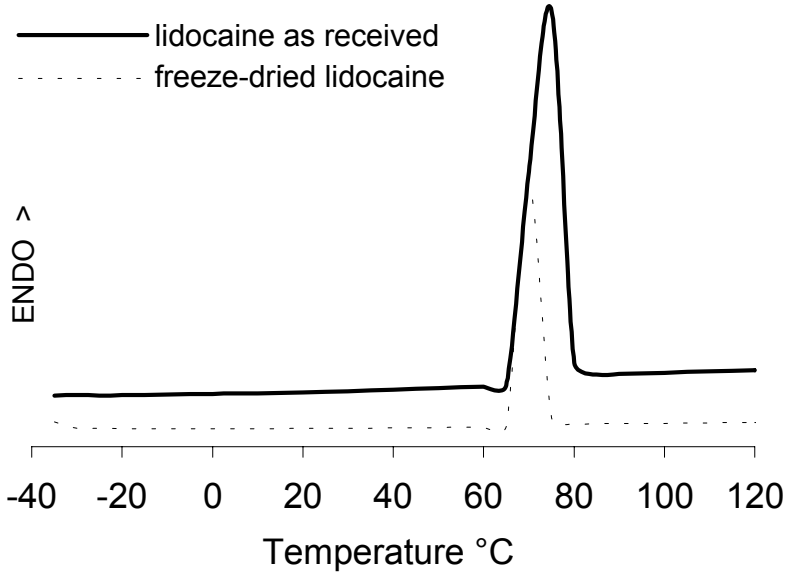
Figure 3.24 Compressive strength (Force) for sponges prepared at 10% RG 503H in 1,4-dioxane and different lidocaine base concentrations.

3.6.2 Drug distribution

The implants were prepared by freeze-drying of drug-containing PLGA solutions. After freezing, the drug could be either within the polymer phase and or within the frozen solvent phase. In the later case, the drug would then be present within the pores. Drug present within the polymer matrix could be either dissolved or dispersed (amorphous or crystalline) in the polymer.

Lidocaine remains crystalline after freeze drying (Figure 3.25A), but sponges prepared by freeze drying a drug-polymer solution in 1,4-dioxane presented no signs of cristallinity (Figure 3.25B and 3.26). Films were clear in appearance, except for the formulation with 40% lidocaine, which presented some drug crystals in the polymer film (data not shown).

A.



B.

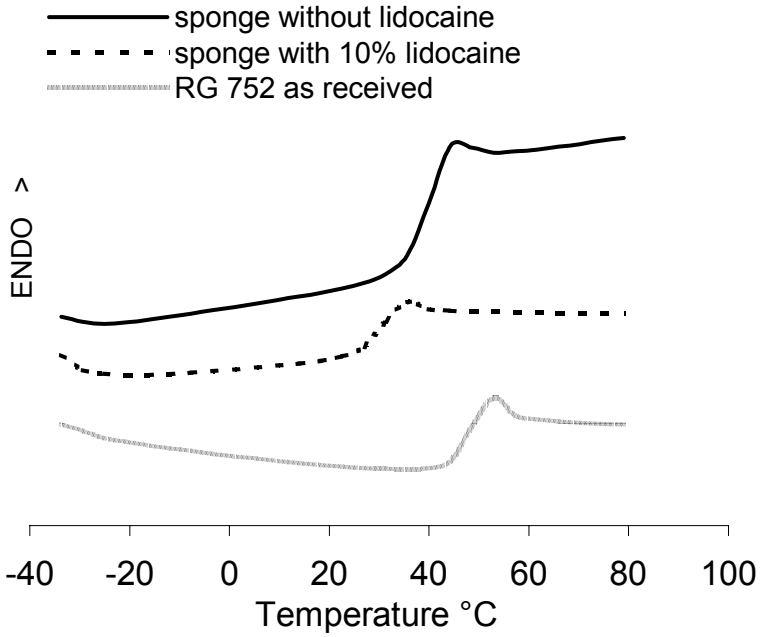


Figure 3.25 DSC thermograms of (A) lidocaine base as received and lyophilized (B) polymer as received and correspondent sponges with and without 10% lidocaine base.

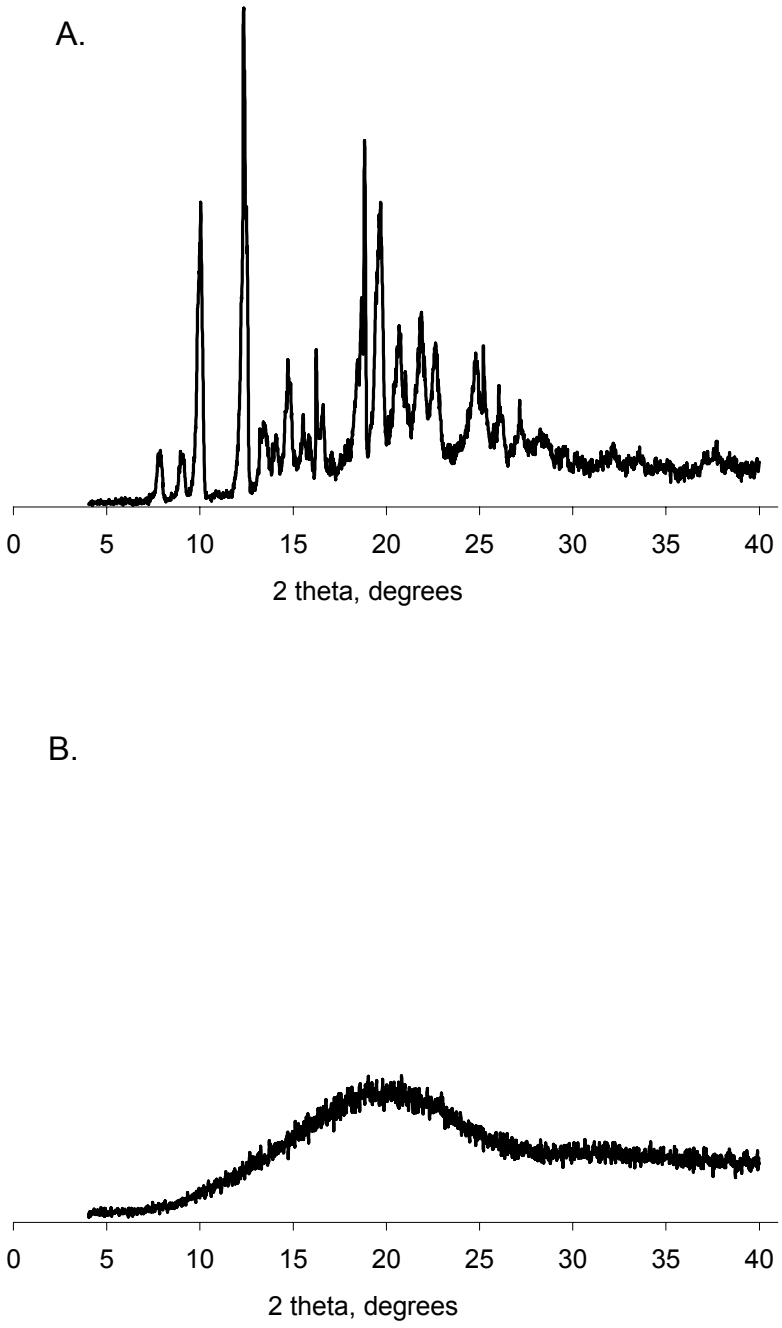


Figure 3.26 XRD of (A) freeze dried lidocaine base and (B) sponge with 10% lidocaine base.

In the first run of DSC thermograms of physical mixtures of drug and polymer, two peaks appeared, at 56°C (polymer Tg) and between 68-70°C (lidocaine base) or between 79–80°C (lidocaine hydrochloride). The area under the melting peak of the drug was directly proportional to the amount of drug present. In the second run no

drug melting peak was observed, except for the sponges with 40% lidocaine base (Figure 3.27).

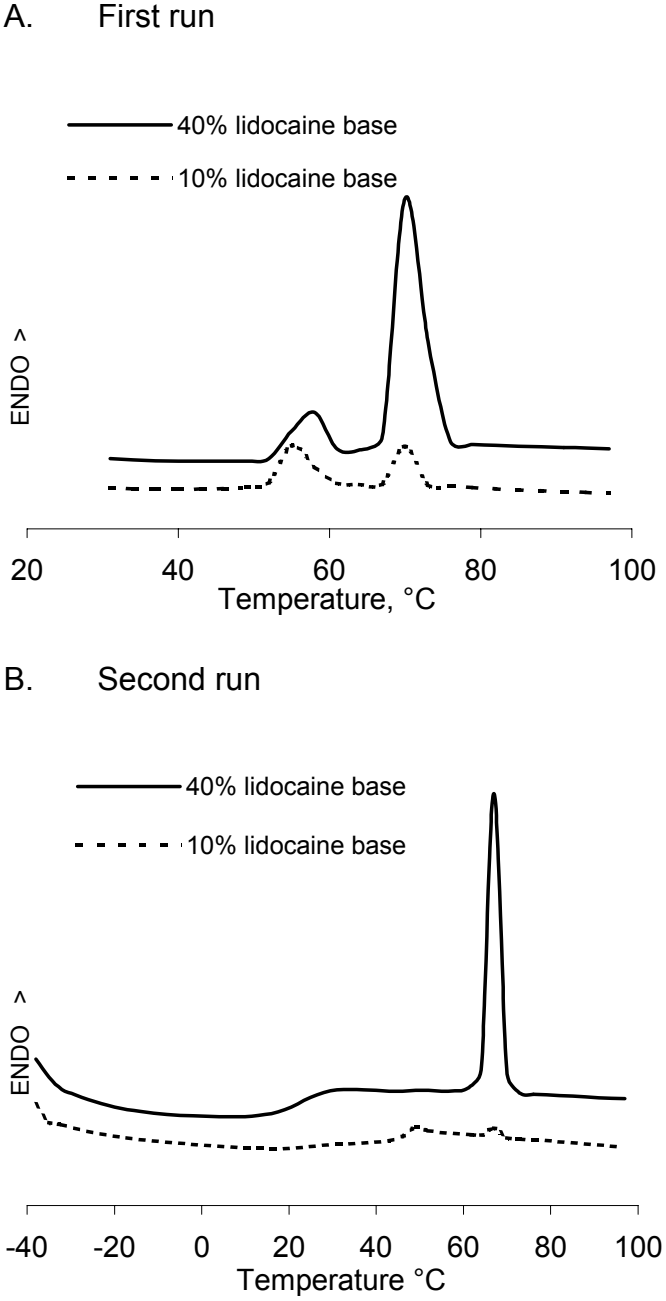


Figure 3.27 DSC thermograms of physical mixtures of sponges of RG 503H with lidocaine base (A) first run (B) second run.

The lowering and broadening of the melting point of the drug, as much as to make it undetectable using DSC, as well as an amorphous state by XRD are signs of solid solubility [120, 121]. The lidocaine / PLGA solubility is confirmed by comparison of their solubility parameters. It was demonstrated that two compounds are likely to be miscible when the difference between their solubility parameters is less than 7 $MPa^{1/2}$ [18]. Lin and Nash reported three lidocaine base solubility parameter values obtained by three different methods, 21.89 $MPa^{1/2}$ (Fedor's group contribution method), 23.52 $MPa^{1/2}$ (Hildebrand solubility parameter determined by the three solvent system) and 22.91 $MPa^{1/2}$ (Hildebrand solubility parameter determined by the five solvent system) [19]. Shivley et al. determined experimentally the solubility parameter value for PLGA, to be 20.05 $MPa^{1/2}$ [12]. Since the solubility parameter value for the lidocaine is very close to that of PLGA, solubility of the drug in the polymer can be assumed.

The presence of drug affected the Tg of the polymer, it decreased from 49.9°C (polymer as received) to 38.5°C for lidocaine-containing sponges (Table 3.9). This suggested a plasticizing effect of the lidocaine. Chen et al. also reported a lidocaine plasticizing effect on PLA-based microspheres [122]. The plasticizing effect was observed also with the physical mixtures. After the second heating run, once polymer and drug have been dissolved, the Tg of polymer decreased. The maximum solubility of the lidocaine base within the polymer was found to be between 25% and 40% loading, since recrystallization was found at 40% drug concentration but not at 25% (Figure 3.28). In the case of lidocaine hydrochloride, sponges even at 40% drug loading presented no recrystallization peak. DSC results of the drug as received showed no recrystallization during the cooling and consequently no melting point after reheating (Figure 3.29). This then presumes that the drug remains amorphous or degrades after melting.

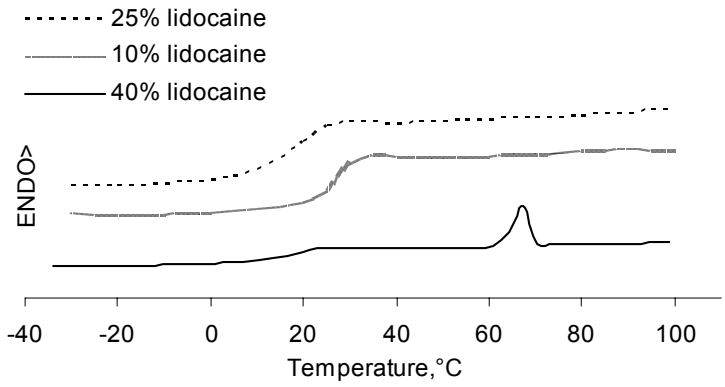


Figure 3.28 DSC thermograms of sponges of sponges of 10% RG 503H in 1,4- dioxane with different lidocaine base concentrations.

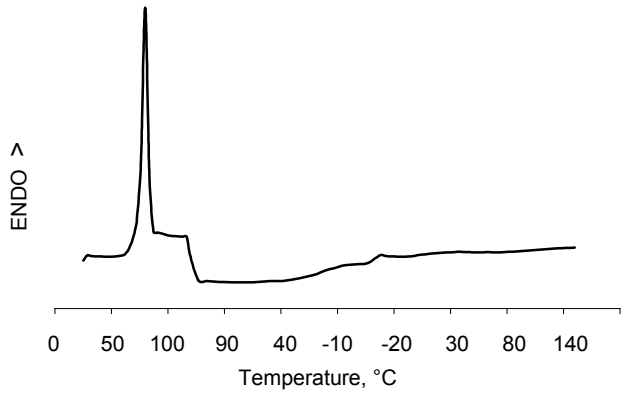


Figure 3.29 DSC of lidocaine hydrochloride as received (25°C to 120°C, 120°C to –40°C and –40°C to 140°C).

3.6.3 Drug release

The lidocaine release from films was compared from that of the sponges, being slower for the sponges (Figure 3.30 and 3.31). Expected was the contrary since the films were prepared with a polymer with a lower degradation rate, RG 752, and the sponges are highly porous facilitating the lidocaine diffusion. But, due to the porosity, the sponges floated and the drug was released only from the surface in contact with the medium. No big differences were observed between different polymer concentrations or drug loadings. In contrast, when the sponges were totally immersed in the medium, the lidocaine was released faster (Figure 3.32).

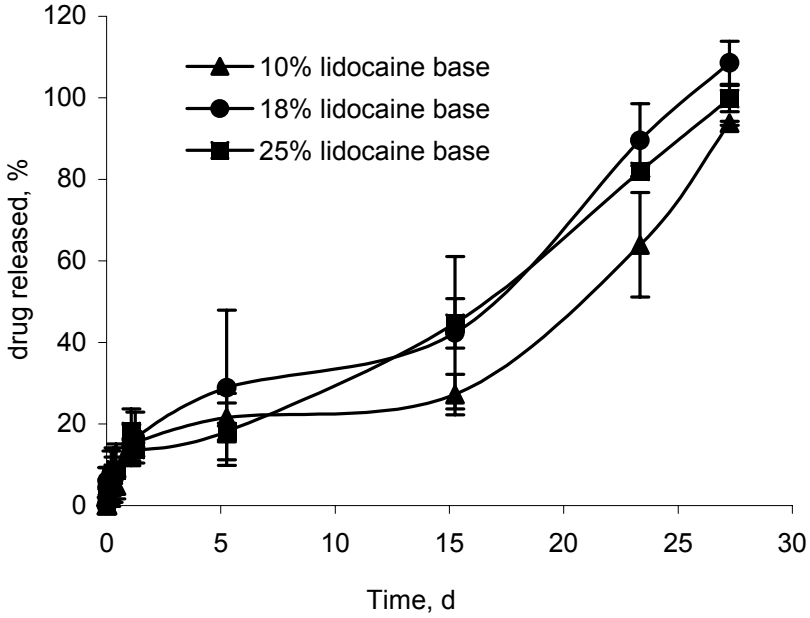


Figure 3.30 Lidocaine release from films prepared with 20% RG 752 in 1,4-dioxane and different lidocaine loading.

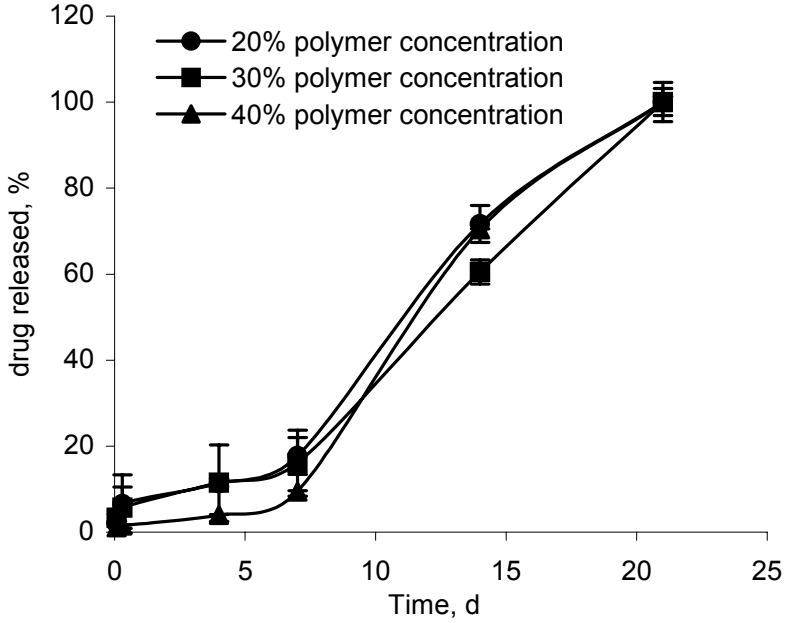


Figure 3.31 Lidocaine release from floating sponges of RG 503H in 1,4-dioxane at different concentrations and 10% lidocaine base.

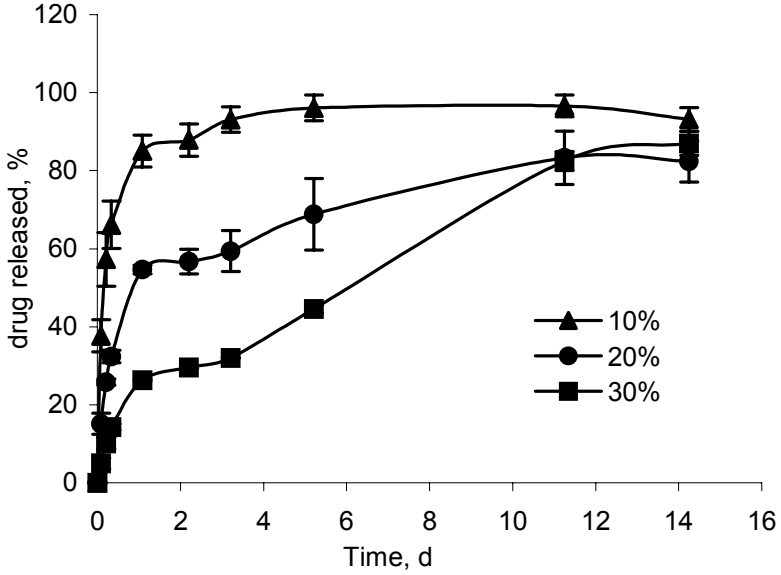


Figure 3.32 Release from non-floating sponges prepared with RG 503H in 1,4-dioxane at different concentrations and 10% lidocaine base.

The faster initial release was due to the high water uptake during the first two days (75%) in presence and absence of lidocaine base (Figure 3.33). However, a continuous increase in the swelling of the sponges was visible during 28 days (Figure 3.34 and 3.35).

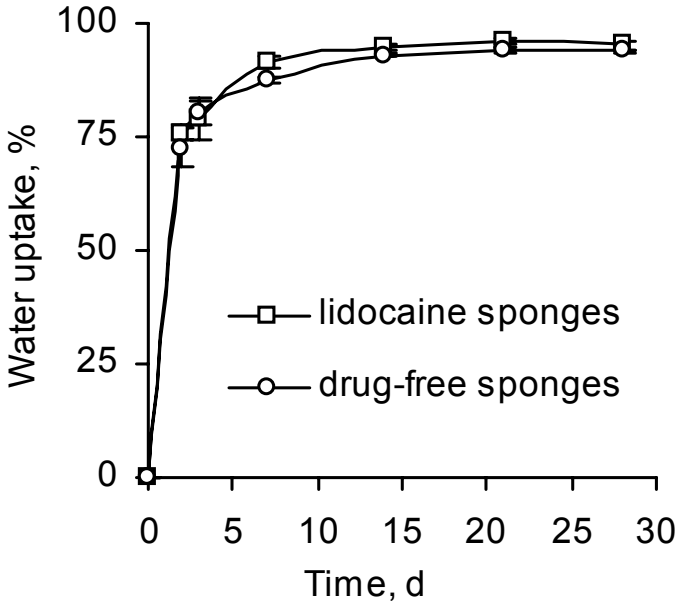


Figure 3.33 Water uptake of sponges (20% RG 503H in 1,4-dioxane and 10% lidocaine base or no drug) immersed in PBS pH 7.4 during 28 days.

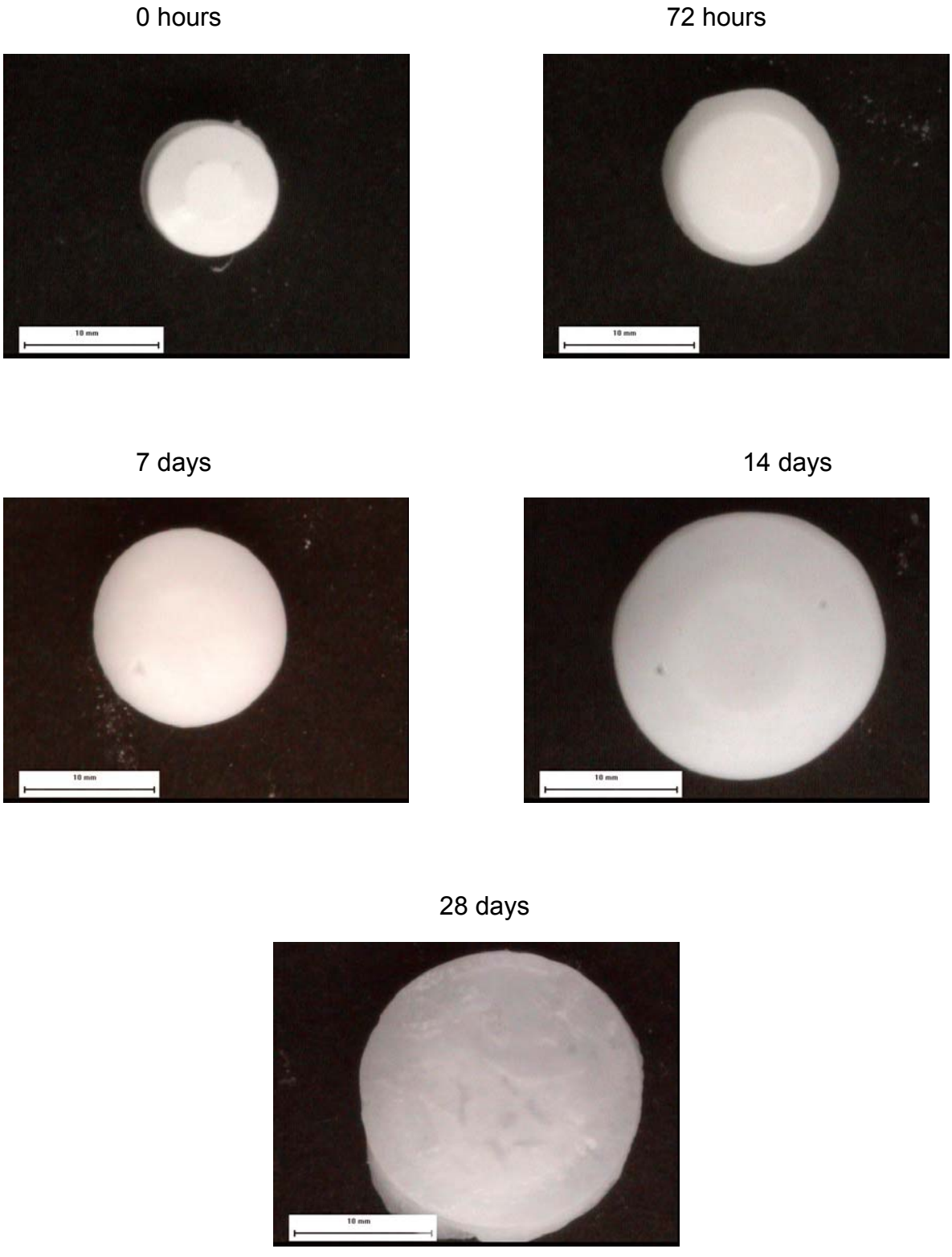


Figure 3.34 Frontal macroscopical pictures of sponges prepared with 20% RG 503H in acetic acid, after different incubation times in PBS pH 7.4.

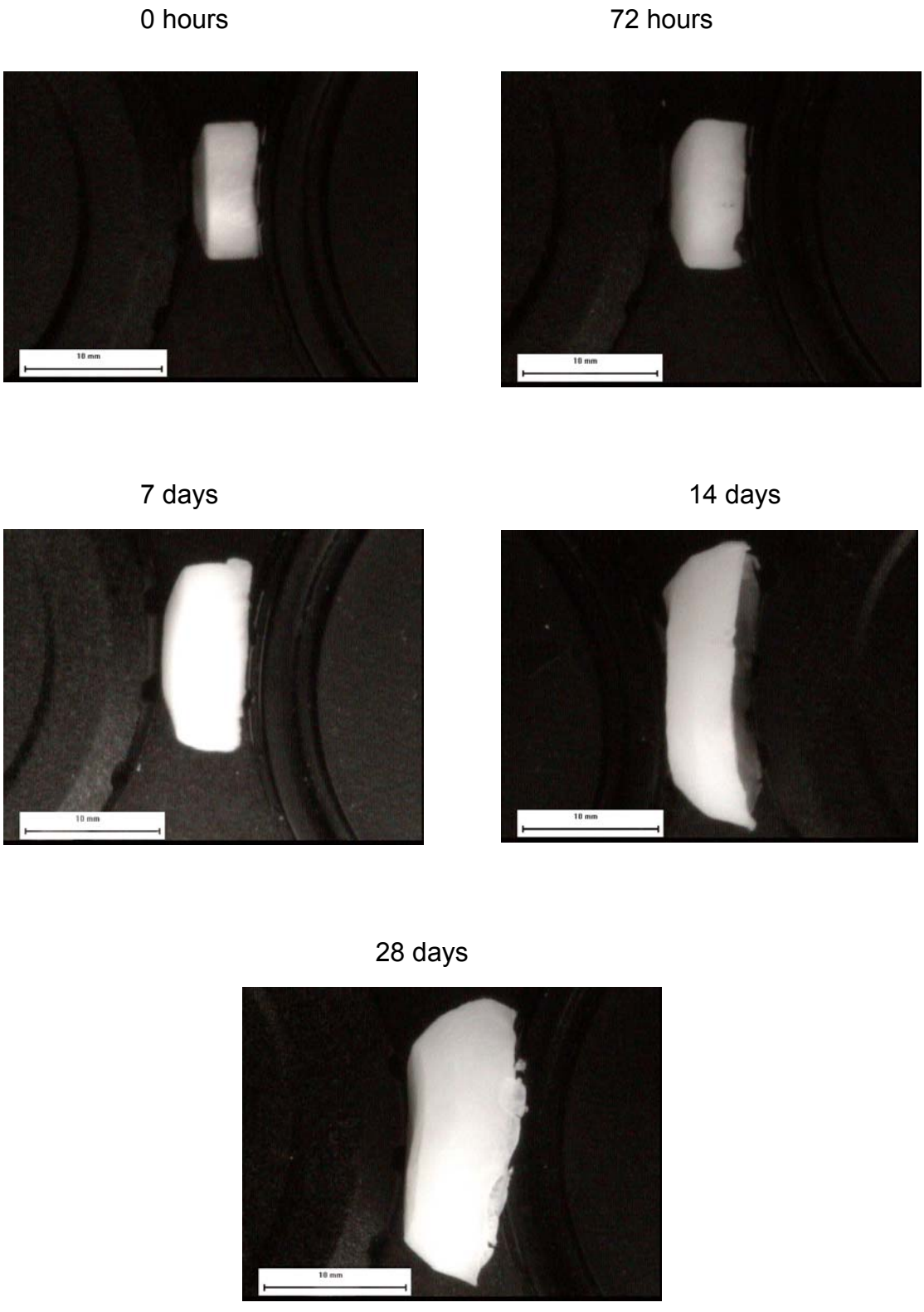


Figure 3.35 Side macroscopical pictures of sponges prepared with 20% RG 503H in acetic acid, after different incubation times in PBS pH 7.4.

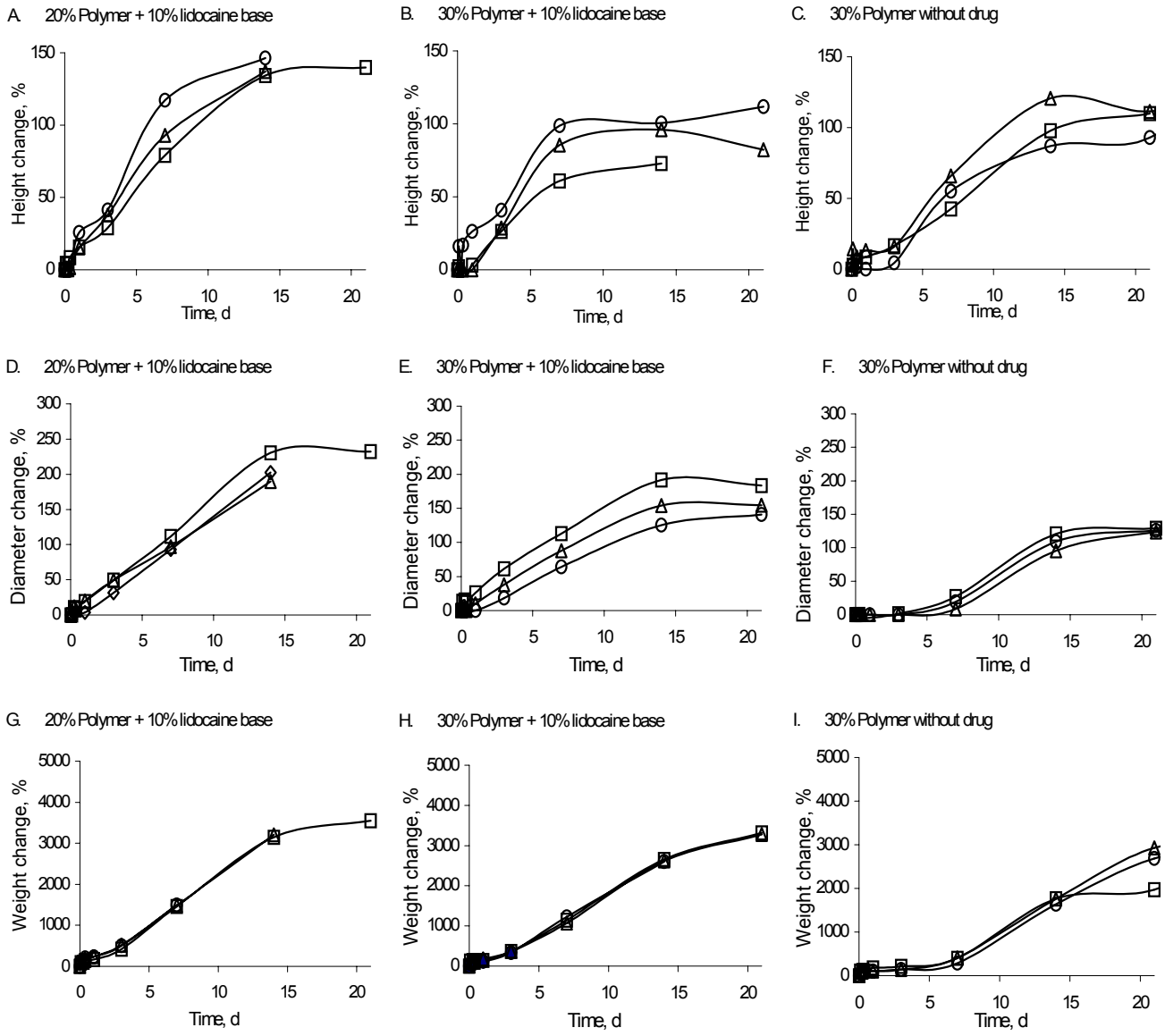


Figure 3.36 Swelling behavior, (A, B, C) height, (D, E, F) diameter and (G, H, I) weight change, of individual samples of sponges (9 mm diameter) prepared with RG 503H at different concentrations in 1,4-dioxane.

The sponges increased in size in a ratio inversely proportional to the polymer concentration, due to the porosity. More porous sponges, e.g. less polymer concentration, absorb more buffer (Figure 3.36). However, in all cases, around the seventh day, changes in size were visible. Since is this the start point of degradation, as observed from the mass loss studies (Figure 3.37).

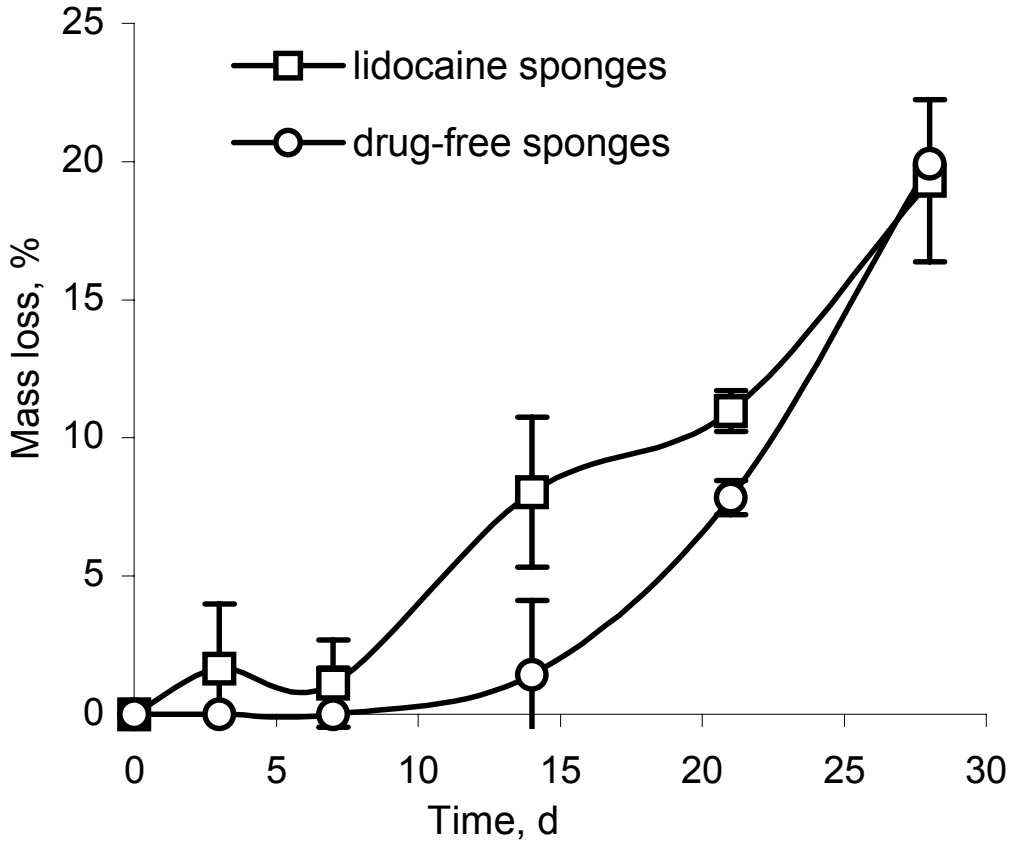
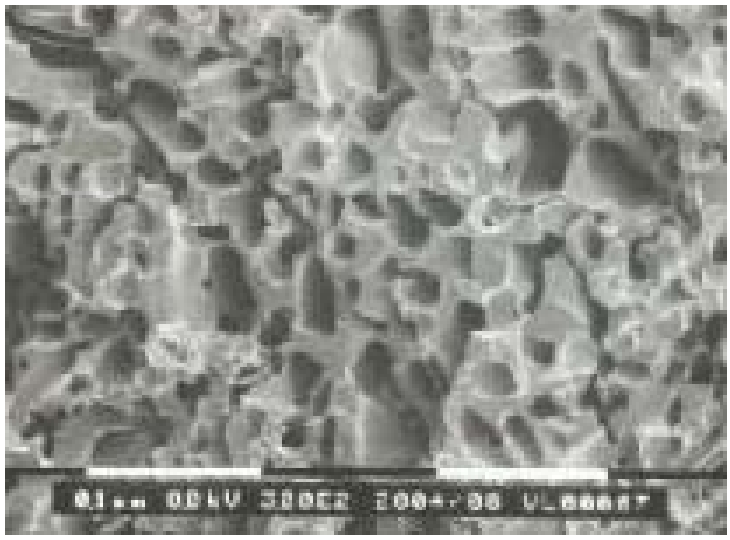


Figure 3.37 Mass loss of sponges (20% RG 503H in 1,4-dioxane and 10% lidocaine base or no drug) immersed in PBS pH 7.4 during 28 days.

The presence of drug is an important factor on the PLGA degradation rate. Drug-free sponges increased in size less than lidocaine-containing sponges (Figure 3.36 C, F, I), they also presented a slower mass loss in comparison with drug loaded sponges (Figure 3.37). This could be attributed to the lower porosity of lidocaine-free sponges (Figure 3.38) and to the polymer-drug interactions, since the basic lidocaine will catalyzed the cleavage of the ester bonds more rapidly [122-124]. This basic catalysis degradation was also confirmed with the DSC thermograms obtained for the freeze-dried samples after swelling (Figure 3.39).

A.



B.

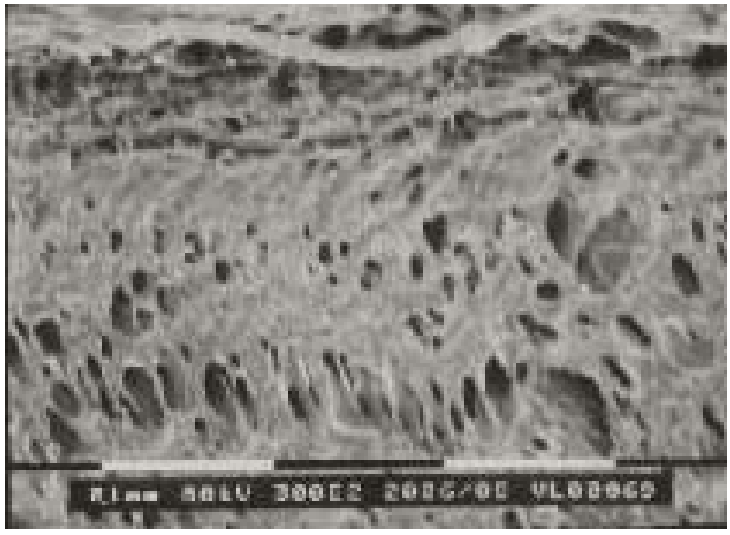


Figure 3.38 SEM pictures of sponges prepared with RG 503H 20% in 1,4-dioxane and (A) 10% lidocaine base and (B) no drug.

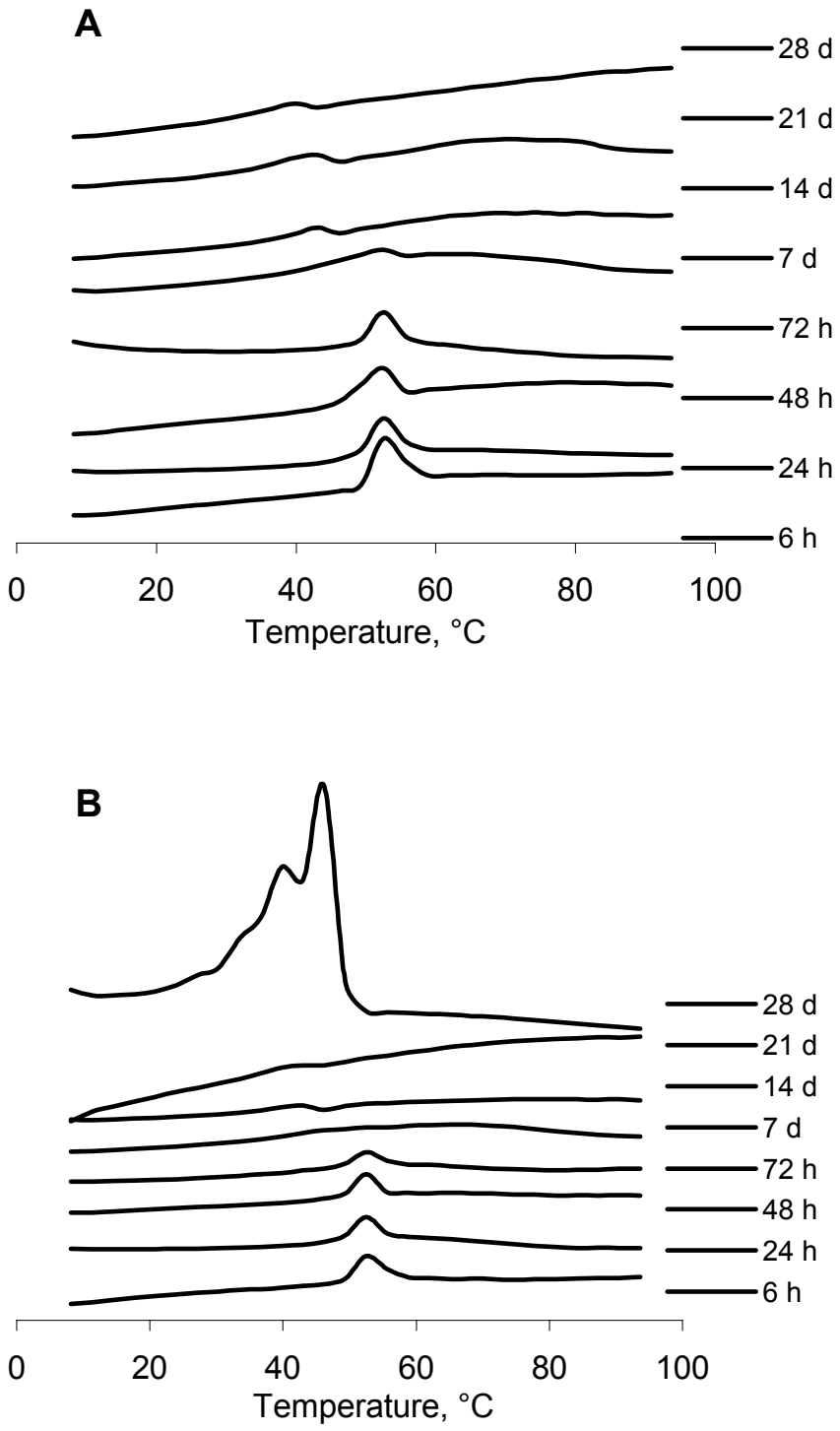


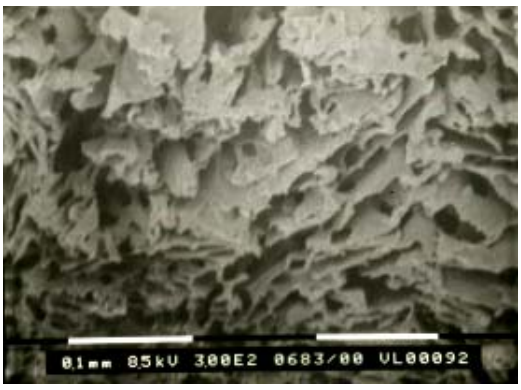
Figure 3.39 DSC thermograms of sponges (20% RG 503H in 1,4-dioxane) as a function of incubation time (A) drug-free sponges and (B) 10% lidocaine base sponges.

Tg for both drug-free and lidocaine sponges, shift to lower temperatures after 7 days of incubation, indicating a decrease in the molecular weight of the polymer backbone. After 28 days only the drug-loaded samples presented a double Tg, corresponding to crystallizable oligomers product of the polymer degradation (Figure 3.39). Similar behavior for poly(D,L-lactide-co-glycolide) microspheres was reported [125]. The start of degradation is evident after 7 days with the increase in porous size, that continuous until 28 days (Figure 3.40).

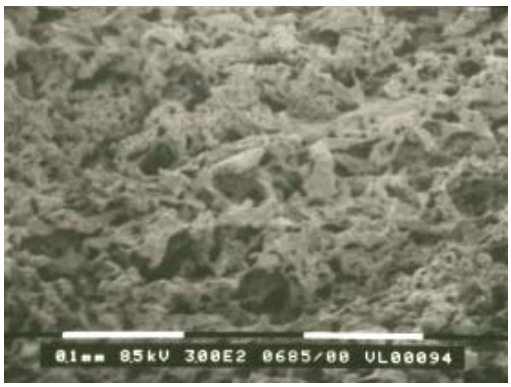
The sponge's size is a factor with big impact on the degradation rate. Small sponges (6 mm diameter) start to decrease in size after 7 days and disintegrate within 20 days, while big sponges (9 mm diameter) continue growing even after 15 days (Figure 3.41 and 3.42). The faster degradation of smaller devices could be related with their larger surface area that controlled the water diffusion and thus hydrolysis [126]. Since the sponges size increased quite a lot, a device to be implanted should be necessarily smaller, to offer more comfort to the patient.

The release of lidocaine obtained during the first 24 hours (time frame for greater drug release), from sponges prepared by varying some formulation parameters was also compared. The polymeric implants prepared with acetic acid released faster the drug than those prepared with 1,4-dioxane, as well lidocaine base was released faster from sponges prepared with RG 502H, a lower molecular weight polymer compared with RG 503H. Since the release was governed by the swelling behavior and not by polymer degradation, the differences are a reflex of the porosity. The release is faster for higher drug loadings (10% vs. 40%) and for the salt, due to the higher solubility in the medium (Figure 3.43)

0 hours



72 hours



7 days

21 days

28 days

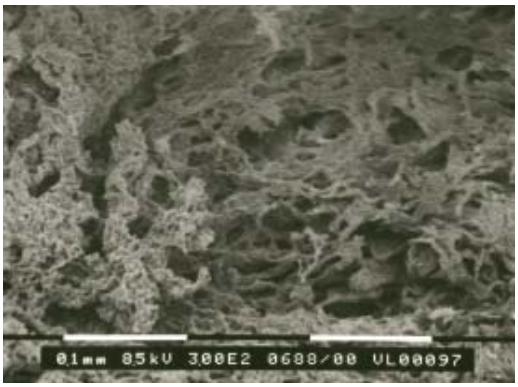


Figure 3.40 SEM pictures of freeze-dried sponges prepared with 20% RG 503H in 1,4-dioxane, after different incubation times in PBS pH 7.4.

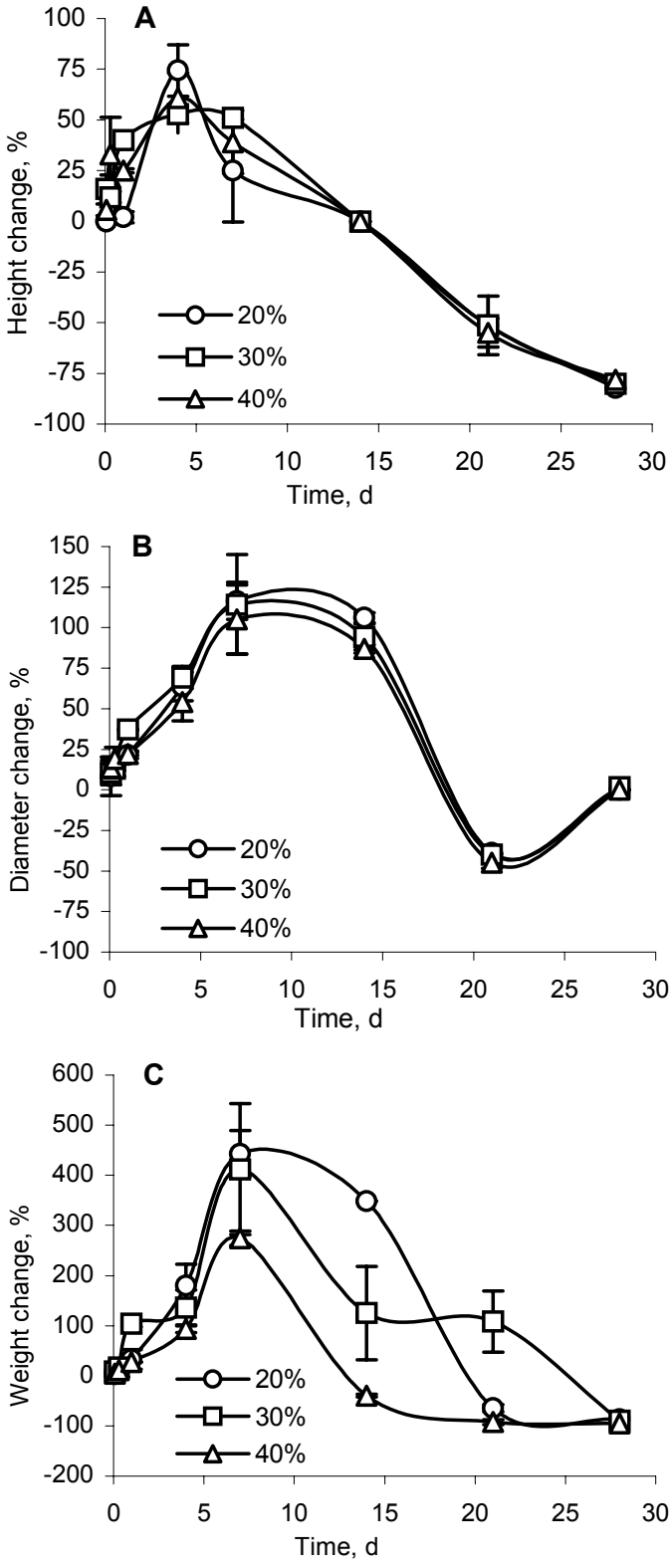


Figure 3.41 Swelling behavior (height, diameter and weight change) of small sponges (6 mm diameter) prepared with RG 503H at different concentrations in 1,4-dioxane.

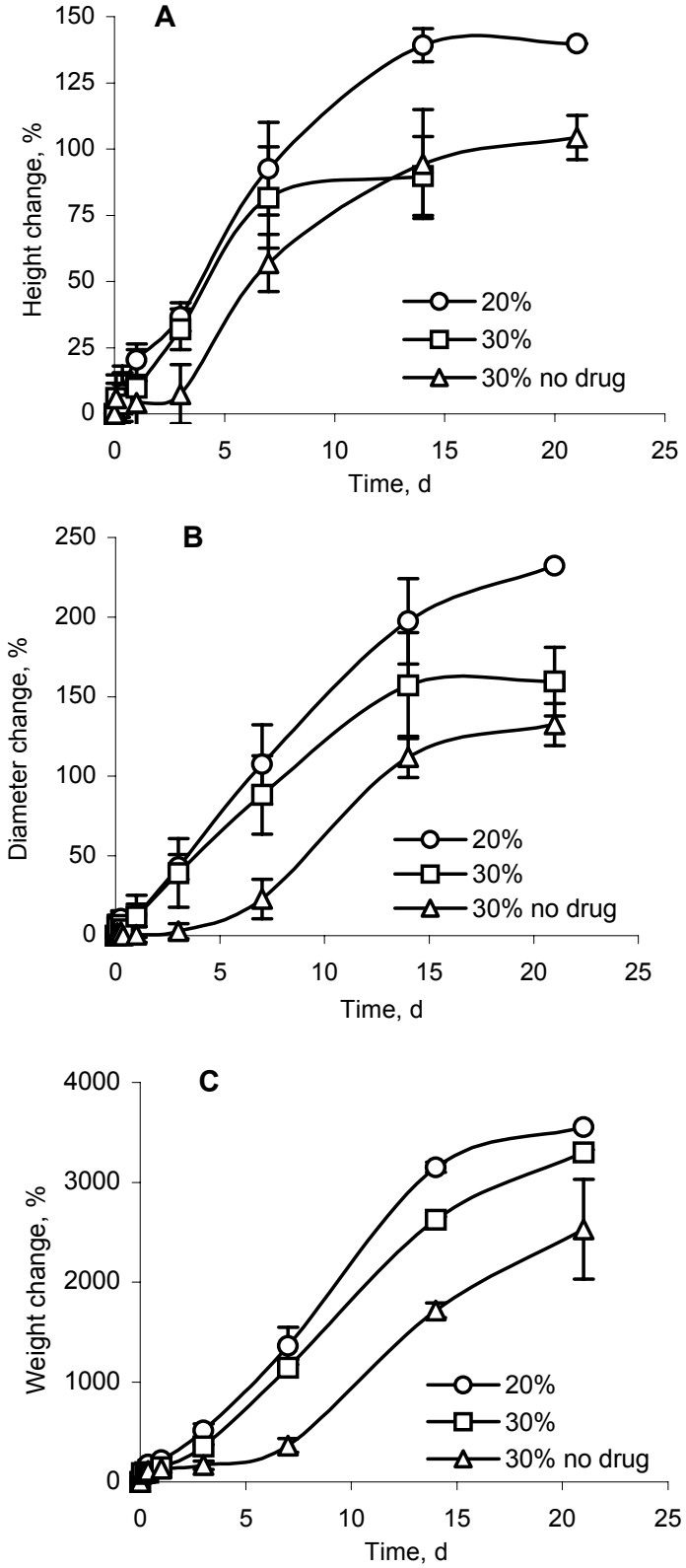


Figure 3.42 Swelling behavior (height, diameter and weight change) of big sponges (9 mm diameter) prepared with RG 503H at different concentrations in 1,4-dioxane.

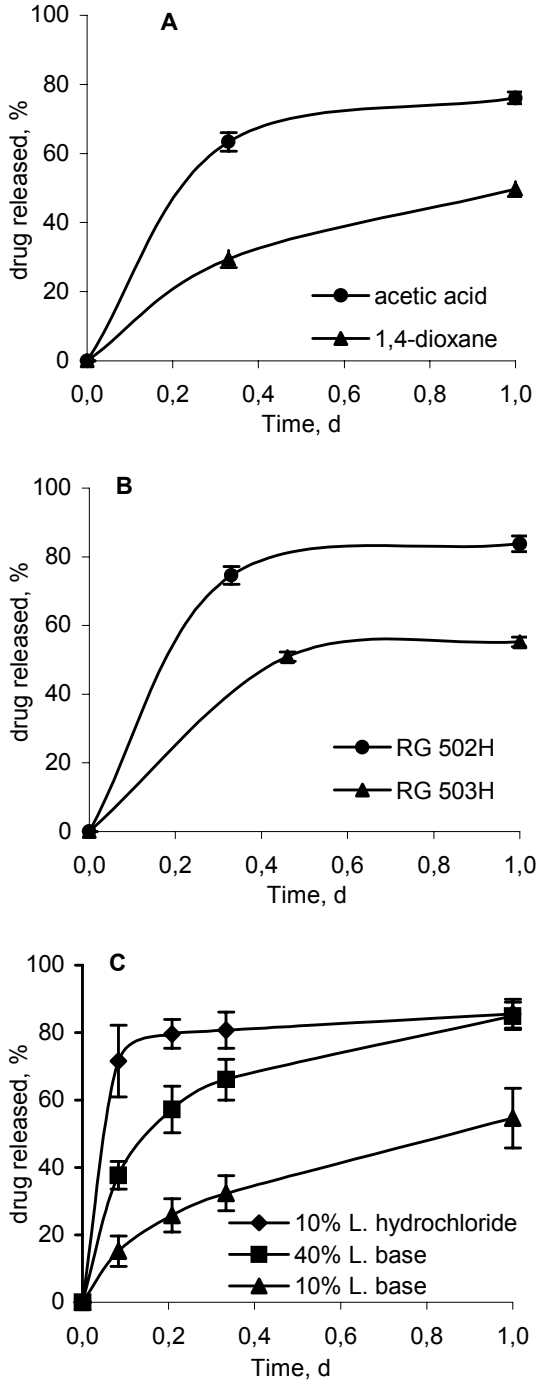


Figure 3.43 One day drug release from sponges prepared with (A) 20% RG 503H in different solvents and 10% lidocaine base, (B) 30% of two different polymers in acetic acid and 10% lidocaine base and (C) 30% RG 503 in 1,4-dioxane and different drug loadings and state (base and salt).

3.6.4 Sponges curing

After freeze drying, the implants were temperature-cured in order to possible slow down the release by closing of pores and to decrease the residual solvent (permitted limits: acetic acid: 0.50% and 1,4-dioxane: 0.03%) [110]. The maximal secondary drying temperature possible in the freeze-drier was 40°C, therefore for 50°C and above a vacuum dryer was used. The residual solvent decreased and the Tg increased with curing temperatures at or above 50°C, indicating a less plasticizing effect because of the less amount of solvent present and leading to an increase in the strength of the sponges (Table 3.12 and Figure 3.44).

Table 3.12 Glass transition temperature, residual solvent and mechanical properties of sponges of 30% RG 503H in acetic acid and 10% lidocaine base, cured at different temperatures.

Curing, °C	Tg, °C	Res. solvent, %	F max., N	Recovery, %
22°C	38.5	2.46	312.28	72.62
30°C	38.7	2.61	---	---
50°C	41.9	1.65	383.82	92.29
65°C	41.6	1.10	---	---
70°C	41.8	0.90	---	---

'--- not measured

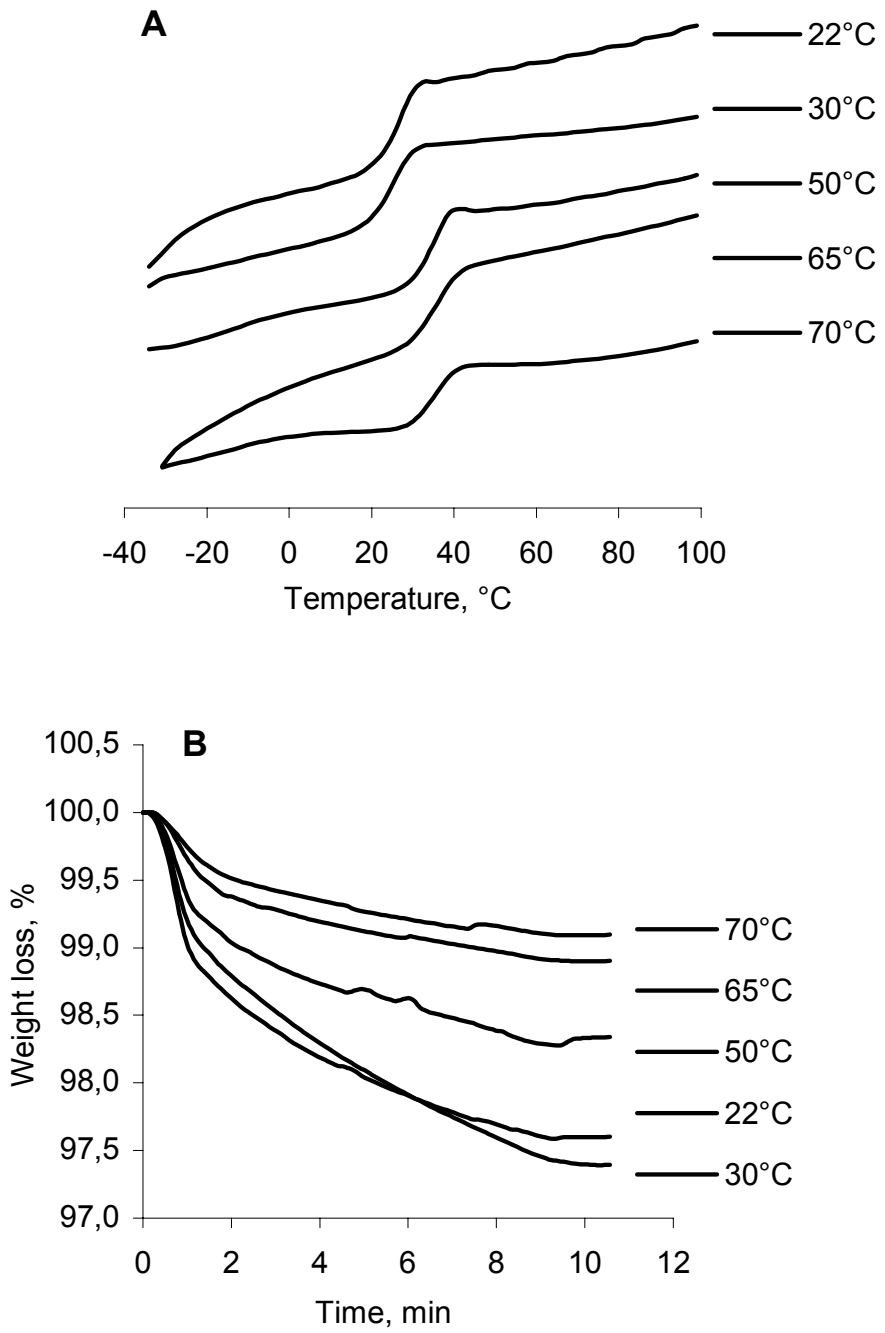
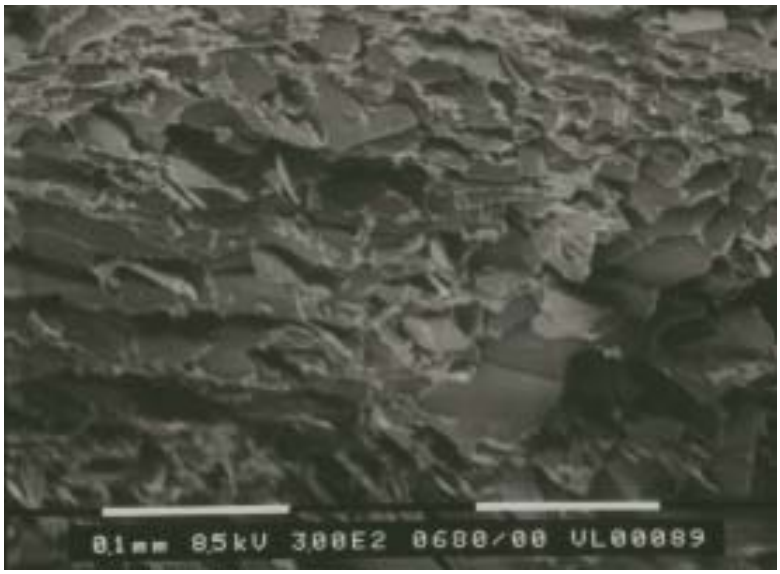


Figure 3.44 (A) DSC thermogram and (B) TGA of sponges of 30% RG 503H in acetic acid and 10% lidocaine base, cured at different temperatures.

A.



B.

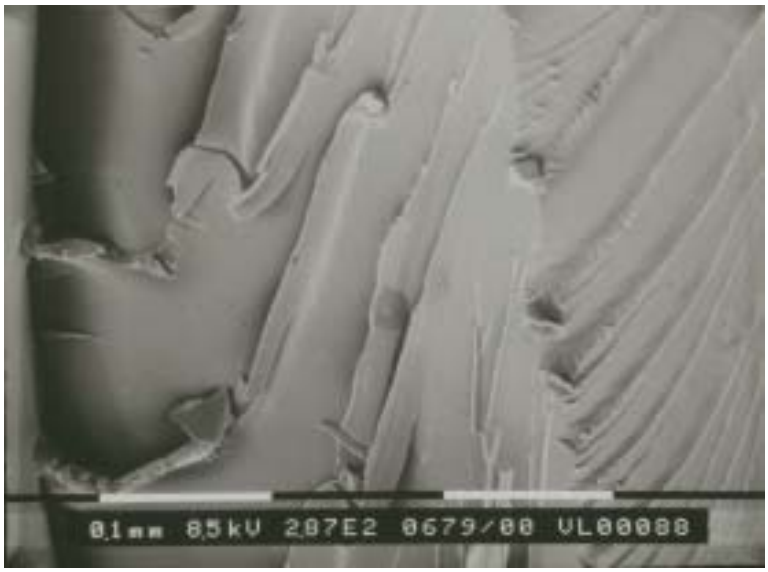


Figure 3.45 SEM pictures of sponges prepared with 30% RG 502H in acetic acid and 10% lidocaine base, before and after curing.

The sponges cured at 50°C were selected for the drug release. After curing, the porosity of the sponges decreased and the polymeric matrix became denser (Figure 3.45). The release of lidocaine was prolonged over 2 weeks by curing and could be even more sustained by increasing the polymer concentration (Figure 3.46).
Due to the short duration of the anesthetic effect, a release of two weeks of duration will be valuable in the treatment of post-operative pain. It will avoid the need of continuous infusion and therefore the hospitalization of the patient.

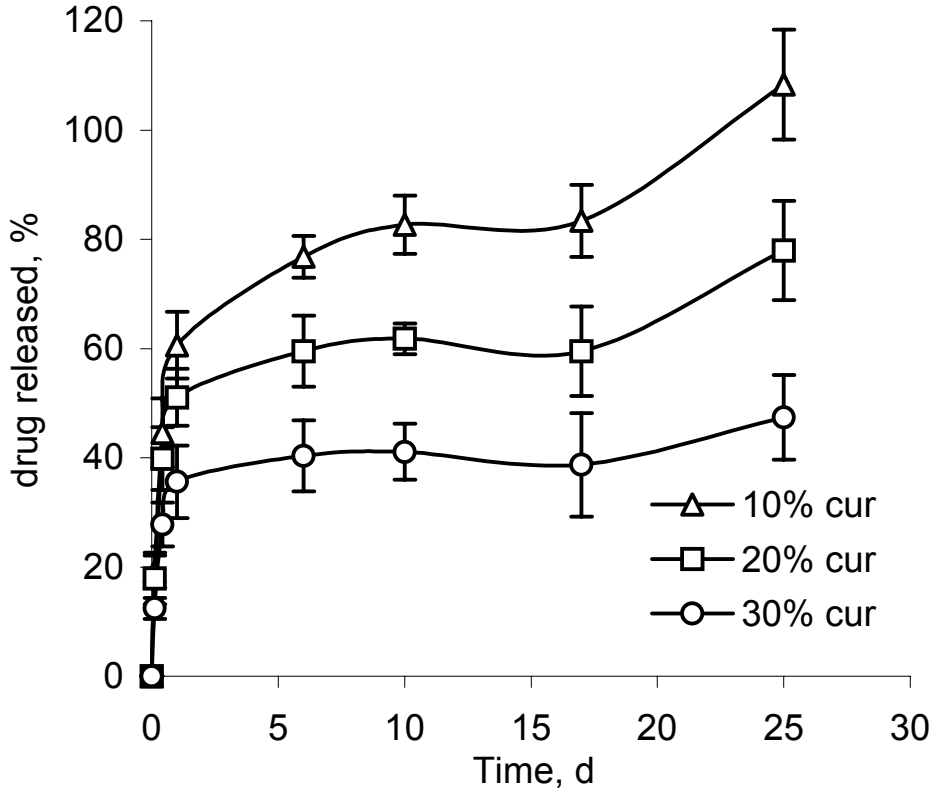


Figure 3.46 Release from cured sponges prepared with RG 503H in acetic acid at two different concentrations.

3.6.5 Follow-up stability

One of the main stability issues with solid dispersions / solutions is the conversion of the drug to the crystalline state after storage [127]. Samples stored at room temperature during 2.5 years were studied by DSC to find out whether the lidocaine was able to recrystallize. The results showed no melting peak, indicating that the samples are physically stable under storage at ambient conditions. Also a shift of Tg to lower temperatures, which could be a sign of polymer degradation, was not observed (Figure 3.47).

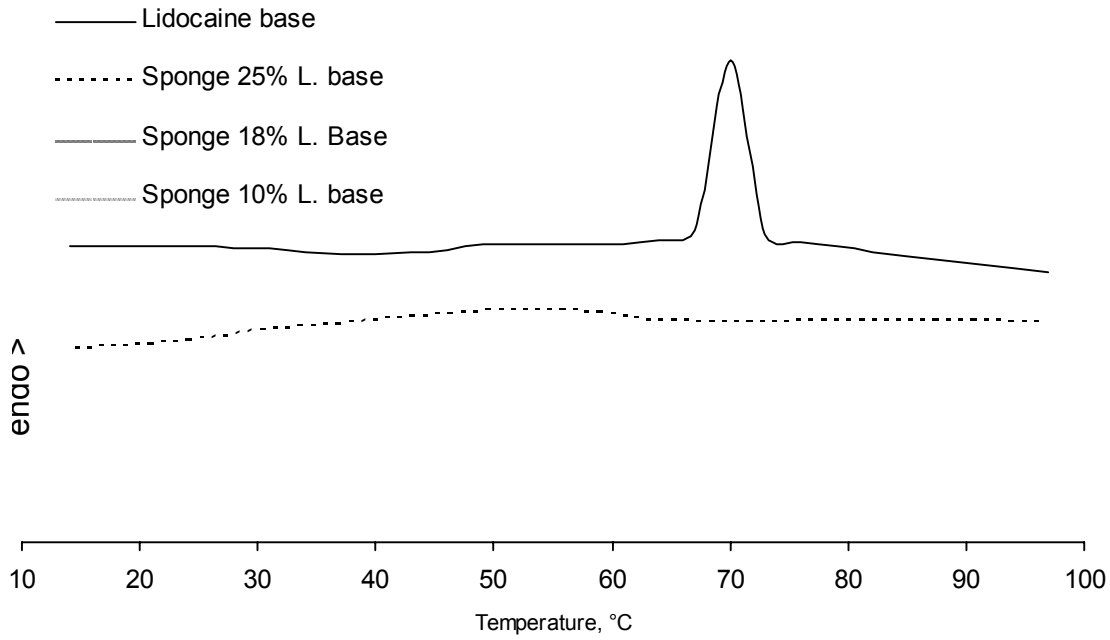


Figure 3.47 DSC thermogram of sponges (10% RG 503H in 1,4-dioxane) after storage at ambient conditions during 2.5 years.

3.7 Conclusion

An improvement in the storage stability of in situ forming systems was achieved by freeze drying the polymer, which in turn improve the dissolution rate. Moreover, since the drug could be simultaneously freeze-dried with the polymer, a simplification of the dosage form was possible without increasing the time needed to form a solution. Furthermore, freeze-drying was an alternative method to prepare biodegradable implants without the need of elevated temperatures and its versatility offers possibilities to manufacture many different products according to the needs.