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Research Paper

Poly(ethyleneglycol) 500 Dimethylether as Novel Solvent for Injectable *In Situ* Forming Depots

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Purpose. Poly(*D,L*-lactide-co-glycolide) (PLGA) solutions in poly(ethyleneglycol)600 (PEG600), N-methyl-2-pyrrolidone (NMP) and poly(ethyleneglycol)500dimethylether (PEG500DME) as a novel solvent, were investigated as suitable for use in injectable *in situ* forming depots (ISFD).
Methods. The hemolytic potential of the solvents was investigated. Viscosimetry was used to determine rheological properties of solvents and PLGA solutions. DSC was used to evaluate the stability of the PLGA solutions through investigation of the melting behavior of semicrystalline PEGs which depended on tempering and glass transition temperature of the PLGA. Phase separation was studied to determine ternary phase diagrams. *In vitro* release kinetics of the solvents and the surrogate methylene blue were investigated.
Results. Significantly less hemolysis was observed for PEG500DME compared to PEG600 and NMP. Newtonian fluid properties were found for all polymer solutions. A melting point depression of the solvents was detected in presence of PLGA. The duration of tempering of the polymer solutions showed no impact on their melting behavior. The initial *in vitro* release of methylene blue was according to the solvent diffusion kinetics.
Conclusions. Low hemolytic potential, suitable viscosity for injection, stability of PLGA solutions in PEG500DME and the correlation between phase separation and *in vitro* release confirmed the potential of PEG500DME as a promising solvent for ISFD.

KEY WORDS: degradation; *in situ* forming depot; poly(D,L-lactide-co-glycolide); stability.

INTRODUCTION

The principle of the injectable *in situ* forming depot system (ISFD) that we were interested in is based on a polymer precipitation system. It is composed of a biodegradable polymer, such as poly(*D,L*-lactide-co-glycolide) (PLGA), dissolved in an organic solvent. After intramuscular (i.m.) or subcutaneous (s.c.) injection, the aqueous environment at the application site induces polymer precipitation and solvent diffusion into the surrounding tissue. Currently, a solution of PLGA in N-methyl-2-pyrrolidone (NMP) is used in the existing Atrigel® technology developed by Dunn (1). However, the NMP content is limited by the ICH guidelines (2) for toxicological reasons. The limited allowable solvent volume therefore places a restraint on the total possible injection volume of the formulation. Poly(ethyleneglycol)s (PEG)s (3), which are allowed up to 65% (*w*/*v*) in parenteral formulations (4,5), can serve as an alternative solvent to overcome this limitation. Since the stability of PLGA dissolved in endcapped PEGs has been found to be superior to that of PEGs (6), we investigated this highly promising solvent for use in ISFDs.

Our investigations concentrated first on the hemolytic potential of the novel poly(ethyleneglycol) 500 dimethylether (PEG500DME) in relation to poly(ethyleneglycol) 600 (PEG600) and NMP to make sure that parenteral application is possible. Next, the thermal and viscous properties of the PLGA solutions were examined to obtain information on storage stability and the acceptable injection force, which would help to determine the appropriate application technique. Controlled release systems comprising PLGA that are currently on the market are all stored at reduced temperature (2–8°C). PEGs show (semi)crystalline structures depending on the storage conditions (7). We therefore investigated the thermal behavior of the three solvents and the polymer solutions in both PEGs and NMP using differential scanning calorimetry (DSC). The semi-crystalline structure of PEGs can also alter the orientation of the polymer chains based on their thermal history (7). This can be observed by changes in the melting behavior of the DSC thermograms.

Of utmost significance for an ISFD is the precipitation of polymer solutions in the presence of water (8,9). Therefore, the phase separation of PLGA dissolved in PEG500DME, PEG600 and NMP was investigated. For this ternary phase, diagrams were developed and compared with literature data for NMP, dimethylsulfoxide (DMSO) and ethylbenzoate (EB) (5).

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A difference in phase inversion was expected for the three water miscible solvents: PEG500DME, PEG600 and NMP. In addition to phase inversion by polymer precipitation, solvent diffusion and water permeation demand appropriate characterization (10–12), since this might have a significant impact on the release of an encapsulated drug substance (13).

The aim of this work was to characterize the end group modified PEG500DME with respect to standard solvents such as PEG600 and NMP, to assess its potential as a solvent for use in an ISFD. To fulfill the requirements of a solvent for parenteral use, hemolysis, viscous properties, storage stability, phase inversion, diffusion of the solvent and the release of the model substance methylene blue were investigated.

MATERIALS AND METHODS

Materials

Poly(*D,L*-lactide-co-glycolide) ($PLA_{50}GA_{50}12$) with 50% *D,L*-lactide and 50% glycolide was purchased from Boehringer Ingelheim Pharma KG (Ingelheim, Germany). The inherent viscosity of $PLA_{50}GA_{50}12$ in a solution of 0.1% in chloroform was 0.16–0.24 dL/g (weight average M_w=12,000 Da). Poly(ethyleneglycol) 600 (PEG600) with an average molecular weight of 600 Da, N-methyl-2-pyrrolidone (NMP), dichloromethane (DCM), di-sodium hydrogen phosphate anhydrous, potassium dihydrogen phosphate, methylene blue, tween 80 and benzalkonium-chloride were purchased from Sigma-Aldrich (Steinheim, Germany). Drabkin's reagent was purchased from Sigma (St. Louis, USA). All chemicals were used without further purification. Poly(ethyleneglycol) dimethylether 500 (PEG500DME) with a molecular weight of 500 Da was a kind gift from Clariant (Burgkirchen, Germany).

Methods

Hemolysis Test of Solvents

NMP, PEG500DME and PEG600 were tested for hemolytic activity in dilutions of 1 : 2, 1 : 4, 1 : 8, 1 : 16, 1 : 32, 1 : 64, 1 : 128 with 0.9% sodium chloride solution. Each dilution was measured in duplicate with clean erythrocytes from male and female blood donors. The test was repeated to ensure the results (n=4). 100 µl of each dilution and 100 µl blood (stabilized with 0.129 M NA-citrate solution) were mixed in 96-well plates and incubated for 10 min at 37°C. The samples were centrifuged at 3,000 rpm for 10 min (Sorvall RC 600B, Thermo scientific, Switzerland). The supernatant was mixed with Drabkin's reagent and measured in duplicate using a multidetection plate reader (Biotek, Synergy HT, Bad Friedrichshall, Germany) at a 540 nm wavelength (14). As a control, 20% ethanol was used for <5% hemolysis and 30% ethanol as control for >10% hemolysis. The values for hemolysis (%) were calculated to a reference standard.

Rheology of Polymer Solutions

Solutions of 20% (*w*/*w*) $PLA_{50}GA_{50}12$ in NMP, PEG500DME, PEG600 and a solution of 40% (*w*/*w*) $PLA_{50}GA_{50}12$ in NMP were investigated to determine their dynamic viscosity using a Haake Rheostress1 rheometer (Tracomme AG, Adliswil, Switzerland) with a linear shear rate increasing from 0.1 to 1,000 s^{-1} in 5 min. After a constant shear rate of 1,000 s^{-1} for one minute, the ramp was again decreased linearly to 0.1 s^{-1}. All measurements were performed in triplicate at 20°C with a cone/ plate geometry of 35 mm diameter and an angle of 1°.

Determination of Injection Forces

The injection force for a solution of 20% $PLA_{50}GA_{50}12$ in NMP and PEG500DME and a solution of 40% $PLA_{50}GA_{50}12$ in NMP was determined with an electronic tensile tester (Zwick Z 2.5, Ulm, Germany). Approximately 0.3–0.4 ml of the polymer solutions were injected with a 1 ml syringe (1 ml syringe with Luer-Lok Tip, BD, Franklin Lakes, NJ, USA) and a 20G (BD Microlance™3 Nr.1, BD, Fraga, Spain), 23G (BD Microlance™3 Nr.16, BD, Fraga, Spain) and 25G (Luer Einmalkanüle 0.50×10 mm 25G×5/8″, Henke-Sass Wolf GmbH, Tuttlingen, Germany) needle into an empty glass vessel. The injection force was measured for three different injection speeds (60, 100 and 150 mm/min) for each needle size and analyzed with the testXpert software (version 3.0, Zwick, Ulm, Germany).

DSC Investigations

Solutions of 20% (*w*/*w*) $PLA_{50}GA_{50}12$ in PEG600, PEG500DME and NMP and a solution of 40% (*w*/*w*) $PLA_{50}GA_{50}12$ in NMP were analyzed for thermal properties using a differential scanning calorimeter (DSC) 2920 from TA instruments (Alzenau, Germany). The objective was to investigate a relation between the time interval for crystallization and the melting behavior. Therefore, the solutions were tempered at −40°C for three different time intervals (0.5 h, 4 h and 8 h) after the first heating and cooling cycle. This time interval allowed PEG600, PEG500DME to re-crystallize, as well as solutions of 20% (*w*/*w*) $PLA_{50}GA_{50}12$ in the two solvents. NMP, 20 and 40% (*w*/*w*) $PLA_{50}GA_{50}12$ solutions in NMP were used as a control. The second heating cycle was performed after the respective tempering time at −40°C with a heating rate of 5°C/min to 100°C.

The tempering time at −40°C was fixed to 0.5 h after the observation of no change in the crystalline structure of PEG500DME and PEG600 and the $PLA_{50}GA_{50}12$ solutions thereof. The determination of the melting point of the $PLA_{50}GA_{50}12$ solutions was performed in the first heating cycle from −40°C to 100°C with a linear heating rate of 5°C/min. Accordingly, a second heating cycle under the same conditions as the first one was performed to determine the glass transition temperature (T_g) of the polymer.

Ternary Phase Diagrams for $PLA_{50}GA_{50}12$ / Solvent / Water Systems

The change from a homogenous polymer solution to a binary system was determined by addition of water. Five different concentrations of $PLA_{50}GA_{50}12$ in NMP [5%, 10%, 20%, 30%, 50% (*w*/*w*)], in PEG500DME [5%,10%, 20%, 30%, 40% (*w*/*w*)] and in PEG600 [5%, 7.5%. 10%, 12.5%, 15%, 20% (*w*/*w*)] were prepared by magnetic stirring (IKA Labortechnik

RET basic, Staufen, Germany) at approximately 400 rpm at room temperature. $PLA_{50}GA_{50}12$ in NMP was dissolved after 2 h; the dissolution in PEG500DME was obtained after 6 h and in PEG600 after 24 h. After equilibration to 37°C in a water bath, solvent/ water mixtures (*w*/*w*) (ratio 3 : 1 and 5 : 1) were added dropwise to the solutions. Solvent/ water mixtures were used due to immediate precipitation when a water droplet contacted the surface of the polymer solution. A homogenous distribution of water in the polymer solutions was only possible with mixtures of solvent with water. The onset of turbidity was determined visually, and, thus, the quantity of water necessary for polymer precipitation was calculated. This experiment was carried out in triplicate for each polymer solution, and all determined values were plotted in ternary phase diagrams.

Solvent Diffusion During Precipitation

The solvent diffusion kinetics were determined by examining the change in refractive index as a function of surface area.

In experiments that allowed control of the precipitate surface area, 20% (*w*/*w*) solutions of $PLA_{50}GA_{50}12$ in NMP, PEG500DME and PEG600, plus a 40% (*w*/*w*) solution of $PLA_{50}GA_{50}12$ in NMP were tested. 0.4–0.5 g (accurately to 0.001 g) of the polymer solutions were weighed on the bottom of a glass vial and filled with 10 ml PBS buffer pH 7.4, equilibrated to 37°C. The samples were kept at 37°C in a water bath. The surface area of the precipitate for the solvent diffusion was limited to 3.5 cm^2. 100 µl samples were taken after 0.16, 0.33, 0.5, 1, 3, 24 and 48 h and replaced with 100 µl buffer.

In a second setup of the experiment, 0.4–0.5 g (accurately to 0.001 g) of the polymer solutions were injected directly into 10 ml PBS buffer pH 7.4 (equilibrated to 37°C) with a 1 ml syringe (1 ml syringe with Luer-Lok Tip, BD, Franklin Lakes, NJ, USA) and a 22G needle (Sterican 0.70×30 mm, B.Braun, Melsungen, Germany) and kept at 37°C in a water bath. The surface area of the resulting precipitate was approximately 9.5 cm^2, but it was strongly dependent on the injection speed, the needle size and the resulting shape of the *in situ* depot. The surface area was calculated for a cylinder based on the diameter and length of the straight polymer precipitate extruded by the aforementioned syringe and needle. With this experiment, the effect of surface area on the release of solvent from the polymer solution into the buffer was investigated. All experiments were performed in triplicate (n=3). The refractive index was determined with a refractometer (No. 16922, Erma optical works, Tokyo, Japan) and the solvent concentration was calculated from a calibration curve in a range from 5 mg/ml to 40 mg/ml solvent in the buffer solution. The change in the refractive index represented the amount of solvent released into 10 ml PBS buffer pH 7.4 at 37°C.

In Vitro Release Study with Model Substance Methylene Blue

0.6% (*w*/*w*) methylene blue was added to a solution of 20% (*w*/*w*) $PLA_{50}GA_{50}12$ in PEG500DME, PEG600 and NMP, plus a 40% solution of $PLA_{50}GA_{50}12$ in NMP. An amount of 0.4–0.5 g (accurately to 0.001 g) of the solutions comprising methylene blue were injected with a 1 ml syringe (1 ml syringe with Luer-Lok Tip, BD, Franklin Lakes, NJ, USA) with a 23G (BD Microlance™3 Nr.16, BD, Fraga, Spain) needle into 50 ml polypropylene Falcon tubes (BD, Franklin Lakes, USA) with 25 ml PBS buffer pH 7.4. The tubes were incubated in a shaking water bath (AD Krauth, Hamburg, Germany) at 37°C at very low frequency. Complete buffer replacement was performed at every sampling point. The samples were taken at t=0, 1, 3, 7, 14, 21, 28, 35, 42 and 49 days and analyzed with a Varian Cary spectrophotometer (Darmstadt, Germany) at a wavelength of 665 nm.

RESULTS

Hemolysis Study

To outline the suitability of PEG500DME as a solvent for parenteral use, a hemolysis study was performed (Table I). 48.8% erythrocytes showed lysis using undiluted NMP. At a dilution of 1 : 2 with isotonic NaCl solution, NMP still shows 7.8% hemolysis. 13.3% hemolysis was observed for undiluted PEG600. For a dilution of PEG600 with isotonic NaCl solution (1 : 2), 2.0% hemolysis was determined. The lowest hemolytic activity of all undiluted solvents was represented by PEG500DME with 5.5%. No significant hemolytic effects were shown for further dilutions of the three solvents.

Viscosity of Polymer Solutions

In order to compare the rheological properties, two polymer solutions using NMP [20% and 40% (*w*/*w*)] and 20% (*w*/*w*) solutions of PEG500DME and PEG600 were investigated. First, the pure solvents were analyzed, then the respective polymer solutions. Dynamic viscosity values for the solvents and polymer solutions are listed in Table II. The dynamic viscosities of PEG500DME and PEG600 were determined to be 28 mPas and 180 mPas, respectively. For NMP, a dynamic viscosity of 1.7 mPas was found. The solution with 20% polymer has a viscosity of 40 mPas as compared to 800 mPas for the solution with 40% polymer in NMP. The solutions exhibited Newtonian fluid properties. A slight change in viscosity with increasing shear rate was noticed for the 20% $PLA_{50}GA_{50}12$ solution in PEG600. Viscosity was shown to be 3,440 mPas at a shear rate of 0.1 s^{-1}, while at an increased shear rate of 1,000 s^{-1}, the viscosity dropped to 3,240 mPas. The initial viscosity was not

Table I. Hemolysis [%] of the Three Investigated Solvents, Tested in Erythrocytes of Female and Male Blood Donors (n=4)

Hemolysis [%]	Dilutions with 0.9% NaCl solution							
solvent	0	1 : 2	1 : 4	1 : 8	1 : 16	1 : 32	1 : 64	1 : 128
NMP	48.8±21.1	7.8±5.6	0.5±0.6	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5
PEG600	13.3±3.8	2.0±1.8	0	0	0	0	0	0
PEG500DME	5.5±4.7	0	0	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5	0.3±0.5

Table II. Dynamic Viscosities of $PLA_{50}GA_{50}12$ Solutions at 20°C Obtained with Linear Shear Rate from 0.1 s^{-1} to 1000 s^{-1}

Polymer	Polymer conc. [%]	Solvent	Viscosity [mPas]
$PLA_{50}GA_{50}12$	20	NMP	40
$PLA_{50}GA_{50}12$	40	NMP	800
$PLA_{50}GA_{50}12$	20	PEG600	3,200
$PLA_{50}GA_{50}12$	20	PEG500DME	580
/	/	NMP	1.7
/	/	PEG600	180
/	/	PEG500DME	28

regained upon subsequent decreasing of the shear rate (3,340 mPas at 0.1 s^{-1}). The polymer solution in PEG600 did not exhibit complete ideal viscous properties. Its pseudoplastic behavior was not further investigated. A 20% $PLA_{50}GA_{50}12$ solution in PEG500DME was reflective of an ideal viscous system with a shear-rate-independent viscosity of 580 mPas. Nearly all polymer solutions seemed to behave like Newtonian fluids, except for the solution of $PLA_{50}GA_{50}12$ in PEG600. Due to the increased polymer content, the solution of 40% $PLA_{50}GA_{50}12$ in NMP showed an increased dynamic viscosity compared to the solution of 20% $PLA_{50}GA_{50}12$ in PEG500DME.

Injection Forces

The required injection force for an ISFD was measured using 1 ml syringes with three different needle sizes. The resistance of subcutaneous tissue at the application site was not taken into account. An injection force lower than 5 N was obtained with a solution of 20% (*w*/*w*) $PLA_{50}GA_{50}12$ in NMP using a 20 or 23G needle (Fig. 1). The 40% $PLA_{50}GA_{50}12$ solution in NMP exceeded this limit at the injection speed of 150 mm/min using a 23G needle. An injection force of more than 20 N was found with the use of a 25G needle. With the 20% solution of $PLA_{50}GA_{50}12$ in PEG500DME, an injection force lower 20 N was possible for a needle size of 20G or 23G. Using a 25G needle, an injection force of 21.3 N was found at a speed of 100 mm/min, while at 150 mm/min, this force increased to 31 N. In general, a linear relationship between the dynamic viscosities of the solutions and the injection forces for the respective

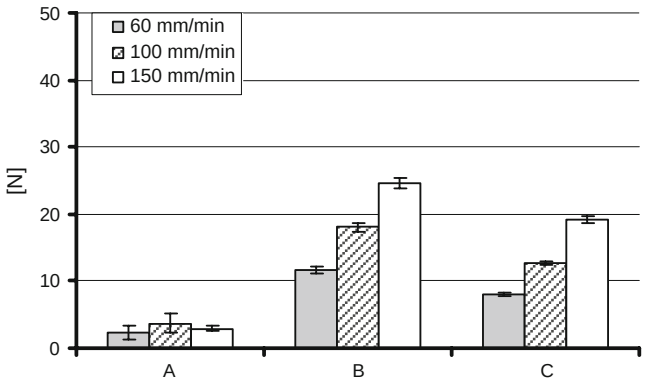

Fig. 1. Comparison of injection forces at three different injection speeds for 20% (*A*) and 40% (*B*) solutions of $PLA_{50}GA_{50}12$ in NMP and a 20% (*C*) $PLA_{50}GA_{50}12$ solution in PEG500DME injected with a 1 ml syringe and 23G needle.

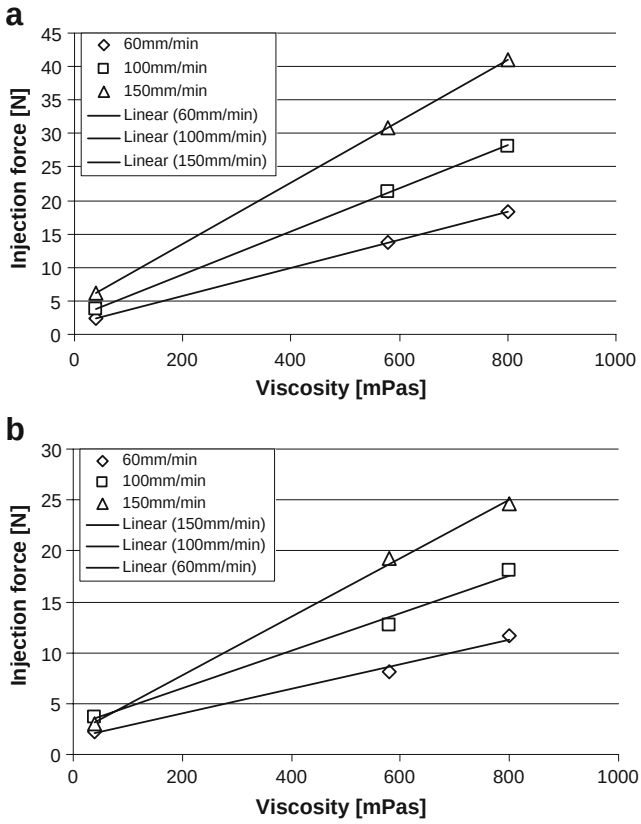

Fig. 2. Linear relationship between injection force [N] and viscosity [mPas] shown for 23G (**a**) and 25G (**b**) needle.

injection speed was observed (Fig. 2). An increased dynamic viscosity of the solution leads to a higher injection force. The limit of 20 N injection force was exceeded for all needle sizes with the solution of 20% (*w*/*w*) $PLA_{50}GA_{50}12$ in PEG600.

Thermal Properties of Polymer Solutions

PEG500DME, PEG600 and solutions of 20% (*w*/*w*) $PLA_{50}GA_{50}12$ in the two PEGs were tempered at −40°C for 0.5, 4 and 8 h after the first DSC heating and cooling cycle. No difference in the melting behavior was observed for the solvents and the polymer solutions during the second heating cycle (data not shown). The crystalline structure of the pure PEGs seemed to be independent of the tempering interval. No change in the melting behavior was found for the polymer solutions due to the tempering duration. This was an important finding with regard to the long-term stability of the $PLA_{50}GA_{50}12$ solutions, because they seemed to be able to maintain their properties throughout the cooling procedure.

The melting points for PEG500DME were 14.7±0.4°C and 21.8±0.7°C for PEG600 (Fig. 3a and b) as determined by DSC. In both thermograms, broad signals for the melting peak in the first heating cycle and a broad signal for the exothermal crystallization during the cooling cycle appeared. The thermal observation of NMP in the same temperature range did not show any first order transitions (Fig. 3c). The thermogram for pure $PLA_{50}GA_{50}12$ is shown in Fig. 3d. The endothermal relaxation of the polymer powder was visible, with an endothermal peak at 42.3°C. No aging was observed during the

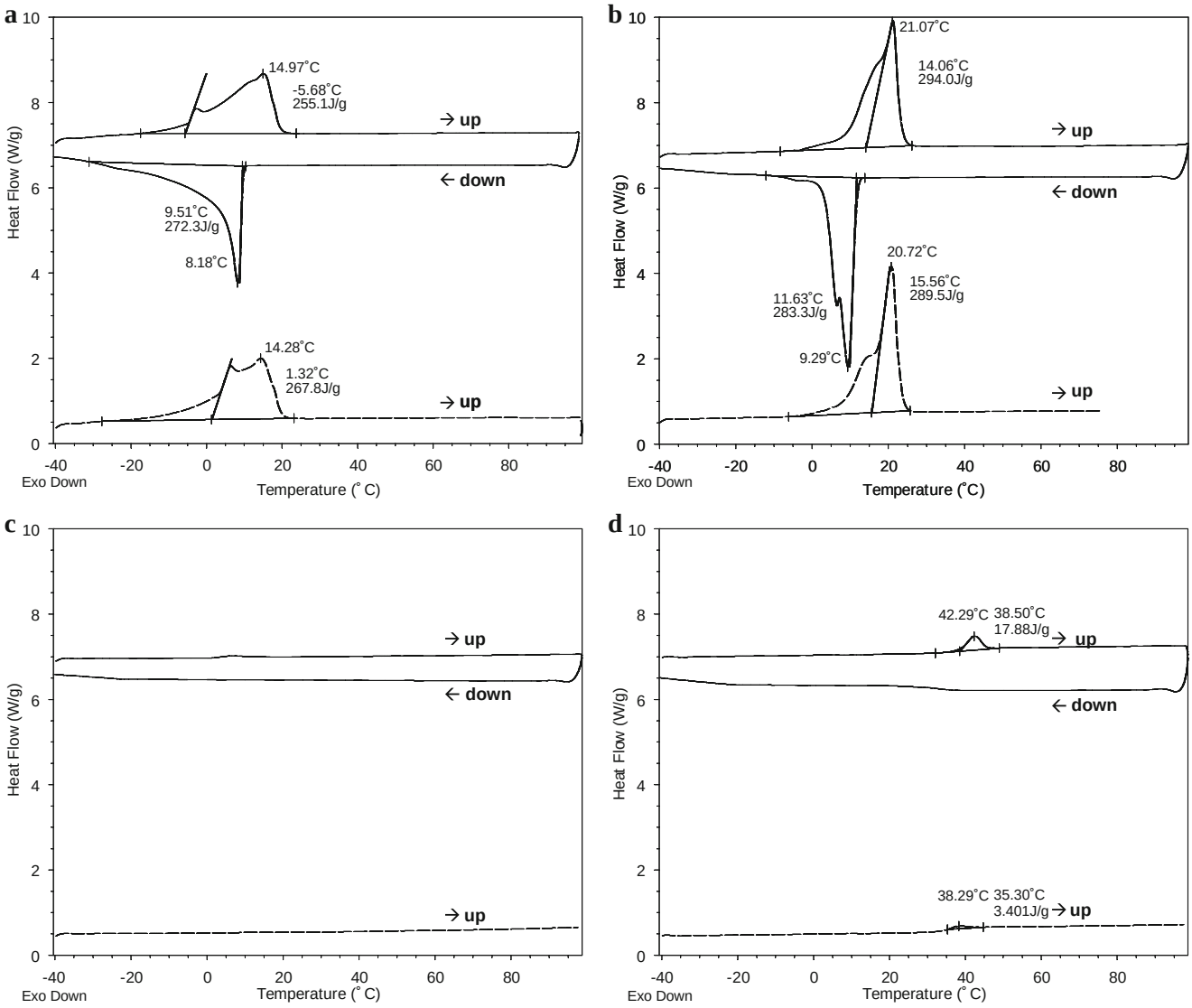

Fig. 3. Thermograms of PEG500DME (**a**), PEG600 (**b**), NMP (**c**) and $PLA_{50}GA_{50}12$ (**d**) with a first heating cycle from −40°C to 100°C, cooling to −40°C with 5°C/min, 30 min at −40°C and a second heating cycle from −40°C to 100°C.

subsequent cooling cycle. In the second heating cycle, the glass transition temperature (T_g) was identified at 39.5±2.6°C.

A melting point depression was observed for the solution of 20% (*w*/*w*) $PLA_{50}GA_{50}12$ in PEG500DME to 10.4±0.9°C (Fig. 4a) as compared to the pure solvent. In the first heating cycle, gradual melting was visible, and this resulted in a broad peak with several shoulders. During the cooling cycle, the onset of crystallization was at 5.8±0.5°C, as noticed with a tailing of the peak. A broad melting peak was again observed during the second heating cycle after 0.5 h tempering at −40°C. No signal for the T_g of $PLA_{50}GA_{50}12$ was detected in the second heating cycle. For a solution of 20% $PLA_{50}GA_{50}12$ in PEG600, again a significant decrease of the melting temperature to 19.8±0.6°C was observed (Fig. 4b). A broad melting peak with two stages in the first heating cycle was visible in the thermogram. The crystallization peak during cooling and the second heating cycle at 5.0±0.8°C showed broad signals. For the solutions of 20% and 40% $PLA_{50}GA_{50}12$ in NMP, no signal for melting, crystallization or T_g was detected in the observed temperature range (Fig. 4c and d).

Phase Inversion in the Presence of Water

$PLA_{50}GA_{50}12$ solutions in NMP, PEG500DME and PEG600 at different concentrations were investigated for the onset of polymer precipitation in presence of water at 37°C (Fig. 5). The straight line in the diagrams was drawn from 100% $PLA_{50}GA_{50}12$ and divided the area into the upper homogenous three-component system and a binary system (Phase separation) below the line. The onset of polymer precipitation was determined for different $PLA_{50}GA_{50}12$ concentrations in PEG500DME by addition of solvent/ water mixtures to solutions of polymer in organic solvent. Polymer precipitation already occurred in presence of 1.0% water for a solution of 32.6% $PLA_{50}GA_{50}12$ in PEG500DME (Fig. 5a), signifying that only a small amount of water was necessary to precipitate the hydrophobic polymer in the solution. 13.4% water was required to precipitate the polymer out of 3.0% $PLA_{50}GA_{50}12$ in PEG500DME. As expected, the addition of an increased quantity of water at lower polymer content was possible (5).

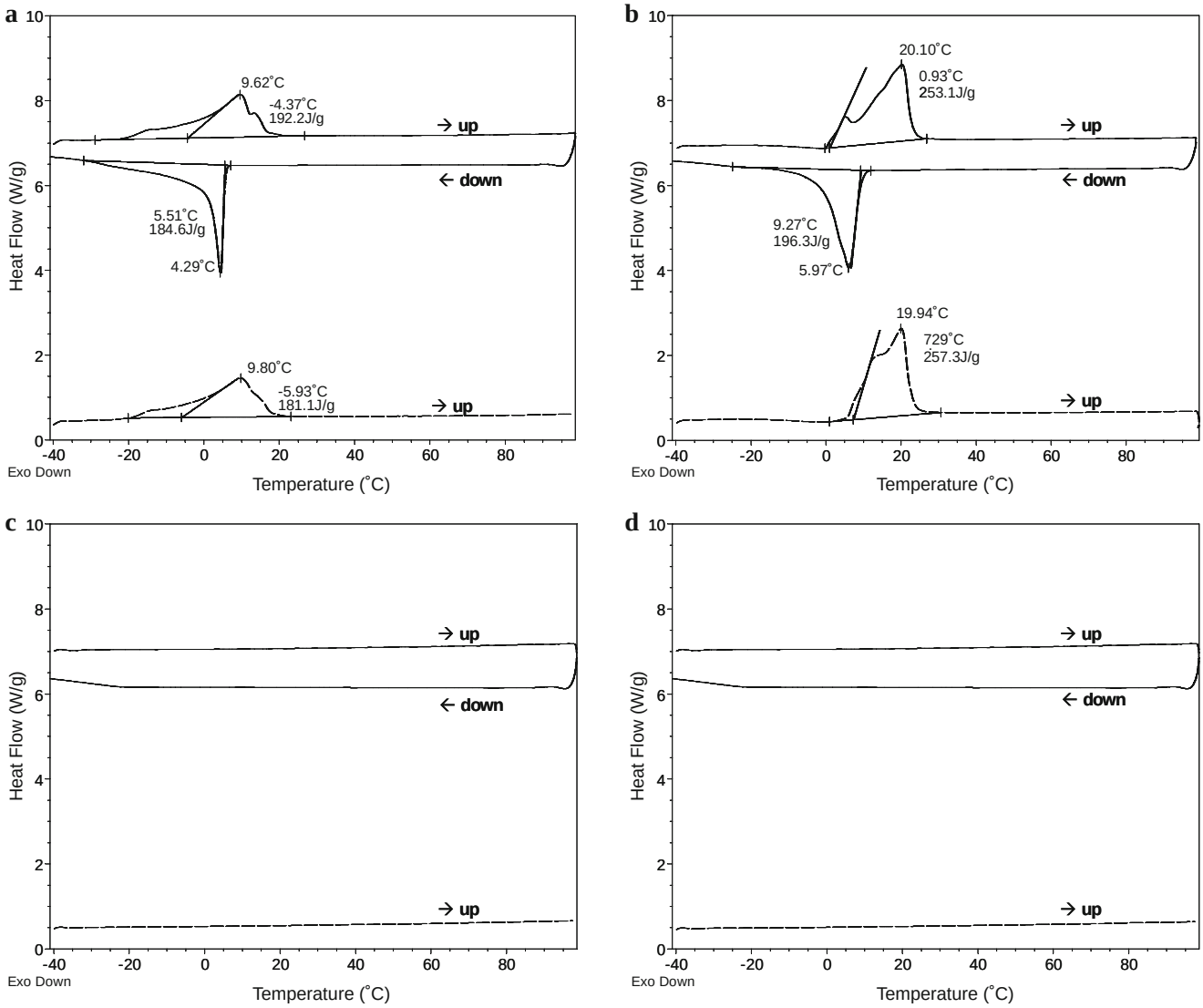

Fig. 4. Thermograms of a 20% solution of $PLA_{50}GA_{50}12$ in PEG500DME (**a**), in PEG600 (**b**) and a 20% (**c**) and 40% $PLA_{50}GA_{50}12$ (**d**) solution in NMP with a first heating cycle from −40°C to 100°C, cooling to −40°C with 5°C/min, 30 min at −40°C and a second heating cycle from −40°C to 100°C.

The ternary phase diagram using PEG600 (Fig. 5b) as solvent reveals that only 0.3% uptake of water is needed to precipitate the polymer at a content of 24.8%. At a low $PLA_{50}GA_{50}12$ content (3.5%), 9.2% water had to be added to induce the onset of turbidity in the polymer solution. The $PLA_{50}GA_{50}12$ solutions in PEG600 behaved similarly to the solutions in PEG500DME, displaying precipitation at increased polymer and low water content. Accordingly, an increased tolerability of water until precipitation was observed at low polymer concentrations.

The ternary phase diagram of $PLA_{50}GA_{50}12$ in NMP (Fig. 5c) shows a miscible homogenous system up to 50% polymer in 50% solvent (*w*/*w*). At a water content of 4.7%, turbidity appeared in a solution of 45.6% $PLA_{50}GA_{50}12$ in 49.6% NMP. The capacity to dissolve $PLA_{50}GA_{50}12$ was significantly increased in NMP relative to PEG500DME and PEG600. 14.1% water had to be added to 2.9% $PLA_{50}GA_{50}12$ in NMP for precipitation to occur. The area of a miscible homogenous mixture above the straight line was increased in the ternary phase diagram using NMP as solvent compared to PEG500DME or PEG600. The capacity to dissolve $PLA_{50}GA_{50}12$ in PEG500DME and PEG600 was lower than in NMP. Overall, the onset of $PLA_{50}GA_{50}12$ precipitation occurred at lower concentrations in PEG500DME and PEG600 compared to NMP.

Solvent Diffusion Process

The influence of a limited, but defined surface area (Fig. 6a) and an increased, but poorly defined surface area (Fig. 6b) of the *in situ* forming depot was compared with two different setups. In the first experiment, the surface area was 3.5 cm^2, similar to the bottom size of a 10 ml vial. Diffusion was only permitted to take place in one direction. Fig. 6a shows the release of 3.5% PEG500DME over 10 min, constantly increasing to approximately 100% of the incorporated solvent after 48 h. 23.9% of the solvent was released in the first 10 min from a 20% polymer solution in NMP. It subsequently continued with a similar release profile to the $PLA_{50}GA_{50}12$ solution in PEG500DME to approximately 100% over 48 h. The

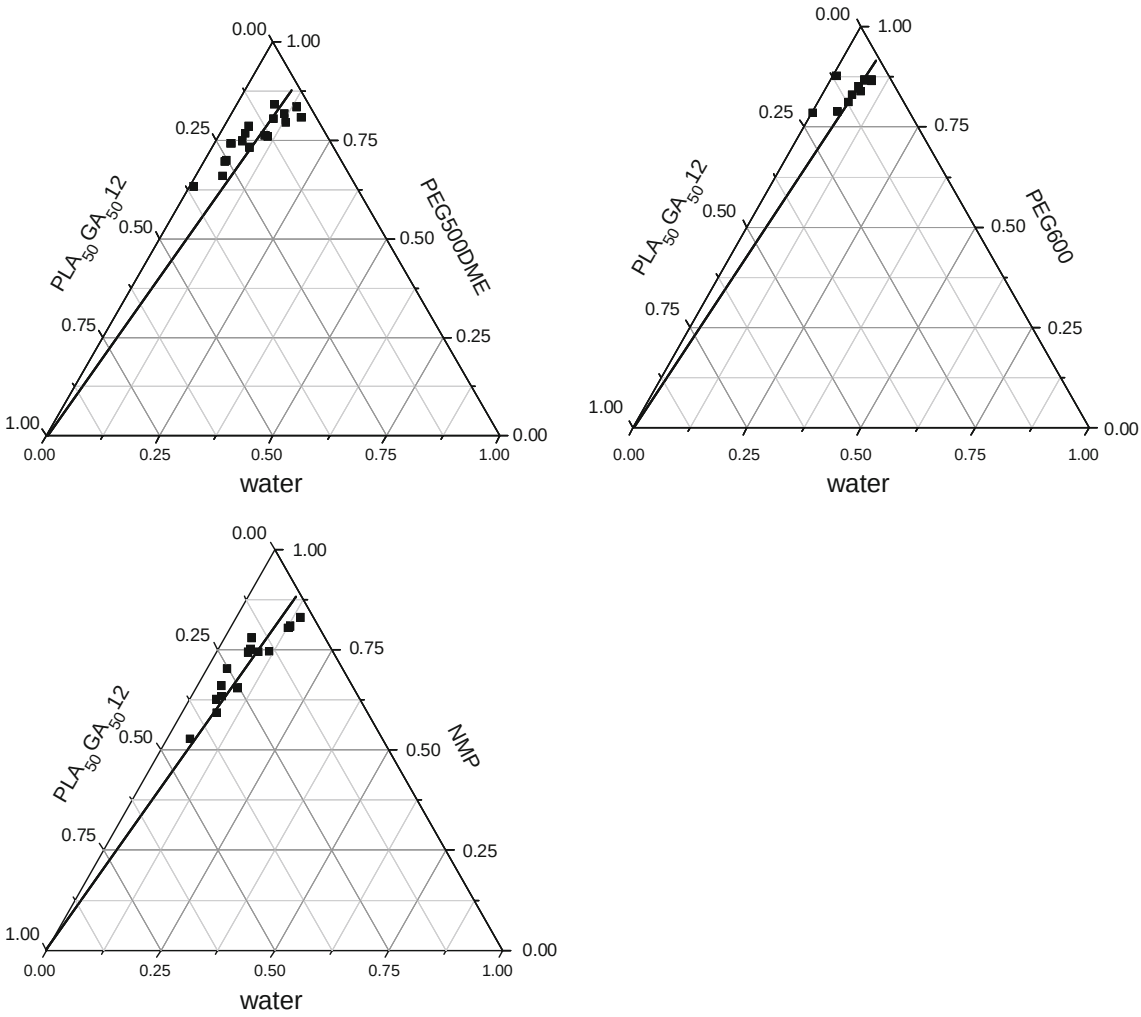

Fig. 5. Ternary phase diagrams at 37°C for $PLA_{50}GA_{50}12$/ NMP/ water (**a**), $PLA_{50}GA_{50}12$/ PEG500DME/ water (**b**) and $PLA_{50}GA_{50}12$/ PEG600/ water (**c**) mixtures are represented by showing the onset of polymer precipitation. A homogenous three-component system is indicated above the straight line and a binary mixture below the line.

diffusion of PEG600 from the polymer precipitate into the buffer was already 14.8% after 10 min and 53.2% in the first 1 h, and it also continued to 100% in 48 h. The 40% $PLA_{50}GA_{50}12$ solution in NMP exhibited a fast solvent release of 12.1% in the first 10 min and a constant release to 48 h, but the precipitate still incorporated NMP. The three ISFD with 20% $PLA_{50}GA_{50}12$ released the solvent almost completely (Fig. 6a). The standard deviation varied for all precipitated polymer solutions due to the addition speed of buffer and therefore the membrane formation on the surface of the polymer solution.

Fig. 6b illustrates the solvent diffusion kinetics out of the polymer precipitate, injected (1 ml syringe, 22G needle) in buffer. A free-floating precipitate was created in the buffer solution. The surface area for the diffusion of the solvent was approximately 9.5 cm^2. The $PLA_{50}GA_{50}12$ solution in PEG500DME showed very slow solvent release of 37.9% during the first hour, increasing to 76.9% after 3 h. After 48 h, the solvent was completely released into the buffer. The solvent diffusion profile of the 20% $PLA_{50}GA_{50}12$ solution in NMP showed a higher initial solvent release of 25.2% in the first 10 min, aligning to the release of PEG500DME with 78.0% after 3 h. The polymer solution with PEG600 showed a lower solvent release of 19.3% in the first 10 min compared to the 20% $PLA_{50}GA_{50}12$ solution in NMP, but an increased release from 0.5 h to 3 h. The release profile of the 40% $PLA_{50}GA_{50}12$ solution in NMP released 13.9% in the first 10 min, to a constant solvent diffusion of nearly 100% after 48 h. Complete NMP release was obtained due to an increased surface area.

All 20% $PLA_{50}GA_{50}12$ solutions with a small surface area (Fig. 6a) released between 62 and 70% within 3 h, compared to the solvent released from the injected systems with increased surface area (76–91%, Fig. 6b).

In Vitro Release of Methylene Blue

Methylene blue was used as highly hydrophilic model substance. In general, constant controlled release was achieved upon polymer precipitation and encapsulation of the substance. Fig. 7 represents the release profiles of four different formulations loaded with the same quantity of methylene blue.

The solution with 20% $PLA_{50}GA_{50}12$ in NMP released 54.8±0.6% of the model substance initially during the first 24 h. Subsequently, methylene blue was slowly released to 100% within 48 days. The initial release of the model

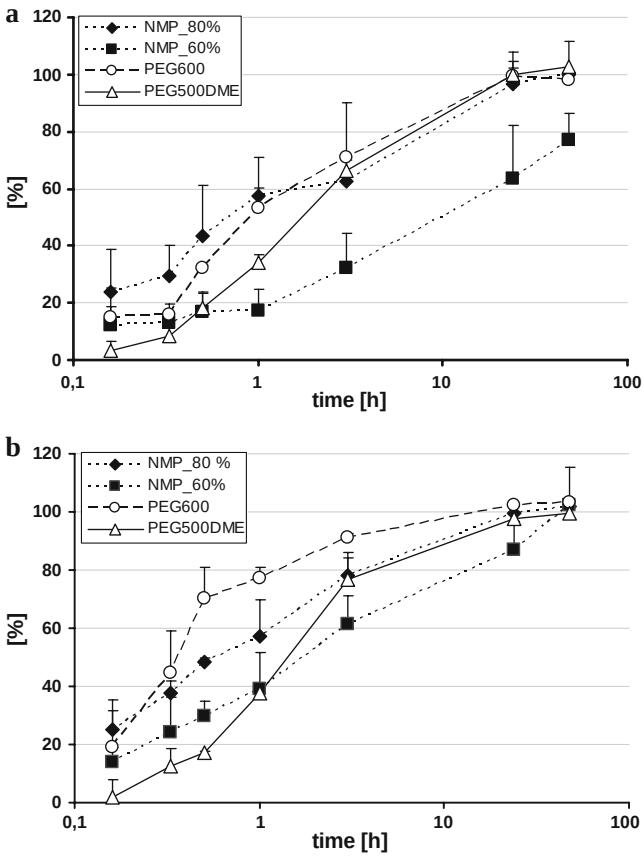

Fig. 6. Solvent release kinetics of 20% solutions of $PLA_{50}GA_{50}12$ in PEG500DME, PEG600 and NMP and a 40% solution of $PLA_{50}GA_{50}12$ in NMP, with a limited surface area of 3.5 cm^2 (**a**) and an increased surface area of approx. 9.5 cm^2 (**b**).

substance from the 40% $PLA_{50}GA_{50}12$ solution in NMP depot was 13.2±2.5%, and this increased to 75.1% during 35 days. An increased $PLA_{50}GA_{50}12$ content of 40% instead of 20% in the formulation using NMP as solvent showed a reduction of the burst and a more long-term release. The solution of $PLA_{50}GA_{50}12$ in PEG600 exhibited 22.7±2.9% initial drug release, which continued to 86.5% released methylene blue over 42 days. A very low burst was shown in the solution of $PLA_{50}GA_{50}12$ in PEG500DME with 6.1± 0.4% being released initially, and 25.5% of the drug substance being released over the duration of the observation time.

DISCUSSION

The common organic solvents used for PLGA depot formulations have numerous limitations for parenteral application (15–17). Therefore, alternative solvents for ISFD are still of great interest. Although PEGs have already been established for use in parenteral formulations (18), the use of PEG500DME as a solvent for parenteral applied ISFD has not been previously investigated. Our results suggest that PEG500DME is a promising solvent for ISFD development.

In general, sustained release formulations are injected subcutaneously (s.c.) or intramuscularly (i.m.) (17). Therefore, the s.c. tissue was envisioned as a prospective application site for an ISFD. The fluid flow in s.c. tissue is known to be in the range of 7–53 ml/ 100 g/ min (5,19). After s.c. injection of an ISFD, the solvent diffuses into the surrounding tissue and is transported via the fluid flow. Accordingly, the solvents' biocompatibility and distribution inside the body need to be critically evaluated.

PEG500DME possesses very limited potential to damage erythrocytes, and it is an effective solvent for $PLA_{50}GA_{50}12$, providing evidence basis to further investigate this excipient for parenteral formulations (Table I). In our study, PEG500DME, PEG600 and NMP were compared in the context of hemolytic activity. The finding confirmed that NMP is associated with a higher risk for parenteral application than either of the other two solvents. PEG500DME showed a clear advantage on hemolysis compared to NMP or PEG600. Despite NMP, DMSO and 2-pyrrolidone exhibiting high myotoxicity (16), sustained release products comprising NMP for parenteral administration have been approved by the FDA (20). The use of less toxic solvents than NMP shows how ISFD can be improved further.

The application route of an ISFD strongly impacts patient compliance. In order to select an appropriate application device, the viscosity of the solvents and the polymer solutions was investigated (Table II). The syringe and needle size and the injection force are parameters correlating with the dynamic viscosity of the $PLA_{50}GA_{50}12$ solutions, which are a function of polymer content and solvent type. Low viscosity allows small needle size and decreased injection force, making the application less painful for the patient. An injection force between 10 and 17 N into s.c. tissue was previously potential for insulin injection devices with a 30G needle (21). Based on this data, we considered an injection force for an ISFD of 20 N to be acceptable. This value was matched by a 20% solution of $PLA_{50}GA_{50}12$ in NMP and PEG500DME using a 1 ml syringe and 23G needle. A formulation of 40% $PLA_{50}GA_{50}12$ in NMP exceeded 20 N at increased injection speeds. Only the solution of 20% $PLA_{50}GA_{50}12$ in NMP did not exceed 20 N injection force, using a 25G needle. Injection forces were recently investigated by Rungseevijiprapa (17), where they described a linear relationship between injection force and viscosity by the Poiseuille equation. This relationship could also be confirmed with our investigations.

The stability of the PLGA formulation in PEG500DME also required further investigation. $PLA_{50}GA_{50}12$ dissolved in endcapped compared to conventional PEGs, has already

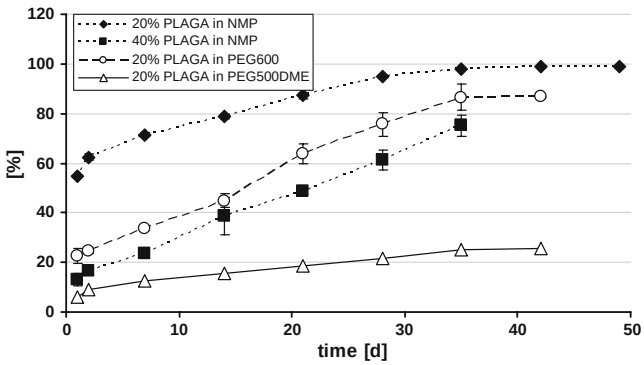

Fig. 7. *In vitro* release of methylene blue of a 20% (◆) and a 40% (■) $PLA_{50}GA_{50}12$ solution in NMP, and a 20% solution of $PLA_{50}GA_{50}12$ in PEG500DME (Δ) and in PEG600 (O).

been demonstrated (6). PEGs, in general, show semicrystalline structures with amorphous and ordered crystalline regions, depending on the orientation of the single chains. This orientation of the chains was shown to be dependent on the thermal history of PEGs with increased M_w (>4,000) (7). The low M_w of the PEG500DME and PEG600 enables them to form a liquid at room temperature and a solid at 2–8°C (Fig. 3). This low solidification point might be useful to stabilize the formulation and prevent PLGA degradation. It might also be advantageous in preventing sedimentation of a dispersed active ingredient. At room temperature, the PEGs are liquid but highly viscous (Table II), allowing the stabilization of a suspension in a specific time-frame prior to the application. Tempering time after the first heating and cooling cycle to rebuild crystalline structure varied from 0.5 to 4 and 8 h. This was investigated to show potential effects of the duration of tempering on the crystalline structure of the PEGs. Differences in the crystal lattice, related to the tempering, can be problematic for the stabilizing effect of the preparation. Changes in the crystalline structure of a drug, affected by the delivery system comprising PEG, can have consequences on solubility, release and bioavailability. However, no effect of tempering on the melting behavior was observed for the pure PEGs and the polymer solutions thereof (data not shown). Since differences in the melting behavior of PEGs with increased M_w were shown (7) due to modifications of different thermodynamic stability, this could also be the case for PEGs with a M_w of 500 to 600 Da. The broad peaks of PEG500DME (Fig. 3a) and PEG600 (Fig. 3b) with several shoulders highlight this. A melting point depression was observed when comparing the solvents with the solutions of 20% $PLA_{50}GA_{50}12$ in the PEGs (Fig. 4). No melting point could be determined for NMP (Fig. 3c) or a polymer solution of NMP (Fig. 4c and d) in the investigated temperature range.

While the stability of the solution is an important factor, the phase separation after injection from a homogenous system to a binary mixture in presence of water is crucial for the functionality of the system and was investigated through ternary phase diagrams (Fig. 5). For all three of the solvents, an increase in water content required for the onset of precipitation with decreasing polymer content was observed. This was previously reported for $PLA_{75}GA_{25}$ in NMP (5). The area representing a homogenous mixture seems to decrease for NMP (Fig. 5c) compared to PEG500DME (Fig. 5a) and PEG600 (Fig. 5b). The polymer precipitation occurred at decreased $PLA_{50}GA_{50}12$ concentrations in PEG500DME and PEG600, compared to NMP. This showed that the solutions of $PLA_{50}GA_{50}12$ in PEG500DME and PEG600 are more sensitive to water.

The phase inversion for the water-miscible solvents—PEG500DME and PEG600—has not been previously published. A solid depot was formed when the solvent content dropped due to the intrusion of water. As a consequence, we observed polymer precipitation after injection of the polymer solution into an aqueous environment. The solvent diffusion kinetics described in Fig. 6 were affected by surface area as well as by the affinity of the solvent to water (22). The effect of the surface area on this process was demonstrated by comparing the release from a small area (3.5 cm^2) (Fig. 6a) with that from an increased surface (approximately 9.5 cm^2) (Fig. 6b). Differences in the solvent release between water-miscible and hydrophobic solvents were shown by Wang (11). One potential reason for the incomplete NMP release (Fig. 6a) might be the increased $PLA_{50}GA_{50}12$ content of 40% in the solution. This may make polymer precipitation occur faster, thus decelerating the solvent release kinetics. Increased polymer content in the solution might also limit the solvent release through the polymer layer, that is created by precipitation. In contrast, PEG500DME showed slow diffusion from the ISFD in the first hour, constantly increasing until complete release after 48 h. This solvent release profile of PEG500DME could be advantageous for drug release.

The release kinetics of the solvents seemed to have an impact on the release of a dissolved hydrophilic compound as well (Fig. 7). The diffusion kinetics of the solvents were observed to be in accordance with the initial release (burst) of methylene blue. This surrogate was chosen to simulate the properties of hydrophilic compounds such as peptides and proteins. The polymer solutions with fast solvent diffusion showed an increased initial drug release. For example, NMP showed a fast solvent release and a high initial burst in a formulation comprising 20% $PLA_{50}GA_{50}12$ (Fig. 7). In alignment to a fast initial PEG600 diffusion, a high burst of methylene blue was observed for the 20% $PLA_{50}GA_{50}12$ solution in PEG600. The findings of the ternary phase diagrams agree well with the fact that the onset of precipitation of the $PLA_{50}GA_{50}12$ in PEG500DME and PEG600 was observed at a lower content than in NMP. Additionally, lower water content was required to precipitate the polymer. The affinity of the solvent to the acceptor media was already described as a major influencing factor for the initial release (22) and could be further supported by our results.

CONCLUSION

PEG500DME is an excellent candidate for the development of an ISFD based on the observations of this study. PEG500DME demonstrates low hemolytic activity, and the PLGA solution in PEG500DME is a stabilizing system that is in the solid state at temperatures of 2–8°C. Furthermore, its high sensitivity to water results in fast phase inversion and polymer precipitation. Finally, extended release profiles with PEG500DME as a solvent displayed a significantly low burst compared to ISFDs that use other typical water miscible solvents.

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