International Journal of Pharmaceutics 434 (2012) 251–256

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Improved physical stability and injectability of non-aqueous in situ PLGA microparticle forming emulsions

M. Voigt, M. Koerber*, R. Bodmeier

College of Pharmacy, Freie Universitaet Berlin, Kelchstrasse 31, 12169 Berlin, Germany

ARTICLE INFO

Article history:
Received 11 January 2012
Received in revised form 19 April 2012
Accepted 15 May 2012
Available online 4 June 2012

Keywords:
Biodegradable microparticles
Emulsion stability
Injectability
In situ forming
Non-aqueous emulsion
Poly(lactide-co-glycolide)

ABSTRACT

The goal of this study was to obtain physically stable non-aqueous in situ forming microparticle (ISM) emulsions capable of forming biodegradable microparticles upon injection. ISM emulsions consist of a biocompatible organic PLGA solution dispersed in a continuous oil phase prepared in a two-syringe/connector system prior to administration. A variety of parenteral approved excipients were tested for a stability-enhancing effect and possible stabilization mechanisms evaluated. Glycerol monostearate (GMS) showed superior stabilizing potential prolonging the emulsion stability from a few minutes to more than 12 h. Flow behavior analysis, differential scanning calorimetry, polarized light- and Cryo-electron microscopy revealed, that the stabilization was caused by an immediate, more than 5-fold viscosity increase in the continuous phase after emulsification and by a stabilized interface through a liquid crystalline GMS layer around the polymer solution droplets. Despite the viscosity increase the injectability of the stabilized ISM emulsion was improved by about 30% compared to the corresponding highly viscous PLGA solution (in situ implant) due to a pronounced shear thinning of the GMS containing oil phase. The injectability improvement allows a faster administration or enables the use of thinner needles and hence reduced patient discomfort.

1. 1 Introduction

Injectable in situ forming biodegradable drug delivery systems have received increasing attention as an alternative to implants and microspheres due to simpler preparation, less stressful manufacturing conditions for sensitive drugs (e.g. proteins) and easy administration (Packhaeuser et al., 2004). Upon injection into the body these systems become more viscous or solidify and thereby encapsulate the drug, which is then released over extended periods of time.

Biodegradable in situ forming microparticle (ISM) systems are injectable emulsions where an internal phase, consisting of a drug-containing polymer solution (e.g. PLGA in DMSO), is emulsified into a stabilizer-containing aqueous or oily continuous phase with a two syringe/connector system prior to administration (Luan and Bodmeier, 2006a). These ISM emulsions showed advantages over the corresponding drug-containing polymer solutions (in situ implants; ISI), such as decreased myotoxicity (Kranz et al., 2001), more reproducible and burst-free drug release patterns (Kranz and Bodmeier, 2007) and better injectability (Rungseevijitprapa and Bodmeier, 2009). This, however, requires that the ISM emulsions are stable until their administration. The preparation of stable non-aqueous emulsions is not trivial and, in contrast to aqueous emulsions, kinetically stable non-aqueous emulsions have rarely been achieved (Suitthimeathegorn et al., 2007). The selection of suitable stabilizers is challenging due to a lack of general knowledge about the underlying stabilization mechanisms in non-aqueous emulsion systems (Cameron and Sherrington, 1996; Suitthimeathegorn et al., 2005; Payghan et al., 2008). The hydrophilic–lipophilic balance (HLB) introduced by Griffin in 1949, for instance, does not apply (Petersen et al., 1964). Stabilization has been achieved by either utilization of a suitable oil-immiscible polar liquid that can substantially replace water, e.g. formamide or by designing surfactants having two incompatible blocks, each of which is selectively soluble in one of the immiscible liquids (Imhof and Pine, 1997). These substances, however, are not approved for parenteral applications.

The lower viscosity of ISM emulsions, due to the dispersion of the highly viscous polymer solution phase in a less viscous continuous phase, resulted in decreased injection forces compared to polymer solutions only (ISI) (Rungseevijitprapa and Bodmeier, 2009). This viscosity-driven evaluation of the "injectability", however, neglected the injection volume to achieve similar drug doses. According to Hagen-Poisseuille's law, even highly viscous liquids (e.g. ISI) could be injected at low forces using for example large needles or low injection speeds (Ravivarapu et al., 2000; Crawford et al.,

* Corresponding author at: FREIE UNIVERSITAET BERLIN, College of Pharmacy, Kelchstrasse 31, 12169 Berlin, Germany. Tel.: +49 30 838 50613; fax: +49 30 838 50707.
E-mail address: koerberm@zedat.fu-berlin.de (M. Koerber).

0378-5173/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijpharm.2012.05.029

2006). However, this would probably decrease the patient acceptance, considering that needle diameter is connected to pain level (Mitchell and Whitney, 2001) and pain exposure time to patient discomfort.

Parameters of an "ideal" injection for both patients and health care professionals are therefore (1) short duration (≤10 s) (Dacre and Kopelman, 2002), (2) small needle size (≥20 G) and (3) low maximal injection force (≤20 N) (Debra et al., 2008; Schoenhammer et al., 2009). The choice of the injection site will determine the limit of the injection volume, e.g. 1 ml for the deltoid site (Rodger and King, 2000), and also set the ideal injection speed (e.g. 1 ml per 10 s). The evaluation of the "injectability" of an injectable depot formulation thus needs to include the aforementioned aspects.

The objectives of this study were therefore to obtain physically stable non-aqueous ISM emulsions, to elucidate the underlying stabilization mechanisms and to characterize the effect of the emulsion stabilization on the injectability of the resulting ISM formulations.

2. Materials and methods

2.1. Materials

The parenterally approved compounds investigated as potential stabilizers comprised poloxamer 188 (Pluronic F68), poloxamer 407 (Lutrol F127), polyethylene glycol 660 12-hydroxystearate (Solutol HS 15), polyoxyl 35 castor oil (Cremophor EL), polyoxyl 40 hydrogenated castor oil (Cremophor RH 40), polyoxyl 60 hydrogenated castor oil (Cremophor RH 60) all from BASF AG, Ludwigshafen, Germany; alpha-tocopherol, chenodeoxycholic acid, cholic acid, cholesterol, polyoxyethylene 20 sorbitan monooleate (Tween 80), sodium dodecyl sulfate from Carl Roth GmbH & Co. KG, Karlsruhe, Germany; aluminum monostearate from Fluka, Chemie AG, Buchs, Switzerland; egg-phosphatide mixtures (Lipoid E80, EPC) and soy-phosphatide mixtures (Lipoid S 100, S45) from Lipoid GmbH, Ludwigshafen, Germany; sorbitan monooleate (Span 80) from Merck, Darmstadt, Germany; glycerol monostearate (Imwitor 900K) from Sasol Germany GmbH, Witten, Germany and ethanolamine from Sigma–Aldrich Company, St. Louis, USA.

Medium chain triglycerides (MCT) from Fagron GmbH & Co. KG, Barsbüttel, Germany and sesame oil from Sigma–Aldrich Company, St. Louis, USA were used as continuous phases. Low molecular weight 50:50 PLGA (capped poly(D,L-lactide-*co*-glycolide; Resomer RG 502S, intrinsic viscosity 0.2 dl/g) was obtained from Boehringer Ingelheim Pharma GmbH & Co. KG, Ingelheim, Germany. Dimethylsulfoxide (DMSO) and tetrahydrofuran were obtained from Carl Roth GmbH & Co. KG, Karlsruhe, Germany. Coloring of the internal phase was performed with methylene blue from Sigma–Aldrich Company, St. Louis.

2.2. Methods

2.2.1. ISM emulsion preparation

The internal phase was prepared by dissolving PLGA Resomer RG 502S (e.g. 300 mg) in DMSO (e.g. 700 mg) under intermittent vortexing to obtain a final polymer concentration of for example 30% (w/w). Separately, 50 mg of potential stabilizers were dissolved or dispersed in 950 mg vegetable oil at 80 °C under vortexing to prepare continuous phases containing 5% stabilizer (w/w). Both phases were stored in a desiccator for 24 h at ambient temperature before further use.

ISM emulsions were prepared in a two-syringe/connector system, comprising the internal and continuous phases each in a 1 ml single-use syringe (B. Braun Melsungen AG, Melsungen, Germany). The syringes were coupled with a connector and the two phases were emulsified by back-and-forth movement of the syringe plungers.

Unless mentioned otherwise, 0.5 ml of the ISM emulsion (internal phase:continuous phase ratio of 1:1, v/v) were prepared by mixing 0.25 ml internal phase (30% PLGA in DMSO, w/w) with 0.25 ml external phase (5% stabilizer-containing sesame oil, w/w) under the following conditions: 1.4 mm connector diameter and 1 mixing cycle per s for 180 s.

2.2.2. Hot-stage polarized light microscopy

A Zeiss Axioscope (Carl Zeiss Jena GmbH, Jena, Germany) equipped with a Mettler Toledo FP82HT hot stage (Mettler-Toledo GmbH, Giessen, Germany) and EasyMeasure analysis software (Inteq Informationstechnik GmbH, Berlin, Germany) were used to study the melting behavior of glycerol monostearate (GMS) and the ISM emulsion droplet size distributions 15 min after preparation ($n = 3$, each with 300 droplets).

Particle size distribution and oil separation ($n = 3$) as a function of time were determined with formulations stored vertically in 1 ml syringes in a desiccator under ambient conditions and were characterized by the arithmetic mean diameter (d_{av}) and the particle size at 10% (d_{10}) and 90% (d_{90}) of the particle size distribution to visualize possible particle size growth.

2.2.3. Electron microscopy

2.2.3.1. Cryo-scanning electron microscopy (Cryo-SEM). The emulsion droplet interface of freshly prepared non-aqueous ISM emulsions was investigated with a Hitachi S-4800 scanning electron microscope at −145 °C. Thereby, emulsions were rapidly frozen in liquid nitrogen slush, fractured at −190 °C and sputtered with platinum.

2.2.3.2. Freeze etching-transmission electron microscopy (FE-TEM). Additionally, the droplet interface was investigated with a Zeiss EM-902 transmission electron microscope. The samples were pretreated with the aid of a Balzers BAF 400T etching unit, i.e. emulsions were rapidly frozen in liquid propane, fractured at −100 °C and sputtered with platinum and carbon. Subsequently, the samples were dissolved in tetrahydrofuran. Transmission electron microscopy was then performed on the corresponding mirror images.

2.2.4. Viscosity

The flow behavior of continuous phases and the corresponding ISM emulsions were investigated with a software-assisted rheometer (Rheostress RS 100 with RheoWin Pro software, Haake Messtechnik GmbH, Karlsruhe, Germany) equipped with a plate-cone setup of 60 mm diameter (1° angle) at 23 ± 0.2 °C. Shear rate controlled ramps (CR) with increasing and decreasing segments (0–150–0 s^{-1}, 4 min per measurement) were used.

The yield point, indicating the gel-network strength of GMS in the continuous phase (sesame oil), was determined using controlled shear stress ramps (CS) with logarithmic distributed data points from 1 to 1000 Pa in 180 s and consecutively double logarithmic data plotting (shear rate against shear stress). The yield point was defined as the shear stress value where the slope of the resulting curve increased.

Prior to all measurements, the samples were treated with a shear rate of 10 s^{-1} for 60 s and rested for 60 s to standardize the sample history.

2.2.5. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was used to investigate the thermal characteristics of GMS in the continuous phase and in the corresponding ISM emulsions. 30–40 mg sample material were

accurately weighed in closed 40 μl aluminum crucibles and analyzed with a DSC 821 (Mettler Toledo AG, Giessen, Germany) under nitrogen atmosphere. The samples were cooled to 15 °C and then heated to 80 °C at a constant rate of 10 °C/min. The thermograms were standardized to a sample weight of 40 mg.

2.2.6. Injectability

The force–distance profile of an injected 35% PLGA 502S containing DMSO (w/w) solution was compared with the corresponding in situ microparticle emulsions using a texture analyzer (50 N load cell) equipped with Texture Expert Exceed software (TA.XTplus, Stable Micro Systems, Vienna Court, UK). The maximal force was set to 45 N. 0.275 ml of the PLGA solution or 0.550 ml of the corresponding ISM emulsions (sesame oil with or without 5% GMS; phase ratio 1:1, connector diameter 0.75 mm and 2 cycles per s for 180 s) were injected from 1 ml syringes (Injekt-F, B. Braun Melsungen AG, Melsungen, Germany) through hypodermic needles (24 G × 1.0 in) in order to compare the formulation at a similar polymer solution volume. The freshly prepared ISM emulsions were kept in horizontal position for 5 min (non-stabilized formulations) or 15 min (stabilized formulations) before injection. The injection speed was set to 5 mm/s, which corresponded to an injection of 1 ml per 10 s. The syringe friction force of 0.49 ± 0.16 N was not subtracted to present real injection forces. The injection energy was calculated from the area under the curve (AUC).

3. Results and discussion

3.1. Stabilizer screening

The selection of stabilizing excipients for non-aqueous emulsions is not as straightforward as for aqueous emulsions. A number of parenterally approved potential stabilizers (Costarelli et al., 2002; Rowe et al., 2005; Dassinger et al., 2009) were screened for a stabilizing effect on non-aqueous ISM emulsions (see Section 2.1). The excipients were added to the continuous phase (medium chain triglycerides (MCT) or sesame oil) and were then emulsified with a PLGA-DMSO solution (internal phase:continuous phase ratio 1:1, v/v) with a two-syringe connector system.

For most samples, two separate phases formed within 5 min. Only ethanolamine in sesame oil, Lipoid S 100 in MCT and GMS using either sesame oil or MCT as continuous phases showed no phase separation over an observation period of 15 min, which is an appropriate time frame to administer the ISM emulsion to a patient. The alkaline ethanolamine formed the emulsifying substances, hydroxyethylammonium carboxylates, in situ via saponification of vegetable oils (McMahon et al., 1963). In general, the use of saponifying agents may be a promising tool to stabilize non-aqueous emulsions. However, ethanolamine can also potentially hydrolyze PLGA and was thus not further studied.

Emulsions containing Lipoid S100 in MCT showed phase separation after about 20 min. Interestingly, emulsions stabilized with GMS showed no signs of phase separation over more than 1 h independent of whether MCT or sesame oil was used as continuous phase. The stabilization of non-aqueous ISM emulsions with GMS was thus more effective compared to the previously used stabilizer Span 80, Pluronic F68 and aluminum monostearate (Jain et al., 2000; Luan and Bodmeier, 2006b; Kranz and Bodmeier, 2007).

3.2. Stability of GMS-stabilized emulsions

Decreasing the internal phase:continuous phase ratio from 1:1 to 1:1.5 further improved the ISM emulsion stability against droplet coalescence. No sign of instability was observed within 12 h of storage in a horizontally positioned 1 ml syringe at room temperature in a desiccator (Table 1). The average particle size (d_{av}) increased from 19.1 μm after 12 h to 21.9 μm at day 1 and steadily further increased to 29.6 μm at day 14. The particle size, where 90% of the emulsion droplets have a smaller diameter (d_{90}) increased slightly faster during storage compared to the particle size where 10% of the droplets have a smaller diameter (d_{10}). This phenomenon was attributed to Ostwald ripening (Imhof and Pine, 1997). Separation of the continuous phase was observed after 1 d, when 5% of the oil could be decanted. This amount increased to about 20% within 14 d. The formation of a separate PLGA solution phase was not observed. The PLGA-containing internal phase thus remained finely dispersed, which indicated that microparticle formation could still be achievable.

Table 1
Stability of glycerol monostearate (GMS)-containing non-aqueous ISM emulsions (internal phase: 30% PLGA 502S in DMSO, continuous phase: 5% GMS in sesame oil, internal phase:continuous phase ratio 1:1.5, volume 0.5 ml) during storage in horizontally positioned 1 ml syringes.

Storage time (days)	Droplet size distribution (μm)			Oil separation (%)
	d_{av}[a]	d_{10}	d_{90}	
0	19.3 ± 3.4	15.8	22.6	0.00
0.5	19.1 ± 3.2	15.4	23.0	0.00
1	21.9 ± 4.1	17.4	25.5	4.9 ± 0.1
2	25.0 ± 5.2	19.9	29.8	9.7 ± 0.3
5	26.0 ± 5.9	20.4	32.6	14.3 ± 0.9
9	28.2 ± 6.5	20.7	35.7	19.2 ± 0.8
14	29.6 ± 6.8	22.4	38.4	19.2 ± 0.8

[a] d_{av} is the arithmetic mean diameter, d_{10} and d_{90} are the droplet diameters at 10% and 90% of the particle size distribution.

3.3. Stabilization mechanisms

Glycerol monostearate formed rod-like crystals embedded in a fine particle matrix upon preparation of the continuous oil phase (Fig. 1A). This was accompanied by a change of the DSC chromatograms from a single melting event for glycerol monostearate, representing the high melting β modification only, to a triple melt endotherm at lower temperatures, indicating the presence of a mixture of modifications (α, β′ and β) in the continuous phase (Fig. 2), which typically occurs upon heating and cooling of glycerol esters (Ojijo et al., 2004; Himawan et al., 2006). Interestingly, the multiphasic melting behavior of the continuous phase disappeared upon preparation of the ISM emulsion, and an endotherm at even lower temperature appeared (Fig. 2).

Furthermore, a birefringent layer around the dispersed droplets was seen in the emulsions with polarized light microscopy (Fig. 1B). This indicated that a liquid crystalline phase of glycerol monostearate was formed at the interface between the oil phase and the PLGA solution, as seen previously with an aqueous emulsion (Mele et al., 2002). Cryo-SEM and freeze etching-TEM confirmed the presence of liquid GMS crystals at the interface. Lamellar, platelet-like structures of GMS were observed on the droplet surface by Cryo-SEM (Fig. 1C) and also by freeze etching-TEM in the corresponding mirror images of the droplet interface (Fig. 1D). In agreement with studies on aqueous systems (Macierzanka et al., 2009), interface stabilization by GMS was identified as one stabilization principle for non-aqueous ISM emulsions based on the highly polar polymer solvent DMSO (relative permittivity of 48 for DMSO vs. 80 for water at 25 °C).

Monoglycerides such as GMS, however, are also able to gel vegetable oils (Chen and Terentjev, 2009), which is known to decrease droplet coalescence in W/O emulsions (Hodge and Rousseau, 2005). The strength of the gel network and thus the stabilization potential can be expressed by the yield point (T_0), which is defined as the minimal shear force required to break down the network structure and hence initiate plastic flow (Lee et al., 2009). The presence of a yield

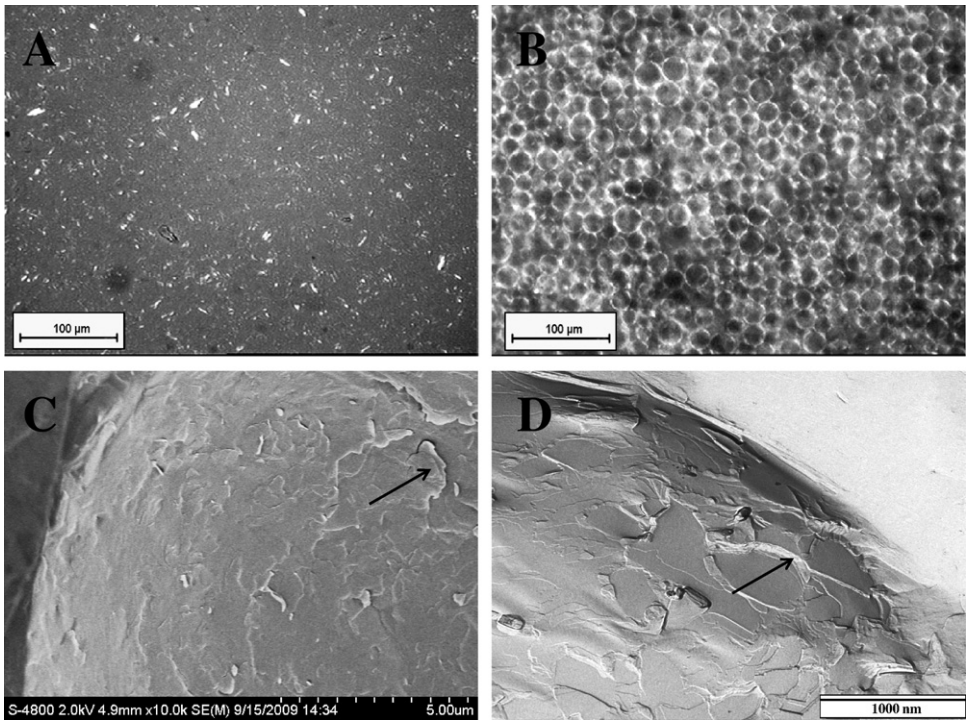

Fig. 1. Microscopic images of (A) the continuous phase (5% GMS in sesame oil) stored 1 day at room temperature and (B) the corresponding ISM emulsion 15 min after preparation; (C) Cryo-SEM (surface) and (D) freeze etching-TEM (interface) images of GMS stabilized ISM emulsion droplets. The arrows indicate the lamellar, platelet like structures of GMS liquid crystals at the interface.

point at GMS concentrations ≥2.5% indicated GMS network formation in sesame oil (Fig. 3). The yield point and hence the gel strength and viscosity increased with the GMS concentration, being 0.5, 2.0 and 8.0 Pa at GMS concentrations of 2.5, 4 and 5%, respectively. The presence of a network structure at 2.5% GMS concentration correlated well with the minimal amount of GMS necessary to form stable ISM emulsions (Table 2), indicating its importance for the stabilization of the emulsion.

A yield point of 6 Pa in the non-aqueous ISM emulsion containing 5% GMS indicated that the GMS network remained intact upon emulsification. The gel strength, however, decreased compared to the 5% GMS-containing sesame oil (8 Pa). This was attributed to the partitioning of a portion of GMS from the continuous phase into a liquid crystalline phase at the interface.

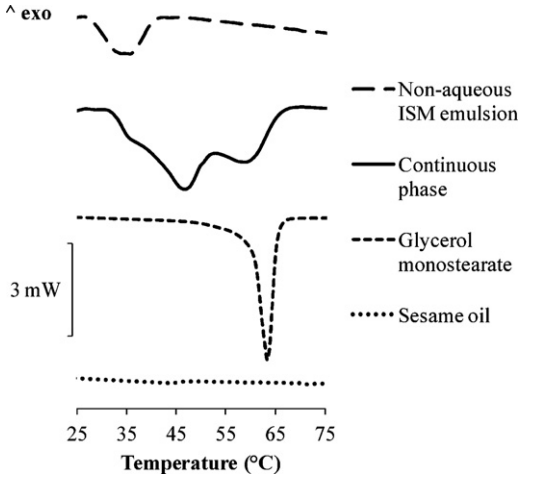

Fig. 2. DSC thermograms of sesame oil, pure glycerol monostearate, continuous phase (5% GMS in sesame oil) stored 1 day at room temperature and the corresponding non-aqueous ISM emulsion.

The gel network forming and viscosity enhancing effect of GMS was probably the dominant emulsion stabilization mechanism in this study, since liquid crystalline layers were already seen at 2% GMS, whereas a stable emulsion required more than 2.5% GMS content in the continuous phase.

Other potential stabilizing effects of GMS, e.g. a reduction of the density difference of dispersed and continuous phase (Derkach, 2009) or a decrease of the surface tension (both 4.5 dynes/cm) could be excluded.

3.4. Flow behavior and injectability

GMS-containing sesame oil showed a shear rate dependent decrease of the viscosity, opposite to the Newtonian flow behavior of pure sesame oil (Fig. 4). The shear thinning was reversible with decreasing shear rate. The hysteresis loop indicated a slightly

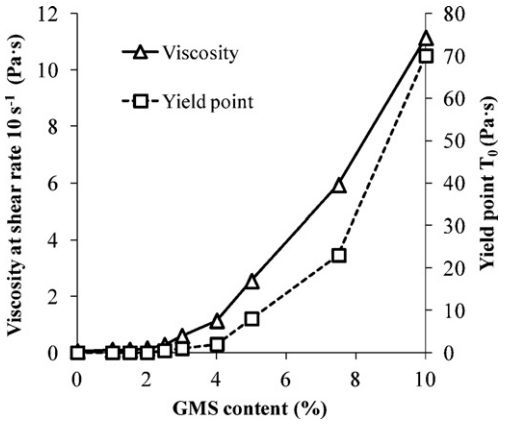

Fig. 3. Effect of GMS concentration in the continuous phase on the yield point (network formation) and the viscosity at a shear rate of 10 s^{-1} (internal phase:continuous phase ratio 1:1.5).

Table 2
Effect of glycerol monostearate (GMS) concentration in the continuous phase on ISM emulsion droplet size (internal phase: 30% PLGA 502S in DMSO, continuous phase: 0–7.5% GMS in sesame oil, internal phase:continuous phase ratio 1:1.5).

GMS concentration (%)	Mean emulsion droplet size (μm)
0	–
2	–
2.5	45.8 ± 11.0
3	32.3 ± 10.4
5	18.9 ± 2.9
7.5	17.9 ± 3.1

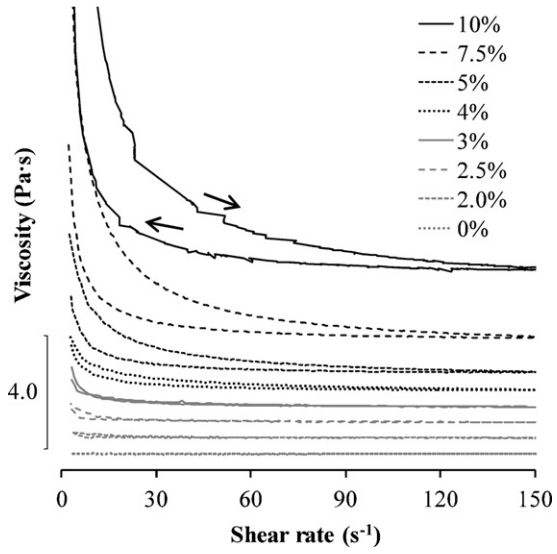

Fig. 4. Flow behavior of sesame oil as a function of GMS concentration under increasing (→) and decreasing (←) shear rates (internal phase:continuous phase ratio 1:1.5).

thixotropic flow behavior, which is typically seen for fluids containing gel networks (Lee et al., 2009).

ISM emulsions showed similar flow properties to the continuous phase (Fig. 5). Applying a high shear rate (100 s^{-1}) to the continuous phase and the corresponding ISM emulsion to mimic the emulsification step resulted in viscosities as low as 0.46 Pa s and 0.34 Pa s,

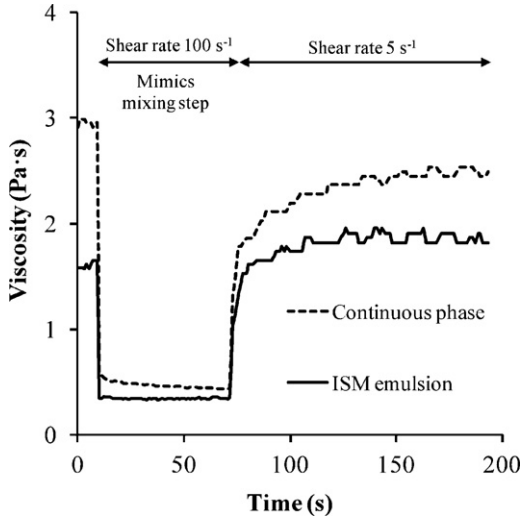

Fig. 5. Flow behavior of the continuous phase (5% GMS in sesame oil) and the corresponding ISM emulsion (internal phase:continuous phase ratio 1:1.5) treated with different shear rates on a rheometer to mimic the emulsification via the two syringe system. During the first 10 s, the samples were exposed to a shear rate of 5 s^{-1}.

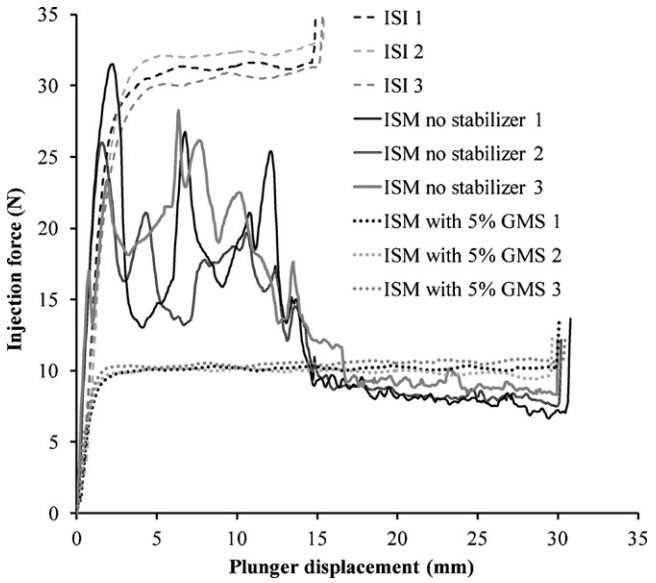

Fig. 6. Required force to inject ISI solutions (0.275 ml of 35% PLGA 502S dissolved in DMSO) and the corresponding ISM emulsions (0.550 ml) stabilized with 5% GMS (after 15 min of vertical storage) or without stabilizer (after 5 min of vertical storage) from 1 ml syringes equipped with 24 G × 1.0 in. hypodermic needles.

respectively. The viscosity increased rapidly when the shear rate was decreased from 100 to 5 s^{-1}. Values of 2.47 Pa s for the continuous phase and 1.87 Pa s for the corresponding ISM emulsion corresponded to a more than 5-fold viscosity increase in both cases. The handling of the ISM emulsion could benefit from shear thinning, if the stability-enhancing viscosity increase (low shear) does not compromise the injection of the emulsions (high shear).

The injectability of ISM emulsions and the corresponding PLGA solution was therefore evaluated using force–displacement profiles of the formulations ejected through thin hypodermic needles (24 G × 1.0 in.). The ejection speed was set to 0.1 ml/s in order to have an acceptable injection time of 10 s (Dacre and Kopelman, 2002) for the maximum dose (1 ml) administrable to the deltoid (Rodger and King, 2000). GMS-stabilized ISM emulsions could be injected at a constant force of 10.2 ± 0.29 N (Fig. 6 and Table 3). The force necessary to inject 5% GMS containing sesame oil was very similar to the emulsion (9.45 ± 0.64 N), which highlighted the viscosity-determining role of the continuous phase in an emulsion containing droplets of the highly viscous PLGA solution, which can pass the needle. As a result of rapid droplet coalescence in unstabilized emulsions, peak forces approaching the injection force of the PLGA solution (31.08 ± 1.40 N) were detected. Such high forces exceeded the upper limit for an acceptable injection of 20 N (Schoenhammer et al., 2009).

The high peak forces, however, were not apparent from the average force of the complete injection (12.84 ± 5.49 N) due to the low force necessary to inject the separated oil portion of the emulsion. Besides this disadvantage, comparing the injectability on the basis of average injection forces ignores, that the injection volume

Table 3
Required force and energy to inject in situ forming implant (ISI) solutions, the corresponding ISM emulsions and the continuous phases of the emulsions through 24 G hypodermic needles.

	Injection force (N)	Injection energy (mJ)
ISI	31.08 ± 1.40	2901 ± 39
ISM no stabilizer	12.84 ± 5.49	2753 ± 180
ISM with 5% GMS	10.20 ± 0.29	2051 ± 60
Sesame oil	5.15 ± 0.23	518 ± 20
5% GMS in sesame oil	9.45 ± 0.64	901 ± 52

required to administer a similar drug dose is lower for the polymer solution (in situ implant) compared to the ISM emulsion, which leads to either a shorter injection time (Fig. 6) or allows for a lower injection speed and hence a lower injection force. In contrast to the average force, an evaluation on the basis of the dose-normalized area under the force–displacement curve (injection energy) overcomes these shortcomings. A 25–30% lower injection energy of the GMS-stabilized ISM emulsion compared to the corresponding PLGA solution and the unstabilized ISM emulsion (Table 3) showed the superior injectability of the stabilized ISM emulsion. Thus, injection times could be shortened by about 30% and handling issues due to peak injection forces would be eliminated.

4. Conclusions

The stability of non-aqueous in situ forming microparticle (ISM) emulsions was significantly improved from a few minutes to more than 12 h through the addition of glycerol monostearate (GMS) to the continuous oil phase due to a viscosity increase of the continuous phase and the formation of a liquid crystalline GMS layer at the interface.

Homogeneous injection profiles with low injection forces were obtained with GMS-stabilized emulsions. The injectability of stabilized ISM emulsions was improved by 30% compared to the corresponding highly viscous PLGA solution, allowing for a faster administration and hence a reduction of the pain exposure time for patients or the use of smaller more acceptable needles.

Acknowledgement

Dr. Brigitte Tiersch (Universitaet Potsdam, Department of Colloid Chemistry, Potsdam, Germany) is thanked for recording the electron microscope images.

References

Cameron, N.R., Sherrington, D.C., 1996. Non-aqueous high internal phase emulsions—preparation and stability. J. Chem. Soc., Faraday Trans. 92, 1543–1547.
Chen, C.H., Terentjev, E.M., 2009. Aging and metastability of monoglycerides in hydrophobic solutions. Langmuir 25, 6717–6724.
Costarelli, V., Key, T.J., et al., 2002. A prospective study of serum bile acid concentrations and colorectal cancer risk in post-menopausal women on the island of Guernsey. Br. J. Cancer 86, 1741–1744.
Crawford, E.D., Sartor, O., et al., 2006. A 12-month clinical study of LA-2585 (45.0 MG): a new 6-month subcutaneous delivery system for leuprolide acetate for the treatment of prostate cancer. J. Urol. 175, 533–536.
Dacre, J., Kopelman, P.G., 2002. A Handbook of Clinical Skills. Manson Publishing, London.
Dassinger, M., Dootz, H., et al., 2009. Rote Liste. Rote Liste Service GmbH, Frankfurt am Main.
Debra, A.I., Opincar, M., et al., 2008. FlexPen® and KwikPen™ prefilled insulin devices: a laboratory evaluation of ergonomic and injection force characteristics. J. Diabetes Sci. Technol. 2, 533–537.
Derkach, S.R., 2009. Rheology of emulsions. Adv. Colloid Interface Sci. 151, 1–23.
Himawan, C., Starov, V.M., et al., 2006. Thermodynamic and kinetic aspects of fat crystallization. Adv. Colloid. Interface. Sci. 122, 3–33.
Hodge, S.M., Rousseau, D., 2005. Continuous-phase fat crystals strongly influence water-in-oil emulsion stability. J. Am. Oil Chem. Soc. 82, 159–164.
Imhof, A., Pine, D.J., 1997. Stability of nonaqueous emulsions. J. Colloid Interf. Sci. 192, 368–374.
Jain, R.A., Rhodes, C.T., et al., 2000. Controlled release of drugs from injectable in situ formed biodegradable PLGA microspheres: effect of various formulation variables. Eur. J. Pharm. Biopharm. 50, 257–262.
Kranz, H., Bodmeier, R., 2007. A novel in situ forming drug delivery system for controlled parenteral drug delivery. Int. J. Pharm. 332, 107–114.
Kranz, H., Brazeau, G.A., et al., 2001. Myotoxicity studies of injectable biodegradable in situ forming drug delivery systems. Int. J. Pharm. 212, 11–18.
Lee, C.H., Moturi, V., et al., 2009. Thixotropic property in pharmaceutical formulations. J. Control. Rel. 136, 88–98.
Luan, X., Bodmeier, R., 2006a. In situ forming microparticle system for controlled delivery of leuprolide acetate: influence of the formulation and processing parameters. Eur. J. Pharm. Sci. 27, 143–149.
Luan, X., Bodmeier, R., 2006b. Influence of the poly(lactide-co-glycolide) type on the leuprolide release from in situ forming microparticle systems. J. Control. Rel. 110, 266–272.
Macierzanka, A., Szelag, H., et al., 2009. Effect of crystalline emulsifier composition on structural transformations of water-in-oil emulsions: emulsification and quiescent conditions. J. Colloids Surf. Physicochem. Eng. Aspects 334, 40–52.
McMahon, J.D., Hamill, R.D., et al., 1963. Emulsifying effects of several ionic surfactants on a nonaqueous immiscible system. J. Pharm. Sci. 52, 1163–1168.
Mele, S., Khan, A., et al., 2002. A didodecyldimethylammonium bromide ternary system: characterization of three-phase stable emulsions by optical microscopy. J. Surf. Det. 5, 381–389.
Mitchell, J.R., Whitney, F.W., 2001. The effect of injection speed on the perception of intramuscular injection pain. A clinical update. AAOHN J. 49, 286–292.
Ojijo, N.K.O., Neeman, I., et al., 2004. Effects of monoglyceride content, cooling rate and shear on the rheological properties of olive oil/monoglyceride gel networks. J. Sci. Food Agric. 84, 1585–1593.
Packhaeuser, C.B., Schnieders, J., et al., 2004. In situ forming parenteral drug delivery systems: an overview. Eur. J. Pharm. Biopharm. 58, 445–455.
Payghan, S.A., Bhat, M., et al., 2008. Non-aqueous emulsion: Versatile vehicle for drug delivery. Pharmainfo.net (http://www.pharmainfo.net/reviews/non-aqueous-emulsion-versatile-vehicle-drug-delivery, July 2011) 6(1).
Petersen, R.V., Hamill, R.D., et al., 1964. Emulsifying effects of some nonionic surfactants on a nonaqueous immiscible system. J. Pharm. Sci. 53, 651–655.
Ravivarapu, H.B., Moyer, K.L., et al., 2000. Parameters affecting the efficacy of a sustained release polymeric implant of leuprolide. Int. J. Pharm. 194, 181–191.
Rodger, M.A., King, L., 2000. Drawing up and administering intramuscular injections: a review of the literature. J. Adv. Nurs. 31, 574–582.
Rowe, R.C., Sheskey, P.J., et al. (Eds.), 2005. Handbook of Pharmaceutical Excipients. APhA Publications, Washington.
Rungseevijitprapa, W., Bodmeier, R., 2009. Injectability of biodegradable in situ forming microparticle systems (ISM). Eur. J. Pharm. Sci. 36, 524–531.
Schoenhammer, K., Petersen, H., et al., 2009. Poly(ethyleneglycol) 500 dimethylether as novel solvent for injectable in situ forming depots. Pharm. Res. 26, 2568–2577.
Suitthimeathegorn, O., Jaitely, V., et al., 2005. Novel anhydrous emulsions: Formulation as controlled release vehicles. Int. J. Pharm. 298, 367–371.
Suitthimeathegorn, O., Turton, J.A., et al., 2007. Intramuscular absorption and biodistribution of dexamethasone from non-aqueous emulsions in the rat. Int. J. Pharm. 331, 204–210.