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Effect of solvent type on porous structure of emulsion templated poly (glycerol sebacate)-methacrylate

María Fernanda Velázquez de la Paz, Mina Aleemardani, Rachel Furmidge, Samand Pashneh-Tala, Frederik Claeyssens *

Department of Materials Science and Engineering, University of Sheffield, Sheffield, UK

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ABSTRACT

Polymerised emulsion templating is a common method for the fabrication of biomaterials with interconnected porous structures. Here, we present the fabrication of poly(glycerol sebacate)-methacrylate (PGSM) porous structures via emulsion templating. The mixing speed and photoinitiator concentration for emulsions were optimised (350 rpm, 16 wt%, respectively). The resulting emulsion separation before/after mixing and pore morphology of PGSM emulsions was then assessed by altering the emulsion formulation using four different types of diluting solvent (chloroform, dichloromethane, dichloroethane, toluene) for the first time. By altering the type and volume of solvents, the overall pore morphology of polymerised emulsions was tuned.

1. Introduction

In 2002, a thermoset elastomer, poly(glycerol sebacate) (PGS), was developed via a two-step condensation reaction between glycerol and sebacic acid [1]. PGS is a promising material as it has attractive biomaterial properties and can be easily functionalised. In this study we functionalised 50% of the hydroxyl groups with methacrylates, to render the pre-polymer photocurable (PGSM) [1]; the produced resin was set rapidly via UV-illumination [2].

Porous materials are used for tissue engineering because they mimic the extracellular matrix to promote three-dimensional (3D) cell organisation, cell migration, and nutrient perfusion [7]. Porous structures produced by emulsions are formed by the involuntary interaction of two phases; a continuous 'oil phase' and an internal 'water phase'. Mixing both phases disperses droplets of internal phase within the continuous phase ('water-in-oil' emulsion), resulting in a porous structure following polymerisation of the external phase. Phase interaction is usually facilitated by a surfactant. Additionally, solvents and/or mineral particles are used to alter the phase interactions, stabilising the mixture, facilitating emulsification. The interaction of individual polar solvents used in emulsion fabrication has been reported [3]; this is the first study investigating the effect of various solvents on creating PGSM emulsions.

2. Experimental section

PGS was synthesised by a two-step polycondensation, then methacrylated (PGSM) (S1), and emulsions were created by stirring a continuous oil phase (PGSM, Hypermer B246 surfactant, solvent, photoinitiator (diphenyl (2,4,6-trimethylbenzoyl)) in a glass vial at room temperature at 350 or 850 rpm, using a magnetic stirrer (Fig. 3). Once homogeneous, water was added dropwise, the emulsion was mixed until of a foam-like viscosity, and photopolymerised under UV light for 10 min (100 W, OmniCure Series 1000 curing lamp). Cured emulsions were washed in methanol for three days and stored in water, prior to characterisation by SEM, lightsheet and confocal microscopy (S2).

3. Results and discussion

3.1. Chemical characterisation

GPC analysis revealed the molecular weight, molecular number, and polydispersity of PGS and PGSM-50% were within the range of previous studies [1,5]. $^{1}H^{+}$ NMR spectra show peaks related to glycerol and sebacic acid [1] and the incorporation of methacrylate groups [4] (Fig. 1).

* Corresponding author.
E-mail address: f.claeyssens@sheffield.ac.uk (F. Claeyssens).

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3.2. Effect of photoinitiator concentration and mixing speed on PGSM emulsion fabrication and crosslinking

The relationship between mixing speed and water intake in PGS emulsions was tested (S3). The oil phase consists of polymer, solvent, and photoinitiator [6]. Two mixing speeds (350, 850 rpm) and three photoinitiator volumes (11, 16, 25% wt) were investigated. Both parameters showed an effect on emulsion photocrosslinking. At 850 rpm, reagents were homogeneously mixed, but not all emulsions mixed at 350 rpm crosslinked successfully, as the photoinitiator must be homogeneously distributed to ensure proper crosslinking [3,6]. The presence of photoinitiator in the continuous phase affects viscosity and density, impacting the morphology of the pores. Smaller pores were created at higher mixing speeds [7]; the higher shear rate breaks up the internal water phase into smaller droplets and produces more viscous emulsions [6]. 350 rpm was chosen as optimal mixing speed because the pore sizes were larger (>50 μm), both pore and windows were distinguishable, and pore morphologies were more rounded, which has shown to be desirable for cell migration and integration in tissue engineering applications [7].

The extent of photo-polymerisation can be affected by the photoinitiator's availability; a minimum volume is required [9]. Emulsions with 11% wt photoinitiator struggled to crosslink; and emulsions with 25% exhibited skin formation, preventing complete curing in the centre; an optimum balance was found at 16% for both mixing speeds. Skin formation has been previously reported, as high photoinitiator amounts can decrease the photocuring efficiency [9] by increasing photoabsorption, reducing light penetration depth [6]. The effect of the mixing speed and photoinitiator on pore geometries of photocured PGSM emulsions is shown in Fig. 2.

Despite all images showing pore interconnectivity, their morphology is mostly heterogeneous; whilst some structures showed large defined pores (>250 μm) and significantly smaller windows (<50 μm) (B,E) [3], others showed irregular cavities; no pores nor windows can be fully distinguished (C,D).

3.3. Effect of solvent type on emulsion formation and crosslinking

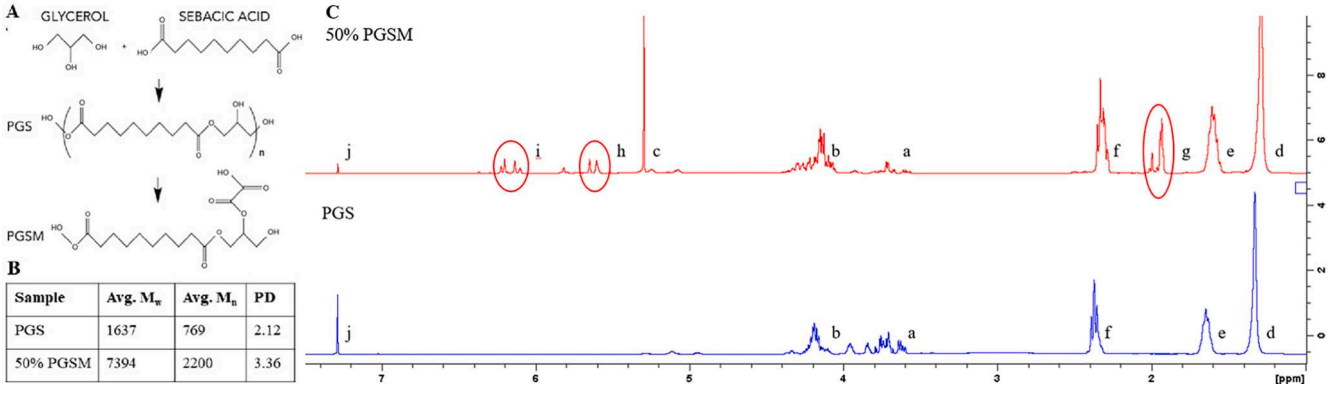
Four different solvents commonly used in polymeric emulsions (toluene, chloroform, dichloroethane (DCE), dichloromethane (DCM)) were studied at different constituent volumes (25–48% wt). Separation during and after mixing was recorded (Fig. 3). The densities and the polarities of the solvents are determining factors for emulsion stability [8]. It should be noted that for all emulsions fabricated, the PGSM polymer solution contained 10% residual DCM following the methacrylation process.

Solvent-free emulsions presented low-viscosity with no apparent difference between phases, inverting into a particulate (water-in-oil-in-water emulsion) [3]. Solvents decreased the viscosity of the organic phase, allowing more droplet travel and increased mixing efficiency of the emulsion [3]. Porous structures were found in 70% of the experiments, reinforcing the suitability of selected solvents to create functional emulsions. At higher DCM and chloroform concentrations, structures lost their porosity and collapsed (due to coalescence of droplets within the prior emulsion). Emulsion separation during mixing was infrequent but most common in chloroform experiments. Separation after mixing occurred more frequently in chloroform, DCM and DCE, but not with toluene. Finally, higher solvent volumes produced separation of the emulsion, mainly after mixing, specifically on DCM and DCE. Results using 70:30 and 80:20 polymer:solvent* solutions are shown in S4-5.

Some emulsions reached a water absorption limit under 3 ml. As emulsions are thermodynamically unstable, emulsion destabilisation is inhibited by the surfactant which decreases the interfacial energy of the emulsion and allows the creation of more and/or smaller droplets. When the polymer layers between droplets thin to a limit coalescence occurs [2]. Additionally, solvents with low interfacial tension have a low 'solvent power' that supports aggregation rather than dispersion [10]. This could be the case for emulsions that separated during mixing (DCM and DCE with water intake limits of 1.5 and 2.5 ml, respectively). Water absorption was mostly optimal at 42% wt solvent (S4).

Chloroform appears to produce emulsions with large pores indicating an unstable emulsion prior to curing (Fig. 4) [3], breaking down via Ostwald ripening [6]. At higher viscosities, droplet transportation is limited (Stoke's Law), hence the formation of larger pores [3]. DCM is also poor in stabilising the emulsion on its own, limiting the water absorbed. As a result, the emulsions inverted above 2 ml of internal phase [3]. DCE had more consistent results; emulsion viscosity and water absorption was optimal for most emulsions with 2.5 ml. DCE emulsions showed round pores with large interconnecting windows (Fig. 4); DCE's density is close to PGSM's (1.32 g/ml), with no impact on the overall oil phase density. PGSM emulsions fabricated with toluene as solvent were the only ones that could incorporate 3 ml of water during mixing without separation. Toluene has the highest interfacial tension with water (35 mN/m), increasing emulsion stability and reducing the risk of coalescence [6,10]. Interestingly, although the toluene-based PGSM emulsion showed the least separation, the structure of the emulsion did not have a typical spherical appearance [7], with well-defined droplets incorporated within the polymer. The structure is still highly porous, with ~ 10–50 μm interconnected pores incorporated within the polymer, making it potentially suitable as tissue engineering scaffolds.

Three imaging techniques were used in this work (SEM, lightsheet and confocal microscopy). Lightsheet and confocal were more suitable for PGSM, as these techniques do not require drying of the samples. PGSM methacrylated to a degree of 50% is a soft polymer that is



Sample	Avg. M_w	Avg. M_n	PD
PGS	1637	769	2.12
50% PGSM	7394	2200	3.36

Fig. 1. A) Representation of PGSM synthesis. B) GPC values show polymers' average molecular weight, number and polydispersity. C) $^{1}H^{+}$ NMR spectra of PGS and PGSM-50%. Peaks at 3.7, 4.2 and 5.2 ppm represent glycerol composition (a–c); at 1.2, 1.6 and 2.3 ppm, sebacic acid (d–f); at 1.9, 5.6 and 6.2 ppm, methacrylate groups (g–j).

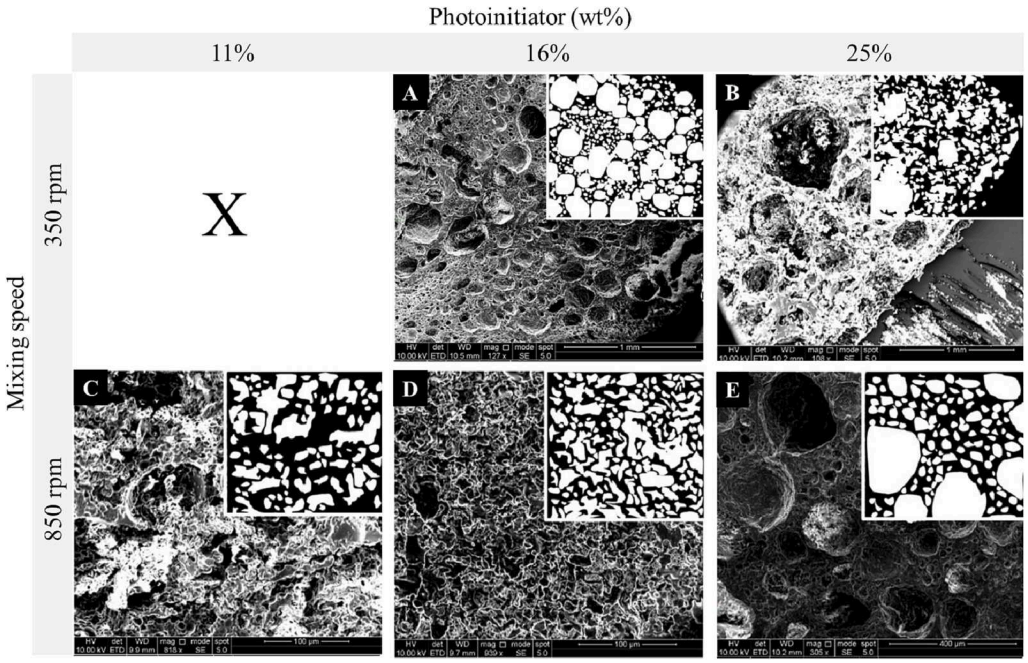


Fig. 2. SEM images for PGSM emulsions: 350 rpm (A, B), 850 rpm (C, D, E), with different amounts of photoinitiator (wt%) and toluene as solvent. Top corners: mimicked pore geometries.

Solvent	V solvent (ml)	V_{oil} phase	ρ_{oil} phase (g/ml)	Separation during mixing	Separation right after mixing	Porous structure
None	0	0.6090	1.2319	N	Y	N

CHLOROFORM

Solvent volume (ml): 0.25 | 0.40 | 0.55 | 0.70

PGSM: solvent ratio * 90:10 — 25% | 35% | 42% | 48% wt

Solvent	V solvent (ml)	V_{oil} phase	ρ_{oil} phase (g/ml)	Separation during mixing	Separation right after mixing	Porous structure
Chloroform	0.25	0.8590	1.2016	Y	Y	P
	0.4	1.0090	1.1907	Y	Y	P
	0.55	1.1590	1.1826	N'	Y	P
	0.7	1.3090	1.1763	N	N	P

TOLUENE

Solvent volume (ml): 0.25 | 0.40 | 0.55 | 0.70

PGSM: solvent ratio * 90:10 — 25% | 35% | 42% | 48% wt

Solvent	V solvent (ml)	V_{oil} phase	ρ_{oil} phase (g/ml)	Separation during mixing	Separation right after mixing	Porous structure
Toluene	0.25	0.8590	1.1254	N	Y	P
	0.4	1.0090	1.0868	N	N	P
	0.55	1.1590	1.0582	N	N	NDP
	0.7	1.3090	1.0362	N	N	NDP

DICHLOROMETHANE

Solvent volume (ml): 0.25 | 0.40 | 0.55 | 0.70

PGSM: solvent ratio* 90:10 — 25% | 35% | 42% | 48% wt — X

Solvent	V solvent (ml)	V_{oil} phase	ρ_{oil} phase (g/ml)	Separation during mixing	Separation right after mixing	Porous structure
DCM	0.25	0.8590	1.2604	N*	Y	P
	0.4	1.0090	1.2708	N*	Y	P
	0.55	1.1590	1.2784	N*	Y	P
	0.7	1.3090	1.2844	Y	Y	N

DICHLOROETHANE

Solvent volume (ml): 0.25 | 0.40 | 0.55 | 0.70

PGSM: solvent ratio* 90:10 — 25% | 35% | 42% | 48% wt

Solvent	V solvent (ml)	V_{oil} phase	ρ_{oil} phase (g/ml)	Separation during mixing	Separation right after mixing	Porous structure
DCE	0.25	0.8590	1.2372	N'	Y	P
	0.4	1.0090	1.2391	N'	Y	P
	0.55	1.1590	1.2405	N'	N	P
	0.7	1.3090	1.2416	N	Y	P

Fig. 3. Lightsheet images: PGSM-50% emulsions with different solvents for emulsification: Chloroform, toluene, DCM, DCE. *: 90:10 solvent ratio refers to the 10% residual DCM in the polymer solution following the methacrylation process (S3). Emulsion parameters: 350 rpm, 3 ml internal phase. P/N: porous/non-porous structure, N/Y: no/yes, NDP: non-defined pores, X: unsuccessful emulsions, N*: watery continuous phase, N': maximum 2.5 ml internal phase. (Scale bars: 500 μm.)

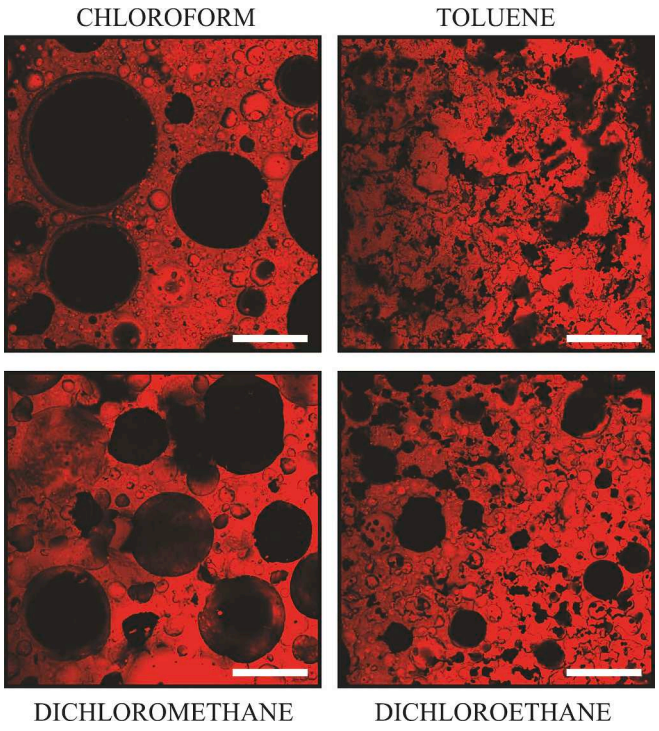


Fig. 4. Confocal microscopy images detailing pore morphology of photo-crosslinked PGSM emulsions fabricated using 0.55 ml of chloroform, toluene, dichloromethane or dichloroethane. Scale: 200 μm.

propense to collapse during the drying process. Furthermore, confocal allowed the creation of high-resolution 3D images through z-stacks.

4. Conclusion

PGS was successfully synthesised and methacrylated to obtain PGSM. Emulsion fabrication parameters were individually assessed to understand their role in the emulsification of PGSM. The relationship between the external variables (mixing speed) and internal variables (photo-initiator, water and solvent absorption) was explored. PGSM templated emulsions with four different formulations (toluene, chloroform, DCM and DCE) resulted in porous, interconnected structures with a range of pore sizes. SEM, lightsheet and confocal microscopy were used to characterise and analyse pore sizes and morphologies. Chloroform resulted in less stable PGSM emulsions with large pores, while toluene created more stable emulsions with smaller pores. This study can help for a better understanding of PGSM emulsions, and these porous templated emulsions have potential applications in tissue engineering.

CRediT authorship contribution statement

Maria Fernanda Velázquez de la Paz: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Funding acquisition. **Mina Aleemardani:** Formal analysis, Investigation, Writing – original draft. **Rachel Furmidge:** Formal analysis, Investigation, Writing – original draft. **Samand Pashneh-Tala:** Methodology, Writing – review & editing. **Frederik Claeyssens:** Conceptualization, Supervision, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare they have no financial interests/personal relationships which may be considered as potential competing interests.

Data availability

Data will be made available on request.

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