of the study were: (i) to modify dissolution of naproxen, a model hydrophobic drug, by solid dispersions using Solutol-HS15, Cremophor-RH40 (non-ionic amphiphilic surfactants) and Synperonic-PE/F68 (triblock copolymer) and (ii) to assess the effects of these carriers on solubility and integrity of naproxen.

Methods Melting and solvent methods were applied to prepare solid dispersions of naproxen with carriers in 50:50, 30:70, 20:80 and 10:90% w/w drug/carrier ratios. In the melting method, naproxen was dispersed in the molten carrier, whereas in the solvent method acetone was used as a solvent to dissolve naproxen and the carrier. Then, acetone was evaporated at 40°C. All solid dispersions (in quantities equivalent to 12 mg drug) were filled into bodies of hard gelatine size 2 capsules and stored in brown glass bottles at ambient conditions. Solid dispersions were investigated, employing drug-solubility studies, dissolution testing (at 100 rpm and 37°C), drug content uniformity, microscopic examination, differential scanning calorimetry (DSC) to study crystallinity of naproxen formulations and Fourier-transform infrared (FTIR) spectroscopy to study structural changes.

Results Solubility studies showed that formulations containing Solutol-HS15 and Cremophor-RH40 in concentrations of 80 and 90% increased naproxen solubility (4-fold), whereas Synperonic-PE/F68 did not show any increase in drug solubility. Increasing carrier concentrations in solid dispersions showed better drug-content uniformity. Naproxen alone showed a slow release (18% of naproxen released after 10 minutes). Formulations containing non-ionic amphiphilic surfactants in concentrations of 80 and 90% enhanced the drug dissolution rate with immediate pulsed release (about 85% of the drug released after 10 minutes). The solid dispersion of naproxen with Synperonic-PE/F68 has not improved drug dissolution; only 17% of naproxen released after 10 minutes. Solid dispersions prepared by melting method seemed to be more effective in improving dissolution than those prepared by solvent method (e.g. for solid dispersions of 30:70% w/w naproxen/Solutol-HS15, the release rate constant was 2.77 ± 0.55 in the case of the melting method versus 1.86 ± 0.46 in the case of the solvent method) probably due to better drug incorporation into the carrier in the molten state than in the solvent method. The highest ($P < 0.05$, analysis of variance) drug dissolution from 90% non-ionic amphiphilic surfactant formulations could be attributed to: (i) conversion of hydrophobic crystalline drug into amorphous state as confirmed by DSC (in thermograms of these formulations, there was no endotherm for naproxen melting at 160°C as shown in the DSC thermogram of pure naproxen) and polarized microscopy or (ii) solubilization and micelle formation effect of surfactants. Hence, drug crystallinity played an important role in governing drug solubility and dissolution. FTIR analysis demonstrated that solid dispersions of naproxen with 80 and 90% w/w non-ionic amphiphilic surfactants exhibited spectral changes with disappearance of the peak at 1680 cm^{-1}, indicating a drug–carrier interaction that resulted in improvement of drug dissolution. The spectra of all other formulations were similar to that of the pure drug.

Conclusions Solid dispersions of naproxen with Solutol-HS15 and Cremophor-RH40 (non-ionic amphiphilic surfactants) show promise for dissolution enhancement of hydrophobic drugs. Synperonic-PE/F68 proved to be a poor choice of carrier.

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Uptake and intracellular trafficking of novel ternary lipoplexes for gene delivery

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Objectives To date a large number of vesicle-forming cationic lipids and peptides have been synthesized and used for the delivery of DNA with varying degrees of success. To meet therapeutic requirements, however, the DNA-delivering efficiency of those non-viral vectors needs major improvement. One possible strategy is to gain better understanding of their mechanisms of entry in relation to their eventual transfection. Endocytosis is thought to be the major internalization pathway for most non-viral gene-delivery vectors; however, the relative contribution of each distinctive endocytic pathway, including clathrin- and caveolae-mediated endocytosis and/or macropinocytosis, is not yet fully understood. In this study the transfection efficiency and intracellular uptake and trafficking of novel lipid ternary vectors composed of a series of C_{14} glycerol-based analogues of *N*-(1-(2,3-dioleyloxy)propyl)-trimethyl-ammonium chloride (DOTMA) were studied using various endocytotic pathway inhibitors.

Methods The novel C_{14} DOTMA analogues (Writer et al 2006), incorporating *cis*, *trans* and alkyne moieties at C-9 and C-11 positions of the alkyl chain, were synthesized, formulated into vesicles with the neutral lipid dioleoylphosphatidyl-ethanolamine (DOPE) and mixed with pGL-3 plasmid in the presence or absence of an integrin-targeting peptide, Pep6. The novel peptide contains a Lys16 domain at the N-terminus designed to bind and condense the DNA, and a cyclic integrin-targeting recognition site known to bind specifically to the cell-surface protein $\alpha5\beta1$ integrin. Lipid–DNA complexation efficiency was assessed using gel electrophoresis, light scattering and zeta potential. Transfection studies of the lipoplexes in the presence or absence of Pep6 were performed in MDA-MB-231 breast cancer cells in the presence of endocytic inhibitors to determine the route of complex internalization and the relative contributions of each endocytic pathway (Khalil et al 2006). Endocytic inhibitor toxicity studies were also carried out using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay.

Results Zeta potentials, particle-size measurements and gel electrophoresis indicated that complete complexation of DNA occurred between lipid/DNA charge ratios of 2:1 and 4:1, offering partial protection of DNA from DNAseI enzymatic degradation. The presence of Pep6 showed highly improved transfection efficiency compared to the lipoplexes alone, showing up to 3-fold higher transfection efficiencies in some of the lipids when compared with Lipofectamine®. The transfection efficiency of lipoplexes with and without Pep6 was totally inhibited in the presence of chlorpromazine, a clathrin-mediated endocytic inhibitor. No inhibition of transfection was observed in the presence of filipin III or nystatin, both used as inhibitors for caveolae-mediated endocytosis. Some concentration and time-dependent cytotoxicity was observed with chlorpromazine (with T_{50} of 25.2 and 9.2 mg/mL for 30 and 60 minute incubation times respectively); however, any reduction in cell viability was accounted for during the transfection experiments.

Conclusions The proposed ternary lipoplexes are promising candidates for gene delivery due to their high transfection efficiency, although slight structural variations in the lipids also play a major role in their eventual transfection. Clathrin-mediated endocytosis appears to be the major pathway for complex internalization in both the presence and absence of Pep6.

Khalil, I. A. et al (2006) *Pharmacol. Rev.* **58**: 32–45
Writer, M. et al (2006) *J. Liposome Res.* **16**: 373–389

Material Science

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Amphiphilic copolymers and hydrogels based on 2-hydroxyethylmethacrylate and 2-hydroxyethylacrylate as potential materials for pharmaceutical applications

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Objectives Hydrogels based on poly(2-hydroxyethylmethacrylate) are widely used as components of biomedical devices and drug-delivery systems due to their biocompatibility and excellent physico-chemical properties. The objective of the present work was to synthesize and characterize soluble copolymers and hydrogels by copolymerizing 2-hydroxyethylmethacrylate (HEMA) with 2-hydroxyethyl-acrylate (HEA) and assess the possibility of their application in drug delivery.

Methods A series of soluble copolymers was synthesized by free-radical copolymerization of HEMA and HEA at different monomer ratios (140 minutes at 60°C). These copolymers were purified by dialysis against distilled water. The hydrogels were synthesized using similar reaction mixtures but the copolymerization was conducted for 18.5 hours. The hydrogels were purified by immersing in deionized water, which was changed daily, for 2 weeks to remove any unreacted chemicals. The compositions of soluble copolymers and their molecular weights were determined by ^{1}H-nuclear magnetic resonance spectroscopy and gel-permeation chromatography, respectively. The behaviour of the copolymers in solutions was studied by dynamic light scattering using a Malvern Zetasizer Nano-S (Malvern Instruments, UK). The mechanical properties of the hydrogels were assessed using a TA XT.plus Texture Analyser (Stable Microsystems, UK) in a compression mode at room temperature. The porous structure of the hydrogels was probed by scanning electron microscopy using FEI Quanta FEG 600 environmental scanning electron microscope. The freeze-dried hydrogel samples were sputtered with gold before analysis.

Results HEMA was found to be more reactive in copolymerization compared with HEA, and all the copolymers had higher HEMA content than in the feed mixtures. The copolymers containing up to 47 mol% of HEMA were soluble in water and their solution behaviour was studied by dynamic light scattering at different temperatures. These copolymers exhibited lower critical solution temperature in aqueous solutions; that is, they underwent a phase separation upon increase in temperature. Similar behaviour was previously reported for copolymers of HEA and butyl acrylate (Mun et al 2007). In ethanol solutions HEMA–HEA copolymers showed the presence of an upper critical solution temperature. The analysis of the copolymers by gel-permeation chromatography

revealed their high polydispersity and branched structure. The mechanical properties of the hydrogels based on HEMA–HEA were dependent on the copolymer composition, and decrease in elastic modulus was observed upon increase in HEA content. The average molecular weight between crosslinks, calculated using the data on mechanical properties, was found to be higher for the samples synthesized from the feed mixtures containing more HEA. The hydrogels containing more HEA in their structure were also more transparent, had high swelling ability and larger pores. The HEMA–HEA copolymers were found to be less irritant than poly(acrylic acid), which is often used in ophthalmic applications.

Conclusions The soluble HEMA–HEA copolymers exhibiting temperature-induced phase transitions can be promising for designing novel *in situ* gelling dosage forms. The crosslinked copolymers (hydrogels) can also be used for developing drug-delivery systems and biomaterials.

Mun, G. A. et al (2007) *Macromol. Chem. Phys.* **208**: 979–987.

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Hydrogel nanoparticles via a water-in-oil microemulsion polymerization: design and synthesis optimization for biomedical applications

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Objectives To prepare hydrogel nanoparticles composed of either Pluronic F127 diacrylate (F127DA) and poly(ethylene glycol) diacrylate (PEGDA), or acrylamide and methylene bisacrylamid,e using an efficient free-radical inverse microemulsion polymerization process. These polymers were chosen for potential pharmaceutical and biomedical applications. For example, the thermoresponsive nature of F127DA and PEGDA together may be useful for *in situ* gelling drug-delivery applications (Missirlis et al 2005) and the hydrophilicity of acrylamide and methylene bisacrylamide may make them suitable for enzyme encapsulation (Poulsen et al 2007). Further objectives included selection of inverse microemulsions for reaction, monitoring polymerization efficiency using nuclear magnetic resonance (NMR), optimization of purification and characterizing nanoparticle size and shape.

Methods Pseudoternary phase diagrams were constructed of Aerosol-OT and Brij 30/hexane/aqueous polymer solution systems to identify the inverse microemulsion region. The aqueous polymer solution was either 9.45% F127DA ($M_n \approx 13000$ Da, synthesized by double acrylation of Pluronic F127 with acryloyl chloride) and 3.15% PEGDA, or 30% acrylamide and 9% methylene bisacrylamide. The aqueous solution was added incrementally to solutions of surfactants in water. Inverse microemulsions were identified visually and characterized by dynamic light scattering (DLS). Selected inverse microemulsions were used as 'reactors' for free-radical polymerizations. The microemulsions were degassed using freeze–thaw cycles, before addition of the free-radical initiator system (ammonium persulphate (24 μl 10% w/w_{aq} solution)/*N,N,N′,N′*-tetramethylethylenediamine (12 μl)) under nitrogen. Reactions proceeded for 16 hours before extraction and purification of nanoparticles. Polymerization efficiency was monitored by NMR analysis of microemulsions before and after reaction. Purification was monitored using infrared spectroscopy and optimized by dialysis (molecular-weight cut-off 15000 Da) and size-exclusion chromatography (SEC) (Sephadex LH-20 column) with online UV spectroscopy (at 210 nm). Nanoparticles were analysed by atomic force microscopy (AFM) and DLS.

Results Construction of pseudoternary phase diagrams successfully identified distinct inverse microemulsion regions for both polymer solutions. Surfactant concentrations ranged between 9 and 18%, and hexane/aqueous solution ratios from 81:5 to 67:5. These were transparent, stable systems with droplet sizes between 10 and 20 nm (measured by DLS). NMR analysis of inverse microemulsions before and after reaction showed complete disappearance of acrylate group peaks at 6.4 and 5.8 ppm, indicating 100% conversion to polymer. Successful purification was achieved by 3-day dialysis then SEC. UV analysis over time throughout SEC showed two well-separated peaks (5–8 and 12.5 minutes). These fractions were analysed by infrared spectroscopy, comparing them with spectra of pure surfactants and with 'macroscopic' gels of fully reacted polymers. Separation of polymeric particles from surfactant was clear. AFM imaging showed surfactant to be visible before purification only. Analysis by DLS and AFM showed spherical nanoparticles with diameters between 50 and 100 nm.

Conclusions A robust, reproducible method for synthesis and purification of hydrogel nanoparticles has been established. Characterization by infrared, NMR, DLS and AFM showed consistent size and purity. Ongoing and future work includes modifying nanoparticle 'strength' by varying monomer ratios, cell toxicity studies and model compound loading/release.

Missirlis, D. et al (2005) *Langmuir* **21**: 2605–2613
Poulsen, A. K. et al (2007) *Anal. Biochem.* **336**: 29–36

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An examination of the drying kinetics and physico-chemical properties of ethylcellulose films from organic solvent casting

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Objectives The main objective of this work was to quantitatively examine and model the effects of drying conditions on the drying process of physico-chemical properties of ethylcellulose films. For this purpose, the drying of ethylcellulose films from organic solutions was studied.

Methods Solutions of ethylcellulose (0.5–10% w/w) were prepared using a range of solvents (100% dichloromethane (DCM), 100% ethanol and mixtures thereof). Following this the polymeric solution (100 μL) was dispensed into a thermogravimetric analysis (TGA) aluminium pan (100 μL volume) and dried at a range of temperatures (15, 20 and 25°C) and different air flow rates (20, 60 and 100 mL/second). Mass transfer during the drying process was continually monitored by TGA and modelled using MATLAB. In addition the polymeric solution (50 mL) was dispensed into a mould and dried using a tray drier, operating at ranges of airflow rates and temperatures. The mechanical properties, thermal properties and morphology of ethylcellulose films were characterized using tensile analysis, differential scanning calorimetry (DSC) and transmission electron microscopy (TEM), and successfully modelled by MATLAB to describe the experimental data using a Fickian second law diffusion drying model. The effects of polymer concentration, drying temperature, air flow rate and solvent composition on the drying rates of the drying process were determined using a three-way analysis of variance ($P < 0.05$).

Results It was found that these drying processes have three distinct regions: (i) a period of constant drying rate, which is controlled by solvent evaporation; (ii) a period in which the drying rate decreased linearly, which was due to increasing diffusion surface area coverage on the top of the solution; and (iii) a period of falling drying rate, which is completely controlled by solvent diffusion and an increased the path length through the predominantly elastic/solid films. It was also noted, however, that the Fickian second law model only demonstrated a high degree of accuracy when the initial boundary condition was chosen as the end of the period of linearly decreasing drying rate, rather than the conventional boundary condition at the end of the period of constant drying rate. From the results it was indicated that the choice of organic solvent is fundamental to the drying process. Furthermore, a gel point was identified (30% solvent for DCM and 15% solvent for ethanol) that marked the beginning of film formation.

Conclusions This study has highlighted and modelled the effects of drying parameters on the rate of drying and quality of ethylcellulose films. The results have implications for the convection drying process, including using hot air in tray dryers, and also for the tablet-coating process.

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The use of quasi-isothermal modulated-temperature differential scanning calorimetry as a means of characterizing the re-crystallization of Gelucire 44/14

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Objectives To use standard and quasi-isothermal modulated-temperature differential scanning calorimetry (DSC) methods to characterize the re-crystallization process of Gelucire 44/14 to determine the effect of cooling rate.

Methods Samples of Gelucire 44/14 in the weight range 2–2.5 mg were prepared in standard aluminium crimped pans and run using standard DSC, melting and cooling at 0.5, 2, 10 and 20°C/minute. The solid fat content of Gelucire 44/14 was calculated as a function of temperature using the area under the re-crystallization traces at various temperature points and expressed as percentages of the total on complete re-crystallization. Samples of Gelucire 44/14, prepared in the same manner as above, after complete melting at 60°C for 10 minutes, were run using quasi-isothermal modulated-temperature DSC with an amplitude of ±1°C and a period of 60 seconds, cooling at 1°C increments from 35 to 5°C with an isotherm of 10 and 40 minutes at each increment.

Results Using standard DSC, re-crystallization of Gelucire 44/14 occurred at decreasing temperatures with increasing cooling rate. Profile measurements were repeated four times each with excellent reproducibility throughout. The solid fat

content technique allowed the visual simplification of the re-crystallization process. By plotting the percentage solid against the temperature for each individual cooling rate it is possible to identify the amount of Gelucire 44/14 present in the solid state at any temperature point during the re-crystallization process. The quasi-isothermal modulated-temperature DSC method allowed the isolation of the temperature at which Gelucire 44/14 re-crystallization occurred by holding the molten sample at each temperature increment for an extended period. This was detected by the use of Lissajous figures, whereby the modulated heat flow is plotted against modulated temperature. This in turn allows observation of the reproducibility of the sine-wave heat-flow modulations within a single isothermal period. The re-crystallization could be observed in real time by noting the deviation of the sine-wave curves from the steady state through the course of the crystallization process, thereby providing a novel means of deconvoluting the heat-flow processes associated with the thermal event as a function of time. It was noted that the re-crystallization temperature of Gelucire 44/14 was 32–31°C with an isotherm of 40 minutes, and 30°C with an isotherm of 10 minutes.

Conclusions Quasi-isothermal modulated-temperature DSC appears to be a very promising new tool in the investigation of the Gelucire 44/14 re-crystallization process. By holding the sample at each temperature increment for an extended period, it is possible to isolate the re-crystallization process. This in turn leads to the possibility of mathematically modelling the associated kinetics; work is ongoing to this effect.

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Rheological investigation of some common natural polymer gels and their synergistic combinations

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Objectives To investigate rheological synergies evident from simple combinations of natural polymers used to produce lyophilized wafers. Sodium alginate (SA) and a selection of natural gums, including xanthan gum (XG), locust bean gum (LBG), guar gum (GG), ghatti gum (GhG) and karaya gum (KaG), alone or in combination, are useful for the production of lyophilized wafers. These wafers have potential use as stable vehicles for the topical delivery of anti-microbial compounds to suppurating wounds and control of their flow properties is essential for optimizing their performance in a variety of wound environments.

Methods All gels were prepared in distilled water at concentrations ranging from 0.5 to 3.5% w/v. Individual gels and simple combinations of two gels (50:50) were prepared and characterized by continuous flow rheometry at 25°C. A cone-and-plate geometry was used (40 mm/2° steel) and flow measurements were conducted between 0 and 600 s^{-1} using an AR1000 dynamic rheometer (TA Instruments). Analysis of flow curves was undertaken with the system software using Newtonian, Power Law and Herschel–Bulkley models, *viz* $\sigma = \eta'\gamma$ (Newton), $\sigma = \eta'\gamma^n$ (Power Law) and $\sigma = \eta'\gamma^n + \sigma_o$ (Herschel–Bulkley), where σ is shear stress (Pa), η' is viscosity coefficient or 'consistency' (Pa·s), γ is shear rate (s^{-1}), n is rate index of pseudoplasticity and σ_o is yield stress (Pa).

Results Results are shown in Table 1.

Conclusions All mixtures showed increased consistencies compared with the respective homopolymers. This is indicative of synergistic interactions between constituent polymers. XG/LBG and KaG/SA exhibited yield stresses of 100.60 and 193.26 Pa respectively. This is in agreement with Higiro et al (2006), who reported strong intermolecular interactions between the trisaccharide side chains of XG and the 1–4-linked β-D-mannan backbone of LBG. The greatest consistency of 53.69 Pa·s was displayed by GG/GhG (1:8) compared with 0.16 and 0.38 Pa·s for the individual gels. Control of the rheological properties of gels formed from these natural polymers and their combinations are critical when producing lyophilized wafers for the topical delivery of therapeutic agents to suppurating surfaces such as chronic wounds.

Higiro, J. et al (2006) *Food Res. Int.* **39**: 165–175

Pharmaceutical Microbiology

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Application of a novel drug-delivery device with photodynamic anti-microbial chemotherapy: potential treatment of methicillin-resistant *Staphylococcus aureus* wound infection

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Objectives Photodynamic anti-microbial chemotherapy (PACT) is one alternative approach to the selective killing of methicillin-resistant *Staphylococcus aureus* (MRSA), whereby a combination of a sensitizing drug and visible light causes the selective destruction of microbial cells via singlet oxygen production. The objectives of this study were to (1) determine the sensitivity of MRSA to methylene blue (MB)- and meso-tetra(*N*-methyl-4-pyridyl)porphine tetratosylate (TMP)-mediated lethal photosensitization and (2) develop a dosage form for delivery of adequate photosensitizer doses to infected wounds.

Methods For TMP and MB release from a novel poly(vinyl alcohol) (PVA; 8% w/w)/borate (2% w/w) hydrogel, gel-loaded inserts (25 mm diameter × 10 mm height) containing 4 g gel (1.0 mg mL^{-1} drug) were placed in 100 mL phosphate buffer. At defined times, 5.0 mL of receiver phase was removed. Photosensitizer concentration was determined spectrophotometrically at their λ_{max}. Light diffusion studies through photosensitizer solutions and newborn calf serum were carried out at 635 nm. Planktonic cultures of a clinical MRSA isolate were prepared by overnight incubation at 37°C. Biofilm samples were prepared by incubation of PVC discs with inocula equivalent to 10^7 organisms for 24 hours before use. Test samples were incubated for 30 minutes with solutions of photosensitizers of concentrations 2, 5, 10, 50 and 250 μg mL^{-1} and irradiated (635 nm, 100 mW cm^{-2}, irradiation time 5 minutes, distance from sample 1.8 cm, total light dose 100 J cm^2). The number of surviving bacteria was determined using the Miles and Misra technique. The experiment was repeated using photosensitizer solutions in newborn calf serum to simulate conditions in wounds. Where appropriate, the Mann–Whitney U test was used for statistical analysis.

Results Photosensitizer release from PVA/borate hydrogels showed receiver compartment concentrations of MB and TMP of more than 10 μg mL^{-1} after a 6-hour release period. Serum depths up to 3.5 mm did not affect light diffusion. The presence of increasing concentrations of photosensitizers caused significant reductions in light transmission. For example, increasing MB concentration from 50 to 500 μg mL^{-1} reduced measured fluence from approximately 1.5 to 0.3 mW cm^{-2} ($P < 0.0001$). In PACT, the kill rate was dependent on photosensitizer concentration, even in the absence of irradiation. For example, in the case of a planktonic culture incubated with 2 μg mL^{-1} MB in phosphate-buffered saline, pH 7.4, the percentage kill was 29.73%, while that for 250 μg mL^{-1} MB was 99.46% ($P = 0.0339$). The growth of bacteria in a biofilm and the presence of serum decreased the kill by MB-PACT. For example, irradiated planktonic MRSA incubated with 10 μg mL^{-1} MB reduced survivors to below detection levels, while the equivalent biofilm culture had a reduction of 88.19% ($P = 0.0209$). TMP-PACT was unaffected. For example, irradiated planktonic culture exposed to 10 μg mL^{-1} TMP reduced survivors to below detection levels while that of the equivalent biofilm culture was 99.71% ($P = 0.0833$).

Conclusions MB- and TMP-mediated photosensitization significantly reduced the bacterial load of both planktonic and biofilm cultures. Factors such as light

Table 1 Viscosity coefficient or 'consistency', η' (Pa·s), and yield stress, σ_o (Pa), for six natural polymers and their binary mixtures (50:50). Note the particularly large yield stresses for the synergistic mixtures of KaG/SA and LBG/XG

Polymer (% w/v)	SA η' (σ_o)	XG η' (σ_o)	LBG η' (σ_o)	GG η' (σ_o)	KaG η' (σ_o)	GhG η' (σ_o)
SA (2.5%)	1.17 (0.00)	–	–	–	–	–
XG (0.5%)	2.00 (0.00)	0.17 (7.71)	–	–	–	–
LBG (0.5%)	9.66 (0.00)	2.08 (100.60)	0.04 (4.87)	–	–	–
GG (0.5%)	6.89 (0.00)	2.35 (23.04)	10.50 (34.43)	0.16 (6.19)	–	–
KaG (1.5%)	22.57 (193.26)	0.94 (22.78)	9.49 (34.01)	3.15 (24.31)	0.03 (5.27)	–
GhG (3.5%)	9.35 (0.00)	3.54 (35.76)	20.58 (70.62)	53.69 (52.49)	4.54 (24.30)	0.38 (16.09)