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A study of microemulsions as prolonged-release injectables through *in-situ* phase transition

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ABSTRACT

Microemulsions (MEs) have been studied extensively as colloidal carriers for the delivery of both water-soluble and lipid-soluble drugs. Our previous study showed that addition of water to ME formulations resulted in phase transition to either liquid crystal (LC) or coarse emulsion (CE). The aim of this study was to investigate whether these MEs could be used as drug delivery vehicles for prolonged release through *in-situ* phase transition following extravascular injection. Three ME formulations from the same pseudo-ternary phase diagram were investigated with respect to their phase transition behavior, and *in-vivo* drug release; a coarse emulsion-forming ME (CE-ME), an oil rich LC-forming ME (LC-ME1), and an oil poor LC-forming ME (LC-ME2). CE-ME was a W/O ME and both LC-MEs were O/W type. The release profiles of ^{99m}Tc labeled MEs following subcutaneous (SC) injection in rabbits were investigated with gamma-scintigraphy. The CE-ME dispersed readily in water, forming a CE, whereas the LC-forming MEs formed 'depots' in water. Polarized microscopy revealed a LC boundary spontaneously formed at the water/ME interface for the LC-MEs with the LC-ME2 forming a substantially thicker LC layer. The CE resulting from the water-induced transition of the CE-forming ME had a higher viscosity than the MEs, but lower than the LCs resulted from LC-MEs. Compared to LC-ME1, LC-ME2 underwent more rapid phase transition and the resultant LC had significant higher viscosity. The LCs formed from both ME formulations exhibited pseudoplastic properties; increasing the shear rate decreased the apparent viscosity exponentially. Following SC injection into the animal thigh, the LC-MEs had more prolonged release of ^{99m}Tc in a first-order manner, than CE-ME. The oil poor LC-ME2 had the slowest release with a $t_{1/2}$ of 77 min, 2.3 times longer than the oil rich LC-ME1; consistent with the thickness of LC layer formation observed *in-vitro* and their relative viscosities. In conclusion, the present *in-vivo* study has demonstrated the application of MEs as extravascular injectable drug delivery systems for sustained release. The retention of the vehicles at the injection site and the release rate were determined predominantly by their phase transition rather than ME type or oil content.

1. Introduction

Conventional parenteral delivery systems provide rapid release of the incorporated drug after administration, thus rapid absorption and onset of action. However, such formulations often require high frequency dosing regimens leading to poor patient adherence and wide variations in drug plasma levels which can result in either toxic drug reactions or therapeutic failure [1]. For poorly water-soluble drugs, fast release from the delivery system can result in post-injection drug precipitation if the concentrations at the injection site exceed the solubility [2,3]. In the last decade sustained release parenteral formulations have undergone major advancement and there has been a significant spike in the number of available sustained release formulations that have gained regulatory approval for both intravenous (IV) and extravascular (intramuscular and subcutaneous) administration [1]. For extravascular administration, sustained release formulations include suspensions, multiple emulsions, microcapsules, liposomes, as well as some recently developed *in-situ* depot-forming systems [4–6]. Among these formulations, *in-situ* depot-forming systems are the least complicated to manufacture and scale up.

Low viscosity is a prerequisite property for injection through a syringe with ease. The *in-situ* depot-forming systems utilize a liquid which the drug is dissolved in that forms a solid or semisolid depot after administration into the tissue [4], drug release rates are reduced due to the high viscosity of the formulation. This research area is rapidly progressing and significant developments are expected in the next few years [1]. Several polymeric *in-situ* forming parenteral sustained release systems have been developed from both biodegradable and non-degradable polymers such as gellan gum, alginic acid, pectin and chitosan [4,7], all based on a gelling response to the physiological microenvironment at the injection site. For example, pH-induced and thermal-

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induced gelling based systems [1,4,7] are available to provide controlled release upon changes in either pH or temperature in the body, respectively.

Microemulsions (ME) have been studied extensively as drug delivery vehicles for various administration routes [8–12], and have gained increasing interest as parenteral delivery vehicles [3,11,13–16] including for extra-vascular injections and intravenous administration. MEs are transparent colloidal systems that are made of surfactant(s), co-surfactant, oil and water. They are easy to prepare, have low viscosity and are thermodynamically stable [8,9]. An essential requirement for the formation and thermodynamic stability of MEs is ultra-low interfacial tension between water and oil which can be achieved through the addition of surfactants [10,12]. The co-surfactant on the other hand is employed to increase interfacial fluidity by penetrating into the surfactant film in order to disrupt its well-arranged structure [10]. One attractive quality of these rather simple formulations is their ability to solubilize both hydrophilic and lipophilic compounds [9,12], which many drug delivery systems fail to achieve [1,5,10]. It has been estimated that 40% of newly discovered drugs have setbacks related to their poor solubility in oil and/or in water [17,10].

We recently demonstrated through an *in-vitro* study that certain ME formulations have the potential to transition to liquid crystals (LC) or coarse emulsions (CE) in an aqueous environment [18]. LCs are semi-solids with crystalline structures combining properties of both solid and liquid states [19]. *In-situ* phase transition, particularly from a ME to a LC upon aqueous dilution was demonstrated to provide sustained release of an incorporated lipophilic drug as well as the hydrophilic compound ^{99m}Tc, attributed to an increase in viscosity in the LC layer, leading to a reduction in diffusion rate [20]. Since MEs can be used as injectable formulations owing to their low viscosity [11], it is conceivable that a LC-forming ME could be injected subcutaneously (SC) or intramuscularly (IM) with ease, then, upon contact with the body fluids transition to a dense LC depot whereby the incorporated drug will be slowly released, becoming available for absorption.

Medical imaging techniques such as Gamma-scintigraphy are commonly used to observe the absorption and distribution behavior of radio-labeled formulations *in vivo* [21–24]. Gamma-scintigraphy allows one to "see" what happens to the formulation after administration to the body. The formulations are normally spiked with a radiolabeled ligand such as sodium (^{123}I) iodide or pertechnetate (^{99m}Tc). These radioactive agents act as tracers allowing not only the retention of the formulations at the injection site but also the absorption to be observed [21,23,24].

The present study, built on our previous *in-vitro* work, aims to evaluate the retention and release from the LC-forming MEs in comparison to a CE-forming ME in a rabbit model to determine whether phase transition of these MEs could be induced by the tissue fluids following extravascular injection. To fully understand the mechanism and key factors that determine phase transition and controlled release an additional LC-forming ME formulation was selected from the pseudo-ternary phase diagram. The three formulations were investigated with regard to phase transition in water, rheology change, and potential for prolonged drug release. While the sustained release properties of a LC-forming ME have been observed *in-vitro* [18], this study is the first to evaluate *in-vivo* performance. Gamma-scintigraphy was employed to monitor the retention of each ME formulation and absorption of the tracing agent ^{99m}Tc following subcutaneous (SC) injection in rabbits.

2. Materials and methods

2.1. Materials

Miglyol 812N, a mixture of medium chain triglycerides, was provided by Sasol GmbH Oleochemicals, Witten, Germany. Macrogol (15)-hydroxystearate, also known as Solutol HS 15 with hydrophilic–lipophilic balance (HLB) value of 15, was provided by BASF, Fine Chemicals Division, Germany. Span 80 (sorbitan monooleate, HLB = 4.3) and Nile Red were purchased from Sigma-Aldrich NZ Ltd, New Zealand. Absolute ethanol was purchased from ECP-Analytical Reagent ECP Ltd, New Zealand. The raw isotope molybdenum (Mo-99) was supplied from ANSTO, Australia, to produce the radioactive agent pertechnetate (^{99m}Tc), using a Gentech Technetium Generator (ANSTO, Sydney, Australia). Milli-Q water was prepared using the Milli-Q water purification system (Millipore Corp., MA, USA). All other chemicals and reagents used were of analytical grade, without further purification.

2.2. Formulation preparation

The CE-forming ME (CE-ME) and LC-forming ME (LC-ME1) reported in our previous study [18] were selected alongside an additional LC-forming ME formulation (LC-ME2) containing less oil from the same pseudo-ternary phase diagram (Fig. 1) and were prepared according to the compositions reported in Table 1. Briefly, Solutol HS 15 (surfactant), Span 80 (adjuvant surfactant) and ethanol (co-surfactant) were premixed at weight ratios of 3:1:0.5 with a blend HLB value of 12.3. Miglyol 812N was then added, followed by Milli-Q water. MEs were formed with gentle mixing.

2.3. Formulation characterization

MEs, LCs, and CEs were identified with the aid of a cross polarized light microscope (DM RXP, Leica DMR, Germany). MEs are clear transparent samples that portrayed an isotropic appearance whereas LCs are gel-like formulations that displayed birefringence when viewed under cross polarizers due to their double refraction attribute. CEs on the other hand do not display any birefringence but show droplet structures under phase-contrast microscopy.

2.4. Visual observation on ME spreadability and phase transition in aqueous media

The various ME formulations were dyed with the lipid soluble Nile Red (10 μg/ml) and each was injected (100 μl) into the center of a Petri dish filled with approximately 10 ml of water, or a beaker filled with 100 ml of water. The increase in diameter of each formulation

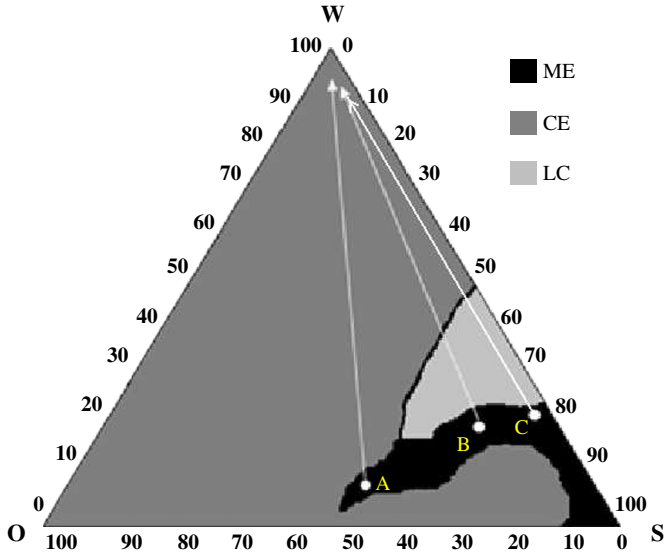

Fig. 1. Pseudo-ternary diagram and phase transition trends of the three different tested microemulsions (ME) to a coarse emulsion (CE) or liquid crystals (LC). The CE-ME (A) and LC-ME1(B) and LC-ME2 (C) characterized *in-vitro* and *in-vivo* are indicated as white dots with lines indicating the trend of the phase transition on addition of water.