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# Composite alginate hydrogels: An innovative approach for the controlled release of hydrophobic drugs

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ABSTRACT

We present an innovative methodology for the sustained delivery of hydrophobic drugs using composite hydrogels, prepared by embedding oil-in-water microemulsions in hydrophilic hydrogels. The hydrophobic nature of the microemulsion core enhances the solubilization of hydrophobic drugs, while the crosslinked matrix could be readily used as a solid controlled delivery vehicle. A microemulsion was formulated from pharmaceutical accepted components; the droplets diameter was shown to be about 10 nm by dynamic light scattering, cryo-transmission electron microscopy and small-angle X-ray scattering (SAXS). Combining the microemulsion with alginate solution and crosslinking with calcium ions resulted in a clear hydrogel. A model hydrophobic drug, Ketoprofen, precipitated from the alginate hydrogel, but the drug–containing composite hydrogel was clear and macroscopically homogeneous. The nanostructure was investigated by SAXS; scattering plots indicate that oil droplets exist in the composite hydrogel. Release profiles of the drug from the composite hydrogel with various concentrations of polymer and crosslinker demonstrate the applicability of this system as a controlled delivery vehicle, and suggest that the release rate is governed not by the microemulsion structure but, rather, by the network properties. Furthermore, it was demonstrated that the release rate could be tailored for a specific application utilizing different alginate and calcium concentrations. The generalization of the methodology of including hydrophobic drugs in composite gels is discussed.



## 1. Introduction

Many of the new medical entities (an estimated 43%) developed since the late 1980s are extremely hydrophobic as a result of new processes of drug development, such as combinatorial chemistry, recombinant DNA techniques and high-throughput screening [1,2]. A lipophilic drug can enter a hydrophobic cell membrane, enabling better targeting and drug efficiency [3]. On the downside, low water solubility is related with low absorption and bioavailability [1]. Special care needs to be taken for the delivery of these drugs as they cannot be simply introduced to an aqueous solution, and may form aggregates upon intravenous administration. The challenge becomes not just to find new drugs, but to find creative ways to deliver them. Many strategies to overcome this problem are investigated, some of which are complexes of drug–cyclodextrin or drug–lipoprotein [4], drug nanocrystals and nanoparticles incorporating the drug [5], such as liposomes, solid lipid particles, dendrimers, quantum dots, micelles and microemulsions.

The concept of microemulsions was introduced by Schulman et al. in 1943 [6]. Although the exact definition of microemulsions is debated, they are generally described as fluid dispersions of oil and water, stabilized by an interfacial film of amphiphilic molecules, which is of low viscosity, single phased, optically isotropic, transparent (or translucent) and thermodynamically stable [7,8]. Microemulsions are attractive drug delivery vehicles, if only for the fact that oil-in-water (o/w) droplets can incorporate poor water soluble drugs. Numerous studies have been published on this subject, including topical, ophthalmic, nasal, oral and parenteral delivery routes [7–9].

Another commonly utilized family of delivery systems is polymers, which can provide mechanical strength, control of physical and chemical properties, and sustained release of drugs. One of the common polymers used in pharmaceutical research is alginate, a biocompatible polysaccharide isolated from brown alga. It is a linear unbranched copolymer, consisted of β-D-mannuronic acid (M) and its C-5 epimer, α-L-guluronic acid (G), arranged in a blockwise pattern [10]. The blocks can be similar (MMM, GGG) or

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strictly alternating (MGMG). The mechanical properties are easily controlled; gel formation is induced by lowering pH or by adding various divalent cations, in particular $Ca^{+2}$, which crosslinks a pair of G blocks within the alginate chains. The shortcoming of crosslinked alginate as a drug carrier is that, because it has a hydrophilic nature, many hydrophobic drugs cannot be solubilized in the hydrogel.

We suggest creating composite hydrogels by embedding an o/w microemulsion carrying a hydrophobic drug in a hydrophilic alginate matrix. This approach will combine the advantages of two known drug delivery vehicles. Thus, drug loading will increase due to its higher solubility in the oil droplets, while the crosslinked alginate matrix could be readily used as a solid controlled delivery vehicle. The addition of polymers to microemulsions has been much studied [11] for various purposes, such as modifying the phase behavior [12] and achieving desired rheological properties [13]. However, the applications in drug delivery are limited; most of the drug delivery studies, entailing the addition of polymers to microemulsions, focus on increasing the viscosity of microemulsions to achieve a cream-like consistency that could be easily spread on the skin [13–26]. Applications in other delivery routes are even more limited, and restricted to ocular [27,28], vaginal [29] and buccal [30] routes. Chauhan and co-workers published a series of studies in which microemulsions were embedded in HEMA hydrogel, forming a clear drug–loaded lens for ocular delivery [27,31–34]. Another interesting approach to ocular delivery is the combination of in situ gelation with a microemulsion containing the lypophilic drug cyclosporine A, resulting in a fluid system that gels upon administration via the eye [28]. D'Cruz and Uckun [29] have developed an gel-microemulsion system as a vaginal spremicides. Rozman and Gasperlin [19] studied mixing alginate with a microemulsion, but the result was of low viscosity or phase separated. To the best of our knowledge, no study has been published so far on the successful mixture of alginate and microemulsions. Since alginate has been extensively studied and is easy to use, a simple method of introducing hydrophobic moieties to it opens the door to a range of drugs or nutraceuticals that could consequently be delivered using alginate. The resulting device can have a high mechanical strength compared to the mechanical strength of microemulsions alone, which are practically liquid, and has the potential to be delivered via various routes, including the oral and mucosal routes. Additionally, the nanometric droplets containing the drug provide a large area of contact with the surrounding medium, which makes this system ideal for short duration delivery, in the course of a few hours to a day.

## 2. Materials and methods

### 2.1. Materials

D(+)-gluconic acid δ-lactone (GDL), ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), sorbitan laurate (Span 20) and isopropyl myristate (IPM) were purchased from Fluka. $CaCl_2$ was purchased from J.T. Baker, Polysorbate 80 (Tween 80) from Merck and the model drug Ketoprofen (KT) from sigma. KT has a log*P* of 0.97 [35]. Alginate (LF 200S) was supplied by FMC Biopolymers, Drammen, Norway. All materials were used as received.

### 2.2. Microemulsion preparation

The microemulsion (ME) was prepared by mixing the surfactants Tween 80 and Span 20 with oil (IPM), followed by dropwise addition of double-distilled water. Drug-containing microemulsion (ME-KT) was prepared by mixing the drug, the surfactants and the oil prior to the addition of water. The existence of microemulsion was initially confirmed by visual inspection. The solution was allowed to equilibrate for 24 h to obtain a clear microemulsion. The ratio of Tween 80:Span 20:IPM:drug was 26:1.25:4:1 and kept constant throughout the study. The concentration of Tween 80 ($\varphi$) was 2.8%, 1.4% or 0.9%.

### 2.3. Gel preparation

Alginate was dissolved in double-distilled water; drug was then added as a powder or in a microemulsion form and stirred with a magnetic stirrer. A calcium source in the form of pre-prepared Ca-EGTA solution was introduced next, followed by fresh GDL solution. GDL induces the slow release of calcium ions from the Ca-EGTA complex, thus allowing gelling of the alginate solution [36]. The $Ca^{2+}$:GDL molar ratio was 1:2. For the preparation of Ca-EGTA solution, an equimolar amount of $CaCl_2$ and EGTA was dissolved in water and the pH was adjusted to 7 by adding 1 M NaOH. Drug release measurements were done at least 24 h after GDL addition, to allow the alginate solution to gel completely [36]. Final compositions were 5–25 mg $ml^{-1}$ alginate, 5.5–20 mM calcium, 1 mg $ml^{-1}$ KT, and microemulsion with $\varphi$ = 2.8%.

### 2.4. Drug release

To 1 ml of gel, 15 ml of double-distilled water were added. The samples were put in a 37 °C bath and shaken at a rate of 100 rpm. At each time interval 0.3 ml of the surrounding medium was sampled and replaced with 0.3 ml of fresh water. The sample was measured in a spectrophotometer at a wavelength of 259 nm. Three types of crosslinked alginate gels were prepared: (i) without drug or microemulsion (control); (ii) with drug added as is; and (iii) with microemulsion containing drug (composite gel). Five samples from each type of gel were prepared. The control gel was used as blank, and drug concentrations were calculated from calibration curves. Ultraviolet spectroscopy was carried out on a 96-well plate with a Synergy HT microplate reader (Bio-Tek Instruments, Winooski, VT, USA).

### 2.5. Dynamic light scattering (DLS)

DLS measurements were performed using a BI-200SM Research Goniometer System (Brookhaven Instruments Corp.). A Compass 415M solid-state laser (Coherent), generating a monochromatic green light of 532 nm wavelength, was used. The detector assembly includes a selected photo-multiplier tube (PMT), a dynode chain and an integral amplifier/discriminator. The BI-9000AT digital signal processor was used as a photon counter for DLS measurements. Samples were placed in a glass cell and immersed in a glass vat containing decalin as the index matching fluid. All solvent were filtered through a Micropore (0.2 μm) pressure filter to dispose of dust particles prior to microemulsion preparation. Several concentrations of surfactants with and without drug were examined.

Windows-based *DLS* software Version 3.19 provided with the instrument was employed for data processing. The relaxation time for each system was obtained from the autocorrelation function using the CONTIN model; one relaxation time was observed, indicating an isotropic system. The relaxation time is related to the apparent diffusion coefficient. For monodisperse diluted systems with aqueous viscosity the diameter of the droplet can be calculated from the diffusion coefficient, by implementing the Stokes–Einstein equation [37].

### 2.6. Transmission electron microscopy (TEM)

TEM micrographs were obtained from ultrafast-cooled vitrified cryo-TEM specimens prepared under controlled conditions of