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

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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, a lipophilic drug can enter a hydrophobic cell membrane, enabling better targeting and drug efficiency [3]. On the downside, 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 block wise pattern [10]. The blocks can be similar (MMM, GGG) or

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 cross-linked 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.
strictly alternating (MGMC). 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 lyophilic 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 spermicides. 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-aminoethyl ether)-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 logP 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 (\(\phi\)) 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 \(\phi = 2.8\%\).

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, Winoo-ski, 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 vial containing decalin as the index matching fluid. All solvent were filtered through a Microprobe (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
37 °C and 100% relative humidity, as described elsewhere [38]. Specimens were examined in a Philips CM120 cryo-transmission electron microscope operating at 120 kV, using an Oxford CT3500 cooling–holder system that kept the specimens at about −180 °C. Low electron-dose imaging was performed with a Gatan Multiscan 791 CCD camera, using the Gatan Digital Micrograph 3.1 software package.

2.7. Small-angle X-ray scattering (SAXS)

SAXS was performed using a small-angle diffractometer (Molecular Metrology SAXS system with Cu Kα radiation from a sealed microfocus tube (MicroMax-002+S), two Göbel mirrors and three-pinhole slits; the generator was powered at 45 kV and 0.9 mA). The scattering patterns were recorded by a 20 × 20 cm two-dimensional position-sensitive wire detector (gas-filled proportional type of Gabriel design with 200 μm resolution) that was positioned 150 cm behind the sample. The scattered intensity I(q) was recorded in the interval 0.07 < q < 2.7 nm⁻¹, where q is the scattering vector defined as q = (4πλ/λ) sin(θ/2), where 2θ is the scattering angle and λ is the radiation wavelength (0.1542 nm). The solution or gel under study was sealed in a thin-walled capillary (glass) of about 2 mm diameter and 0.01 mm wall thickness; measurements were performed under vacuum at ambient temperature. The scattering curves were corrected for counting time and sample absorption.

3. Results and discussion

3.1. Formulation

Several considerations were taken into account when choosing the ingredients of the microemulsion. Primarily, all components have to be non-toxic, in order to be used in a drug release system. Although microemulsions are more readily stabilized by hydrocarbon oils, they are not considered suitable for pharmaceutical application [8]. One common approach to stabilize microemulsions uses co-surfactants, such as short-chained alcohols, which are less favorable [7]. Alternatively, several authors have suggested using a mixture of surfactants with different hydrophilic–lipophilic balance values [8]. The final microemulsion formulation described in this manuscript was derived following initial attempts to achieve a clear solution with different oils and ratios between surfactants. The formulation is based on a biocompatible oil, IPM, and a mixture of Tween 80 and Span 20, which were used in a previous study to create o/w microemulsions [39].

Like many polysaccharides, alginate is considered to be a hydrophilic polymer. Therefore it is not surprising that the model hydrophobic drug used in this study, Ketoprofen, is insoluble in alginate hydrogels and solutions; it either forms crystals or precipitates, thus leading to a turbid gel (Fig. 1B). Use of this gel as a sustained release matrix is problematic, as the size and size distribution of the drug crystals formed are very sensitive to the preparation conditions, such as mixing time and temperature. In turn, the crystals’ size could influence the release profile [40,41]. Mixing the drugfree microemulsion with alginate resulted in a clear solution, with no observable phase separation. Furthermore, when the microemulsion contained drug, there were no visible crystals or precipitates. Crosslinking did not affect the clarity of the gel (Fig. 1C). The fact that the gel does not scatter light suggests that any structure that might exist is no larger than 100 nm. Therefore the release in this case is potentially more reproducible. Similar results were obtained for all degrees of crosslinking and alginate concentrations used in this study. Assessment of these matrices as sustained release vehicles will follow.

3.2. Structure

A composite gel composed of nanodroplets and a polymer is a complex system. In order to begin deciphering its structure and consequently the drug release mechanism, the microemulsion without added alginate was investigated first. Initially, the existence of microemulsion droplets was established by cryo-TEM. An image of a formulation with surfactant concentration of 2.8% (Fig. 2) shows that fairly spherical particles are formed, with a
diameter of ~10 nm. A more accurate estimate for the dimensions of the spherical oil droplets was provided by DLS measurements performed on microemulsion with various surfactant concentrations, with and without drug. The data in Table 1 reveal that the presence of the drug and changes in the surfactant concentration hardly affect the size of the droplets as seen by light scattering. At the highest surfactant concentration (\(\varphi = 2.8\%\)) there is a small but significant difference between the diameter of the microemulsion containing drug and the drug-free microemulsion (\(\varphi < 0.001\)), suggesting that the drug influences the structure of the microemulsion but that this influence is very small. Differences in diameter for other surfactant concentrations are not statistically significant.

The location of the drug within the microemulsion was investigated using the fluorescent probe method, which can detect the microenvironment near a substance. This technique is commonly used for revealing phase changes and structure in microemulsions and micelles [42], and is also employed to study the site of the probe in the microemulsion [43]. Upon changing the solvent, the maximum absorbance or fluorescence wavelength of solvatochromic probes may shift to the blue or red. This shift corresponds to the microenvironment of the probe, mainly the polarity or the viscosity. Since KT contains a chromophore, and is prone to peak to the microenvironment of the probe, mainly the polarity or the microenvironment near a substance. This technique is commonly significant.

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spherical or from the nature of the shell, which is not constant in density due to radial concentration gradients. The parameters of the model agree with the findings of the light-scattering studies.

The presence of drug and changes in the surfactant concentration do not alter the core size ($R_c$) or the overall size ($R_s$). Nonetheless, the density of the shell depends on these factors, reinforcing the finding, derived from the absorbance spectra, that the drug is located in the interface of the droplets.

It is worth noting that the oil component, although small in quantity, has a strong influence on the structure of the microemulsion. Without the oil the solution was not stable and phase separated after a short time. The scattering profile of the surfactants in water with no oil component (dotted line in Fig. 4) is different in both the intensity and position of the peak. The oil is essential for creating the microemulsion structure.

The addition of a polymer to a microemulsion system can change its structure, and possibly break the spherical droplets. Theoretically, if there is no interaction between the microemulsion and alginate, the scattering curve of the mixture of both should be simply an addition of the scattering curve of each of them alone. Fig. 5a shows that, although this is not the case, the differences are mainly in the low $q$ region. This could imply that the interaction between the microemulsion droplets changes with the addition of alginate, or that there is an interaction between alginate and the microemulsion. Nevertheless, the peak of the form factor is shifted slightly to higher distances but has a similar shape, thus suggesting that at least some of the droplets maintain their structure. In order to get a more accurate picture, a small-angle neutron scattering experiment with varying contrasts should be performed.

Since the drug delivery system consists of crosslinked alginate, it is also important to examine the structure of the microemulsion in these circumstances. The SAXS curves of alginate crosslinked with two calcium concentrations (Fig. 5b) are very similar to the arithmetic addition of the curves from crosslinked alginate and the microemulsion, revealing that the change in structure of the microemulsion when combined with alginate gel is even less pronounced than the combination with alginate solution.

The structure and phase diagram of microemulsions depend strongly on temperature. Since application of this system as a drug delivery vehicle in the body implies that this system would be in an environment of 37 °C but the structure studies were conducted at room temperature, we repeated the SAXS measurements of the microemulsion and alginate separately and in combination at 37 °C (data not shown). The differences in the plots are small; the size and shape of droplets are practically unaffected. Therefore it is safe to assume that the suggested structure is preserved at 37 °C.

### 3.3. Drug release

Having demonstrated that the structure of the microemulsion is unchanged upon mixing with alginate, whether it is gelled or not, the influence of the gel's properties on the drug release rate was studied, as the existence of microemulsion droplets in the gel could modify the release properties markedly. Since drug release studies of microemulsions embedded in crosslinked polymers are rather scarce, both the timescale and the behavior of the release are unknown and need to be explored. In Ref. [27] the release of lidocaine from poly(hydroxyethyl methacrylic) acid was of order of days, and in Ref. [28] 80% release of cyclosporin A from deacylated gellan gum was attained after 12 h [33]. Fig. 6 shows a release profile collected for a gel with an alginate concentration of 25 mg ml⁻¹ and calcium concentrations of 5.5, 7.5, 10 and 20 mM. The timescale for the release is hours, consistent with previous studies, which demonstrate that substances are released from alginate gels, beads or tablets from around an hour to a few days [52–55]. A complete release within 24 h was observed for all formulations in this study. As expected, the release depends on the concentration of crosslinker. A 80% release can be acquired within 2 or 6 h; release within several hours is advantageous for certain drugs (e.g. analgesics). A distinctive profile can be observed for the three
lower calcium concentrations, in which the release is at a constant rate with no burst effect. Generally, a higher calcium concentration increases the crosslinking density of alginate; high crosslinking in gels typically indicates smaller pores and slower diffusion. As anticipated, when the calcium concentration is increased from 5.5 to 7.5 mM the release decelerates. However, as the concentration increases further, there is a surprising acceleration of the release rate; this anomaly is not common for alginate gels and will be investigated in future studies.

It is noted that quantifying the release profiles is not straightforward. Simple power laws could not be fitted to Figs. 6–8. Throughout the experiment, the gel swells and in a few cases disintegrates, both of which cause a change in the cross-section of the gel. Fitting release profiles to diffusion equations should be accompanied by further experiments.

The first step towards unveiling the release mechanism is understanding if differences in release profiles of these formulations occur due to changes in the network structure or due to structural alteration of the microemulsion at high calcium concentrations. If the changes in the alginate network are indeed accountable for the release rates, changes in the rates as a function of alginate concentration could be also expected. Indeed, Fig. 7a and b confirms that the concentrations of both alginate and calcium play a role in determining the release rate, and that the slowest rate depends on these two concentrations. At the low calcium concentration, for alginate concentrations above 20 mg ml\(^{-1}\), the initial release rate in the first 6 h approaches zero-order kinetics (Fig. 7a). However, in the case of the higher calcium concentration the release is more complicated, does not follow a power law and has a burst effect (Fig. 7b).

Given that SAXS results imply that the structure of the microemulsion does not vary significantly upon mixing with alginate or adding calcium, it is not likely that these large variations in the release rate are due entirely to changes in the structure of the droplets. To corroborate this, the experiment in Fig. 6 was repeated with one change – the drug was dispersed in the matrix as is, without incorporation into a microemulsion (Fig. 8). As stated before, this scheme is not favorable as a drug delivery vehicle since the size of the drug crystals depends on the preparation and storage conditions. However, it can assist in understanding the diffusion from these gels. Fig. 8 illustrates that the general timescales and calcium dependency are similar to those obtained in Fig. 6. A possible quantitative attribute for discrimination between different formulations is the time to achieve a certain release percentage.

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<tr>
<th>[Ca-EGTA]</th>
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<tr>
<td>KT</td>
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<tr>
<td>5.5</td>
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<td>7.5</td>
<td>3.9 (0.45)</td>
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<td>10</td>
<td>1.5 (0.1)</td>
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<td>20</td>
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Alginate concentration, 25 mg ml\(^{-1}\); Ca-EGTA concentrations, 5.5, 7.5, 10 and 20 mM. The standard error is indicated in brackets.
[57], e.g. 50% (t1/2). Table 3 compares the time to 50% release of KT from composted hydrogels vs. hydrogels with the dispersed solid drug. It is apparent that the microemulsion does not have an impact on the t1/2, with the exception of the hydrogel with 10 mM Ca-EGTA, where the release from the composite hydrogel is slower. Thus, it can be deduced that the contribution of the microemulsion droplets to the release rate is small; it is thus more conceivable that the alginate network governs the release. The release data imply that adjustments in both the order of the kinetics and the time to complete release could be obtained by employing different amounts of polymer and crosslinker. However, a better understanding of the release mechanism, which will be the subject of our future studies, is essential for tailoring these systems for specific applications. At this point we can only conclude that differences in release probably derive from the network properties and not from the structure of microemulsions.

4. Conclusions

We have presented a novel method for the solubilization of hydrophobic drugs in alginate hydrogel using a microemulsion loaded with a model hydrophobic drug. Precipitation of the drug from the alginate gel was observed, whereas no phase separation was visualized in the composite gel, inferring that the model drug is more soluble in the gel–microemulsion composite than in the gel alone. The existence of intact microemulsion droplets in the gel was verified by SAXS. Control over the release profile was gained using variations in the alginate and calcium concentrations, thus allowing fine tuning of the release rate. The methodology of incorporating microemulsion droplets into alginate hydrogels for the purpose of increasing drug solubility could be applied to a range of drugs. Moreover, various hydrophobic polymers can be utilized to create composite hydrogels. Other systems have to be examined carefully, as the polymer and the drug could affect the stability of the microemulsion. However, we believe that composite hydrogels hold great potential for enhancing the solubility of hydrophobic drugs.

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References


