Rheo-Dissolution: A new platform for the simultaneous measurement of rheology and drug release

F. G. Senjoti[1], M. U. Ghori[1], R. Diryak[1], B. R Conway[1], G. A. Morris[2] and A. M. Smith[1*]

[1]*Department of Pharmacy, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK.*

[2]*Department of Chemical Sciences, University of Huddersfield, Queensgate, Huddersfield, HD1 3DH, UK.*

*Correspondence:

Prof. Alan M. Smith

Tel: +44-1484-472-305

Fax: +44-1484-472-305

a.m.smith@hud.ac.uk

Abstract

There is great potential to improve drug delivery through the use of *in-situ* gelling delivery systems. Here we demonstrate a technique capable of measuring changes in rheology (gelation and/or dissolution) of *in-situ* gelling delivery systems on contact with physiological fluid, while simultaneously analysing drug release. An ocular *in-situ* gelling formulation (gellan and timolol maleate) and an *in-situ* gelling oral liquid (alginate and metronidazole) were used as exemplar formulations. The method allowed profiling of increasing gellan concentration resulting in a reduction of timolol maleate released into simulated lacrimal fluid. When alginate was used as an *in-situ* gelling oral formulation there was a rapid increase in G′ on contact with simulated gastric fluid. When this was changed to simulated intestinal fluid, drug release rate increased rapidly, coinciding with alginate gel dissolution. This work highlights the potential of this technology as a tool in development and optimisation of these increasingly popular delivery systems.

Keywords: Rheology; drug delivery; in situ; hydrogel; rheo-dissolution

1. Introduction

Physiologically responsive polymers are useful materials for the design of innovative drug delivery systems, in particular, polymers that undergo sol-gel transitions in the presence of physiological fluids have been incorporated into pharmaceutical formulations acting as functional excipients to control release or to increase retention time at the site of drug uptake. Release from such systems is generally governed by drug diffusion through the polymeric material and by erosion/dissolution of the gel. The rate of drug release therefore, is strongly related to the mechanical properties that include gelation kinetics, gel strength and gel dissolution (Mahdi, Conway, & Smith, 2014). *In situ* sol-gel transitions can take place as a

result of changes in temperature as occurs with polymers such as methylcellulose (Bain, Bhowmik, Ghosh, & Chattopadhyay, 2009), poloxamer (Amiji, Lai, Shenoy, & Rao, 2002; Edsman, Carlfors, & Petersson, 1998) or PLGA (He, Kim, & Lee, 2008) by changes in pH and/or presence of electrolytes Carbopol® (Srividya, Cardoza, & Amin, 2001), gellan gum (Diryak et al., 2018; Rajinikanth & Mishra, 2008), alginate (Miyazaki, Kubo, & Attwood, 2000), pectin (Kubo, Konno, Miyazaki, & Attwood, 2004), and carrageenan (Endo, Watanabe, Matsumoto, & Shirotake, 2000). During development, thermal transitions can be accurately measured using a temperature-controlled rheometer. However, when the sol-gel transitions are the result of a change in pH or ionic strength/species, measurements of sol-gel transitions are considerably more challenging. Indeed, sol-gel transitions triggered by pH or crosslinking ions tend to be rapid processes. This is best illustrated with sodium alginate which undergoes almost instantaneous gelation reaction on exposure to divalent cations. Furthermore, sodium alginate undergoes a similarly rapid transition in acidic media such as gastric fluid, forming an alginic acid gel. Recently in our laboratories we have demonstrated the experimental monitoring of the external gelation of alginate by modifying a commercially available rheometer (Mahdi, Diryak, Kontogiorgos, Morris, & Smith, 2016) and have since adapted this modification as an experimental simulation of gelation on contact with various physiological fluids (Diryak et al., 2018).

Many modifications have been applied to rheometers recently that have been developed from a fundamental characterisation perspective or to simulate an application/industrial process, further extending their usefulness. These adaptations include appending spectroscopic techniques to rheological equipment such as NMR (Callaghan & Gil, 2000) , rheo-SAXS (Somani et al., 2002) , Rheo-FTIR (Boulet-Audet, Byrne, & Kazarian, 2014), Rheo-Raman (Chevrel et al., 2012). From a more application focused perspective, rheometer adaptations include a light curing lower plate (Lee, Cole, Palmiter, & Koh, 2000), electro-rheology

accessories (Stanway, Sproston, & El-Wahed, 1996), and relative humidity accessories. These adaptations have been successful for informing the design of materials for specialized applications.

Despite the widespread use of *in situ* gelling materials as drug delivery systems, methods to enable mechanical testing during gelation and dissolution of polymer gels while simultaneously measuring the release of active molecules *in situ* have yet to be developed, therefore the mechanisms controlling release from such systems are poorly understood. To overcome these problems, we have developed a rheo-dissolution cell that can be attached to the lower plate of a commercially available rheometer and enables the examination of dynamic changes in rheological behaviour and drug release simultaneously. We have demonstrated the power of this technique using an ocular *in situ* gelling formulation (gellan gum and timolol maleate) and an *in situ* gelling oral liquid (sodium alginate and metronidazole). These gelling materials were chosen as they are both routinely used as *in situ* gelling agents in commercially available pharmaceutical formulations and are delivered to different physiological target sites.

2. Materials and Methods

2.1 Materials

Low acyl gellan gum (Gelrite®) and sodium alginate was purchased from Sigma-Aldrich (Poole, UK) and had a M:G ratio of 0.39:0.61 and molecular weight of 120,000-190,000 g/mol. Metronidazole, hydrochloric acid (37.5%), sodium hydroxide, calcium chloride and sodium chloride were purchased from Sigma-Aldrich (Poole, UK). Sodium bicarbonate was purchased from Fisher Scientific (Loughborough, UK). Timolol maleate was purchased from Tokyo Chemical Industry (Oxford, UK). All chemicals were used without further purification.

2.1.1 Rheo- dissolution cell

The computer-aided design (CAD) of the rheo-dissolution cell was developed using Solidworks® (version 25, 2017) and constructed from acrylonitrile butadiene styrene using a Makerbot Replicator™ 2 3D printer (New York, USA). The cell was designed as a circular reservoir with an opening on the top that was covered with a stainless steel mesh during the experiments. The cell was designed with inlet and outlet ports for loading and sampling from the reservoir. The inlet was used to load solutions or buffers in to the reservoir and the outlet allowed withdrawal of samples for analysis. The cell was attached to the lower plate of a commercially available rheometer thus enabling examination of the rheological behaviour of the formulation and drug release simultaneously **(fig. 1)**. The reservoir of the rheo-dissolution cell was designed to be capable of holding a volume of 55 ml. The mesh (80 mesh count) which was placed on the top of the reservoir (where test samples are loaded) can be removed, and interchanged with different mesh sizes. If required, (particularly with very low viscosity samples) a semipermeable membrane can be placed on the surface of the mesh to prevent the sample flowing into the reservoir.

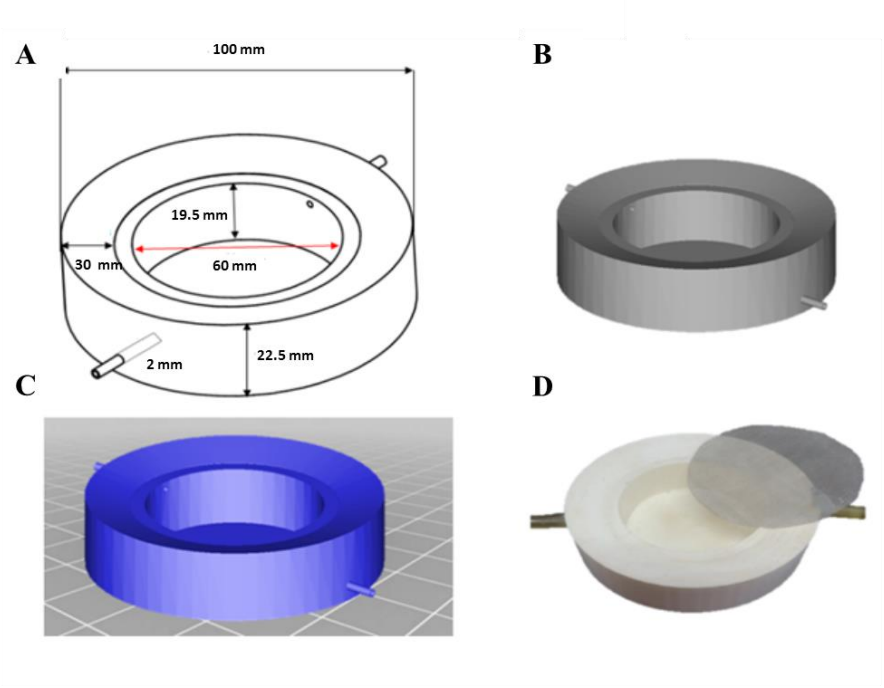

Figure 1: (A) Dimensions of rheo-dissolution cell (B) CAD model (C) Stl file model and (D) 3D printed rheo-dissolution cell showing removable mesh.

2.2 Preparation of *in situ* gel forming ophthalmic formulations

To demonstrate the concept of this method with an *in situ* gelling ocular formulation, a gel forming eye drop solution based on a formulation that is currently marketed (Timoptol LA® 0.5%) was prepared. Different concentrations of low acyl gellan gum (0.3%, 0.4%, 0.6%, and 0.8%) were used as the gel former with 6.8 mg/ml timolol maleate (TM) used as the active. TM (6.8 mg/ml) was dissolved in deionized water at room temperature and then heated to 85 °C, low acyl gellan gum was then added while stirring until fully dissolved. The solutions were allowed to cool quiescently to room temperature prior to further analysis. The pH of the formulation was 4.5.

2.3 Preparation of *in situ* gel forming oral solutions

An *in situ* gelling oral formulation was designed containing metronidazole (MNZ, 200 mg/5 ml) and contained 2% w/v sodium alginate as the gel former. Sodium alginate (2%) was dissolved in deionized water at room temperature and stirred until it was fully dissolved. MNZ (200 mg/5 ml) was then added to the alginate solution and stirred until a uniform dispersion was formed. There was no notable difference in the rheological properties of the alginate when MNZ was added indicating that there was no interaction between the drug and the polymer. The prepared suspension was then stored at room temperature for 2 h prior to further analysis.

2.4 Preparation of simulated fluids

Simulated lacrimal fluid (pH 7.5) was prepared according to the formulation reported by Marques et al. (2011). This formulation has previously been used to evaluate *in vitro* release of pilocarpine from hydrogels (Anumolu, Singh, Gao, Stein, & Sinko*, 2009) and nanoparticles (Lin, Yu, Lin, & Wang, 2010). *In vitro* release of indomethacin from a sodium alginate gel has also been reported where the release medium used was simulated lacrimal fluid formulated with the same recipe (Pandit et al., 2007). 0.1M HCl, pH 1.2 was used as simulated gastric fluid. This was prepared without pepsin as this enzyme does not have any effect on polysaccharides.

2.5 Comparison of rheological measurements using a standard parallel plate geometry and the rheo-dissolution cell

Rheological measurements of *in situ* gelling ophthalmic and oral formulations were performed using a Kinexus rheometer (Malvern Panalytical, UK). Measurements of elastic modulus (G′) and viscous modulus (G″) were taken as a function of time using a 40 mm serrated parallel plate geometry with a fixed gap of 0.8 mm. Freshly prepared sample solutions were loaded onto the rheometer at room temperature and measurements were performed (within the linear

viscoelastic region) using 0.5% strain and a frequency of 1 $rads^{-1}$. Silicone oil was added to the periphery of the samples to prevent evaporation during the measurements.

Rheological measurements were also performed using the rheo-dissolution cell as a lower plate to compare the performance of the system with that of the standard geometry. The rheo-dissolution cell was attached to the Kinexus rotational rheometer as a lower plate. A stainless steel woven wire mesh (mesh count 80) was placed on top of the reservoir and attached securely to the surface of the cell. A 40 mm serrated parallel plate geometry was attached to the rheometer and the gap was fixed at 0.8 mm. The samples were loaded on to the mesh and the volume of the sample was determined by the set gap. Oscillatory measurements of G′ and G″ were measured as a function of time using the same parameters (0.5% strain, 1 $rads^{-1}$ angular frequency) as used with the standard parallel plate geometry. When measuring the rheological changes of the *in situ* gelling oral formulation, a dialysis membrane (14000 mwco) which had been soaked in deionised water, was placed on the surface of the mesh before loading sample. A solvent trap was also used to avoid evaporation of the samples during measurements.

2.6 Rheo-dissolution measurements for *in situ* gel forming ophthalmic formulation

Rheo-dissolution measurements were conducted using Kinexus rotational rheometer with the rheo-dissolution cell replacing the lower plate of the rheometer. A circulating peristaltic pump maintained the flow of simulated lacrimal fluid in the reservoir to maintain sink conditions, to provide a source of ions and to facilitate sampling. Two pieces of silicone tubing was securely attached to the inlet and outlet of the rheo-dissolution cell and then connected to a pump. A 3-way sampling port was attached to the tube for collecting and replacing samples **(fig. 2A)**. The circulatory system consisted of 2 pieces of tubing (45 ml) and the cylindrical reservoir (55 ml); which altogether was capable of holding 100 ml volume. A stainless steel woven wire mesh (mesh count 80) was placed on top of the reservoir **(fig. 2B)** and was secured with adhesive to

prevent disturbance of the cell during the experiment. The aperture of the mesh was 180 µm which was sufficient to prevent the sample passing through the mesh. Receiver fluid, in this case lacrimal fluid (90 ml), was added and once the circulatory system had settled, a 40 mm serrated parallel plate geometry was attached to the rheometer (0.8 mm gap) and the test was started immediately using the parameters described in section 2.5. Measurements of storage modulus (G′) and loss modulus (G″) were recorded as a function of time at room temperature **(fig. 2C)**. Immediately after the test had commenced, 10 ml of simulated lacrimal fluid was injected through the sampling port; this volume was sufficient to enable contact with the formulation, initiating the gelling process. As the inner diameter of the reservoir was 60 mm and the geometry was 40 mm, a solvent trap was used to prevent evaporation of the fluid.

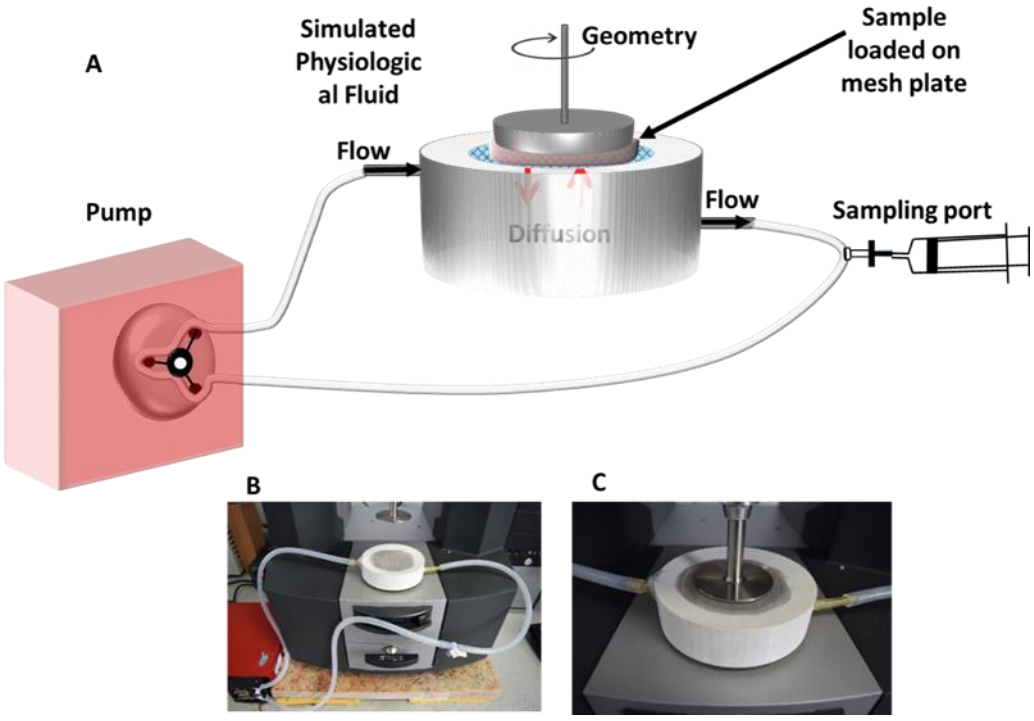

Figure 2: (A) Schematic diagram of the experimental set up of rheo-dissolution cell (B) rheo-dissolution cell attached to the lower plate of rheometer prior to loading sample and (C) rheo-dissolution experiments in process

Samples (0.5 ml) of the simulated lacrimal fluid in the reservoir were collected via the sampling port at regular time intervals (2, 4, 6, 8, 10, 30, 60, 90, 120, 150, 180 min) and were replaced

with same volume of fresh simulated lacrimal fluid. The volume of simulated lacrimal fluid was maintained 100 ml throughout the system, so that the fluid remained in contact with the sample. Collected samples were then analysed for the drug released from the gels. All experiments were performed in triplicate. To validate the method against conventional dissolution testing, release studies were also preformed using a dissolution bath containing 100 ml inserts and applying the same sampling regime as used in the rheo-dissolution tests.

2.6.1 Analysis of TM

Reverse-phase high performance liquid chromatography of TM was performed following the method of Nasir et al. (2011). Briefly, a C18 HPLC column (Phenomenex, Macclesfield, UK) was used to analyse TM released from the *in situ* gel forming ophthalmic formulation, the mobile phase was comprised of methanol:0.2% triethylamine (60:40, v/v), pH 2.75, adjusted with 85% phosphoric acid. The flow rate was 1 ml/min with a run time of 5 min. TM was detected with a UV detector (Cary 60, Agilent Technology, Cheadle, UK) at a wavelength of 295 nm at a 3 min retention time. The linearity of the proposed method was determined from the calibration curve constructed at five concentration levels by plotting peak areas against their respective concentrations.

2.7 Rheo-dissolution measurements for *in situ* gel forming oral formulation

Rheo-dissolution experiments were also performed on an alginate-MNZ formulation to monitor drug release during dissolution of the alginate gel that occurs at varying pH (acidic and alkaline). The tests were performed using the experimental set up described in section 2.5 adapted by the addition of a dialysis membrane (14000 mwco) on the surface of the mesh which had been soaked in deionised water. This adaptation was applied to facilitate measurements of the pre-gelation state due to the extremely rapid gelation kinetics of alginate. The addition of the dialysis membrane ensured that the alginate did not flow through the mesh pores prior to

the commencement of the rheological measurements and allowed greater control of measurements of the alginate in the pre gelled state. The flow through system contained 90 ml of simulated gastric fluid at the beginning of the test and the liquid level was below the mesh not in contact with the sample. The pH of simulated gastric fluid was 1.2. The gap was fixed at 0.8 mm and after zeroing the geometry. Required volume of alginate-MNZ *in situ* gelling formulation was placed on the top of the dialysis membrane. Oscillatory measurements of G′ and G″ were performed as a function of time (0.5% strain, 1 $rads^{-1}$ frequency). The sample was measured in the pre-gelled state for 10 min before inducing gelation (which enabled the capture of the whole gelation event) by the addition of 10 ml of simulated gastric fluid (pH 1.2) to the 90 ml present in the reservoir. This was introduced through the sampling port and allowed the formulation to come in contact with the simulated gastric fluid. The experiment was continued for 7 hours and samples of the simulated gastric fluid (0.5ml) were withdrawn at 4, 8, 12, 16, 20, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390 and 420 min. The simulated gastric fluid sample was replaced with the same volume of fresh fluid at each time point via the sampling port. The pKa values of guluronic acid and mannuronic acid residues of the alginate are 3.65 and 3.38 respectively. So when the pH value is below the pKa of the uronic acid, the negative charge is removed, resulting in gel formation stabilized by intermolecular hydrogen bonding. Therefore, the alginate gels are stable in acidic environment in stomach but broken down in alkaline environment of intestine as the acidic groups become charged causing electrostatic repulsion between the polymer chains.(Francis et al., 2013; Pawar & Edgar, 2012; Rasel & Hasan, 2012).

Therefore, a further rheo-dissolution experiment was also performed on the alginate-MNZ formulation to monitor drug release during dissolution of the alginate gel that occurs at alkaline pH. The rheo-dissolution experiment as previously described was allowed to proceed for 120 min at pH 1.2. At 120 min, 9ml of the media was then replaced with same volume of 1M NaOH

to increase the pH of gastric media to pH 8, to observe release behaviour of MNZ while the gel was dissolving. The rheo-dissolution study was continued until the *in situ* gel was broken down and identified by a reduction in modulus. The samples were withdrawn every 30 min and replaced with fresh medium (pH 8).

All collected samples were then analysed using UV spectrophotometry (Agilent technology, Cary 60) at a wavelength of 277 nm. The percentage of metronidazole released from the *in situ* gel was determined from linear regression equation obtained from the UV standard calibration curve. All experiments were done in triplicate.

MZN release data obtained during the rheo-dissolution experiments were curve fitted to the zero order model using equation 1.

$$Q = Q_o + K_0 t \quad \text{(Eq.1)}$$

where Q is the amount of drug released at time t, Q_o is the initial amount of drug and K_0 is the zero order release constant.

2.7.1 Solubility profile of MNZ

Solubility of MNZ was determined at room temperature (22 ± 1°C). Excess amounts of MNZ were added to 0.1M HCl at pH 1.2 and pH 8 (adjusted by addition of 1M NaOH) to prepare saturated solutions. The solutions were stirred overnight to attain equilibrium. The solutions were then filtered and diluted as necessary to analyse the MNZ content spectrophotometrically at 277 nm. The experiments were performed in triplicate.

3. Results and Discussion

3.1 Comparison of rheological measurements with standard geometry and the rheo-dissolution cell

Measurements of viscoelastic properties against time were performed for *in situ* gelling ophthalmic and oral formulation using Kinexus rheometer fitted serrated parallel plate geometry. Measurements were also performed replacing the lower serrated plate with the rheo-dissolution cell covered with a stainless steel mesh. In the case of the ophthalmic formulation, the G′ value was 0.57 Pa at the end of the test performed with a conventional lower plate **(fig. 3A)** which was comparable with the G′ value (0.68 Pa) when performed with rheo-dissolution cell **(fig. 3B).**

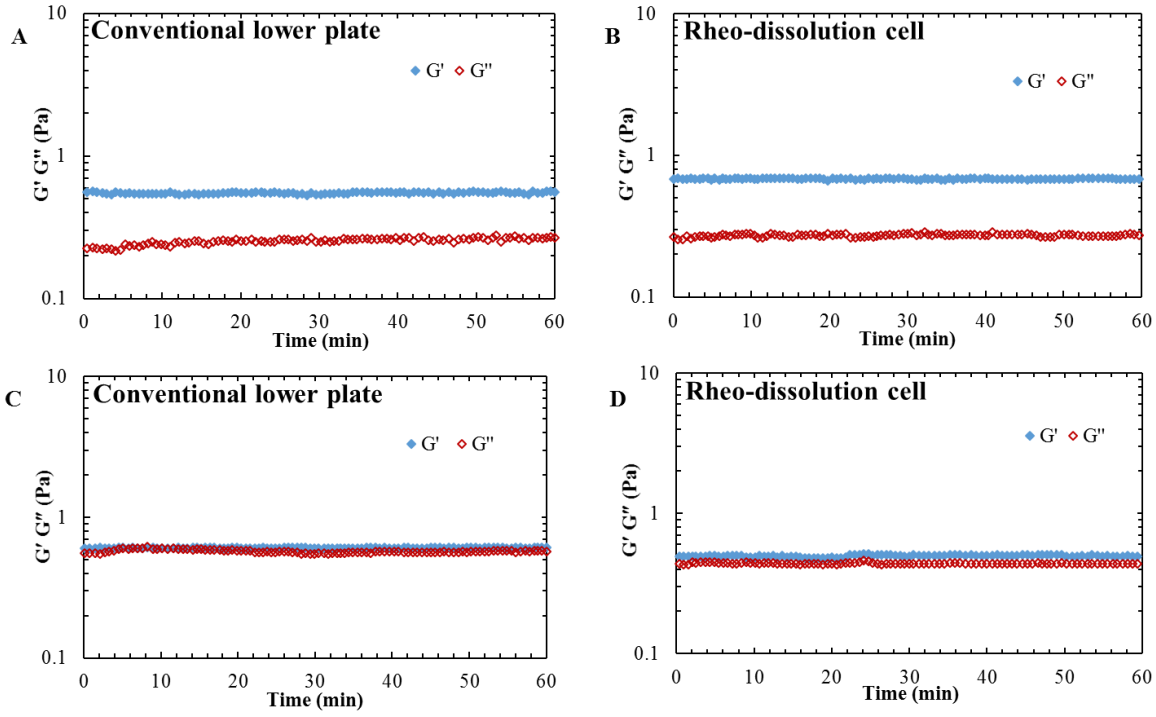

Figure 3: Viscoelastic measurements of G′ and G″ (Pa) over time of: *in situ* gelling ophthalmic formulation of gellan-TM performed with (A) conventional lower plate (B) rheo-dissolution cell and *in situ* gelling oral formulation of alginate-MNZ performed with (C) conventional lower plate (D) rheo-dissolution cell

Similarly, viscoelastic measurements were performed for the *in situ* gelling oral formulation of alginate-MNZ. It was clear from the measurements that the moduli were almost identical in both experimental settings **(fig. 3C and 3D)**.

3.2 Rheo-dissolution measurements of *in situ* gel forming ophthalmic formulation

The simultaneous measurements of rheological changes and drug release were performed for 4 different concentrations of gellan gum to investigate the impact of increasing gel strength on gelling behaviour and ensuing drug release. The formulation containing 0.3% w/v gellan showed a rapid increase in modulus over first 30 min of exposure to the simulated lacrimal fluid as gelation occurred, with G′ reaching ~ 300 Pa and G″ ~50 Pa once fully gelled. Both moduli then plateaued for the remainder of the test **(fig. 4A)**. The release of TM showed initial burst release with 23 % released in the first 10 min of the test as the gellan gel was undergoing its structuring process. Following this period, once the gel was fully formed, release slowed with the total release at 3h only 48%. As the concentration of gellan was increased, gelation occurred more rapidly and the final gel strength also increased G′ (1248 Pa) and G″ (134 Pa) for 0.4% gellan gum **(fig. 4B),** G′ 2275 Pa and G″ 346 Pa for 0.6% gellan **(fig. 4C).** The initial burst release of TM that was apparent in the 0.3% gellan formulation gradually disappeared with increasing concentration and had a significant effect reducing the overall release after 3h with 0.6% gellan releasing ~36% of the drug. When the concentration was increased further to 0.8% gellan, the rheo-dissolution indicated that a strong gel had already formed at the onset of the test with G′>>G″throughout the duration of the experiment **(fig. 4D).** This resulted in a release only~24% TM after 3h. At the end of the test, all gels could be removed as a solid gel in a single piece. It is important to note however, that the gel strength may not be the critical factor that controls the release of the TM from a gellan gum based formulation as there is potential electrostatic interaction that could occur between the amino group of the TM and the carboxylate groups of the gellan gum. The amino group in the TM has a pK_a 9.21 therefore would be in the ionized form in the formulation, which is likely interact with the negative charge of the gellan preventing complete release. Indeed, incomplete release of cationic drugs from negatively charged polysaccharides (Bonferoni et al., 2004; Rupenthal, Green, & Alany, 2011) and phospholipids (Lindell, Engblom, Engstrom, Jonströmer, & Carlsson, 1998) have

been previously reported to be due to electrostatic interactions between the anionic carrier and the drug. Furthermore, the initial work on gellan as an ophthalmic vehicle for the sustained release of TM also reported incomplete release following 6 h submerged 650 ml of simulated lacrimal fluid (Rozier, Mazuel, Grove, & Plazonnet, 1989). A similar study in our laboratory also showed incomplete release and was comparable with data obtained using the rheo-dissolution cell **(fig. S1).** It is however, interesting to witness in real time, that release of TM occurs more rapidly in samples where the gel strength is weak (first 10 min of exposure to simulated lacrimal fluid), clearly evident in the 0.3% and 0.4% gellan formulation **(fig. 4A and B)**. This behaviour is dramatically reduced in the 0.6% **(fig. 4C)** and disappears completely in the 0.8% gellan formulations where G′>>G″ throughout the experiment **(fig. 4D)**. Moreover, when plotting release at 180 min vs gellan concentration (from 0.3% to 0.8%) there was a liner reduction in timolol release as the concentration was increased **(fig. 5)**.

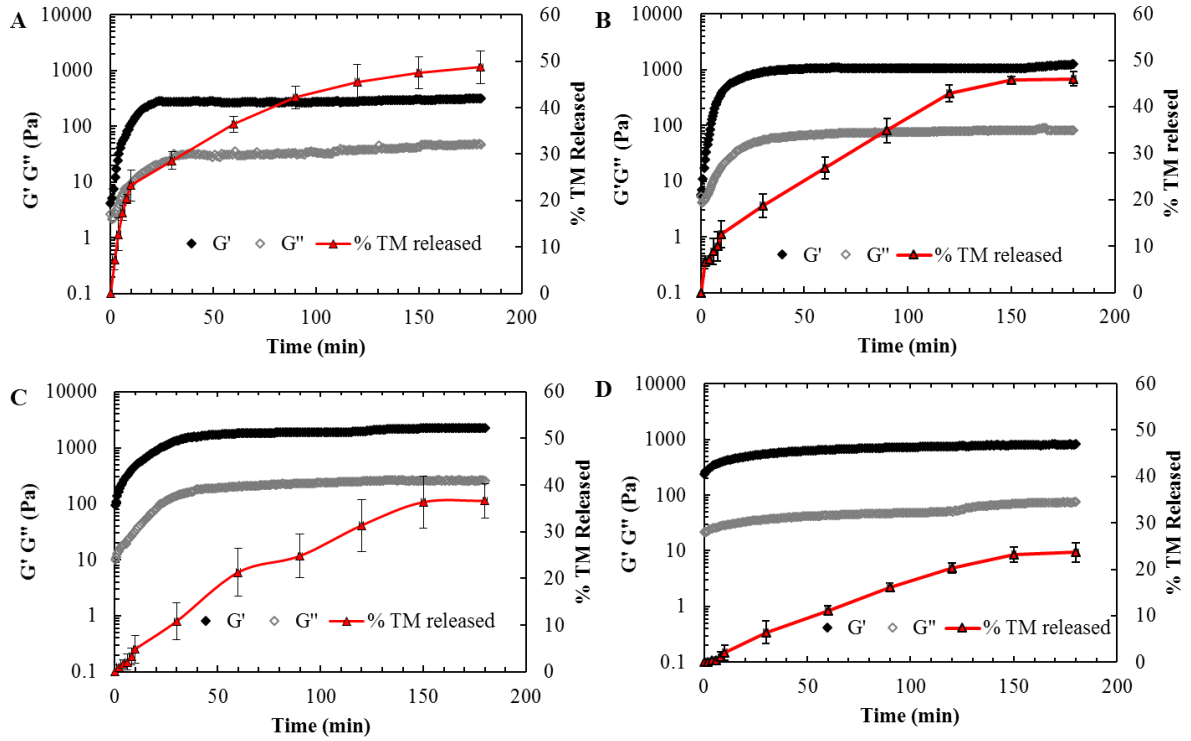

Figure 4: Rheo-dissolution experiments of in situ gel forming ophthalmic formulation containing 6.8mg/ml TM and (A) 0.3% (B) 0.4% (C) 0.6% (D) 0.8% low acyl gellan gum

These large changes in drug release with only relatively small changes in polymer concentration could ultimately impact the performance of *in situ* gelling formulations.

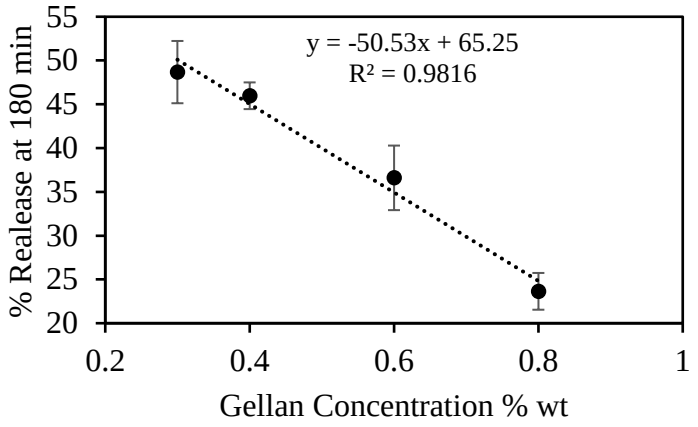

Figure 5: Release of timolol maleate at 180 min with increasing gellan concentration.

Therefore, the development of this *in vitro* technique which provides real-time correlation between the rheological behaviour and drug release is important, as the molecular interplay between the polymer molecules during gelation and the impact that has on drug release and subsequent bioavailability, is poorly understood especially in ophthalmic formulations. The technique however could have uses at other physiological target sites. To demonstrate this a similar approach was investigated for an *in situ* gelling oral liquid.

3.3 Rheo-dissolution measurements for *in situ* gel forming oral formulation

The rapid gelation of sodium alginate on exposure to acid is a well-known and attractive property that has been utilised in oral liquid formulations to increase gastric retention time or to control the release of drugs in the gastrointestinal tract. Here, we formulated a MNZ (200 mg/5ml) oral liquid using 2% w/w alginate as an *in situ* gelling agent to examine the suitability of the rheo-dissolution for oral liquids that are rheologically responsive to changes in pH. At the start of the test, prior to exposing the sample to acidic media, rheological measurements were taken to ascertain the behaviour that would be apparent prior to administration. G′ and

G″ at this stage were very low (~0.6 Pa), indicative of an entangled polymer solution. When the formulation was exposed to 0.1M HCl (pH 1.2), by injecting the simulated gastric fluid into the rheo-dissolution system, there was an almost instantaneous increase in G′ of almost 3 orders of magnitude, similar to that what was previously reported by Diryak et al. (2018) (Diryak et al., 2018) and is in line with expected behaviour (**fig. 6A)**.

Following this initial rapid gelation both moduli continued to increase steadily as the alginate continued to develop its structure until the end of the test at 7 h where the value of G′ was 1286 Pa and G″ 210 Pa. During the first 30 min of the test, release of MNZ was 12%, this occurred while the rheological measurements were at low moduli values and during the gelation process. Once the alginate had formed a strong gel however, only 50% MNZ was released in the following 3.5 h (50% at 210 min) and plateaued until the end of the test (53% in 420 min) with the gel remaining physically stable. To demonstrate the feasibility of changing dissolution media during the course of an experiment, and to analyse changes in release behaviour as the sol-gel reaction is reversed, the pH of the media in the rheo-dissolution cell was raised to pH 8 following 120 min at pH 1.2 by replacing 9 ml of the simulated gastric fluid with 1M NaOH **(fig. 6B)**. Over the first 2 h when exposed to the simulated gastric fluid release of MNZ followed the same release behaviour as was observed in **fig. 6A** with ~34% released at 120 min. On changing the dissolution media to pH 8, however, MNZ continued to be released at the same rate **(fig. 6C)**. This coincided with a rapid reduction in the gel strength as the gel began to dissolve observed by a fall in modulus at 190 min at pH 8, where G′ value reduced from ~801 Pa at 190 min in acid to ~30 Pa at 370 min indicating that alginate gel had broken down at which point the rheological measurements were stopped. The MNZ release curve continued to show a zero order release with a total of 96% released over the duration of the test **(fig. 6B).** Indeed, zero order kinetic modelling of the drug release data **(fig. S2)** revealed negligible variations of release constant K_0 across the entire profile in the sample where the

media was changed to pH 8 **(Table 1)**. Whereas the sample that was maintained at pH 1.2 fitted well to the zero order model first 120 min ($R^2 = 0.98$) then deviated sharply from the zero order model over the remainder of the release curve ($R^2 = 0.77$).

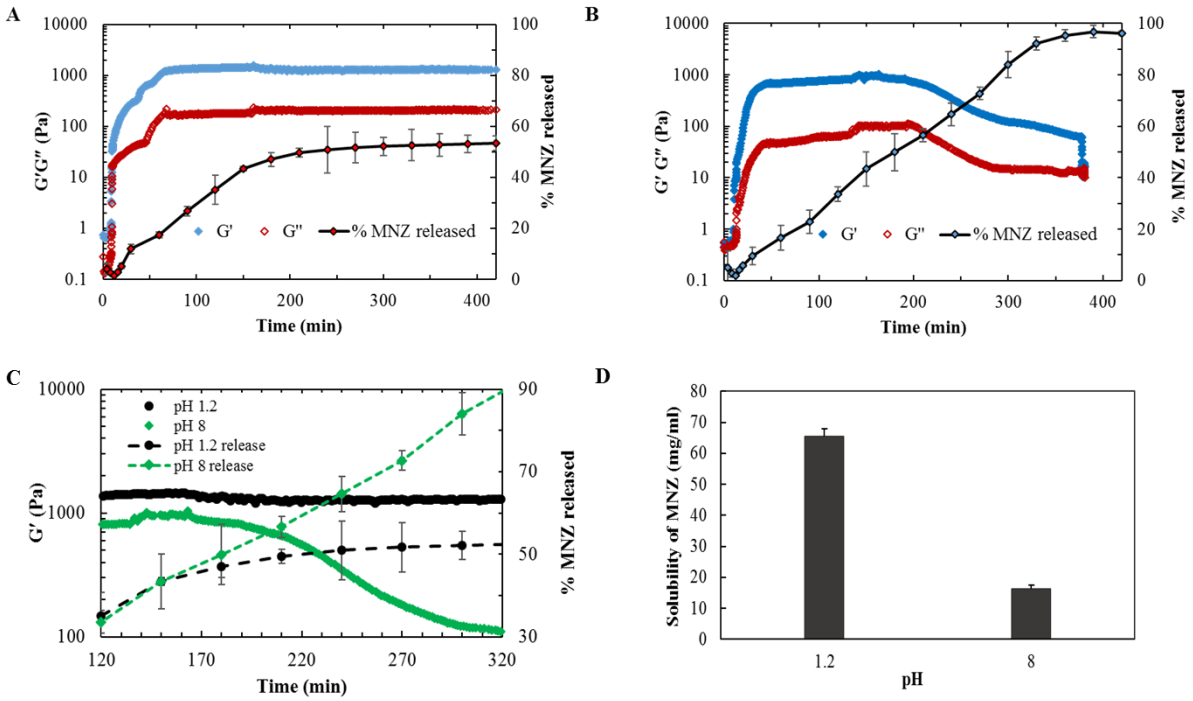

Figure 6: Rheo-dissolution experiment of in situ gel forming oral formulation containing MNZ (200mg/5ml) and 0.2 % sodium alginate at (A) pH 1.2 (B) pH 1.2 and pH 8 (C) comparison of release and elastic modulus following 120 min at pH 1.2 when either maintaining the pH at 1.2 or adjusting to pH 8 and (D) The effect of pH 1.2 and 8.0 on solubility of MNZ (n=3)

To ensure this continuous release of MNZ at pH 8.0 was a result of degradation of the gel and not a solubility effect, solubility tests of MNZ at pH 1.2 and pH 8.0 were performed. MNZ is weak base (pKa 2.62) and it is highly soluble in at pH ≤ 2.0. The highest solubility of MNZ was observed at pH 1.2 (65 mg/ml) which is comparable to previously reported values (Wu & Fassihi, 2005) At pH 8 solubility was reduced to 16 mg/ml **(fig. 6D)** indicating that the release witnessed at pH 8 was not a result of increased solubility of MNZ and more likely a result of the dissolution of the alginate gel.

Table 1 Summary of zero order drug release kinetic parameters

Media Maintained at pH 1.2	K_0	R^2	Media changed to pH 8	K_0	R^2
Full release profile	0.169	0.79	Full release profile	0.261	0.98
0-120 min (pH 1.2)	0.295	0.98	0-120 min (pH 1.2)	0.273	0.98
120-420 min (pH 8)	0.165	0.77	120-420 min (pH 8)	0.260	0.92

The apparent time lag from changing the media to the observed reduction in G′ **(fig. 6C)** can be explained by the time required for diffusion of the release media into the gelled sample. Moreover, the lower surface of the sample, in contact with the media, will begin to dissolve first as the media diffuses causing the gel to become anisotropic (stiffer at the upper surface and more fluid at the lower surface) allowing the continued drug release from the gel at the surface in contact with the release media. The diffusion of the media into the gel progresses until ultimately causing a reduction in G′. It intuitive to assume however, that this effect would have greater significance on thicker gel samples.

It is clear from the data that the rheo-dissolution cell attached to the lower plate of a rheometer has the potential to measure the real time gelation on exposure to a cross linking medium and simultaneously allow measurements of drug release from the formulation. This has the major advantage of being a single experiment rather than performing separate release experiments and rheological tests, thus, providing both of these important sets of data from the same sample, which is not possible using conventional apparatus. Moreover, this new platform has the ability to analyse the rheology and drug release in different environmental pH that can be changed in process. It is believed that this system could also be developed further, and become semi-

automated by connection to a UV spectrophotometer, similar to commercially available semi-automated dissolution testing apparatus.

4. Conclusion and future perspectives

Here we have demonstrated a novel method to simultaneously measure rapidly changing rheological behaviour and drug release from polymeric drug delivery systems. We believe this apparatus can be utilized in designing more efficient *in situ* gelling formulations earlier in the development process and can be modified to target different physiological sites. Furthermore, this technique has the potential to be translated beyond pharmaceutical applications and could be utilised in any system where polymers gel in the presence of metal ions, small molecule crosslinkers or by changes in pH and release an entrapped compound. Moreover, the flow through system provides the option to change the media while rheological measurements are in process and therefore could be used to track and correlate changes in physical behaviour with changing chemical environments, helping to understand how this can impact release of entrapped molecules. The wider application of this system therefore, is the ability to test any polymer for many different industrial applications where there may be a need for rapid or slow gelation while monitoring entities that are released, with the advantage of performing these experiments on the same sample in real time.

5. References

Amiji, M. M., Lai, P. K., Shenoy, D. B., & Rao, M. (2002). Intratumoral administration of paclitaxel in an in situ gelling poloxamer 407 formulation. *Pharmaceutical Development and Technology*, *7*(2), 195–202. http://doi.org/10.1081/PDT-120003487

Anumolu, S. S., Singh, Y., Gao, D., Stein, S., & Sinko*, P. J. (2009). Design and evaluation of novel fast forming pilocarpine-loaded ocular hydrogels for sustained pharmacological

response. *J Control Release*, *137*(2), 152–159. http://doi.org/10.1126/scisignal.2001449.Engineering

Bain, M. K., Bhowmik, M., Ghosh, S. N., & Chattopadhyay, D. (2009). In situ fast gelling formulation of methyl cellulose for in vitro ophthalmic controlled delivery of ketorolac tromethamine. *Journal of Applied Polymer Science*, *113*(2), 1241–1246. http://doi.org/10.1002/app.30040

Bonferoni, M. C., Chetoni, P., Giunchedi, P., Rossi, S., Ferrari, F., Burgalassi, S., & Caramella, C. (2004). Carrageenan-gelatin mucoadhesive systems for ion-exchange based ophthalmic delivery: In vitro and preliminary in vivo studies. *European Journal of Pharmaceutics and Biopharmaceutics*, *57*(3), 465–472. http://doi.org/10.1016/j.ejpb.2003.12.002

Boulet-Audet, M., Byrne, B., & Kazarian, S. G. (2014). High-throughput thermal stability analysis of a monoclonal antibody by attenuated total reflection FT-IR spectroscopic imaging. *Analytical Chemistry*, *86*(19), 9786–9793. http://doi.org/10.1021/ac502529q

Callaghan, P. T., & Gil, A. M. (2000). Rheo-NMR of semidilute polyacrylamide in water. *Macromolecules*, *33*(11), 4116–4124. http://doi.org/10.1021/ma9918203

Chevrel, M. C., Hoppe, S., Falk, L., Nadeìge, B., Chapron, D., Bourson, P., & Durand, A. (2012). Rheo-raman: A promising technique for in situ monitoring of polymerization reactions in solution. *Industrial and Engineering Chemistry Research*, *51*(49), 16151–16156. http://doi.org/10.1021/ie302054k

Diryak, R., Kontogiorgos, V., Ghori, M. U., Bills, P., Tawfik, A., Morris, G. A., & Smith, A. M. (2018). Behavior of In Situ Cross-Linked Hydrogels with Rapid Gelation Kinetics on Contact with Physiological Fluids. *Macromolecular Chemistry and Physics*, *219*(8). http://doi.org/10.1002/macp.201700584

441 Edsman, K., Carlfors, J., & Petersson, R. (1998). Rheological evaluation of poloxamer as an
442 in situ gel for ophthalmic use. *European Journal of Pharmaceutical Sciences*, *6*(2), 105–
443 112. http://doi.org/10.1016/S0928-0987(97)00075-4

444 Endo, H., Watanabe, Y., Matsumoto, M., & Shirotake, S. (2000). Preparation and evaluation
445 of heat-sensitive melting gel–acetaminophen gel. *Jpn. J. Hosp. Pharm*, *26*, 250–258.

446 Francis, N. L., Hunger, P. M., Donius, A. E., Riblett, B. W., Zavaliangos, A., Wegst, U. G.
447 K., & Wheatley, M. A. (2013). An ice-templated, linearly aligned chitosan-alginate
448 scaffold for neural tissue engineering. *Journal of Biomedical Materials Research - Part*
449 *A*, *101*(12), 3493–3503. http://doi.org/10.1002/jbm.a.34668

450 He, C., Kim, S. W., & Lee, D. S. (2008). In situ gelling stimuli-sensitive block copolymer
451 hydrogels for drug delivery. *Journal of Controlled Release*.
452 http://doi.org/10.1016/j.jconrel.2008.01.005

453 Kubo, W., Konno, Y., Miyazaki, S., & Attwood, D. (2004). In situ gelling pectin
454 formulations for oral sustained delivery of paracetamol. *Drug Development and*
455 *Industrial Pharmacy*, *30*(6), 593–599. http://doi.org/10.1081/DDC-120037490

456 Lee, J. Y., Cole, T. B., Palmiter, R. D., & Koh, J. Y. (2000). Accumulation of zinc in
457 degenerating hippocampal neurons of ZnT3-null mice after seizures: evidence against
458 synaptic vesicle origin. *The Journal of Neuroscience : The Official Journal of the*
459 *Society for Neuroscience*, *20*(11), RC79. http://doi.org/20004230 [pii]

460 Lin, H. R., Yu, S. P., Lin, Y. J., & Wang, T. S. (2010). High pH tolerance of a chitosan-PAA
461 nanosuspension for ophthalmic delivery of pilocarpine. *Journal of Biomaterials Science,*
462 *Polymer Edition*, *21*(2), 141–157. http://doi.org/10.1163/156856209X410274

463 Lindell, K., Engblom, J., Engstrom, S., Jonströmer, M., & Carlsson, A. (1998). Influence of a

charged phospholipid on the release pattern of timolol maleate from cubic liquid crystalline phases. *The Colloid Science of Lipids*, 111–118. http://doi.org/10.1007/BFb0117968

Mahdi, M. H., Conway, B. R., & Smith, A. M. (2014). Evaluation of gellan gum fluid gels as modified release oral liquids. *International Journal of Pharmaceutics*, *475*(1), 335–343. http://doi.org/10.1016/j.ijpharm.2014.08.044

Mahdi, M. H., Diryak, R., Kontogiorgos, V., Morris, G. A., & Smith, A. M. (2016). In situ rheological measurements of the external gelation of alginate. *Food Hydrocolloids*, *55*, 77–80. http://doi.org/10.1016/j.foodhyd.2015.11.002

Marques, M. R. C., Loebenberg, R., & Almukainzi, M. (2011). Simulated biological fluids with possible application in dissolution testing. *Dissolution Technologies*, *18*(3), 15–28. http://doi.org/10.14227/DT180311P15

Miyazaki, S., Kubo, W., & Attwood, D. (2000). Oral sustained delivery of theophylline using in-situ gelation of sodium alginate. *Journal of Controlled Release*, *67*(2–3), 275–280. http://doi.org/10.1016/S0168-3659(00)00214-5

Nasir, F., Iqbal, Z., Khan, A., Ahmad, L., Shah, Y., Khan, A. Z., … Khan, S. (2011). Simultaneous determination of timolol maleate, rosuvastatin calcium and diclofenac sodium in pharmaceuticals and physiological fluids using HPLC-UV. *Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences*, *879*(30), 3434–3443. http://doi.org/10.1016/j.jchromb.2011.09.021

Pandit, J., Bharathi, D., Srinatha, A., Ridhurkar, D., Singh, S., & Singh, S. (2007). Long acting ophthalmic formulation of indomethacin: Evaluation of alginate gel systems. *Indian Journal of Pharmaceutical Sciences*, *69*(1), 37. http://doi.org/10.3934/dcdss.2010.3.61

Pawar, S. N., & Edgar, K. J. (2012). Alginate derivatization: A review of chemistry, properties and applications. *Biomaterials*. http://doi.org/10.1016/j.biomaterials.2012.01.007

Rajinikanth, P. S., & Mishra, B. (2008). Floating in situ gelling system for stomach site-specific delivery of clarithromycin to eradicate H. pylori. *Journal of Controlled Release*, *125*(1), 33–41. http://doi.org/10.1016/j.jconrel.2007.07.011

Rasel, M. A. T., & Hasan, M. (2012). Formulation and evaluation of floating alginate beads of diclofenac sodium. *Dhaka University Journal of Pharmaceutical Sciences*, *11*(1), 29–35. http://doi.org/10.3329/dujps.v11i1.12484

Rozier, A., Mazuel, C., Grove, J., & Plazonnet, B. (1989). Gelrite®: A novel, ion-activated, in-situ gelling polymer for ophthalmic vehicles. Effect on bioavailability of timolol. *International Journal of Pharmaceutics*, *57*(2), 163–168. http://doi.org/10.1016/0378-5173(89)90305-0

Rupenthal, I. D., Green, C. R., & Alany, R. G. (2011). Comparison of ion-activated in situ gelling systems for ocular drug delivery. Part 1: Physicochemical characterisation and in vitro release. *International Journal of Pharmaceutics*, *411*(1–2), 69–77. http://doi.org/10.1016/j.ijpharm.2011.03.042

Somani, R. H., Yang, L., Hsiao, B. S., Agarwal, P. K., Fruitwala, H. A., & Tsou, A. H. (2002). Shear-induced precursor structures in isotactic polypropylene melt by in-situ rheo-SAXS and rheo-WAXD studies. *Macromolecules*, *35*(24), 9096–9104. http://doi.org/10.1021/ma020785o

Srividya, B., Cardoza, R. M., & Amin, P. . (2001). Sustained ophthalmic delivery of ofloxacin from a pH triggered in situ gelling system. *Journal of Controlled Release*, *73*(2–3), 205–211. http://doi.org/10.1016/S0168-3659(01)00279-6

512 Stanway, R., Sproston, J. L., & El-Wahed, A. K. (1996). Applications of electro-rheological
513 fluids in vibration control: A survey. *Smart Materials and Structures*.
514 http://doi.org/10.1088/0964-1726/5/4/011

515 Wu, Y., & Fassihi, R. (2005). Stability of metronidazole, tetracycline HCl and famotidine
516 alone and in combination. *International Journal of Pharmaceutics*, *290*(1–2), 1–13.
517 http://doi.org/10.1016/j.ijpharm.2004.10.015

518