The influence of non-ionisable excipients on precipitation parameters measured using the CheqSol method.

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Abstract

Objectives: The aim of this study was to determine the influence of non-ionisable excipients hydroxypropyl-β-cyclodextrin (HPβCD) and poloxamers 407 and 188 on the supersaturation and precipitation kinetics of ibuprofen, gliclazide, propranolol and atenolol induced through solution pH shifts using the CheqSol method.

Methods: The drug's kinetic and intrinsic aqueous solubility was measured in the presence of increasing excipient concentrations using the CheqSol solubility method. Experimental data for example rate of change of pH with time was also examined to determine excipient induced parachute effects and influence on precipitation rates.

Key Findings: The measured kinetic and intrinsic solubilities provide a determination of the influence of each excipient on supersaturation index and the area under the CheqSol curve can measure the parachute capability of excipients. The excipients influence on precipitation kinetics can be measured with novel parameters for example the precipitation pH or percentage ionized drug at the precipitation point which provide further information on the excipient induced changes in precipitation performance.

Conclusion: This method can therefore be employed to measure the influence of non-ionisable excipients on the kinetic solubility behavior of supersaturated solutions of ionisable drugs and to provide data, which discriminates between excipient systems during precipitation.

Introduction

Solubility

A drug's aqueous solubility is a key parameter controlling its pharmaceutical development [38], formulation [32] and biopharmaceutical properties [28]. For oral administration the effect of solubility on bioavailability has been encapsulated in the biopharmaceutics classification system [1] where it is recognized that drugs with a low solubility with respect to dose will potentially have a limited bioavailability [18]. For parenteral therapy, the issue of precipitation after injection of a poorly soluble drug [47] inducing phlebitis [23] is a recognized problem. The current trend is towards the generation of molecules with increasing lipophilicity and consequently decreasing aqueous solubility [17], which necessitates enhanced solubility formulations for parenteral [39] and oral [32] administration using a variety of techniques [43]. The experimental measurement of drug solubility and solution physical stability is therefore an important activity during development and three key solubility measures are recognized; the intrinsic, equilibrium and kinetic solubilities.

Measures of Solubility

Intrinsic solubility is a measure of the neutral (non-ionised) molecule's maximum solubility [46] in solution, which for ionizable molecules requires suppression of ionization by the use of an appropriate pH. Equilibrium or thermodynamic solubility includes solubility of both un-ionised and ionised species using a defined aqueous system (pH and presence of other salts) and employing the most stable solid form in the solution. Either value can be measured using classical shake-flask methods [13] where an excess of solid drug is mixed with a buffered solution phase until equilibrium between the solid and solubilized drug is achieved. Kinetic (or apparent) solubility is a measure of the maximum solubility achieved by creating supersaturated aqueous solutions with concentrations greater than the equilibrium solubility (see [7] for definition of supersaturation) either by dissolution of a high energy solid form [16], dilution of high concentration water miscible solvent solutions [44] or via aqueous solution pH shifts [5] (see Figure 1a), which alter the ionization ratio and therefore equilibrium solubility. Since these supersaturated systems exceed the equilibrium solubility they are thermodynamically unstable, have to be created dynamically and have a tendency to precipitate to the equilibrium solubility value with time [16].

Investigation of Supersaturation

Assisted solubility formulations therefore present drug, irrespective of route of administration, in a supersaturated state [47; 21; 7] with respect to the biopharmaceutical environment in an attempt to increase delivery and or absorption. However, this can result in precipitation upon administration and the bioavailability or toxicity problems detailed above. Multiple experimental methods have been introduced to evaluate the precipitation performance of supersaturated systems [10] for intravenous therapy [27] or solid oral dosage forms [41]. For the oral route, drug is administered

generally as a solid form, which requires dissolution prior to absorption and therefore the initial creation of the supersaturated solution occurs during the dissolution process. This has led to the proposal of the "spring" and "parachute" hypothesis where the drug's physical form or presence of formulation excipients springs the drug into supersaturated solution with subsequent precipitation inhibited (parachute) by the formulation excipients [16]. The spring effect or supersaturation can be quantified by determining the maximum supersaturation index achieved [7], which measures the apparent solubility increase over equilibrium solubility. The parachute effect can be determined using the recently proposed excipient gain factor [3], which measures the increased area under the solubility time curve due to the excipients inhibiting precipitation to the equilibrium solubility value. Several studies have applied this model to screen excipients for solubility improvements, using solvent induced [41], solvent evaporation [11] or solid form [16] techniques to create the initial supersaturated solution.

One alternative method of inducing supersaturation is to shift the pH of an already saturated solution of an ionisable drug to increase the percentage of non-ionised species present, which reduces the system's equilibrium solubility and creates supersaturation [46]. This property is utilized in the chasing equilibrium solubility or CheqSol approach [40] which relies on pH changes produced by the addition of acid or base, to change the ratio of non-ionised to ionised drug present and to alternate between supersaturated and subsaturated conditions permitting the determination of both the kinetic and intrinsic solubility (see Figure 1a). This technique, which was initially aimed at early drug development and candidate screening studies [5], should however be applicable to situations where determination of solubility is required such as parenteral formulation development [35] or crystallization [22]. Recently we have employed this technique to determine solubility increases associated with the solubility enhancing excipients hydroxypropyl-β-cyclodextrin (HPβCD) and the poloxamers 407 and 188 [14]. Since these excipients are non-ionisable the CheqSol method was able to measure their influence on the solubility of test ionisable drugs, determining excipient concentration dependent increases in both intrinsic and kinetic solubility values. For the intrinsic solubility these measurements were comparable to previously published traditional shake flask methods [15]. However during the conduct of this study it was noted other experimental parameters were changing [14] and indicated that since the CheqSol method was dynamic [5], it may also be determining spring and parachute type behavior and the precipitation kinetics. In this paper we have employed the CheqSol method to examine the influence of the non-ionising excipients hydroxypropyl-β-cyclodextrin and the poloxamers 407 and 188 on the precipitation behavior of four test drugs; the acids ibuprofen and gliclazide and the bases atenolol and propranolol. Results indicate that the method can measure parachute behavior and provide novel insight into an excipients ability to maintain supersaturation and influence precipitation behaviour.

Materials and Methods

Materials

Propranolol HCl (base pKa 9.54), atenolol (base pKa 9.48), ibuprofen (acid pKa 4.35), gliclazide (acid pKa 5.54) and poloxamers 407 and 188 were obtained from Sigma-Aldrich Company Ltd (Dorset, UK). HP-β-CD (Cavitron 82004) was obtained from Cargill (Cedar Rapids, Iowa, USA). 0.5M Potassium hydroxide (KOH) (Fisher Scientific, Loughborough, Leicestershire, UK) was prepared from 1 mole vial of KOH diluted to 2 L with HPLC grade water. 0.5M Hydrochloric acid (HCl) (Fisher Scientific, Loughborough, Leicestershire, UK) was used as purchased. HPLC grade water was obtained using an Elga UHQ2 system (Marlow, Buckinghamshire, UK).

Methods

Experiments were carried out using the Sirius T3 titrator (Sirius Analytical Instruments Ltd, East Sussex, UK), in ionic strength adjusted (ISA) water containing 0.15M potassium chloride, obtained from VWR (Leuven, Belgium), in HPLC grade water. Experiments were conducted at a temperature of 25^{o}C ± 0.5^{o}C under an argon atmosphere. The apparatus was connected to and run from Sirius software on a Dell desktop. The T3 set up includes a Ag/AgCl double junction reference pH electrode, a Peltier temperature control device, with thermocouple temperature probe, an overhead stirrer (variable speed, computer controlled). The spectrophotometer was a MMS UV–VIS Carl Zeiss Microimaging spectrophotometer with an ultra-mini immersion probe attached (Welwyn Garden City, Hertfordshire, UK.).

Potentiometric determination of solubility

Solubility determination was carried out using the CheqSol assay [40]. The drug was weighed into a vial and HPβCD or poloxamer was added if required. The mass of drug used varied depending on the expected solubility. ISA water volumes of 1.5 mL (propranolol HCl, gliclazide and ibuprofen) or 1 mL (atenolol) were added before the pH was adjusted to solubilise the drug at a pH where ionisation was expected. After dissolution of the sample was complete detected by pH changes, the sample was titrated from ionisation to non-ionisation through addition of acid or base until precipitation of the non-ionised form was detected. The concentration at precipitation, determined using the principles of mass and charge balance, is equivalent to the kinetic solubility. After this point more titrant was added to increase precipitation. Thereafter, as the solubility re-adjusts towards the equilibrium solubility, alternating aliquots of acid and base were added to induce subsaturation and supersaturation. This would be continued until twenty crossing had been titrated. A crossing point is where the pH change versus time ($\Delta pH/\Delta t$) is zero and the system would be at equilibrium [4]. The concentration at each crossing point is calculated using a series of mass and charge balance equations, [40], intrinsic solubility is the mean of the crossing point concentrations. Crossover plots display the concentration of unionised drug during subsaturation and supersaturation and each crossing point shows the intrinsic solubility. The

solubility was determined for the drugs on their own and in the presence of HPβCD at molar ratios of 1:0.1, 1:0.5, 1:1, 1:1.5 and 1:2 (for exact detail see results) and poloxamer surfactants 407 and 188 at concentrations of 0.5, 1.0, 2.0. 5.0 and for poloxamer 188 additionally at 10 % w/v. The kinetic information associated with each determination, equilibrium chasing cross over plot, precipitation rate graph and neutral species concentration profile were extracted.

Determination of the intrinsic and kinetic solubilities of a drug solubiliser system permits calculation of the supersaturation index (Equation 1) [34], which maybe calculated using the intrinsic solubility in the absence or presence of the excipient. Note that due to the dynamic nature of the CheqSol measurement technique the determination is not thermodynamically competent since the kinetic and intrinsic solubilities are not measured under identical conditions [40]. Nevertheless, useful trends in supersaturation behaviour can be observed.

Equation 1. $$\text{Supersaturation Index} = \frac{\text{Kinetic Solubility-Intrinsic Solubility}}{\text{Intrinsic Solubility}}$$

The area under the solubility time curve of the CheqSol measurement was calculated using a trapezoidal rule on the determined neutral species concentration with time (s) (see Figure 1a). CheqSol experiments have durations of around 5,000 s with the majority of time related to the determination of equilibrium solubility. To standardise results and provide a balance of supersaturation and equilibrium contributions areas under the curve out to 2,500 s were calculated. Excipient gain factors were determined (Equation 2).

Equation 2. $$\text{Excipient Gain Factor} = \frac{\text{Drug+ Excipient AUC}}{\text{Drug AUC}}$$

Statistical Analysis of Results

Values were compared using a one-way non-parametric Kruskal-Wallis test followed by a Dunn's multiple comparison test against the value for the drug alone using Prism 6 for Mac OSX.

Results

Supersaturation Index

The calculated supersaturation index values based on the measured kinetic and intrinsic solubility for all drug excipient combinations tested are presented in Table 1. For HPβCD the supersaturation index based on aqueous intrinsic solubility (see Table legend) increases for ibuprofen, gliclazide and propranolol as the HPβCD molar ratio or concentration increases, and is statistically significant at the higher excipient concentrations/ratios tested. For atenolol there is no significant change with HPβCD concentration increases. A similar pattern is observed for both poloxamers. If the supersaturation index is calculated using each solution's measured intrinsic solubility value (instead of intrinsic solubility in the absence of excipient), a different pattern emerges. In most cases the supersaturation index value remains constant but in some cases the value is significantly lower than the value for drug alone, for example gliclazide with poloxamer 188, and HPβCD with ibuprofen and propranolol.

Excipient Gain

The CheqSol method relies on measuring solution pH changes induced by the addition of acid or base to determine solubility [40] starting with drug in solution and then shifting pH to create a supersaturation which precipitates and is then followed by a chasing phase measuring rates of change of pH with time (dpH/dt). Typical results for propranolol are presented in Figure 1, with full experimental traces in the inset graph and the main graph expanded to cover the initial supersaturation and equilibrium phases. Calculated area under the curve (AUC) and excipient gain factors are presented in Figures 2a and b and whilst not identical to the solubility based supersaturation index results presented in Table 1 the values follow the same trends. Ibuprofen (Figure 2a) exhibits the greatest area increases and therefore excipient gains followed by propranolol and gliclazide, atenolol does not register an excipient gain greater than 2 with any of the excipients.

Precipitation Parameters

During the chasing portion of the CheqSol analysis acid and base additions are cycled to force the system to dissolve or precipitate allowing determination of the intrinsic solubility. During this phase the rate of change of pH with respect to time (dpH/dt) is measured and sample results for propranolol are presented in Figure 3 for the drug on its own and in the presence of selected concentrations of each of the three excipients employed. It is obvious that the presence of the excipients increases the measured solubility by shifting the equilibrium chasing point to higher concentrations [14]. Based on the dpH/dt data the CheqSol method determines precipitation parameters (precipitation concentration i.e., x-axis crossing point (μM), precipitation rate (μM/min) and precipitation pH) for each experiment and this data is collectively presented in Figure 4a and b. HPβCD significantly increases the precipitation concentration of ibuprofen, gliclazide and

propranolol (Figure 4a, first panel, middle graph) in a concentration dependent manner, whilst the results for atenolol are highly variable (Figure 4b) with a trend of increasing precipitation concentration as HPβCD concentration increases. For all drugs and both poloxamers a statistically significant increase in precipitation concentration is evident as excipient concentration increases (Figures 4a and b, second and third panels, middle graphs), which is comparable to the effects of HPβCD. This change is associated with a significant decrease in the HPβCD precipitation pH (Figure 4a first panel, lower graph) for the acidic drugs (ibuprofen and gliclazide) or increase for the basic drug (propranolol). The effect of poloxamers on precipitation pH is complex with a significant reduction in pH for atenolol and some propranolol ratios whilst for ibuprofen and gliclazide there is an increase at low poloxamer concentrations followed by a general increase in precipitation pH.

HPβCD significantly reduces the precipitation rate of ibuprofen, gliclazide and propranolol in a concentration dependent manner (Figure 4a first panel, top graph) whilst the effect of poloxamers is complex with atenolol exhibiting no change and for ibuprofen and propranolol (Figure 4a second and third panels, top graph) an increase at low excipient concentrations followed by a minimum then further increases to values significantly greater than those of the drug alone. For gliclazide the precipitation rate increases at low poloxamer concentrations and then reduces as the excipient concentration is increased.

Excipient Characteristics

Further data comparisons are possible, since the CheqSol mass balance calculation also provides the percentage of drug ionization at the cross over point and Figure 5 presents the percentage ionization plotted against the precipitation rate for each drug. This reinforces the differences between the excipients presented above with HPβCD decreasing the precipitation rate and percentage ionization required for precipitation for propranolol, gliclazide, and ibuprofen, whilst the poloxamers exhibit a different profile for each drug. The latter result indicates differential interactions between the drugs and poloxamers that would not be detected using the excipient gain analysis.

Discussion

The majority of published studies on HPβCD [29; 15] or poloxamer solubilisation [25] involve equilibrium methods, which determine intrinsic/equilibrium solubility not supersaturation and indicate that HPβCD and poloxamer excipients have the ability to enhance the intrinsic solubility of the drugs tested. This study indicates that the excipients can also provide supersaturated solutions of ibuprofen, gliclazide and propranolol during pH induced intrinsic solubility changes with the measured supersaturation index excipient concentration dependent. Atenolol is the exception with no appreciable change, possibly because this drug has the highest aqueous solubility [14] of

all the drugs tested which provided a practical experimental limit based around drug solubility, excipient concentration, reaction volume and acid/base strengths. Recent studies involving HPβCD and non-ionic surfactants [41; 6; 7] also illustrate the ability of these excipients to maintain supersaturated systems created using solvent based methods. The supersaturation values reported in this study are similar, however the extent of supersaturation reported in the cited studies is large and ranges from zero to greater than one thousand, and only a single excipient concentration is tested limiting the applicability of the comparison. Supersaturation values reported for a dypridamole/Cavasol™ combination using the CheqSol technique [4] ranged from twenty to nine again indicating comparability with this study. It has been reported for a topical formulation system consisting of ibuprofen and propylene glycol [21] that increasing the excipient concentration increases the intrinsic solubility and degree of supersaturation with comparable supersaturation values to those reported in this study. Finally the supersaturation value measured within a solution using the solution's intrinsic solubility significantly decreases in some systems as the excipient concentration increases. This decrease is also present for the ibuprofen/propylene glycol and dypridamole/Cavasol systems as the excipient concentration increases and indicates an interesting limitation with respect to drug, excipient ratio and potential supersaturation achievable as intrinsic solubility increases.

It is obvious that the excipients increase the pH induced supersaturation (higher peak neutral species concentration) and equilibrium solubility but changes to the experimental curve's shape are also evident. These changes are similar to those presented for "spring and parachute" type systems [16] and therefore amenable to a comparative area under the curve (AUC) analysis to determine excipient gain factors. Due to the nature of the CheqSol method AUC results incorporate supersaturation and equilibrium solubility with excipient free experiments providing the drug's supersaturation factor [3]. In addition the initial pH shift is employed as the "spring" to create supersaturation not addition of a water miscible solvent solution or high solubility amorphous form. All three excipients are known to assist supersaturation and Guzman et.al., [16] reports that poloxamers can inhibit precipitation of celecoxib for over 60 minutes. Hsieh et.al., [20] have reported on the precipitation behaviour of weak bases and reported similar curves to this study but did not examine the influence of excipients. The results indicate that for ibuprofen, gliclazide and propranolol, HPβCD and poloxamer 407 and 188 can act as parachutes increasing the measured AUC in a concentration and drug excipient combination dependent manner. For atenolol there is very little relative change in area indicating that none of the excipients are influencing behaviour in this system.

The shift of pH in the presence of excipients has been previously employed in potentiometric studies of cyclodextrin inclusion complexes [9] to determine binding constants and to study the interaction of drugs with surfactants [33; 12] and lipid phases [8] to determine partition coefficients.

Changes in the measured slope of the dpH/dt line indicate an excipient effect on the assay's kinetic behaviour, which is to be expected since both excipient types are known to influence the solution behaviour and solubility of small molecules [24; 30; 14]. Although precipitation and determination of precipitation rate is an integral feature of the dynamic nature of CheqSol [40], which has been employed to determine the solubility of multiple drugs [36] and the influence of polymers on precipitation [4; 20; 14; 19], no data on the measured precipitation rates have been presented. The previously mentioned potentiometric studies [33; 9; 8; 12] utilized systems at equilibrium and precipitation rates were not determined. This study therefore is the first examination of the influence of excipients on precipitation rate and the results indicate interesting differences in behaviour between HPβCD and the poloxamers with the former's effect due most likely to its formation of an inclusion complex whilst for the latter micellar solubilisation provides a reservoir that can easily precipitate once solution conditions allow.

The precipitation from supersaturated pharmaceutical system has been extensively investigated [10; 45] however, literature comparisons with this study are limited for a range of reasons. The majority of methods create a "standard" meta-stable supersaturated system, then examine the influence of excipients on time taken for a precipitate to form "naturally" [3] and report results based on an excipient induced precipitation time delay when compared to a free drug system (for example [16]). CheqSol continuously changes pH in the required direction until precipitation is induced [40] to determine kinetic solubility and then cycles pH to determine intrinsic solubility. In addition most studies report the influence of a single excipient concentration [16; 6] and not the influence of a concentration range. However, despite these differences it is obvious that the method is capturing and providing quantitative information on the excipients influence on precipitation behaviour and providing data, which discriminates between HPβCD and the poloxamers. Both excipients for example provide an excipient gain (Figure 2), which has been reported in the literature but with very different effects on precipitation rates (Figure 4) and percentage drug ionization (Figure 5) at precipitation, which has not been previously reported. This data may be useful to determine a drug excipient combination's applicability for a particular setting, for example the slower precipitation rate of HPβCD might be better suited to conditions where rapid changes inducing precipitation is expected and the poloxamer to situations where a more stable environment can support supersaturation.

The theoretical equations describing the interactions between weak acids or bases with surfactants or cyclodextrins were published in the 1960's [33; 9] and several studies have employed these to determine binding constants [37]. However, these have been conducted under equilibrium conditions usually over a range of pH values covering at least 2 pH units either side of the pKa and not always in the presence of solid drug. The CheqSol experiment dynamically explores this system with a limited pH range (see Figure 4 lower graphs), which does not permit determination

of the binding constants. In addition the presence of solid drug, excipient and dynamic precipitation and dissolution events present a complex system. It is known for example that excipients in solution can interfere with precipitation and crystal growth [42; 19] and that buffer physicochemistry can influence cyclodextrin complexation [31]. Analysis to provide rate constants from the results is therefore not sensibly feasible, however modification of the analysis to for example cover a wider pH range, coupled with control experiments which do not involve precipitation [14] could permit a full kinetic analysis.

The investigation of precipitation from supersaturated systems is to determine and understand behaviour that can be related to biopharmaceutical performance, which for pH and parenteral administration is a shift to pH 7.4 [27] and for oral from stomach acid to the higher intestinal pH [26]. Since CheqSol utilizes pH induced supersaturation the direction of pH change is automatically appropriate for weak bases for intestinal transfer but not for weak acids. In the former case the propranolol experiments were conducted around pH 8-9 and atenolol at pH 10-11, which do not represent typical intestinal pH [2] values and therefore not biorelevant, an argument which can also be applied to the parenteral situation for both acids and bases. Nevertheless, results are determined for precipitation of the unionised species and total solubilities could be calculated for other pH values. The atenolol results also highlighted an experimental limitation with respect to the balance of intrinsic drug solubility, pKa, instrument titration volume (1-5mL) and acid/base titration strengths. Modification of the experimental parameters or utilization of a different experimental platform could alleviate some of these issues.

Conclusion

The results presented in this paper indicate that for non-ionisable excipients and ionisable drugs the CheqSol method can be employed to determine the influence of excipients on supersaturation and precipitation. Determination of parachute and excipient gain properties are possible with results that are broadly comparable to related published methods but which provide a degree of quantitative measurement. The measured precipitation rates provide the ability to discriminate between excipients, which both exhibit parachute type activity but produce this through different mechanisms. Modification of the method to permit a more comprehensive kinetic analysis, expand drug applicability and increase biorelevance is also possible. Overall the method represents a useful orthogonal approach to assessing precipitation, provides expanded excipient performance information and could be employed as a quantitative and qualitative excipient screening system.

Table 1

Title: Supersaturation index calculated from solubility analysis results in the presence of non-ionisable excipients.

Legend: Supersaturation index calculated using Equation 1 and the measured kinetic and instrinsic solubility values. Solution Intrinsic column calculated using the measured intrinsic solubility value for each system. Aqueous Intrinsic column calculated using the measured intrinsic solubility for the aqueous system in the absence of excipients. Mean ± standard deviation, n as stated in Table, * Statistically significant difference $P < 0.05$ compared to control result with no excipient.

Figure 1.

Title: CheqSol Solubility Determination.

Figure 1a.

Title: Schematic of CheqSol solubility determination.

Legend: Figure 1a. Progress of CheqSol solubility determination indicating measurement of kinetic and instrinsic solubilities and area under the curve.

Figure 1b.

Title: Propranolol CheqSol determination in the absence and presence of selected concentrations of non-ionisable excipients.

Legend: Figure 1. Propranolol: ○ Free drug; □ Hydroxypropyl-β-cyclodextrin 1:1 drug:cyclodextrin molar ratio; △ poloxamer 407 2% w/v; ◇ poloxamer 188 2% w/v. Inset graph, identical symbols, complete experimental data presented.

Figure 2a and b

Title: Effect of non-ionisable excipient concentrations on CheqSol curve areas and calculated excipient gain factors of Propranolol, Ibuprofen, Gliclazide and Atenolol.

Legend: Figure 2a: ∆ Propranolol; o Ibuprofen; □ Gliclazide, mean ± standard deviation (n = see Table 1), * statistically significant difference from drug alone $P < 0.05$.

Figure 2b: o Atenolol, mean ± standard deviation (n = see Table 1), * statistically significant difference from drug alone $P < 0.05$.

Figure 3

Title: Measured dpH/dt values for propranolol during CheqSol determination in the absence and presence of selected concentrations of non-ionisable excipients.

Legend: Figure 3. Propranolol: ■ Free drug; ▼ Hydroxypropyl-β-cyclodextrin 1:0.2 drug:cyclodextrin molar ratio; ● poloxamer 407 5% w/v; ◆ poloxamer 188 10% w/v. Closed symbols measured dpH/dt values, open symbols determined crossing points.

Figure 4a and b

Title: Effect of non-ionisable excipient concentrations on CheqSol precipitation parameters of propranolol, ibuprofen, gliclazide and atenolol.

Legend: Figure 4a: ∆ Propranolol; o Ibuprofen; □ Gliclazide, mean ± standard deviation (n = see Table 1), * significantly different from drug alone $P < 0.05$. Figure 4b: Atenolol, mean ± standard deviation (n = see Table 1), * significantly different from drug alone $P < 0.05$.

Figure 5.

Title: Effect of Excipient Concentration on Precipitation Rate and Percentage Ionisation.

Legend: Figure 5: ● Excipient Free Drug; o Hydroxypropyl-β-cyclodextrin; ∆ Poloxamer 407; □ Poloxamer 188. Plot symbol size represents excipient concentration, values indicate concentration in mM.

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Figure 1

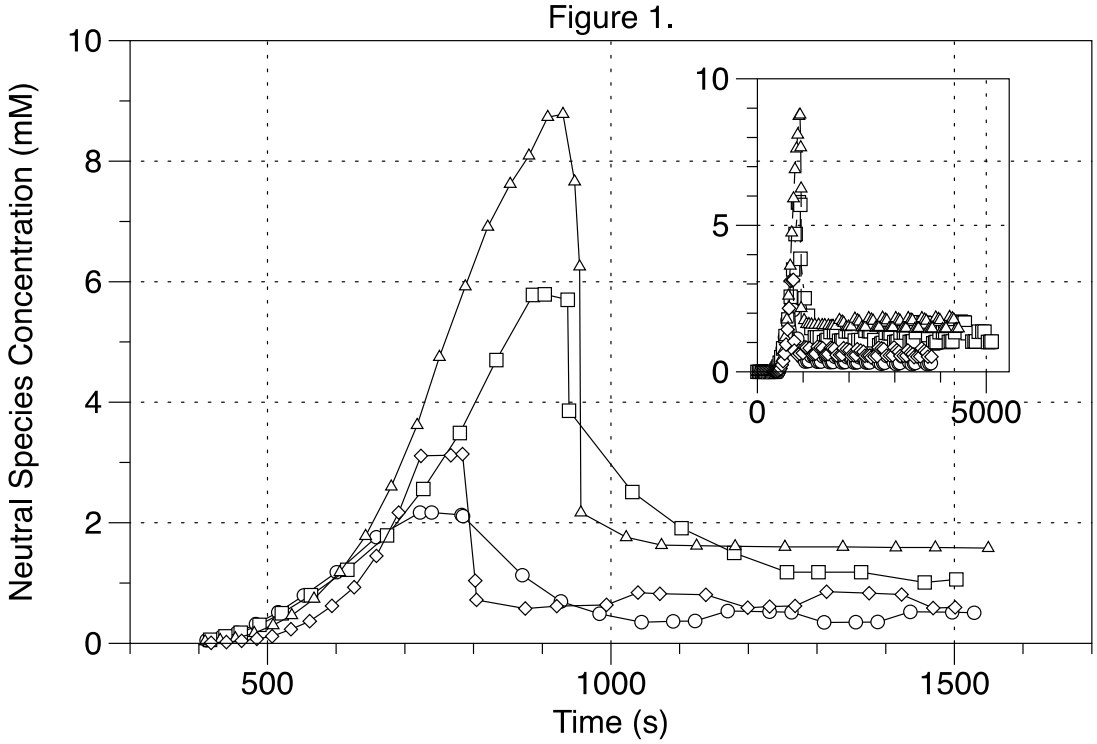


Figure 2a.

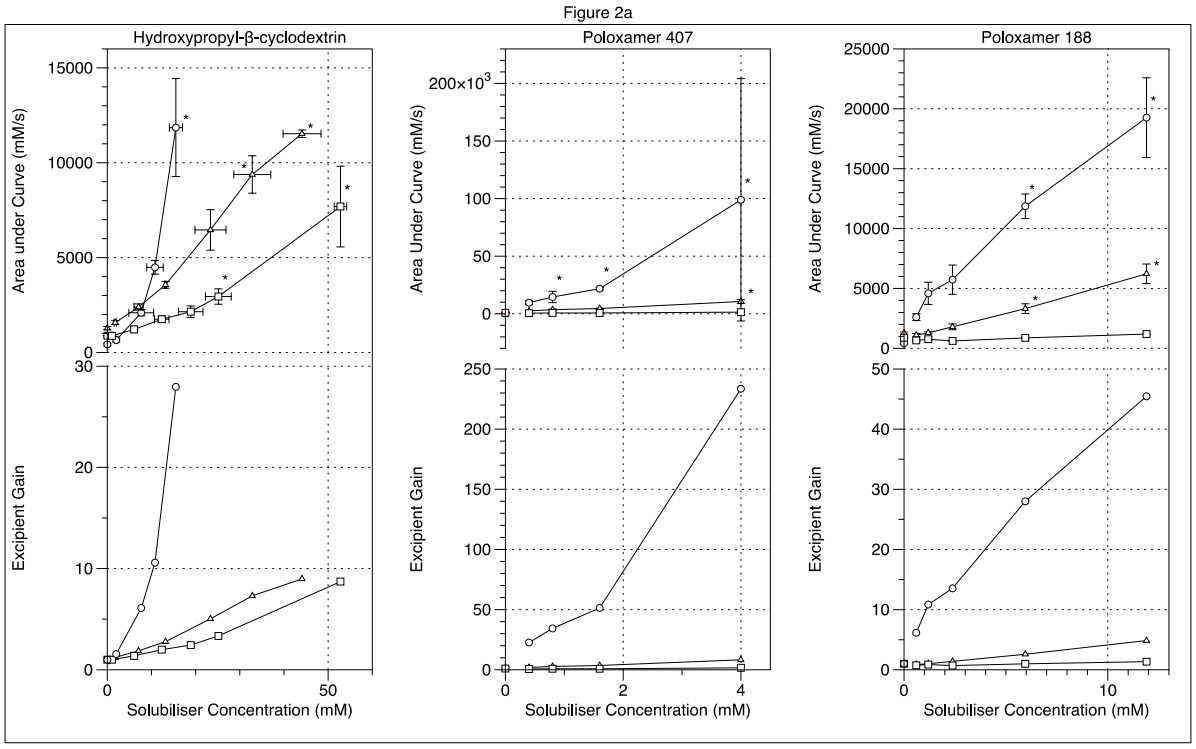


Figure 2b

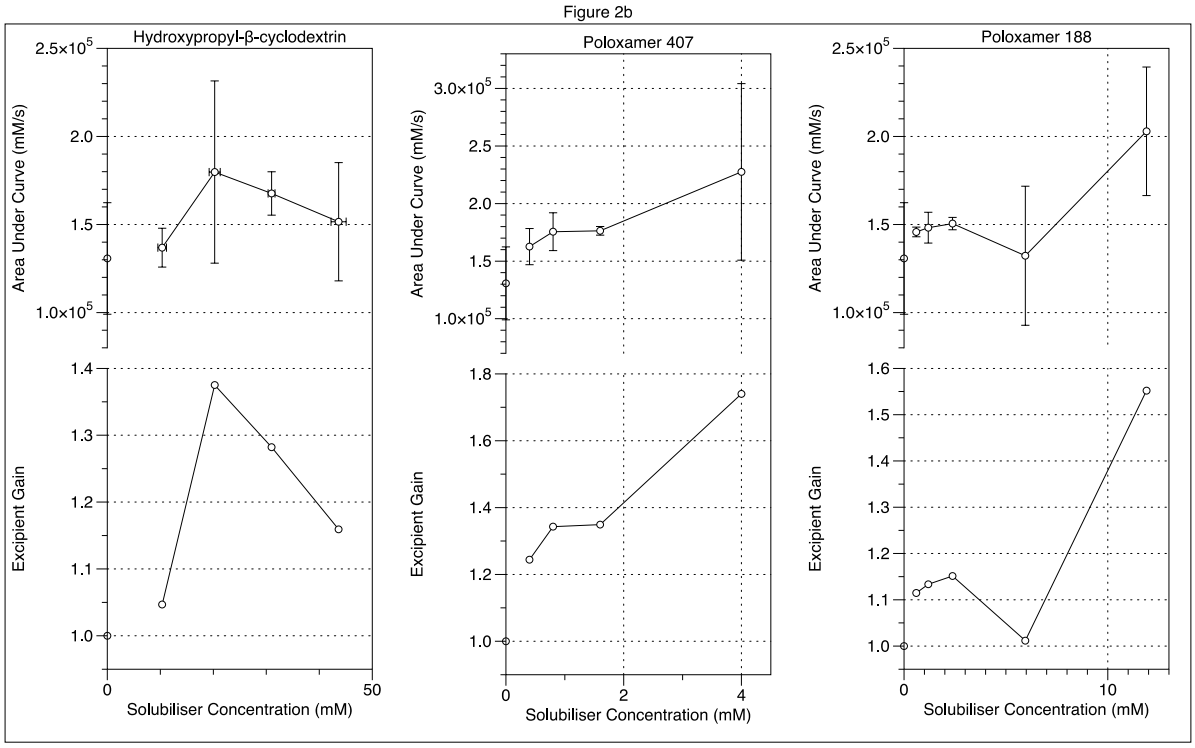


Figure 3

Figure 3. Measured dpH/dt values for propranolol during CheqSol determination in the absence and presence of selected concentrations of non-ionisable excipients.

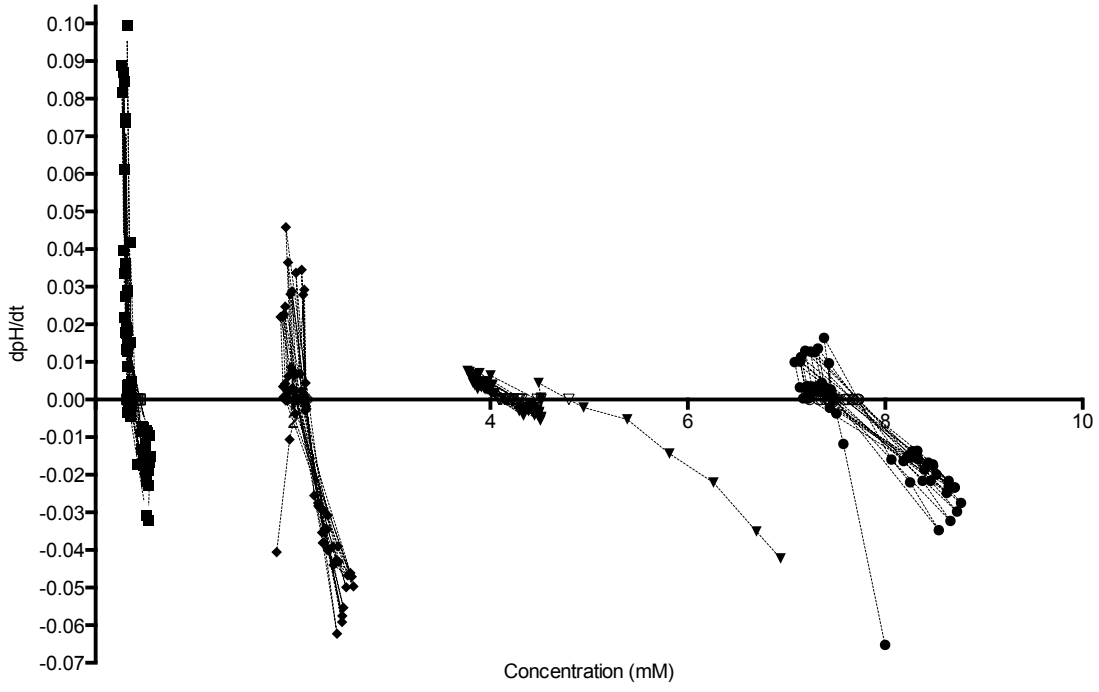


Figure 4a

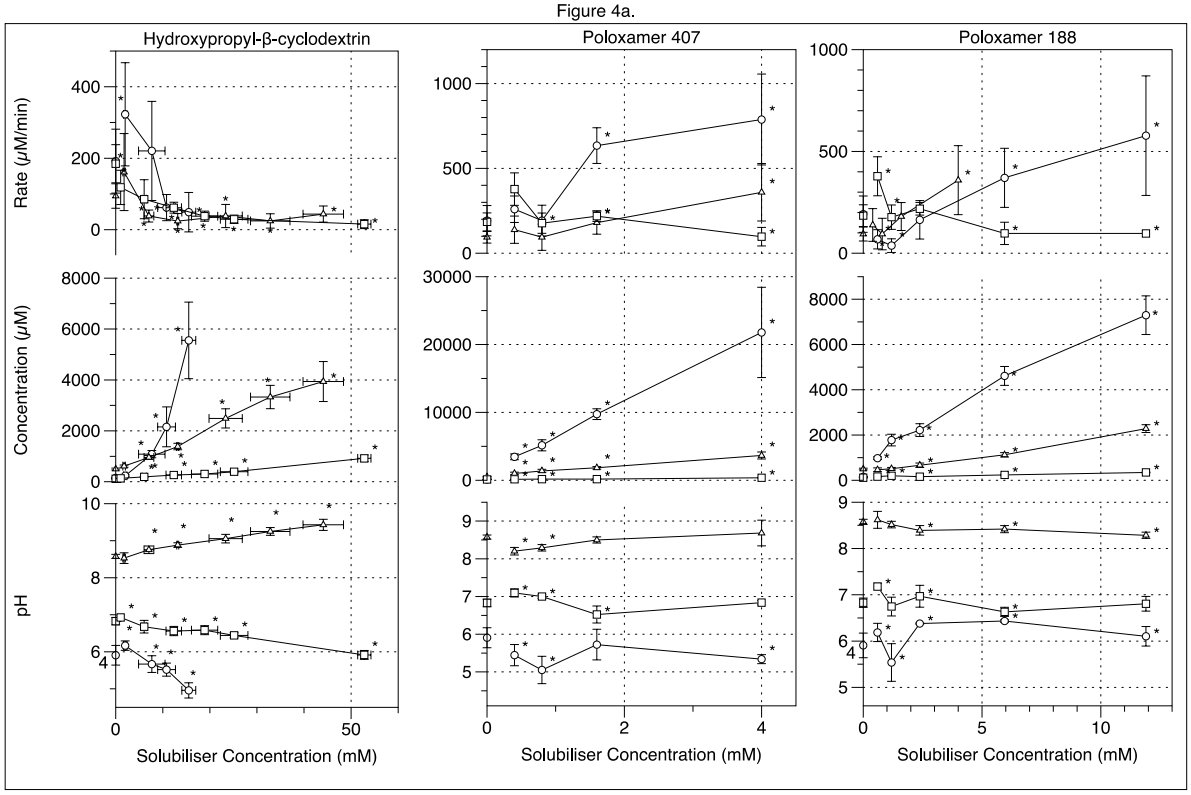


Figure 4b

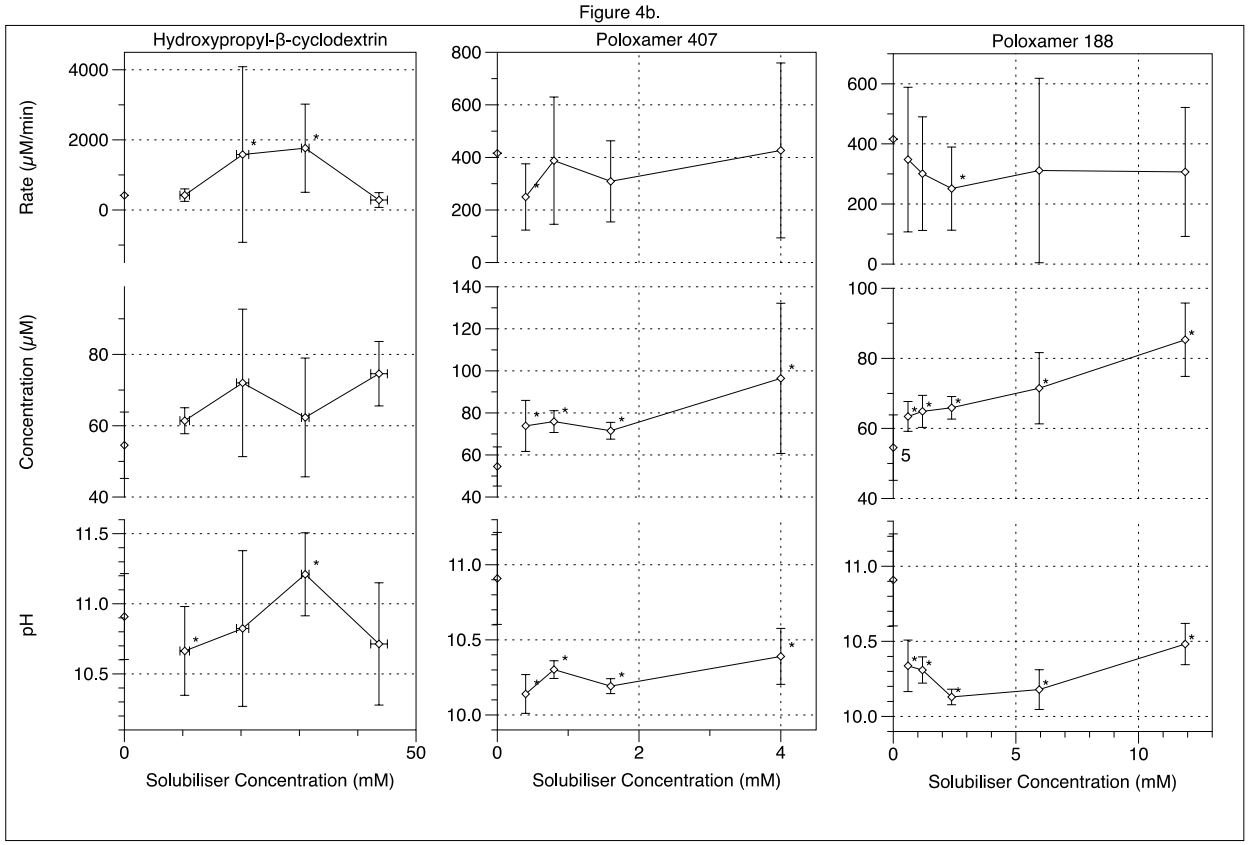
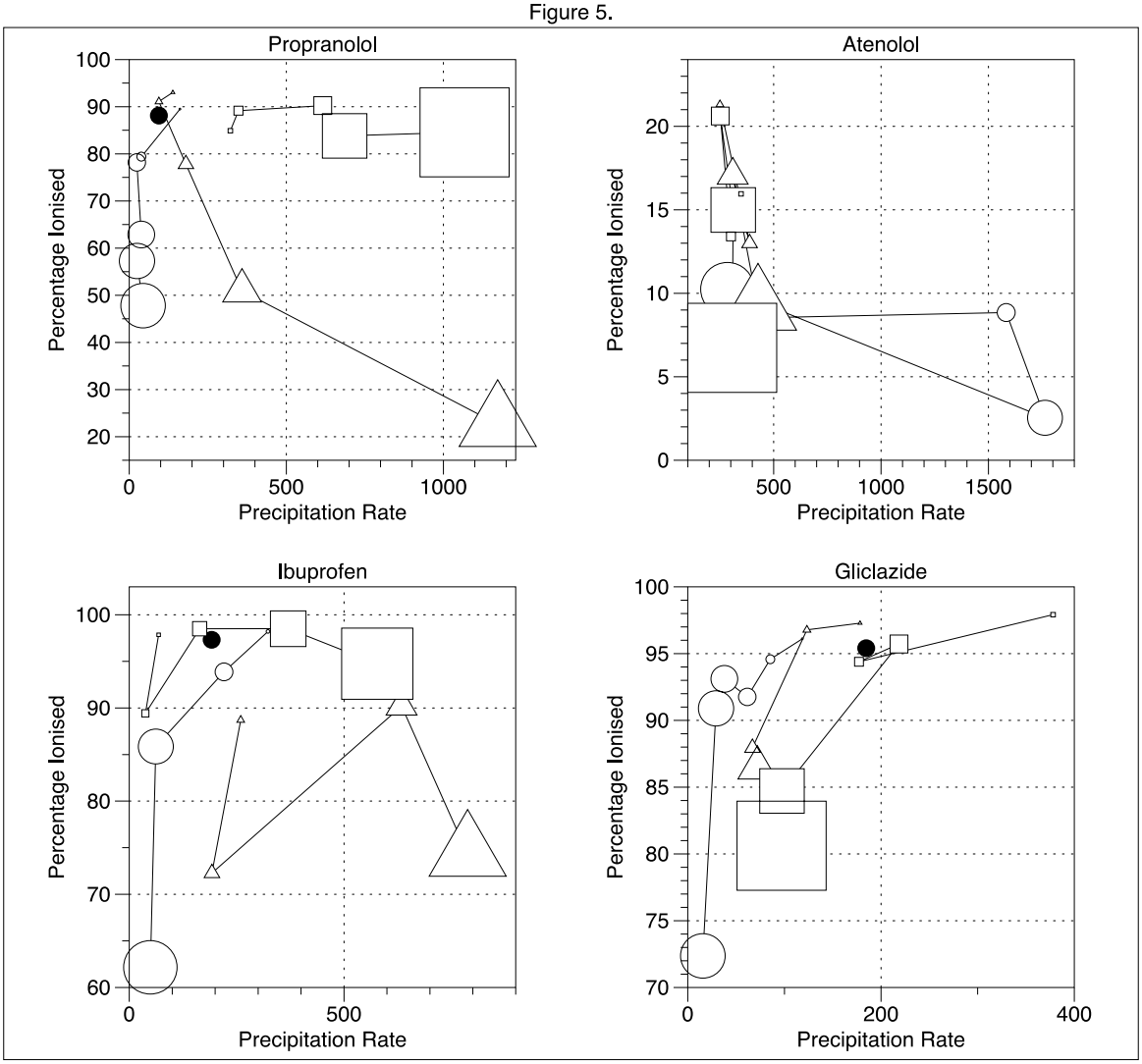


Figure 5

Figure 5.



Table&1.&&Supersaturation&ndex&calculated&from&CheqSol&solubility&analysis&esults&n&he&presence&of&non;ionisable&excipients.&

Hydroxypropyl;β;cyclodextrin&

Ibuprofen&			&	Gliclazide&			&	Atenolol&			&	Propranolol&		
Molar& ratio&	Solution& intrinsic&	Aqueous& intrinsic&	&	Molar& ratio&	Solution& intrinsic&	Aqueous& intrinsic&	&	Molar& ratio&	Solution& intrinsic&	Aqueous& intrinsic&	&	Molar& ratio&	Solution& intrinsic&	Aqueous& intrinsic&
0.00(n=4)&	3.98±0.50&	3.98±0.50&	&	0.00(n=3)&	27.50±5.78&	27.50±5.78&	&	0.00(n=5)&	2.63±2.19&	2.63±2.19&	&	0.00(n=3)&	4.47±0.05&	4.47±0.05&
0.10(n=5)&	3.84±0.69*&	6.02±1.08&	&	0.11(n=3)&	6.73±0.78&	16.22±1.88&	&	0.05(n=3)&	1.36±0.11&	1.69±0.14&	&	0.10(n=3)&	1.16±0.08&	5.78±0.37&
0.50(n=5)&	2.70±0.20&	18.07±1.33&	&	0.50(n=3)&	32.70±1.38&	39.52±1.67&	&	0.10(n=4)&	2.24±0.93&	2.48±1.03&	&	0.50(n=3)&	7.17±0.35&	9.09±0.45&
0.74(n=3)&	2.80±0.18&	33.27±2.14*&	&	0.99(n=3)&	11.38±1.93&	43.32±7.38&	&	0.15(n=4)&	3.32±3.14&	4.40±4.15&	&	0.98(n=3)&	0.87±0.04&	13.22±0.64&
0.99(n=4)&	1.95±0.24*&	68.20±8.58*&	&	1.50(n=4)&	31.41±6.98&	55.54±12.34&	&	0.20(n=3)&	1.06±0.92&	1.35±1.17&	&	1.50(n=4)&	9.89±1.18&	21.73±2.60&
&	&	&	&	2.00(n=5)&	26.95±3.67&	77.20±10.51*&	&	&	&	&	&	1.98(n=4)&	4.32±0.49*&	28.38±3.21*&
&	&	&	&	4.84(n=3)&	15.27±7.77&	128.59±65.46*&	&	&	&	&	&	2.50(n=3)&	0.38±0.37*&	11.54±11.11&

&

Poloxamer&P407&

Concentration& (%&w/v)&	Ibuprofen&		&	Gliclazide&		&	Atenolol&		&	Propranolol&	
&	Solution& intrinsic&	Aqueous& intrinsic&	&	Solution& intrinsic&	Aqueous& intrinsic&	&	Solution& intrinsic&	Aqueous& intrinsic&	&	Solution& intrinsic&	Aqueous& intrinsic&
0&	3.98±0.50& (n=4)&	3.98±0.50&	&	27.50±5.78& (n=3)&	27.50±5.78&	&	2.63±2.19& (n=5)&	2.63±2.19&	&	4.47±0.05& (n=3)&	4.47±0.05&
0.5&	4.33±1.08& (n=3)&	97.89±24.46&	&	14.87±4.2& (n=3)&	18.83±5.32&	&	2.12±0.35& (n=3)&	2.65±0.44&	&	3.08±1.00& (n=3)&	7.49±2.43&
1&	3.95±2.69& (n=3)&	130.36±88.59&	&	19.64±10.49& (n=3)&	29.48±15.74&	&	2.26±0.41& (n=3)&	2.95±0.53&	&	4.11±0.27& (n=3)&	14.48±0.95&
2&	2.40±2.12& (n=3)&	155.47±136.8&	&	20.58±2.13& (n=3)&	32.62±3.38&	&	2.62±0.14& (n=3)&	3.26±0.17&	&	4.01±0.51&	19.97±2.55&
5&	12.24±16.24& (n=3)&	1705.73±2263*&	&	13.90±1.88& (n=3)&	49.51±6.71&	&	2.71±0.73& (n=3)&	3.88±1.04&	&	&	&
10&	&	&	&	&	&	&	&	&	&	3.20±1.14& (n=3)&	75.29±26.87*&

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