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Investigation into Drug Solubilisation Potential of Sulfonated Calix[4] resorcinarenes

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Abstract

Increasing importance is being placed on the role of drug solubilisation in the drug development pipeline. In order for drugs to successfully pass through the pre-clinical studies required before clinical trials they often require addition of excipients or solubility modifying agents. This study highlights the use of sulfonated calix[4]resorcinarenes as drug solubilising agents. The rigid nature of these compounds form cone-like structures with hydrophobic interiors which are capable of accommodation of hydrophobic entities such as drugs. The calix[4]resorcinarenes in this work have varied length of alkyl chains attached to their lower rim. This work investigated the effect of chain length (C = 4 (SC(4)RC4), C = 7 (SC(4)RC7)) on the degree of solubilisation of two model hydrophobic drugs: propofol and griseofulvin. The data showed that the compounds were capable of solubilising both drugs up to 8 $mgmL^{-1}$ (SC(4)RC7-propofol) and 3 $mgmL^{-1}$ (SC(4)RC4-griseofulvin). The size measurements carried out using photon correlation spectroscopy indicated that the SC(4)RC4 was likely to form 1:1 interactions with drug molecules whilst the SC(4)RC7 formed supramolecular structures capable of increased drug loading in the case of propofol. In the case of griseofulvin it is postulated that similar structures were formed however these exceeded the limit of the filter used and may have been lost. Additionally, the supramolecular structures appeared more stable with a reduction in drug release. *In vitro* testing on BxPC-3 cells indicated that the calix[4]resorcinarenes were relatively non-toxic. These studies highlight the potential of these systems in drug delivery.

Keywords: Hydrophobic drug solubilisation; Drug delivery; Calixarene; Resorcinarene

Introduction

Over the past few decades, the development of solubilisers for hydrophobic drugs as drug delivery systems has attracted much attention among researchers in both pharmaceutical and biomedical fields [1,2]. More than 40% of therapeutic agents identified in combinatorial libraries are classified as hydrophobic [3]; in the UK drugs which possess aqueous solubility below 0.1 $mgmL^{-1}$ at 25°C are classed as practically insoluble according to the British Pharmacopoeia [4]. Such drugs which exhibit poor physicochemical properties may be significantly limited in their progress through the drug discovery pipeline. Particularly importantly are those drugs which show promising clinical relevance which may suffer from low bioavailability as a result of their inability to dissolute into aqueous media before further absorption. Formulation strategies are thus required to overcome these issues and improve drug efficacy and efficiency. It is well recognised that commonly used excipients possess undesirable properties in terms of their viscosity, excipient to drug rations required for drug solubilisation and their stability in aqueous media. Hence, novel technologies for drug solubilisation are required which can increase drug solubilisation and overcome the issues of traditional excipients. Calix[n]arenes are one such technology which hold potential as drug solubilisers due to their conformation and ability to form complexes with drug molecules.

Calix[n]arenes have been studied extensively as host molecules in supramolecular chemistry [5]. Derived from phenols, they are cyclic oligomers which can be envisaged as cone-shaped molecules with a hydrophobic interior. Calix[n]arenes themselves are inherently hydrophobic but possess an upper- and lower-rim which may be functionalised readily to modify their properties (Figure 1a). Calix[4] resorcinarenes are related to the calix[n]arenes in that they can also possess a cone-shaped structure with a hydrophobic interior and an upper- and lower rim [6] (Figure 1b). However, since resorcinol will react with a wide range of aldehydes under the conditions required for their synthesis, an extensive library of structurally diverse calix[4]resorcinarenes exists. The calix[4]resorcinarenes have found widespread use in host-guest and supramolecular chemistry, exemplified in the synthesis of cavitands and carceplexes [7,8]. They have been demonstrated to form stable complexes with small molecular species but notably the majority of these studies have been undertaken in non-polar media - which would not be suitable for drug delivery applications.

Of the few examples in the literature, structurally modified calix[n] arenes have been shown to form stable complexes with small molecular species in aqueous media. In particular, there is an established evidence base confirming that water-soluble calix[n]arenes which possess sulfonate groups in the 4-position on the upper rim are capable of solubilising drug molecules which otherwise possess poor solubility in aqueous media [9-21]. However, few investigations into the properties and utility of simple calix[4]resorcinarenes which bear sulfonate groups in the 2-position on the upper rim have been reported.

Kazakova *et al.* first reported the synthesis of three sulfonated calix[4]resorcinarenes and their formation of 1:1 complexes with amino acids in aqueous media [22]. The group later reported a comparative study into the interactions of sulfonated calix[4]resorcinarenes bearing

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alkyl chains of either 1 carbon (methyl group) or 5 carbons (pentyl) on the lower rim with small molecules capable of multiple non-covalent interactions in aqueous media [23]. This study presented evidence that sulfonated calix[4]resorcinarenes are able to aggregate with the hydrophobic pentyl moieties entering the hydrophobic inner core of neighbouring calyxes even in the presence of small molecular guests. More recently, Menon has reported detailed studies into the use of a sulfonated calix(4)resorcinarene derived from acetaldehyde for the solubilisation of pharmaceutical agents mycophenolate mofetil [24] and lamotrigine [25].

In this study two sulfonated calix[4]resorcinarenes which have alkyl chains on the lower rim of varied length (4 & 7 carbon) are compared for their ability to act as drug solubilisers. The chemical structure of the butylated (4 carbon) calix(4)resorcinarene (SC(4)RC4) and the heptylated (7 carbons) calix(4)resorcinarene (SC(4)RC7) are shown in Figure 2a. The potential of SC(4)RC4 and SC(4)RC7 as drug solubilisers is investigated using two model hydrophobic drug molecules: propofol and griseofulvin. Propofol (2,6-diisopropylphenol) (Figure 2b) is a commonly used anaesthetic, licensed for both induction and maintenance of anaesthesia [26,27]. Due to its poor solubility in aqueous media (100 μgmL^{-1} at 25°C), propofol is currently formulated in soybean oil (100 $mgmL^{-1}$) forming an oil-in-water emulsion [26]. Despite the market success of propofol emulsions, the concomitant use of painkillers and antimicrobial agents are required due to its viscosity causing pain on administration and the high infection risk [28]. Additionally, hyperlipidemia, rhabdomyosis and metabolic acidosis have been reported and linked to propofol-fat parental emulsions (propofol-Related Infusion Syndrome) [29]. Griseofulvin ((2*S*)-trans-7-chloro-2',4,6-trimethoxy 6'-methylspiro(benzofuran-2[3H],1'-[2] cyclohexene)3,4'-dione) (Figure 2c) is an orally administered anti-fungal agent, used for the treatment of dermatophytoses. The high degree of lipophilicity of griseofulvin (aqueous solubility of 30 μgmL^{-1} at 25°C), results in long dissolution rates and consequently poor bioavailability in the GI tract [30]. Significant intersubject and intrasubject variations in bioavailability have also been reported, not only between different branded preparations but also between different physiological stages of the stomach, indicating clinical failure with griseofulvin therapy in most patients [31]. Moreover, a dosing regime is difficult to be established considering fluctuations in the drug's bioavailability profile as it is currently formulated.

Materials and Methods

All materials were purchased from Sigma Aldrich (UK) unless otherwise stated.

Synthesis of sulfonated calix[4]resorcinarenes

Sulfonated calix[4]resorcinarenes SC(4)RC4 and SC(4)RC7 were prepared from the parent calix[4]resorcinarenes C(4)RC4 and C(4)RC7 using established procedures [22,32]. In brief, concentrated hydrochloric acid (12.5 mL) was added dropwise to a solution of either n-pentanal (for C(4)RC4) or n-octanal (for C(4)RC7) (90 mmol) and resorcinol (90 mmol) in ethanol (75 mL). In each case the mixture was heated to reflux for 4 h and then allowed to cool to ambient temperature. The product precipitated upon cooling. Water (50 mL) was added slowly to the cooled mixture to complete the precipitation. The crude products were isolated by vacuum filtration and recrystallized from ethanol to give C(4)RC4 (yield 85%) and C(4)RC7 (yield 78%) as colourless solids.

C(4)RC4 or C(4)RC7 (0.01 mol), formaldehyde (37% solution, 0.05 mol) and sodium sulfite (0.05 mol) were combined with water (30 mL) and the stirred mixture was heated at 90°C for 16 h. The mixture was cooled to ambient temperature. Dilute hydrochloric acid (1 molar aqueous solution) was added to the cooled mixture until pH 7 was reached. Acetone (~50 mL) was added to the mixture to precipitate sulfonated calix(4)resorcinarene. The precipitate was isolated by filtration, washed with acetone on the filter and then dried under vacuum to give SC(4)RC4 (yield 53%) and SC(4)RC7 (yield 85%) as yellow solids.

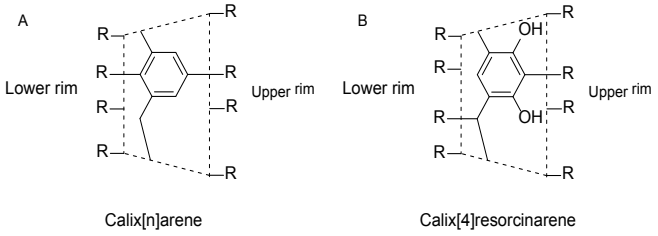

Figure 1: General structures of A) a calix[n]arene and B) a calix[4]resorcinarene.

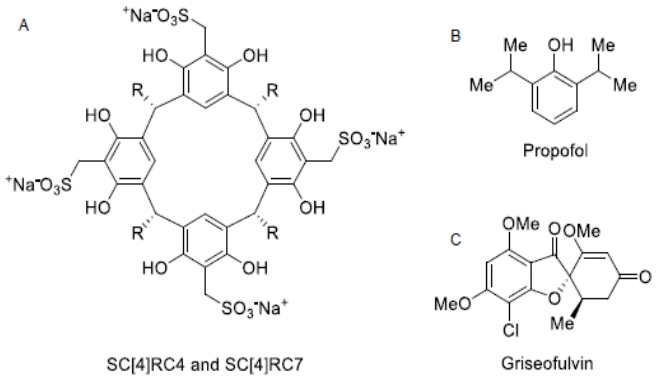

Figure 2: Structures of A) SC(4)RC4 (R = C_4H_{11}) and SC(4)RC7 (R = C_7H_{15}), B) propofol and C) griseofulvin.

Chemical characterisation of calix[4]resorcinarenes

^{1}H Nuclear Magnetic Resonance (NMR) spectra of SC(4)RC4 and SC(4)RC7 were acquired by making a solution of each compound in d6-dimethylsulfoxide (d6-DMSO) (Goss Scientific Instruments Limited) and irradiating the solutions at 400 MHz using a Bruker DMX-400 NMR instrument.

FTIR was carried out on solid powder samples of SC(4)RC4 and SC(4)RC7 using a NICOLET iS5 fitted with a iD5 ATR laminated diamond crystal tip (Thermo Scientific™, UK). The average spectra of 64 scans was recorded.

Physical characterisation of calix[4]resorcinarenes in aqueous phase

The surface activity of SC(4)RC4 and SC(4)RC7 was determined by measurement of surface tension using a torsion balance (OS Instruments). Solutions of SC(4)RC4 and SC(4)RC7 were prepared over a concentration range 0.039 $mgmL^{-1}$ - 5$mgmL^{-1}$ in deionised water. Samples were probe sonicated for 5minutes using a Soniprep 150 (MSE Ltd, UK). The samples were coolied to room temperature, and the surface tension measured. Measurements were conducted in triplicate for each sample, and an average value was obtained over three independent samples.

The hydrodynamic diameter and polydispersity index (PDI) was measured using photon correlation spectroscopy (Zetasizer Nano-ZS, Malvern Instruments, UK). Aqueous solutions of SC(4)RC4 and SC(4)RC7 (1mgmL^{-1}) were probe sonicated for 5 mins and once cooled were filtered using a 0.45 µm syringe filter. The mean particle size and PDI values were attained by averaging values of three measurements. Samples were measured at 24.9°C and pH 7.

Drug incorporation studies

Solutions of SC(4)RC4 and SC(4)RC7 at varied concentration (1 mgmL^{-1}, 3 mgmL^{-1} and 6 mgmL^{-1}) were prepared in deionised water and probe sonicated for 10 min. Samples were cooled to room temperature and the appropriate volume of drug was added in 1:1, 5:1 and 10:1 initial drug: calixa(4)resorcinarene ratio. The solutions were probe sonicated for 5 min and allowed to cool to room temperature. The solutions were filtered using 0.45 µm syringe filter to remove any excess free drug. Drug quantification was carried out using UV-Vis spectroscopy (Cary 50 Bio, Agilent Technologies U.S). Propofol was quantified at 272 nm and griseofulvin at 242 nm. Calibrations were run of the free drugs dissolved in acetonitrile and methanol (propofol and griseofulvin respectively), both obtaining R^2 of 0.999. The formulations were diluted in the appropriate solvent to liberate the free drug and enable measurement. Drug quantification was carried out in triplicate at room temperature.

Physical characterisation of formulations

Optimal formulations were measured for hydrodynamic radius as described above. The formulations were freeze dried and 64 scans run in order to qualitatively show drug presence as previously described.

In vitro drug release studies

Optimal formulation for each calix(4)resorcinarene and drug were dialysed against water to harness their drug release profile. Briefly, 2 mL of formulation was pipetted inside a visking tube (12-14 kDa) (Medicell, UK) and dialysed against distilled water (200 mL) at room temperature. The volume of dialysis fluid was in excess to "mimic" the "in sink" conditions experienced after injection into the blood stream. A 2 mL sample of the exterior solution was obtained at selected time points (5 min, 10 min, 15 min, 20 min, 30 min, 40 min, 50 min and 60 min) and replaced with deionised water. Drug quantification was carried out as previously described. All measurements were carried out in triplicate at room temperature.

Cytotoxicity of calix[4]resorcinarenes

An MTT assay was carried out using human pancreatic adenocarcinoma (BxPC-3) cells. Cells were cultured in RPMI medium containing 10% FBS and 1% *penicillin streptomycin* (Penstrep). Cells were subcultured into a 96-well plate at 15 million cells per well. SC(4)RC4 and SC(4)RC7 of varied concentration (0.019 – 10 mgmL^{-1}) were prepared in media. Once 80% confluency had been reached the cells were exposed to the calix[4]resorcinarenes (100 µL) for 24 h before being washed with fresh media (100 µL three times) and incubated for 4 h with media (100 µL) and MTT solution (20 µL). The absorbance of the plate was read at 570 nm and cell viability was calculated relative to positive and negative controls.

Results and Discussion

Sulfonated calix[4]resorcinarenes SC(4)RC4 and SC(4)RC7 were characterised using ^{1}H NMR spectroscopy and Fourier transform infrared spectroscopy. The ^{1}H NMR spectrum of SC(4)RC4 was acquired as a solution in d6-DMSO and showed characteristic signals at 7.21 ppm (proton on a pentasubstituted aromatic ring) and 4.09 ppm (methylene group bridging two aromatic rings. Similarly, the ^{1}H NMR spectrum of SC(4)RC7 as a solution in d6-DMSO and showed these characteristic signals at 7.30 ppm and 4.27 ppm, respectively (data not shown). This indicated that both compounds had adopted the crown conformation solely.

The FTIR spectra of SC(4)RC4 and SC(4)RC7 (Fig. 3A1 & B1) both show common peaks relating to the O-H stretch of the phenols between 3500-3200 cm^{-1}. Other peaks identified at 1585-1600 cm^{-1} were due to the C-C in the aromatic ring, further C=C stretches were also identified at 1400-1500 cm^{-1}. It was difficult to distinguish between the samples to identify chain attachment, perhaps because detection of vibrational motions of the very restricted regions within a nano-scale sample is more likely to occur with a micro- FT-IR rather than a simple FTIR apparatus [33]. The peaks assigned to the alkyl C–C group, present in either stretching or bending bands are also possibly too weak or too low in frequency to be detected by IR spectroscopy. However, the spectra in combination with the ^{1}H NMR indicate that the calix[4]resorcinarenes were successfully synthesised (Figure 3).

The surface activity of both SC(4)RC4 and SC(4)RC7 was determined via surface tension measurement in water (Figure 4). These studies can give an indication of supramolecular formation in bulk solution and are often used in polymer studies [34]. Calix[n]arenes themselves exist as rigid structures which does not require micellation or aggregation to occur to satisfy themselves stability wise in aqueous media. However, our calix[4]resorcinarenes possess hydrophobic chains on their lower rim which could give rise to aggregation due to reduction in Gibbs' free energy. The short chained SC(4)RC4 did not appear to alter the surface tension of water at most of the concentrations tested (0.039 mgmL^{-1} – 1.25 mgmL^{-1}). However above these concentrations the surface tension of the water was reduced from 0.072 Nm^{-1} to 0.054 Nm^{-1}. This suggests that the SC(4)RC4's do not form aggregates and exit in a singular basis. The reduction of surface tension at higher concentrations may be due to location of these particulates in the bulk, whereby, at increased concentrations the particles are closer to each other and are forced to occupy the surface layer thus interfering with the natural surface tension of the water. The hydrodynamic diameter data for SC(4)RC4 (Table 1) indicated that at all concentration ranges tested only one size population of approximately 78 nm was detected. The polydispersity index readings were also very low which indicated the samples were monodisperse. These finding are in agreement with the surface tension measurements suggesting that the short chained calix[4]resorcinarenes exist as individual entities in solution (Figure 5).

In contrast, the SC(4)RC7's caused a reduction in the surface tension of water at low concentration (Figure 4). The point of inflexion in the surface tension graph is an indication of aggregation in the bulk solution, a technique often used in amphiphilic polymer characterisation to denote critical aggregation concentration. For SC(4)RC7 the surface tension graph showed two points of inflexion and hence two CAC's were evident. In polymer chemistry this would suggest formation of both intramolecular and intermolecular aggregates [34]. However, as calix[4]resorcinarenes are not capable of intramolecular aggregation it is postulated that the first CAC at 0.0195 mgmL^{-1} is due to two particulates coming together with their lipiphilic chains forming weak hydrophobic-hydrophobic interactions (Figure 5). At increased concentrations, above the second CAC value (0.625 mgmL^{-1}) larger supramolecular structures are likely to form, as groupings of increased number (three or more) of calix[4]resorcinarenes come together to

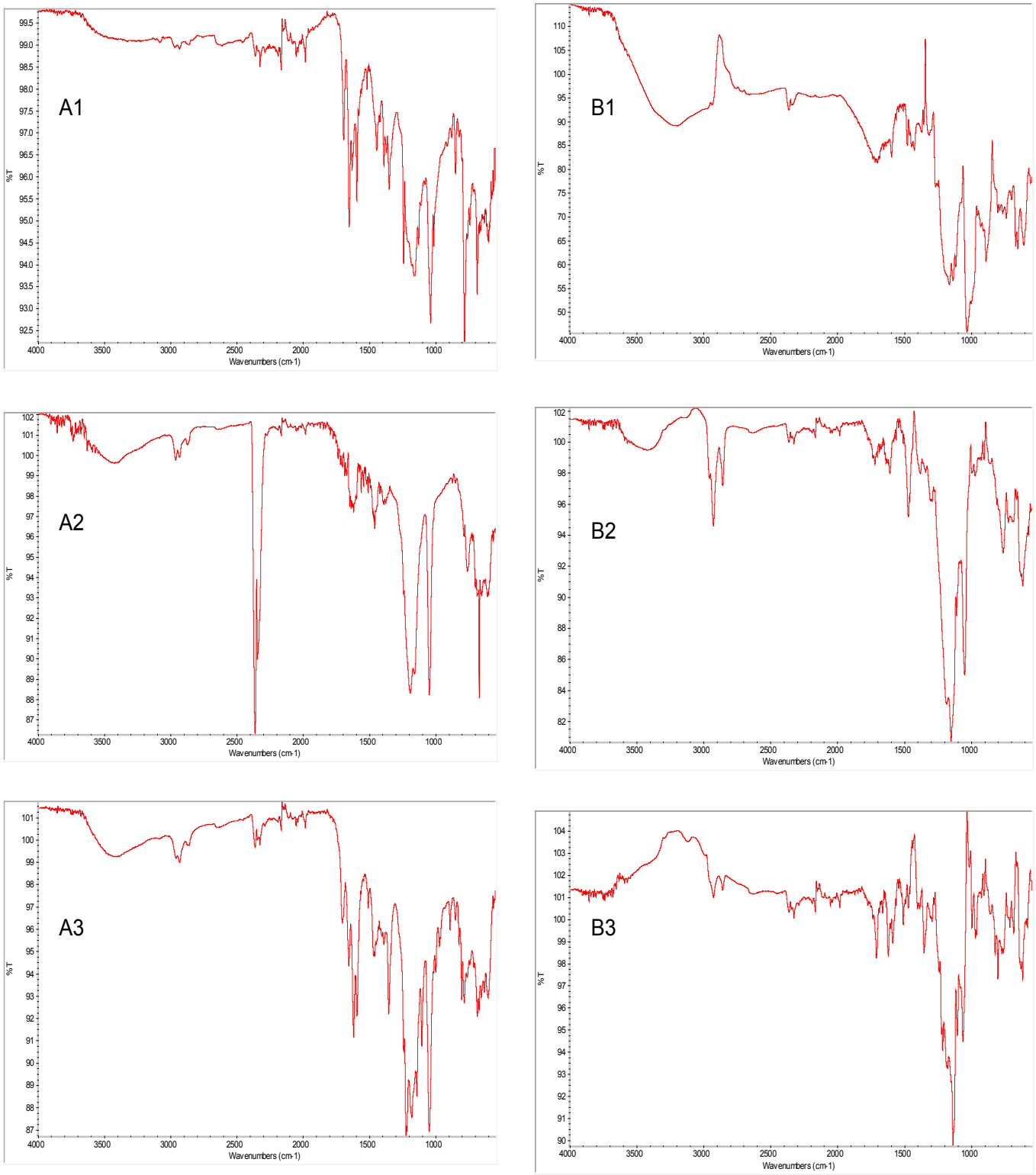

Figure 3: FTIR spectra of freeze dried calix[4]resorcinarenes / formulations. FTIR run using a diamond tipped ATR and run over 64 scans of A) SC(4)RC4 and B) SC(4) RC7, 1) in the absence of drug and after complexation with 2) propofol and 3) griseofulvin.

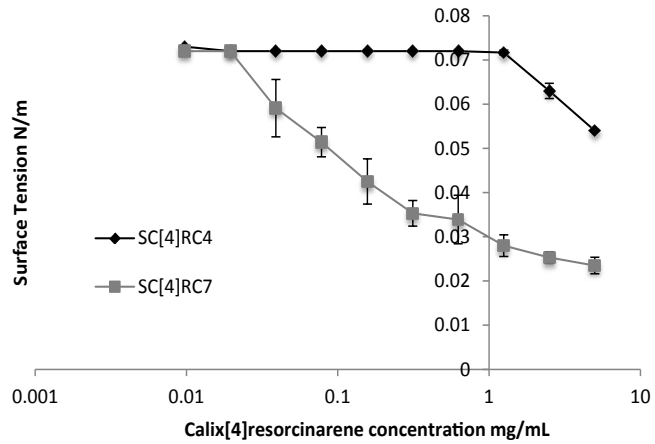

Figure 4: Surface tension of SC(4)RC4 and SC(4)RC7 measured at 25 °C using a torsion balance (n = 3 ± SD).

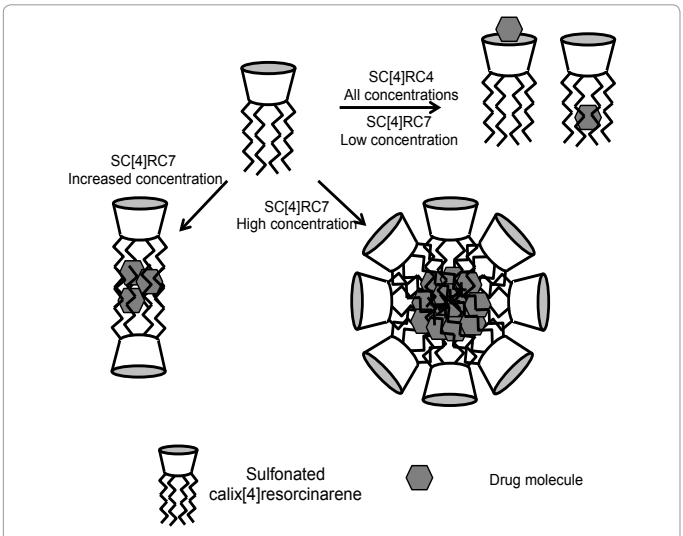

Figure 5: Possible drug incorporation and aggregation mechanisms of calix[4]-resorcinarenes.

Particulate	Concentration mgmL^{-1}	Size nm (± SD): percentage occurrence	PDI (± SD)
SC[4]RC4	0.1	77 (3.43): 100%	0.123 (0.453)
	1.0	78 (3.54): 100%	0.134 (0.065)
	5.0	78 (2.54): 100%	0.111 (0.012)
SC[4]RC4-Propofol	N/A	82 (4.22): 100%	0.211 (0.036)
SC[4]RC4-Griseofulvin	N/A	85 (2.32): 100%	0.152 \(0.333)
SC[4]RC7	0.1	92 (2.42): 100%	0.154 (0.091)
	1.0	86 (4.32): 41% 240 (2.31): 59%	0.345 (0.122)
	5.0	89 (1.23): 20% 363 (3.34): 70% 5095 (12.74): 10%	0.564 (0.098)
SC[4]RC7-Propofol	N/A	124 (1.23): 24% 421 (6.88): 76%	0.434 (0.073)
SC[4]RC7-Griseofulvin	N/A	229 (5.43): 32% 473 (3.45) : 68%	0.345 (0.232)

Table 1: Hydrodynamic radius and polydispersity index of calix[4]resorcinarenes and optimal drug formulations. Samples recorded in triplicate at 25°C.

shield their lipophilic tails from the 'hostile' aqueous environment. Particle size measurements showed that SC(4)RC7 forms particles with a diameter of 92 nm at a concentration of 0.01 mgmL^{-1} in water (Table 1). However, in agreement with the surface tension findings, larger diameter species form at higher concentrations: at a concentration of 1 mgmL^{-1} the dominant species being 240 nm which increases further at increased concentration 5 mgmL^{-1} the predominant species has a diameter of 363 nm. This indicates that supramolecular structures are indeed formed at these higher concentrations as discussed.

The difference in these two calix[4]resorcinarenes is the length of their alkyl chains attached to their lower rim. It is therefore deduced that the short butyl chain with only four carbons does not possess the degree of hydrophobicity of the heptyl chain with seven carbons and hence the different mechanisms of solution existence and stabilisation.

Figure 6 shows the drug loading data for both SC(4)RC4 (A1&2) and SC(4)RC7 (B1&2) with propofol and griseofulvin compared with the intrinsic drug solubility. The study used varied excipient (calix(4)resorcinarene) concentration and initial drug:excipient feed concentration. In general all studies showed that increasing the excipient concentration resulted in increased drug loading. Additionally, the drug feed ration impacted drug incorporation with increased feed ratios increasing drug content. For the propofol studies, the SC(4)RC4 was capable of reduced drug solubilisation compared with the SC(4)RC7 as maximum concentrations of 4 mgmL^{-1} were achieved compared with 8.4 mgmL^{-1} respectively. Given the size and surface tension data, this result is rational based on the SC(4) RC7 forming larger aggregates capable of drug solubilisation (Figure 5). One of the perceived disadvantages to the use of calixarenes and calix[4]resorcinarenes is their rigid structure which can hinder drug incorporation. Often structures such as polymers can experience core expansion upon drug incorporation, however this is not likely to be possible for these rigid structures existing singularly. This is identified in the size data for SC(4)RC4-propofol formulation where the size is increased only marginally in the presence of drug. However, with the SC(4)RC7-propofol formulation, drug solubility was increased up to 84-fold compared to intrinsic levels. Here, the size data of the optimal formulation of SC(4)RC7-propofol (6 mgmL^{-1}, 10:1) showed that core expansion had occurred with a noteable increase in core size compared with the empty vehicle. This may either be due to hydrophobic stabilisation of the lipophilic chains once drug is in the large core resulting in core expansion or the addition of further molecular units forming even larger supramolecules. This formation of stabilised supramolecular structures may increase applicability of such technologies to a wide range of drug molecules compared with the one-to-one interaction of the SC(4)RC4.

Propofol is a small molecule and therefore, testing with a second more bulky drug molecule such as griseofulvin is advantageous. Griseofulvin incorporation did not follow the same trend, infact the short chained SC(4)RC4 was capable of incorporation of concentrations up to 3 mgmL^{-1} compared with only 0.75 mgmL^{-1} for the SC(4)RC7. Additionally, no significant difference was found between 3 mgmL^{-1} and 6 mgmL^{-1} loading. The rationale for this is due to core expansion occurring. As mentioned griseofulvin is a bulky molecule and great steric hindrance would be experience whilst drug incorporation is occurring. In fact, visually before filtration the SC(4)RC7 appeared to have solubilised greater drug content compared with the SC(4)RC4. Therefore, it is postulated that the core expansion experienced with the longer lipophilic chains is such that the aggregates exceeded the maximum cut off for the 0.45 µm filter. Hence, the larger aggregates did not pass through resulting in this reduction of drug concentration.

In the case of griseofulvin, it is therefore more advantageous to use the one-to-one interaction of the short chain SC(4)RC4 (Figure 5). This work highlights the importance of looking at more than one excipient before final decision in drug solubilisation as in the case of these calix[4] resorcinarenes universal drug solubilisation is not achieved with every vehicle and they are dependant on factors such as hydrophobicity, molecular weight and architecture of the drug being solubilised.

Numerous evidence suggests that drug release rate is driven by drugs' intrinsic aqueous solubility [35-37]. A drug molecule with low aqueous solubility, denotes a highly hydrophobic molecule which will experience high affinity for the similarly hydrophobic core of calix[n] arenes. Thus, a slow release profile will be expected. The release data for SC(4)RC4 support the above statement, as griseofulvin with significant lower aqueous solubility (0.03 $mgmL^{-1}$) than propofol (0.1 $mgmL^{-1}$) shows a much lower release profile with only 15% being released after 1 h compared with 30% for propofol (Figure 6A3). Intrinsic hydrophobicity of nano-aggregates is also a determinant key in drug release rate. A notable lower drug release rate is observed when comparing SC(4)RC4 to SC(4)RC7 formulations. Consequently, propofol encapsulated in SC(4)RC4 with the shorter less lipophilic chain is released more rapidly (30% after 1 h) than when held in the more lipophilic supramolecular structure of the SC(4)RC7 (5% after 1 h) (Figure 6A3 and B3). In several drug formulation studies, low and sustained drug release is favoured over rapid release due to significant advantages of the former including; reduced local toxicity and increased *in vivo* half life [36,37]. From this aspect, SC(4)RC7 may be more desirable than SC(4)RC7 as this infers increased stability under *sink* conditions.

In order for any drug delivery system to be clinically relevant it must itself be safe for administration. Therefore it is important to determine whether the empty vehicle exhibits any cytotoxic nature. This study was carried out on human pancreatic adenocarcinoma cell lines (BxPC-3) after exposure for 24 h. The data in Figure 7 shows that even up to extremely high (non clinically relevant) concentrations the calix[4]resorcinarenes remained relatively non-toxic. In fact, up to 10 $mgmL^{-1}$, an IC_{50} value (at which 50% of the cells were no longer viable) was not observed with the SC(4)RC7. In contrast the SC(4)RC4 exhibited an IC_{50} at approximately 7.5 $mgmL^{-1}$, however, this is extremely high compared with other excipients such as polymers [38]. The data is encouraging and shows the potential of these systems to not only carry drugs but to do so more safely and efficiently than traditional systems. Further biological work is required in order to fully appreciate their biosafety.

Conclusion

Both SC(4)RC4 and SC(4)RC7 have demonstrated to improve aqueous solubility of both hydrophobic drugs tested, suggesting the great potential of these molecules as novel drug solubilisers. Nonetheless, parameters affecting formation of inclusion complexes, not explored in this study need to be further investigated, including physical characteristics of aqueous environment (e.g. pH and temperature). Moreover, further toxicology, stability and *in vivo* studies need to be designed, to establish safety clinical relevance of these systems.

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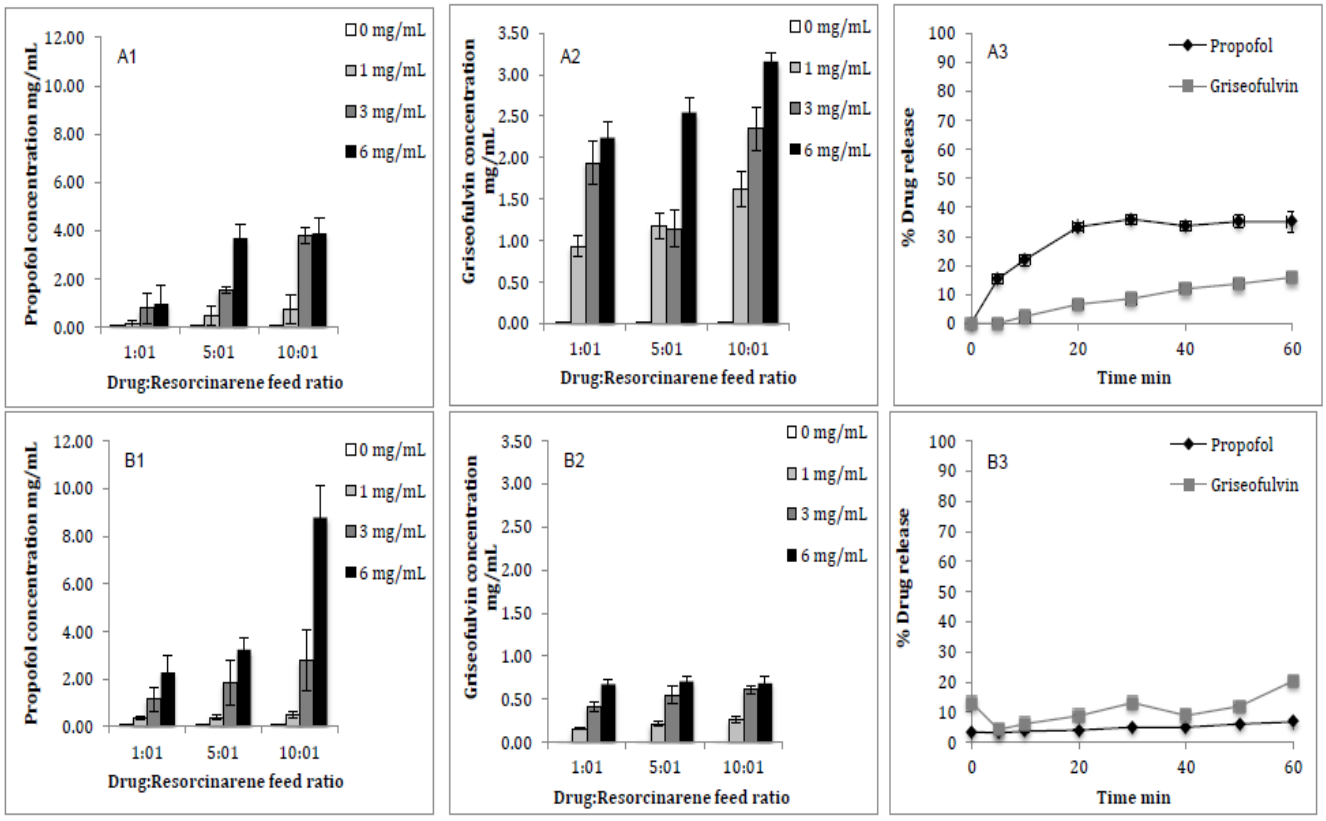

Figure 6: Drug incorporation studies using A) SC(4)RC4 and B) SC(4)RC7 with 1) propofol and 2) griseofulvin. 3) *In vitro* drug release over 1 h. All experiments carried out at room temperature (n = 3, ± SD).

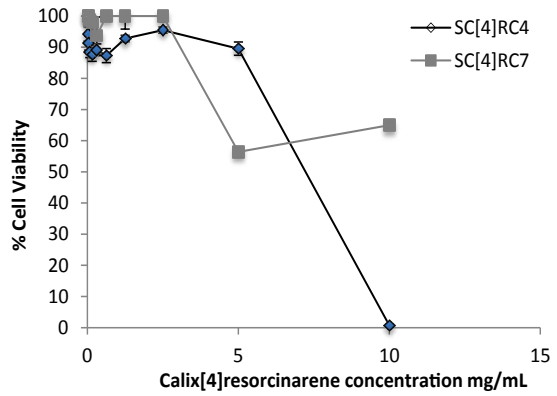

Figure 7: Cell viability of BxPC-3 cells after 24 h incubation with resorcinarenes measured via MTT assay at 570 nm.

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