Understanding polysorbate-compound interactions within the CMC region

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Abstract

Non-ionic surfactants such as polysorbates, known as Tween™ 20 and Tween™ 80, are routinely used within the healthcare and pharmaceutical industry to enhance solubility. This work focuses on analysing the two aforementioned polysorbates, each considered at three purity levels with four model compounds, across the critical micellar concentration (CMC) range for each surfactant. Such data is of interest to investigate the influence of micelle formation upon compound-polysorbate interaction. Two analytical techniques were utilised, namely spectroscopic solubility determination and micellar liquid chromatography (MLC). In all cases it was apparent that the maximum solubility for all four compounds increased substantially at concentrations greater than the CMC and that, in most cases, a different retention profile was observed using MLC once the CMC had been exceeded. This paper is the first to have used such techniques to investigate the behaviour of these polysorbates over a series of concentrations and three levels of polysorbate purity. The findings indicate that the solubilisation potential of polysorbates differs once the CMC has been surpassed and is dependent upon the level of purity selected, i.e. compound-surfactant interactions are partially a consequence of the presence of micelles rather than monomer as well as polysorbate purity. Thus, formulators should include such polysorbates at optimised concentrations and purity if they wish to maximise their solubilisation potential.

Keywords

CMC; critical micellar concentration; micellar liquid chromatography; MLC; solubility; Tween

1. Introduction

Tween 20 (polysorbate 20) and Tween 80 (polysorbate 80) are two non-ionic surfactants routinely used in the cosmetic, healthcare and pharmaceutical industries to enhance solubility and function as emulsifiers in complex mixtures. These polysorbates have previously been analysed by researchers with respect to their degradation [1-3], composition [4, 5] and properties [6-8]. Several purities of each polysorbate are commercially available; including

standard compendial grade, 'High Purity' and 'Super Refined™'. According to the manufacturers [9], the High Purity range was developed to address the purity and control requirements of the pharmaceutical industry with the High Purity range having limits on peroxide value (< 2.0 meq.), 1,4 dioxane (<5 ppm) and ethylene oxide (<1 ppm), a low moisture content (< 0.2 %) and packaged under nitrogen to prevent peroxide escalation. For the Super Refined polysorbates the manufacturers datasheets [9] state that they have undergone a further proprietary process that improves clarity and removes polar impurities such as peroxides, aldehydes and ketones, without altering the surfactants chemical composition, and therefore enhancing the stability of the solute and/or vehicle itself. This is particularly beneficial for minimising levels of dioxane and ethylene oxide which may be present in the polysorbate following manufacture along with peroxide which is known to form over time. For any surfactant, it is well known that when the concentration exceeds the critical micelle concentration (CMC) the surfactant molecules undergo self-aggregation to form micelles [10]. Micelle formation is a dynamic process, with an equilibrium being established between the monomer and micelle. Micelles have an average lifetime of milliseconds to seconds, with the exchange of small amounts of monomers between micelles occurring constantly [11, 12]. It can be said that micellisation is governed by four sets of interactions, those between the hydrophobic tails and water, between adjacent hydrophobic tails, between head groups and the solvation of head groups in water. Surfactant molecules are commonly used in a variety of products because of their ability to reduce interfacial tension between two liquid phases, this enables normally immiscible liquids such as oil and water to become miscible. The reduction in surface tension is brought about by the molecule's preference to orientate itself so that its hydrophilic head is directed towards water and the hydrophobic tail is directed away. The addition of more surfactant molecules further reduces the interfacial tension allowing for mixing between the two phases until the interface is saturated, at this point further increasing the concentration of surfactant has no effect on interfacial tension. Instead, surfactant molecules begin to aggregate and form micelles [10].

Micellar liquid chromatography (MLC) is a reversed-phase form of liquid chromatography with an aqueous solution of surfactant as the mobile phase. MLC has gained popularity for being environmentally friendly, with only very small amounts of organic solvent required, as well as the enhanced speed of analysis and ability to predict physicochemical properties [13-19]. The addition of a surfactant to the mobile phase has several implications, the most noticeable being on the stationary phase whereby saturation of the surface occurs, thus creating

a modified stationary phase with a structure similar to that of the inside of a micelle. This has a large impact on the retention behaviour of compounds, along with the fact that micelles provide alternative sites of interaction to the stationary phase, thus the compound is involved in two separate equilibria – one with the modified stationary phase and one with the micellar phase. Solutes that partition in micelles experience a microenvironment that is different to that of the bulk solvent, which can lead to a change in physiochemical properties, as reflected in retention [20]. For this work, the effect of surfactant concentration on retention time is considered through normalisation using the retention factor term, k wherein 'dead' time has been allowed for.

Polysorbates are mild yet very powerful multi-functional non-ionic surfactants consisting of mixtures of polyoxyethylene sorbitan fatty acid esters, used extensively as emulsifiers, stabilisers, dispersants, wetting agents and solubilisers in pharmaceuticals and food products. Dosage forms include intramuscular, intravenous, nasal, subcutaneous and topical. Tween 20 and Tween 80 are frequently used in formulations, such as ocular products [21] where only a comparatively small volume of solvent can be applied to the patient to administer a drug, to enhance the solubility of a drug to acceptable levels. Maximum solubility is fundamental to this concept and is defined as the maximum quantity of solute that can dissolve in a certain quantity of solvent at a specified temperature. It is of great importance in pharmaceutical products that enough drug can be solubilised in a suitable volume of solvent to allow the drug to be delivered at a therapeutic dose to the patient [22, 23].

Considering the importance of such additives, little is currently known regarding the effect of the concentration of polysorbate on solubility enhancement and physicochemical behaviour in solution, which is especially crucial within the CMC region for each surfactant. This study therefore attempts to further the understanding of solute behaviour in the presence of polysorbates within this critical surfactant concentration range using MLC and determination of maximum solubility.

2. Materials and Methods

2.1 Materials

Polyoxyethylene (20) sorbitan monolaurate (Tween 20) and polyoxyethylene (20) sorbitan monooleate (Tween 80) were kindly donated by Croda Europe Ltd. Three levels of purity were

analysed, firstly standard purity, referred to as 'standard' grade for Tween 20 (SD05530) and Tween 80 (SD02355). Secondly, high purity Tween 20 (SD40271) and Tween 80 (SD43361). Finally, super refined Tween 20 (SR40606) and Tween 80 (SR48833). Four compounds were selected for their structural similarity (to avoid multiple variables during data analysis) yet differing solubilities, namely: acetaminophen (99 %, λ_{max} = 243 nm), benzamide (99 %, λ_{max} = 227 nm), 4-hydroxybenzamide (98 %, λ_{max} = 252 nm) and hydrocortisone (>98 %, λ_{max} = 240 nm (with 265 used for Tween 80 to avoid impurity interference)), purchased from Sigma Aldrich, Dorset, UK and used as received. Chemical structures of the polysorbates and compounds used in this work can be found in the Supplementary Material section (S1). The critical micelle concentration (CMC) has previously been reported to be within a small range of values and is particularly known for its temperature dependence [24]. In this work the CMC was assumed to be consistent throughout the study, and analysis within this study was based upon a recently published value close to the temperature of this research of 9.4 x 10^{-4} M for Tween 20 and 4.4 x 10^{-4} M for Tween 80 [7] .

2.2 Methods

2.2.1 Solubility determination

A stock solution of 20 mg/mL for each compound in methanol was prepared, from this the following dilutions were made using the relevant mobile phase (50:50 methanol and water for acetaminophen, 80:20 10 mM phosphate buffer and acetonitrile for benzamide and 4-hydroxybenzamide and 70:30 methanol and water for hydrocortisone): 10 mg/mL, 5 mg/mL, 1 mg/mL, 0.5 mg/mL, 0.1 mg/mL, 0.05 mg/mL and 0.01 mg/mL), to construct calibration plots. To determine maximum solubility 200 mL solutions of 20 mM Tween 20, Tween 20 High Purity, Super Refined Polysorbate 20, Tween 80, Tween 80 High Purity and Super Refined Polysorbate 80 were prepared followed by serial dilution to create 10 mL solutions of 1x10^{-2}, 1x10^{-3}, 1x10^{-4}, 1x10^{-5}, 1x10^{-6}, 1x10^{-7} and 1x10^{-8} M, i.e. 10 mM to 1 x 10^{-5} mM. 1 mL of each solution was placed into a centrifuge tube and an excess of compound was added, then placed onto a rotating wheel (4 rpm at 25 ºC) for 48 hours, centrifuged and the resulting solution placed into a filter centrifuge tube and centrifuged for a second time. After this, samples were diluted by a factor of 4 with the relevant mobile phase as used previously. All samples were analysed using an Agilent 1260 Infinity II HPLC with an Eclipse XDB-C18 5 µm, 4.6 mm x 50 mm column at a flow rate of 1 mL/min at 31 ºC, an Infinity Diode Array Detector HS, at the λ_{max} for each compound stated in Section 2.1.

2.2.2 Micellar Liquid Chromatography

800 mL of stock solution of each surfactant (0.02 M) was prepared using ultra-pure water from a Thermo Scientific Barnstead Nanopure unit (resistivity = 18.2 MΩ-cm). The stock solution was diluted with ultra-pure water to produce 200 mL solutions of concentration $1x10^{-2}$, $1x10^{-3}$, $1x10^{-4}$, $1x10^{-5}$, $1x10^{-6}$, $1x10^{-7}$ and $1x10^{-8}$ M. The solutions were placed into a sonic water bath (VWR Ultrasonic Cleaner USC – T) for 10 minutes. A 2 mM solution of each compound and each surfactant concentration was prepared in a 10 mL volumetric flask. The resulting solution was then placed into a sonic water bath for 10 minutes. The solution of surfactant was then loaded onto the C18 column (Spherisorb ODSB cartridge, 80 Ä, 5 µm, 4.6 mm x 150 mm) which was kept at 31 ºC at a flow rate of 1.35 mL/min (using an Agilent Binary Pump G1312A). For hydrocortisone a C1 column was used (Spherisorb 3.0 µm C1, 4.6 mm x 50 mm) also at 31 ºC with a flow rate of 3.00 mL/min. This column was selected to optimise the retention time separations acquired, owing to the low aqueous solubility of the compound. A UV spectrophotometer (PerSeptive Biosystems UVIS – 205 Absorbance Detector) was set to the λ_{max} for each compound as stated in Section 2.1 and the compound/surfactant solution was then injected using a 10 µL syringe. After a peak was observed a cleaning solution consisting of 75 % acetonitrile and 25 % ultra-pure water was pumped through the system for 15 minutes, followed by 100 % ultra-pure water for a further 5 minutes. This process was then repeated for each surfactant/compound solution to avoid issues concerning potential irreversible adsorption on the stationary phase. A sample chromatogram can be found in Supplementary Material (S2).

3. Results and Discussion

3.1 Solubility determination

For all three purities of Tween 20 a clear correlation was found between exceeding the CMC of the surfactant and an increase in the maximum solubility for all four compounds as summarised in Figure 1.

FIG. 1

The observed increase in solubility upon exceeding the CMC for the surfactant is as expected in that the hydrophobic compounds will interact with the micelles that form above the CMC, compared with only monomer below the CMC, as hydrophobic regions within the micelles ‘protect’ the compound from the surrounding aqueous solution thus enhancing solubility. Interestingly, differences were observed for the four compounds when comparing the results obtained from the three purities of the surfactant studied. In all cases a more dramatic increase in maximum solubility was observed when using the highest level of purity, namely Super Refined Polysorbate 20 for all four compounds compared with standard Tween 20.

Furthermore, Tween 20 High Purity also enhanced maximum solubility more so than standard Tween 20 yet not as dramatically as that seen for Super Refined Polysorbate 20. Particularly for acetaminophen and hydrocortisone a clear pattern of increase in maximum solubility could be seen from standard Tween 20 < Tween 20 High Purity < Super Refined Polysorbate 20. The most substantial increase in solubility was observed for hydrocortisone - the least soluble compound studied, with an approximate doubling of solubility from the lowest polysorbate concentration to the highest. This finding implies that the solubilising potential of the polysorbate increases as the solubility of the compound decreases.

For Tween 80 a similar relationship was observed in that the maximum solubility significantly increased upon surpassing the CMC, as displayed in Figure 2.

FIG. 2

Comparing results displayed in Figure 1 with Figure 2 it can be seen that in some cases there was a small increase in solubility, with respect to which surfactant was used. A possible explanation is that the higher level of purity, thus lower levels of peroxide and other impurities, helps encourage more of the compound to be solubilised within the micelles, i.e. polysorbate purity contributes to solubilisation as well as polysorbate selection. As seen previously in Figure 1, the four compounds all experienced an increase in solubility in the increased presence of polysorbate, particularly above the CMC. For both polysorbates the greatest increase in maximum solubility when comparing pre and post CMC data was observed for hydrocortisone which is unsurprising as it is the most hydrophobic of the four compounds thus most likely to preferentially favour the micellar environment when available. An increase in solubility for all four compounds was observed when comparing solubility data (even at the lowest concentrations of added polysorbate) with values determined experimentally and those previously published in literature for the compounds in purely aqueous solutions. Experimental and literature solubility values for the four compounds are listed in Table 1.

TABLE 1

This confirms that even below the CMC, the presence of polysorbate enhances the solubility of each compound to a small extent through their interaction within solution. Interestingly, for the first three compounds the increase in solubility in the presence of the lowest concentration of polysorbate was similar to the increase recorded between the lowest and highest polysorbate concentrations even though the CMC was exceeded within this range. This finding can be explained by considering that the three compounds are all comparatively soluble when considered alongside the far more insoluble hydrocortisone which more than doubled in solubility upon exceeding the polysorbate CMC.

3.2 Micellar Liquid Chromatography

For Tween 20, MLC results indicate that a variety of compound-specific interactions occurred as the concentration of surfactant increased, as displayed in Figure 3. In theory it can be postulated that it is only after the CMC has been passed (and therefore a second equilibrium introduced (compound partitioning within micelles in the mobile phase)) that increasing the concentration should have any effect on the resultant profile. Prior to attaining the CMC, surfactant will be present yet only as the monomer form, including a layer upon the stationary phase to create a modified stationary phase.

Fig. 3

For acetaminophen no significant difference in retention behaviour was recorded for the standard purity surfactant as the concentration exceeded the CMC yet a more appreciable decrease was observed for the two remaining purities. With respect to purity for all four compounds, these findings fit well with those reported earlier for maximum solubility in that the higher purity polysorbates have a more pronounced effect on the compound compared with using standard Tween 20, i.e. Tween 20 High Purity and Super Refined Polysorbate 20 displayed a greater reduction in retention compared with standard Tween 20 in most scenarios. One of the possible reasons to explain differences between the results observed, in addition to the difference in purity, is variations in the manufacturing processes for the three surfactants. The generally observed decrease in retention in these cases is as expected in that the hydrophobic compound will interact with the micellar phase, compared with only monomer below the CMC whereupon compound interacts more with the modified stationary phase. A similar trend was also observed for all three purities of surfactant with benzamide although a far more significant change in retention was seen. This reflects that the retention time of the compound within the column has more dramatically decreased compared with acetaminophen as surfactant concentration increased as a result of the compound being more hydrophobic than acetaminophen thus more strongly favouring the created micellar phase, in agreement with that previously seen in Figure 1. A more complicated profile was observed for 4-hydroxybenzamide as surfactant concentration increased. Previous studies have discussed how according to their elution behaviour with a micellar mobile phase, solutes can be classified into three categories: solutes binding to micelles, and non-binding and anti-binding solutes [18] . Solutes that interact with micelles show decreased retention when the concentration of micelles in the mobile phase is increased, as seen for the first two compounds analysed. However, this third compound's

retention initially decreased yet then increased with increasing micelle concentration beyond the CMC. The initial decrease in retention as binding to micelles occurred is often observed, yet the subsequent antibinding event is far less common, indicating the compound firstly encourages the compound to be retained within the micelles then discouraging interaction at higher concentrations. Finally, hydrocortisone is known to be the least soluble of the four compounds and was seen to have the most significant decrease in retention factor over the surfactant range studied. This finding was as expected, indicating that the more insoluble the drug, the more dramatic the effect on retention as the drug more obviously favours the micellar phase when it is available.

For Tween 80, a similar trend to that observed for each compound with Tween 20 was apparent, as displayed in Figure 4.

FIG. 4

Acetaminophen displayed a small decrease in retention after the CMC was surpassed, benzamide a more dramatic decrease than that seen for acetaminophen yet less so for both compounds than when using Tween 20. Therefore, it can be postulated that for these two compounds Tween 20 has a greater propensity to incorporate the compound within the micelle compared with Tween 80 when analysed using MLC. For 4-hydroxybenzamide a similar mixed profile to that observed for Tween 20 was observed, most noticeably considerably decreasing then increasing for Tween 80 High Purity. These findings imply that interactions with Tween 80 micelles (after an initially favourable interaction) were less preferential to the modified stationary phase, thus further increasing the surfactant concentration shifted the compound-micelle vs. compound-stationary phase equilibrium more towards the modified stationary phase. This resulted in the solute interacting with modified stationary phase more preferentially and therefore an increase in retention time, as was observed for Tween 20, was apparent. As was seen previously for hydrocortisone in Figure 3, this compound exhibited the greatest change in retention factor over the polysorbate concentration range studied, as expected with the compound having the lowest aqueous solubility of all four compounds.

Previously published findings by Rukhadze *et al.* investigated how altering the concentration of Tween 80 in the mobile phase between 0.75 - 4 % influenced retention time using a C18 column [27]. It was found that increasing the concentration of Tween 80 from 0.75 % - 1.5 % w/v reduced the retention factor for all compounds analysed whereas increasing it to 4 % increased retention in some cases. A similar reduction in retention was observed for acetaminophen, benzamide and hydrocortsione in this study. MLC with other notable surfactants such as SDS has also demonstrated a decrease in retention time with increasing

surfactant concentration. For example, Hadjmohammadi and Salary found that increasing SDS concentration from 0.07 M to 0.09 M reduced the retention time for fourteen model compounds [28]. Furthermore, in other papers it has been reported that increasing SDS concentration reduced the retention time of the compounds analysed [29, 30], further supporting the results presented in this study.

4. Conclusion

In conclusion, this is the first study to investigate the behaviour of two well-known polysorbates at three levels of purity within their CMC region with respect to maximum solubility and chromatographic retention behaviour for four model compounds. It was found that solubility increased substantially at concentrations greater than the CMC and that, in most cases, a different retention profile was observed using MLC once the CMC had been exceeded implying the compound-surfactant interaction differs above and below the CMC and between the type and purity of polysorbate selected. These findings are particularly useful to those in the manufacturing industry when creating surfactant-based formulations to help optimise the products formulated with regards to the quantity of compound solubilised and quality of formulation achieved.

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