Probing the Molecular Interactions between Pharmaceutical Polymeric Carriers and Bile Salts in Simulated Gastrointestinal Fluids using NMR Spectroscopy

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ABSTRACT

The number of poorly soluble new drugs is increasing and one of the effective ways to deliver such pharmaceutically active molecules is using hydrophilic polymers to form a solid dispersion. Bile salts play an important role in the solubilisation of poorly soluble compounds in the gut prior to absorption. When a poorly water-soluble drug is delivered using a hydrophilic polymer based solid dispersion oral formulation, it is still unclear whether there are any polymer-bile salt interactions, which may influence the drug dissolution and solubilisation. This study, using two widely used hydrophilic model polymers, Hydroxypropyl methylcellulose (HPMC) and polyvynilpirrolidone (PVP), and sodium taurocholate (NaTC) as the model bile salt, aims to investigate the interactions between the polymers and bile salts in simulated fed state (FeSSIF) and fasted state (FaSSIF) gut fluids. The nature of the interactions was characterised using a range of NMR techniques. The results revealed that the aggregation behaviour of NaTC in FaSSIF and FeSSIF is much more complex than in water. The addition of hydrophilic polymers led to the occurrences of NaTC-HPMC and NaTC-PVP aggregation. For both systems, pH and ionic strength strongly influenced the aggregation behavior, while the ion type played a less significant role. The outcome of this study enriched the understanding of the aggregation behaviour of bile salts and typical hydrophilic pharmaceutical polymers in bio-relevant media. Due to the highly surface-active behaviour of the bile salts, such aggregation behaviour is expected to play a role in drug solubilisation in the gut when the drug is delivered by hydrophilic polymer based dispersions.

**Keywords:** Polymer-surfactant interaction, DOSY NMR, solid dispersions, bile salts, poorly water-soluble drugs
Introduction

The use of hydrophilic polymeric carriers for the delivery of poorly water-soluble drugs has been the subject of extensive research in formulation science,[1] leading to the establishment of polymer based solid dispersions with the primary role in improving the dissolution rate and subsequent bioavailability of poorly soluble drugs.[2,3] The accurate assessment of the \textit{in vitro} dissolution performance of solid dispersions is one of the most important aspects of the formulation development.[4,5] Both the choice of carrier polymer and the dissolution medium can strongly affect the dissolution study outcome of a solid dispersion containing poorly soluble drugs.[6,7] Previously Qi and her co-workers reported on the potential role of HPMC/surfactants interactions in the drug solubilisation from solid dispersions during the dissolution process. [8] Recently, in the work by Deshpande \textit{et al}, surfactants were found to negate the drug stabilizing effects exerted by the polymer. [9] The use of fluids mimicking the gastro-intestinal condition is vitally important to move towards obtaining good \textit{in vitro/in vivo} correlation.[10–14] However, the possible intermolecular interactions occurring between the polymeric carrier and the components of the bio-relevant medium, such as bile salts, cholesterol and other digestive enzymes, are still poorly understood. While the drug solubilizing role of the nano-aggregates formed by these bio-molecules has widely been investigated,[15–17] much less is known about the impact of the possible interactions occurring between polymeric carriers and these components on poorly water-soluble drug dissolution behavior. This study investigated the interaction between bile salts and hydrophilic pharmaceutical grade polymers with the aim to contribute new insights into the impacts of bile salt-polymer interaction on drug solubilisation. Such knowledge would benefit the further exploitation of solid dispersions in the delivery of poorly water-soluble drugs.
Bile salts are produced by the oxidation of cholesterol in the liver, stored in the gallbladder and excreted into the intestinal lumen,[18] and have been shown to interact with different kind of drug delivery systems, such as β-Cyclodextrins,[19,20] zein protein [21] and gold nanoparticles.[22] They have a rigid tetracyclic steroid ring structure with the convex side of the ring being hydrophobic and the concave side of the ring being hydrophilic.[23] This unique ring structure gives rise to bile salts’ peculiar aggregation behavior. [24] The main aggregation driving force is hydrophobic interaction of the more exposed hydrophobic portion. [25] The hydroxyl and the acid groups in the hydrophilic portion of the molecule create an intermolecular hydrogen-bonding network that further contributes to the micelle formation, renders micelles more rigid and can lead to the formation of secondary micelles. [18,26] Bile salts play a crucial role in the digestion and the solubilisation, via transport in micelles, of poorly soluble nutrients and drugs to facilitate absorption.[27]

In this study, sodium taurocholate (NaTC) was chosen as representative bile salt, due to its established role as model intestinal surfactant in the development of biorelevant fluids and its low pKa (<2), which determines its high solubility in the proximal small intestine pH range.[10] NaTC self-associative behavior has been widely studied, but its aggregation pattern is still debated in the literature and different critical micelle concentration (CMC) values have been reported (Table 1).[28–30] A few research groups have described the aggregation pattern of NaTC being a “progressive process”, even at very low concentrations, rather than a sharp transition of aggregation behavior at a critical concentration point.[15,31,32] The presence of excipients in the formulations used for delivering the poorly soluble drugs may affect the aggregation behavior of the bile salts and their solubilisation functions. The present work focuses on investigating the
intermolecular interplay occurring between most commonly pharmaceutical polymers used in solid dispersion based formulations and bile salts as representatives of the biosurfactants present in the gastro-intestinal media.[33] In particular, this study aims to provide a fuller understanding of how interactions of bile salts with the excipients employed in the delivery systems could influence the aggregation behavior of these bio-surfactants in the gut. For this purpose, hydrophilic polymers commonly used in oral solid dispersions, polyvinylpyrrolidone (PVP) and hydroxypropyl methylcellulose (HPMC) were used as the model polymers. $^1$H chemical shift measurements and diffusion-ordered spectroscopy (DOSY) NMR techniques were employed to study the NaTC aggregation behavior, with and without the presence of polymer, in conditions reproducing the intestinal pH and osmolarity.

**Table 1. Summary of NaTC CMC values reported in literature**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Technique</th>
<th>Experimental conditions</th>
<th>CMC (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[34]</td>
<td>Light Scattering</td>
<td>$37^\circ$C, pH 7.4, [Na+] 0.15M</td>
<td>8.5</td>
</tr>
<tr>
<td>[35]</td>
<td>Fluorescence</td>
<td>$20\pm1^\circ$C, water</td>
<td>8-12</td>
</tr>
<tr>
<td>[36]</td>
<td>Electrode measurements</td>
<td>$25^\circ$C, [NaCl] 0.012M</td>
<td>12</td>
</tr>
<tr>
<td>[37]</td>
<td>Chromatography</td>
<td>PBS pH 7.4, [Na+] 154mM</td>
<td>Not defined</td>
</tr>
<tr>
<td>[38]</td>
<td>Fluorescence</td>
<td>$25^\circ$C, water</td>
<td>8-12</td>
</tr>
<tr>
<td>[39]</td>
<td>Light scattering</td>
<td>$25^\circ$C, water</td>
<td>12</td>
</tr>
<tr>
<td>[40]</td>
<td>Fluorescence</td>
<td>$25^\circ$C, water</td>
<td>3-5</td>
</tr>
<tr>
<td>[41]</td>
<td>NMR</td>
<td>$25\pm1^\circ$C, D$_2$O</td>
<td>6.5-8.3</td>
</tr>
<tr>
<td>[42]</td>
<td>Fluorescence, Light scattering</td>
<td>$15\pm1^\circ$C, [NaCl] 15-300mM</td>
<td>1-15</td>
</tr>
</tbody>
</table>
Materials and Methods

Materials

HPMC-K4M and PVP-Kollidon 30 were kindly donated by Colorcon (UK) and BASF (Germany), respectively. Sodium Taurocholate with 99% purity was purchased from Prodotti chimici e alimentari SPA (Italy). Deuterium oxide with 99% purity was purchased from Apollo Scientific (UK). All reagents and chemical including sodium hydroxide with purity 97%, sodium chloride with purity 99%, sodium dihydrogen phosphate with purity 99% and glacial acetic acid with purity ≥ 99% were purchased from Sigma Aldrich (UK).

Preparation of biorelevant media

Two different media simulating intestinal were used in this study: fasted state simulating intestinal fluid (FaSSIF) and fed state simulating intestinal fluid (FeSSIF). The FeSSIF and FaSSIF media were prepared by following the method developed by Kostewicz et al,[6,43] but without the addition of lecithin. Lecithin can self-associate and lead to significant interference to the analysis of results. Therefore, in order to primarily focus on investigating the interaction of pharmaceutical polymers and bile slats in these media, lecithin was not added to any media. The FaSSIF media used in the NMR experiments were phosphate buffers with pH 6.5 and an ionic strength of 0.190; whereas the FeSSIF media used were acetate buffers with pH 5, an ionic strength of 0.389. The detailed step-by-step calculations of the ionic strength values of both media can be found in the Supplementary Material. Both sets of the FaSSIF and FeSSIF media contained NaTC with the concentration in the range 1-90 mM. All media were prepared using milli-Q water.

NMR Spectroscopy
Two different NMR techniques, $^1$H NMR and diffusion-ordered spectroscopy (DOSY) were employed in this part of the study. Both experiments were performed using a Bruker Ultrashield Plus 400 MHz Spectrometer (Bruker, BioSpin Corporation, The Woodlands, TX).

$^1$H-proton NMR

$^1$H proton NMR measurements were carried out using 5mm NMR tubes. All experiments were performed at 37 °C, in order to simulate the physiological temperature. 124 scans were acquired, relaxation delay was set at 1s and the pulse angle was 90° and a pulse length of 13 µs. All the samples were prepared in D$_2$O. In order to investigate the NaTC aggregation, $^1$H proton NMR was performed on NaTC/D$_2$O samples with NaTC concentrations from 1 to 90 mM. This concentration range was employed to allow the study of the system from below to well above the bile salt CMC (3-12 mM). The same experiments were performed in phosphate and acetate buffers having pH 5 and 6.5, respectively. In order to make the NMR experiments feasible, buffers were prepared in D$_2$O. The actual pD values of the deuterated buffers were 4.89±0.06 for the buffer at pD 5 and 6.48±0.34 for the buffer at pH 6.5 (n=3). The spectra of NaTC were measured in D$_2$O, in the two deuterated buffers with and without the presence of PVP and HPMC (0.5 mg/mL), in order to evaluate the impact of the polymer on the surfactant chemical shift. Samples were prepared by weight and were allowed to equilibrate for 1 hour before taking the measurement. All the samples were measured in triplicate (n=3). Chemical shifts were referenced to the H18 of NaTC as this group of protons had the lowest chemical shift and were the least likely to be affected by micellisation. A standard chemical shift reference was not used, as it is not clear that it would remain external to micellar system and be itself affected by micellisation. Data were analysed using TopSpin 3.2 Software.
DOSY NMR

DOSY NMR experiments were performed using 5mm NMR tubes. Experiments were carried out at 37 °C. 16 scans were acquired for each sample. Relaxation delay and the pulse length were set at 1s and 90°, respectively. Diffusion was measured using stimulated echo and Eddy current compensation; applying bipolar gradient pulses for diffusion and 2 spoil gradient pulse. The gradient pulse duration and the diffusion delay length were set at 2.5 s and 0.2 s, respectively. Data were analysed using TopSpin 3.2 Software. No restricted diffusion was observed. Experiments were performed in triplicate (n=3).

Results and discussion

$^1$H proton and $^1$DOSY NMR of raw materials

Figure 1 shows the $^1$H NMR spectrum of NaTC. $^1$H peaks were assigned according to literature data.[44,45] The detailed peak assignment, referenced to TMS is summarized in Table S1 for measurements of chemical shift changes chemical shifts were referenced to the H18 of NaTC as this group of protons had the lowest chemical shift and were the least likely to be affected by micellisation. The H12 signal exhibited a larger change compared to the other protons when NaTC concentration was varied, showing chemical shift variation and peak broadening. For this reason, its chemical shift was used as an indicator of the impact of NaTC concentration on micelle formation in milli-Q water and buffered solutions, with and without the presence of polymers (HPMC and PVP).
Figure 1. (a) Chemical structure and (b) $^1$H NMR spectrum of NaTC

The signal-to-noise ratios of the high resolution NMR spectra of large molecules, such as polymers, are often poor.[46] This gives rise to signals that are weak compared to small molecules. In order to exclude possible interference of the PVP and HPMC signals with the NaTC spectrum, $^1$H NMR was performed on different concentrations of the two polymers. As shown in the supplementary materials (Figure S1) the signal of the two polymers is very weak in comparison with the proton signal from residual protons in D$_2$O. No significant interference with the NaTC signal was observed.

Figure 2 shows DOSY NMR spectrum of NaTC in D$_2$O at a concentration of 30 mM. Self-diffusion coefficient ($D$) values were obtained from the DOSY NMR experiments. The log$D$ values were plotted for the different chemical shifts. It can be seen that two self-diffusion processes
are observed, one related to NaTC and the other to D₂O. D₂O self-diffusion coefficient $D$ was $1.58 \times 10^{-9}$ m²/s, which is in agreement with literature data.[42,47] Self-diffusion coefficient measurements were performed also for HPMC and PVP (data not shown). However, due to slow diffusion of the polymers in solution, it was not possible to obtain the self-diffusion coefficient values of the polymers alone.

![Figure 2. DOSY NMR spectra of NaTC 30mM in D₂O. (The unit of $D$ is m²/s)](image)

**1H NMR and DOSY studies on the aggregation behaviour of NaTC**

1H proton NMR was employed to assess NaTC aggregation in D₂O, in phosphate and in acetate buffers, which are commonly employed for FaSSIF and FeSSIF media preparation, respectively. Buffers were prepared in D₂O. The NaTC H12 chemical shift ($\delta$) was plotted as a function of
NaTC concentration. Figure 3a shows the results obtained using three different media. It is clear that NaTC H12 δ does not change significantly within the NaTC concentration range of 1-15mM in D2O. However when NaTC concentration was further increased to 30mM, a significant δ change is observed. Therefore, 15mM can be assigned as the CMC of NaTC. This value is slightly higher than the NaTC CMC value obtained in D2O reported by previous work using NMR, [47] but within the similar range of the the CMC values measured by other methods. [34,35,42]

Experiments performed in two deuterated buffers (d-buffer) showed a different trend from the result obtained using D2O. In both buffered solutions the δ values changed at much lower NaTC concentrations (about 3mM in the two buffers), indicating a dramatic impact of the presence of salts on the NaTC aggregation. This result agrees well with existing literature describing salt effect on the aggregation behavior of surfactants.[48] Taurine-conjugated bile acids are strong sulfonic acids with pKa values <2.[49] Thus the taurocholate ions resulting from NaTC dissociation in the aqueous media, do not lead to the formation of their undissociated acidic form at pH’s of 5 and 6.5. Therefore, pH condition used in the study are not expected to influence the aggregation behavior by altering the state of ionization of the salt as such an effect is only likely to be observed at pH values close to the NaTC pKa.[50]
Figure 3. (a) NaTC H12 chemical shift and (b) NaTC self-diffusion coefficients as a function of NaTC concentration in D$_2$O, phosphate buffer pH 5 and acetate buffer pH 6.5 (Filled circles NaTC in D$_2$O; filled diamonds NaTC at pH5; open squares NaTC at pH 6.5; n=3).
Another important factor that can influence bile salt aggregation is the ionic strength of the medium. Ionic strength increase leads to a reduction of the electrostatic repulsion between charged groups. Such an effect is more significant for micellar than monomeric bile salt molecules, as the charges carried by the molecules are closer in micelles than in bulk solution. Therefore increasing the ionic strength favors aggregation and decreases the CMC.[51] The two buffers used in this study differed significantly for their ionic strength and osmolarity values. Despite these differences (much higher osmolarity at pH 5, (670 mOsmol/l), than at pH 6.5, (270 mOsmol/l) the effects of the two buffers on the NaTC aggregation were very similar. Thus, this may suggest that the effects of the buffer ions on the surfactant anions were already saturated in the phosphate buffer (pH 6.5) with an osmolarity of 270 mOsmol/l, and the presence of higher salts concentration in the acetate buffer (pH 5) could not further impact on the surfactant aggregation.

The aggregation behaviour of NaTC in different media was further probed by measuring their self-diffusion coefficient ($D$). The $D$ values of NaTC in D$_2$O, acetate and phosphate $d$-buffers were measured using the DOSY NMR technique. In Figure 3b, $D$ values are plotted as a function of NaTC concentration. It can be seen that the $D$ value of NaTC does not vary significantly in D$_2$O at NaTC concentrations ranging between 1-15 mM. This indicates that no aggregation between the NaTC monomers occurred in this concentration range, as there is no change in the diffusion of NaTC. For the samples with NaTC concentration of 15 mM, the $D$ reduced from 0.268±0.022 to 0.181±0.005 ($10^{-9}$m$^2$/s), indicating the occurrence of NaTC aggregation (most likely to be micellization). The CMC of NaTC measured by the DOSY NMR experiments is 3 mM in both $d$-buffers, which agrees well with the results from the $^1$H chemical shift.
For both chemical shift and diffusion, there is no evidence of separate signals from NaTC in micelles and free NaTC. We therefore hypothesize the existence of fast exchange, on the NMR time scale, between aggregates and free molecules. Under these circumstances, the observed NMR parameter $Q$ (chemical shift or diffusion coefficient) will be given by:

$$Q_{\text{obs}} = P_m Q_m + P_f Q_f$$  \hspace{1cm} \text{Eq. 1}

Where $Q_{\text{obs}}$ is the observed value, $P_i$ is the fraction of spins in state $i$ and the subscripts $f$ and $m$ refer to populations in the micellar and free state. Above the CMC the fractional population of micelles increases and therefore the value of $Q_{\text{obs}}$ tends towards the value $Q_m$.

Considering the micellization as the process in which, above the CMC, the concentration of free NaTC remains constant and the size of the micelles remains constant but the number of both micelles and free NaTC molecules increase with increasing concentration, then the diffusion coefficient of both micelles and NaTC remains constant. In such case Eq. 1 predicts that, above the CMC, the diffusion coefficient is proportional to the reciprocal of the total concentration of NaTC.

Figure 4 shows the reciprocal plots for chemical shift and diffusion coefficient of NaTC in buffers. Both samples follow similar curved trends, therefore the diffusion data implies that the simple micellization model does not apply and the downward trend of $D$ with concentration indicates that micelle aggregation (which is the further aggregation between micelles that lead to the formation of secondary micelles) or an increase in size of individual micelles may be taking place. This leads to a decrease of the diffusion coefficient of the micellar component, as a consequence of the increase in size. However, the chemical shift changes follow a similar pattern indicating that, if micelle aggregation is occurring, it also involves some change in the environment.
of the NaTC molecules in the micelle. This cannot be attributed to changes in diamagnetic susceptibility, as the reference proton is part of the molecule in the micelle.

Assuming a value for the CMC, it is possible to calculate the micelle diffusion coefficient and hence the apparent hydrodynamic radius of the using the Stokes Einstein equation,[52] provided the microviscosity of the medium was known. For the solutions without polymer, and assuming the viscosity is that of pure water, values for the radii can be calculated and such calculations are included in the Supplementary Material. However, given the assumptions of the Stokes Einstein equation, it is not clear that the values of the radii reflect the physical reality of the system and can only be regarded as a reflection of the change of the diffusion coefficient. When polymer is present, the macroscopic viscosity of the solution would not represent the microscopic viscosity of the microenvironment in which diffusion of micelle is occurring. As the direct measurement of microenvironment viscosity is not available, the accurate calculations of the radii of the micelles using Stokes Einstein equation are not possible.
Figure 4. Plots of the diffusion coefficient of NaTC at pH 5 acetate buffer (open squares), pH 6.5 phosphate buffer (open circles) and the chemical shift at pH 5 acetate buffer (filled squares), pH 6.5 phosphate buffer (filled circles; n=3)

1H and DOSY NMR studies on NaTC-polymer interactions

1H and DOSY NMR experiments were used to study the molecular interaction between NaTC and the two model polymers that are widely used in the preparation of oral solid dosage forms, HPMC and PVP. A polymer concentration of 0.5 mg/ml was used in order to work in a dilute regime, which is close to the polymer concentration in intestinal conditions after the dissolution of a polymer based solid dispersion formulation. NaTC H12 chemical shifts were measured in D2O at different NaTC concentrations in the presence of PVP and HPMC and plotted as a function of NaTC concentrations. According to the literature, the nature of the interactions between polymer
and surfactant changes with the surfactant/polymer ratio.[53] Initially, at low surfactant concentration, there is binding of individual surfactant molecules to the polymer. At the critical aggregation concentration (CAC), micelles are formed along the polymer chain. Increasing surfactant concentration increases the number of micelles until all interaction sites on the polymer chain are saturated with surfactant micelles. At this point, no further surfactant-polymer interactions occur and free micelles of the surfactants are formed and co-exist with the polymer-surfactant aggregates. This model would predict two changes of slope in the chemical shift or diffusion curves.[54] As seen in Figure 5a, in the cases of HPMC and PVP, the presence of polymer in D$_2$O led to the change in slope of the chemical shift and diffusion coefficient curves at around 3 to 6 mM, indicating the reduction of CMC value of NaTC. These values are lower than the CMC of NaTC measured in alone in D$_2$O. No further significant changes in slope are observed.

NaTC interaction with HPMC and PVP in D$_2$O was also investigated by measuring the self-diffusion coefficients ($D$) of the NaTC species using DOSY NMR technique. As seen in Figure 5b, in the presence of polymers the NaTC self-diffusion coefficient values show a significant decrease at a NaTC concentration of about 6 mM. This effect is observed at a similar concentration to the one measured by the chemical shift measurements. Plots of D versus reciprocal concentration show similar behavior to that shown in Figure 4 indicating that either micelle aggregation or an increase in individual micelle size is occurring (data not shown).
Figure 5. (a) NaTC H12 chemical shift and (b) self-diffusion coefficient values as a function of NaTC concentration in D$_2$O, HPMC 0.5 mg/ml and PVP 0.5 mg/ml solutions (filled circles NaTC in D$_2$O; filled diamonds NaTC-PVP in D$_2$O; open squares NaTC-HPMC in D$_2$O; n=3).
$^1$H NMR experiments on NaTC-PVP and NaTC-HPMC systems were also performed in acetate (FeSSIF) and phosphate (FaSSIF) d-buffers in order to investigate the impact of pH and ionic strength of the buffers on the NaTC-polymer aggregation. As previously discussed, the presence of salts had a critical impact on NaTC aggregation in both acetate and phosphate buffers. The data presented in Figure 6 shows differences in behavior between the pH 5 and pH 6.5 samples. In the pH 5 sample there is a clear break in slope in the 3-6 mM region and there is no clear difference can be seen between NaTC, NaTC-PVP and NaTC-HPMC systems. The break is less apparent in the pH 6.5 sample.
Figure 6. (a) NaTC H12 chemical shift as a function of NaTC concentration in buffer pH 5 acetate (FeSSIF), HPMC 0.5 mg/ml and PVP 0.5 mg/ml solutions and (b) pH 6.5 phosphate (FaSSIF), in HPMC 0.5 mg/ml and PVP 0.5 mg/ml solutions (buffer pH 6.5) (filled circles NaTC; filled diamonds NaTC-PVP; open squares NaTC-HPMC; n=3).
DOSY NMR experiments were also performed for the NaTC-polymer-\(d\)-buffer systems. As reported in Figure 7, in the buffer systems, the presence of polymer shows little impact on the \(D\) value of NaTC. When \(D\) is plotted versus reciprocal concentration, the results (data not shown) demonstrate the same nonlinear behavior as discussed above, indicating that, if the concentration of free surfactant remains constant, the size of the aggregates must be increasing. In Figure 7 any break corresponding to a critical aggregation point is less apparent than in both the pH 5 acetate (FeSSIF) and pH 6.5 phosphate (FaSSIF) samples. The gradual decrease of NaTC \(D\) values indicates a progressive increase in degree of aggregation but no clear critical aggregation concentration can be identified. This is in agreement with the trend obtained from the chemical shift measurements.

Both HPMC and PVP are non-ionic polymers, therefore the presence of salts is not expected to impact behavior of the polymers. Nevertheless, previous studies have demonstrated that ions can affect polymer behavior in solution, exerting salting-in and salting-out effects.\cite{55} The effect of salt on NaTC-polymer association is likely to be related to the ionic charge screening effect on the surfactant anions. As the charge screening between NaTC is reduced, the interactions between NaTC molecules will dominate over interactions between NaTC and polymer. Nevertheless, there is still some evidence that at low NaTC concentrations (similar to the physiological NaTC concentration in the gastric region during the transit time of the drug formulations) there is some level of NaTC-polymer interactions, especially with HPMC, which could be driven by hydrophobic interactions. However, as the NaTC concentration approaching the CMC, the micelle population dominates the diffusion and any interactions of NaTC with the polymer are disrupted.
and overtaken by NaTC self-association. The effect of ions on polymer solubility is complex and may result from a number of different types of interaction,[53] but it is interesting to note that in most of the cases reported here ion type seems to have much less effect than simply the presence of ionic materials.
Figure 7. NaTC self-diffusion coefficients as a function of NaTC concentration in HPMC 0.5 mg/ml and PVP 0.5 mg/ml (a) pH 5 acetate (FeSSIF) buffer solutions and HPMC 0.5 mg/ml and
PVP 0.5 mg/ml (b) pH 6.5 phosphate (FaSSIF) buffer solutions (filled circles NaTC in D$_2$O; filled diamonds NaTC/PVP; open squares NaTC/HPMC; n=3).

**Conclusions**

This study investigated the NaTC-HPMC and NaTC-PVP aggregation behaviour under FaSSIF and FeSSIF conditions using a range of NMR techniques. The occurrence of an interaction between hydrophilic polymers and ionic surfactants such as bile salts in aqueous media has widely been discussed in the literature [56,57] and previous study from our group suggested the possible role of such interaction in poorly water-soluble drugs solubilisation. [8] However the existence of interaction in biorelevant fluid used in pharmaceutical product development including simulated gastric and intestinal fluids are still poorly understood. We hypothesized that the ionic strength and pH of the biorelevant media could affect the aggregation between of bile salt and impact on the interactions between bile salt and hydrophilic polymers. [45] Using NaTC as the model bile salt, the present work aimed to test this hypothesis and provide new insights into the aggregation behavior of NaTC and its interaction with typical hydrophilic pharmaceutical polymers in biorelevant media. Although the ion type had minimal effect on the aggregation behavior, the polymer-bile salt interactions were highly influenced by the ionic strength of the media.[58] The results demonstrated the complexity of interactions between the polymer and bile salts in biorelevant conditions. In particular, obtained data highlighted the formation of either NaTC aggregates or individual micelles growing in size, which are expected to play a role in drug solubilisation in the gut, when the drug is delivered by hydrophilic polymer based dispersions. In particular, NaTC-polymer aggregates are expected to contribute to the drug solubilisation under
FaSSIF condition (before meals), when the bile salt concentration is low and NaTC-polymer aggregates formation is favored with respect to NaTC self-association.

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Supporting Information.

$^1$H NMR spectrum of HPMC and PVP, NaTC $^1$H NMR peaks assignment.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interests.

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