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Drug Solubility in Luminal Fluids from Different Regions of the Small and Large Intestine of Humans

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Abstract: The purpose of this work was to study the solubility of two drugs with different physicochemical properties in luminal fluids obtained from various regions of the human gastrointestinal (GI) tract and to determine the most important luminal parameters influencing their solubility. Jejunal fluids were aspirated from healthy volunteers via an oral intubation tube. Ileal and colonic fluids were obtained from patients undergoing GI surgery. Stoma fluids were also retrieved from patients. pH and buffer capacity of all fluids were determined. Saturation solubility of prednisolone (unionisable) and mesalamine (5-aminosalicylic acid) (zwitterionic) was measured. Mean solubility of prednisolone in the different luminal fluids was 0.50 mg/mL (±0.05) and did not vary significantly between the different regions of the GI tract (ANOVA, $p > 0.05$). No correlation between prednisolone solubility and jejunal bile salt content was found. Mesalamine solubility increased down the GI tract: 1.97 (±0.25), 3.26 (±0.08), 6.24 (±1.13) and 7.95 (±0.21) mg/mL in jejunal, ileal, ascending and transverse/descending colonic fluids respectively. Buffer capacity also increased and in one patient was observed to range from 6.4 to 28.6 reaching 44.4 mM/L/pH unit in ileal, ascending and transverse/descending colon fluids respectively. Mesalamine solubility was found to be dependent on both buffer capacity and pH, with buffer capacity being the most important (standardized coefficient $\beta = 0.849$, $p < 0.0001$) compared to pH ($\beta = 0.219$, $p < 0.05$). For drugs delivered as modified release formulations it is important to consider solubility in different regions of the GI tract as significant differences can arise which will ultimately influence drug bioavailability.

Keywords: Biopharmaceutics Classification System (BCS); controlled release; lumen; ileostomy; colostomy; surfactant; mesalazine

1. Introduction

The solubility of a drug in situ is a major determinant of its dissolution rate from a dosage form, and is one of the two factors that are used by the Biopharmaceutics Classification System (BCS). *In vivo* solubility is dependent on the physicochemical properties of the drug and the composition of the dissolution medium it is exposed to. The luminal environment is complex, and the pH, buffer capacity, surfactant concentration, fluid volume and viscosity can vary greatly in different regions of the gastrointestinal (GI) tract.[1,2] Some of these can have significant impact on the solubility of a drug *in vivo*. Bile salts and phospholipids may improve the solubility/dissolution of drugs through wetting which increases the effective surface area and/or through micellar solubilization.[3] It is important to elucidate how the GI

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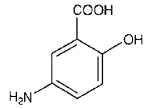
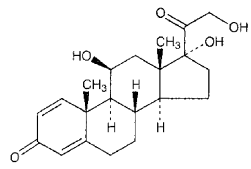
Table 1. Aqueous Solubility, Lipophilicity and Ionization Constants of Mesalamine (5-ASA) and Prednisolone

	5-aminosalicylic acid (5-ASA)	Prednisolone
Structure		
Water solubility[a] (mg/ml)	1.32	0.223
pKa	2.3 and 5.69[b]	N/A
Log P	0.98[c]	1.59[d]

[a] Solubility in water at 37 °C (measured in our laboratory). [b] French and Mauger (1993).[27] [c] National Library of Medicine (2007).[28] [d] Machatha and Yalkowsky (2005).[29]

luminal environment changes along the GI tract, particularly for modified release formulations, as this will have a bearing on drug bioavailability.

There are relatively few studies in the literature on the solubility of drugs in duodenal and jejunal human fluids.[4−6] To our knowledge, however, no attention has been devoted to determining the solubility of drugs in ileal and colonic fluids. It is therefore the objective of this study to elucidate how the solubility of drugs changes in luminal fluids from different regions of the GI tract. Here we investigate how the solubility of two model drugs changes along the GI tract in jejunal, ileal, ileostomy and colonic fluids (Table 1). 5-Aminosalicylic acid (5-ASA, mesalamine) and prednisolone were selected as the model drugs as they are predominantly administered as enteric, modified release formulations. In the case of 5-ASA formulations, the pH-trigger for drug release is typically pH 6−7, for targeting of the distal gut. Both drugs are also administered rectally. It is therefore of relevance to determine their solubility in the small and large intestine. pH and buffer capacity of luminal fluids from the different GI regions are characterized here, in addition to bile salt measurements in jejunal fluids. The relative contribution of these physiological variables in determining solubility of two model drugs is assessed.

2. Materials

Mesalazine (mesalamine, 5-ASA) > 99% purity was obtained from Sigma Aldrich Chemicals (Poole, U.K.). Micronized prednisolone was obtained from Sanofi-Aventis (Romainville, France). All salts to prepare the buffers were of analytical grade and purchased from VWR Chemicals Ltd., Poole, U.K. Sodium taurocholate, 95% pure, batch # 115K1109, was purchased from Sigma-Aldrich Chemicals (Poole, U.K.). Egg phosphatidylcholine (Lipoid E PC, >99% pure), batch # 105038-2/908, was a gift from Lipoid GmbH (Ludwigshafen, Germany). Methylene chloride (dichloromethane), analytical grade, was purchased from Fisher Scientific, Loughborough, U.K. Solvents used in HPLC were water, methanol, acetonitrile and peroxide-free tetrahydrofuran. All were of HPLC grade and purchased from Fisher Scientific, Loughborough, U.K.

3. Methods

3.1. Jejunal Fluid Collection. Jejunal fluids were aspirated from healthy, fasted volunteers via an oral intubation tube (Loc-I-Gut, Synectics Medical, Sweden). The tube is 175 cm long with an external diameter of 5.3 mm. It is a multichannel polyvinyl tube with two inflatable balloons 10 cm apart and a tungsten weight at the tip.[4] The position of the tube was checked fluoroscopically,[7] and once the desired location was reached, only the lower balloon was inflated with 25 to 30 mL of air to prevent fluid from passing down the gastrointestinal tract, thus achieving complete sampling of jejunal fluids. Fluids were collected from the jejunum by continuous vacuum drainage. A separate tube was positioned in the stomach to drain gastric fluid to prevent nausea. The jejunal fluid aspirated was collected on ice, pooled and stored at −70 °C. Four different batches of jejunal fluid were studied, and each batch was pooled from 15 different volunteers. Ethics approval was granted for the fluid sampling.

3.2. Ileal and Colonic Fluid Collection. Fluid was collected from the small bowel and excised sections of the large intestine in patients undergoing hemicolectomy. Regions of the bowel from which fluid was drained were not inflamed. Luminal fluid was collected from the ascending colon of one patient suffering from sigmoid colon carcinoma. In another patient suffering from polyposis, fluid was collected from both the ascending and transverse/descending colon; ileal fluid was also aspirated during the surgical procedure. These patients were fasting overnight and on polyethylene glycol bowel cleansing solutions. The collected fluids were divided into portions and stored in tightly sealed containers.

3.3. Ileostomy and Colostomy Fluid Collection. Fluids were collected from stoma bags in patients who were undergoing a routine change of their stoma bag or who were undergoing surgery for reversal of stoma. Ileostomy fluids

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were collected from five different patients and colostomy fluid only from one patient. Patients did not undergo any dietary restrictions prior to changing of the stoma bag. These fluids were analyzed individually and not pooled.

3.4. pH and Buffer Capacity Measurements. pH values were measured using a pocket sized pH meter (MiniLab IQ125, IQ scientific, Carlsbad, CA) which only required a drop of fluid. Measurements were performed immediately on fluid collection where possible. Buffer capacity in all media was measured at a pH change of 0.5 unit. This was performed by the addition of accurate amounts of HCl to 100 μL of GI luminal fluids and measuring the pH change. Buffer capacity measurements were only performed in one direction due to the limited fluid availability. Furthermore, the anionic species of 5-ASA predominates at pH values above 6. Therefore, it is more relevant to measure buffer capacity by titrating with acid. Measurements were performed at least in triplicate for each sample and prior to addition of drug. Buffer capacity measurements performed immediately on fluid collection and after storage were compared, and no significant changes were observed.

3.5. Characterization of Bile Salt Levels in Jejunal Fluids. Jejunal fluids were used as a representation to determine if a correlation exists between the solubility of the model drugs and intestinal fluid bile salt levels. A solid phase extraction procedure using C18 column for the separation of bile salts was adopted. The levels were subsequently analyzed by reverse phase HPLC with evaporative light-scattering detectors. High recoveries (>90%) with low coefficient of variation (<5%) were observed. For methodology details refer to Persson et al. 2007.[8]

3.6. Physiological Solubility Media. To explore the influence of biological surfactants on the solubility of prednisolone and 5-ASA, pH 6.8 phosphate buffer (28.69 mM NaH_2PO_4 and 106 mM NaCl) with the bile salt sodium taurocholate (NaTC) and phospholipid lecithin was prepared.[9] These physiological media typically contain 3 mM of NaTC and 0.75 mM lecithin to simulate jejunal fluids. Here we also explore different concentrations of these surfactants and their influence on drug solubility. High concentrations (7.5 mM NaTC, 1.875 mM lecithin) were used to represent bile salt levels in the upper ileum as this is where they are highest before enterohepatic recycling commences.[10] Intermediate levels (3 mM NaTC, 0.75 mM lecithin) represent the jejunum, and lowest levels (1.2 mM NaTC, 0.3 mM lecithin) mimic the lower ileum as this is the site at which bile acid absorption arises.

3.7. Solubility Measurements. Solubility measurements were performed in centrifuged intestinal luminal fluids and stoma fluids. Centrifugation at 10,000 rpm for 10 min was performed to remove particulate matter and thus reduce sources of variation between the different fluids. An excess of drug was added to microcentrifuge tubes (Eppendorf AG, Hamburg, Germany) containing 0.2 mL of the different media and placed in a shaking water bath at 37 °C and speed of 400 shakes per min. In the initial preliminary experiments equilibration was found to be achieved within five hours; therefore, following five hours the saturated drug solutions were centrifuged at 13,000 rpm for 10 min. The supernatant was transferred to microcentrifuge filter tubes (polysulfone 0.2 μm filters) (VectaSpin Micro, Whatman, England) and centrifuged at 10,000 rpm for 10 min (Centrifuge 5415D, Eppendorf AG, Hamburg, Germany). Aliquots of the resultant filtrate were removed and subjected to the appropriate dilution with mobile phase. Solubility was determined using HPLC-UV ($n = 3$ from each sample). Spiking the different media with known concentrations of drug showed a recovery beween 95 to 100%. Thus the solubility results were not compromised by degradation in biological fluids or binding to the filters.

3.8. Analytical Methods for Drug Quantification. Drug concentrations were determined by reversed-phase HPLC analysis with UV detection. An aliquot of the drug solution was injected into an integrated HP 1050 series HPLC system comprising a HP1050 autosampler, a HP 1050 pump and a HP 1050 multiple wavelength detector system, a UV−vis spectrophotometric detector. The detector was interfaced with a pc with PC/Chrom + Software (H & A Scientific Inc., Greenville, NC). Separation of 5-ASA was achieved with a C_{18} column (LiChrospher100, Merck, Darmstadt, Germany) at 40 °C. The mobile phase used for analysis consisted of 5% methanol and 95% water with 0.05% trifluoroacetic acid (TFA), and the flow rate was 1 mL/min. The injection volume was 20 μL and detection wavelength 228 nm. Drug retention time was 4.4 min. The separation of prednisolone was achieved with a modification of the HPLC−UV method of prednisone proposed by the United States Pharmacopoeia (2006).[11] The analytical column was a C_8 (5 μm) (Water Symmetry, Waters, MA), maintained at 40 °C during the separation. The mobile phases used for the analysis consisted of 68.8% water, 25% peroxide-free tetrahydrofuran and 6.2% methanol, and the flow rate was 1 mL/min. The injection volume was 20 μL and detection wavelength 254 nm. Drug retention time was 9.9 min.

Calibration curves were prepared in the corresponding mobile phase as the saturated drug solutions were subjected to at least 30-fold dilutions with mobile phase. A comparison of the 5-ASA and prednisolone peak areas for known drug concentrations in GI fluids with those in the mobile phase was made. The relative standard deviations (RSD) ranged

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from 0.033 to 2.6%. The coefficient of variation for prednisolone and 5-ASA was in the range of 1.76% and 3.96% respectively.

4. Results and Discussion

4.1. pH and Buffer Capacity of Jejunal and Ileostomy Fluids. The mean pH and buffer capacity (±SD) of the four different batches of jejunal fluids were measured to be 7.08 ± 0.54 and 3.23 ± 1.26 mM/L/ΔpH unit respectively. These are in agreement with the values reported in the literature whereby a pH of 7.1 ± 0.6 and a buffer capacity of 2.4 mM/L/ΔpH were reported for jejunal fluids aspirated from fasted healthy individuals.[4,12]

The pH and buffer capacity of luminal fluids from different regions of the GI tract of one patient suffering from polyposis were evaluated. The pH changed from 7.75 to 8.5 and down to 8.3 in the ileum, ascending colon and transverse/descending colon respectively. These colonic pH values are higher than those typically recorded.[13,14] Nevertheless, there is evidence for colonic pH values reaching as high as 8 and 9.0.[15−17] The buffer capacity of luminal fluids also increased significantly down the GI tract. In the same individual, the buffer capacity (mM/L/ΔpH) transition was from 6.4 in the ileum to 28.6 in the ascending colon, reaching 44.4 mM/L/ΔpH in the transverse/descending colon. The high buffer capacity of colonic fluids arises from the presence of short chain fatty acids (SCFA) (predominantly acetate, propionate and butyrate) produced by the breakdown of carbohydrate by anaerobic microflora. These levels have been measured to be 123 ± 12 mmol/kg in the ascending colon and falling progressively to 117 ± 9 and 80 ± 17 mmol/kg in the transverse and descending colon respectively.[18] Despite these decreasing levels of SCFA down the large intestine, their concentrations (mM/L) will in effect be increasing due to the lower proportion of fluid in the luminal contents. Moreover, the absorption of SCFA is linked to the accumulation of bicarbonate in the lumen, which is explained by the presence of an acetate−bicarbonate exchange at the surface of the mucosal cells.[19]

Table 2. Summary of pH, Buffer Capacity, Bile Salt Levels, 5-ASA and Prednisolone Solubility (mg/mL) in Luminal Fluids and Stoma Fluids from Different Regions of the Gastrointestinal Tract

			solubility (mg/mL)	
fluid type	pH	buffer capacity (mM/L/ΔpH)	5-ASA	prednisolone
jejunal	6.3	1.7	1.6	0.49
jejunal	7.1	4.5	2.1	0.52
jejunal	7.4	2.7	2	0.51
jejunal	7.5	4	2.2	0.54
ileal	7.8	6.4	3.3	0.46
ascending colon	7.5	35.6	5.4	0.55
ascending colon	8.5	28.6	7.0	0.41
transverse/ descending colon	8.3	44.4	7.9	0.54
ileostomy	5.8	11.1	2.9	0.50
ileostomy	7.1	16.7	4.2	0.52
ileostomy	7.6	14.4	3.5	0.56
ileostomy	8.0	53.6	8.8	0.36
ileostomy	7.7	18.7	4.5	0.41
colostomy	6.4	20	3.6	0.53

The mean pH and buffer capacity (±SD) of ileostomy fluid were measured to be 7.24 ± 0.87 and 22.9 ± 17.39 mM/L/ΔpH respectively (Table 2), while the pH and buffer capacity of colostomy fluids were measured to be 6.4 and 20 mM/L/pH unit respectively. These pH values are lower than that for ileal and colonic fluids respectively; this is partially because these are fluids from individuals who are on their normal diet and not fasted.[20] It has been shown that luminal pH is lower postprandialy compared to the fasted state in the small intestine[21] and the colon.[17] Another major difference between ileal and ileostomy fluids is that about 1.5 L of fluid passes through the ileocecal valve each day, yet average ileostomy contents are less than a third of this.[20,22] Ileostomy fluids would therefore be expected to be more concentrated, another contributing factor to their higher buffer capacity.

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4.2. Solubility Changes of 5-Aminosalicylic Acid down the Small and Large Intestine. Mean 5-ASA solubility in fasted jejunal fluids was measured to be 1.97 ± 0.25 mg/mL. Solubility was however statistically different in each batch of pooled fluid (ANOVA, $p < 0.05$). 5-ASA solubility in ileostomy fluid was 2.3-fold higher compared to jejunal fluid; the mean was 4.78 ± 2.33 mg/mL (Table 2). Again the solubility difference was statistically significant between ileostomy fluids obtained from different patients (ANOVA, $p < 0.05$). This large variability is not surprising considering that these patients are not in the fasted state and all have different dietary intakes. Moreover, patients have stomas for different reasons, and depending on the region of the gut with impaired function, this will affect the absorption and secretion of ions, bile salts and fatty acids, all of which will influence the solubility of ionizable drugs.

In luminal fluids from the same individual (patient with polyposis), solubility changes by 2.4-fold from the ileum to the transverse/descending colon. Table 2 is a summary of pH, buffer capacity and 5-ASA solubility in luminal fluids and stoma fluids from different regions of the GI tract. The trends in 5-ASA solubility observed are likely to be representative of in vivo results; the absolute values, however, may potentially be influenced by the dietary and health status of the individual as these affect the luminal electrolyte balance.

4.3. Relative Importance of pH and Buffer Capacity of Human Intestinal Fluids on Solubility of 5-Aminosalicylic Acid. Standard multiple linear regression (SPSS statistics software release 17.0.0, SPSS Inc., Chicago, IL) was used to explore the influence of pH and buffer capacity of intestinal fluids on the solubility of 5-ASA. No violation of the assumption of normality, linearity, multicolinearity and homoscedasticity was found. In this initial analysis, stoma fluids were not included as all other fluids were from fasted volunteers and obtained directly from the GI lumen. Mesalamine solubility was found to be dependent on both buffer capacity and pH, with buffer capacity being the most important (standardized coefficient $\beta = 0.739$, $p < 0.005$) compared to pH ($\beta = 0.324$, $p < 0.05$). Equation 1 obtained by multiple regression enables the prediction of solubility of 5-ASA in the human GI fluids based on knowledge of pH and buffer capacity ($R^2 = 0.97$, $p < 0.0005$).

$$\text{solubility (mg/mL)} = 1.173(\text{pH}) + 0.106(\beta) - 6.609 \quad (1)$$

Including ileostomy and colostomy fluids still shows buffer capacity to have a predominant affect (standardized coefficient $\beta = 0.849$, $p < 0.0001$) on solubility. The influence of pH is also significant (Standardised coefficient $\beta = 0.219$, $p < 0.05$). The correlation between 5-ASA solubility and either pH or buffer capacity of intestinal fluids is illustrated in Figures 1a and 1b respectively.

Measurements of intestinal fluid pH postequilibration with 5-ASA revealed a drop in pH in the range of 0.5 to

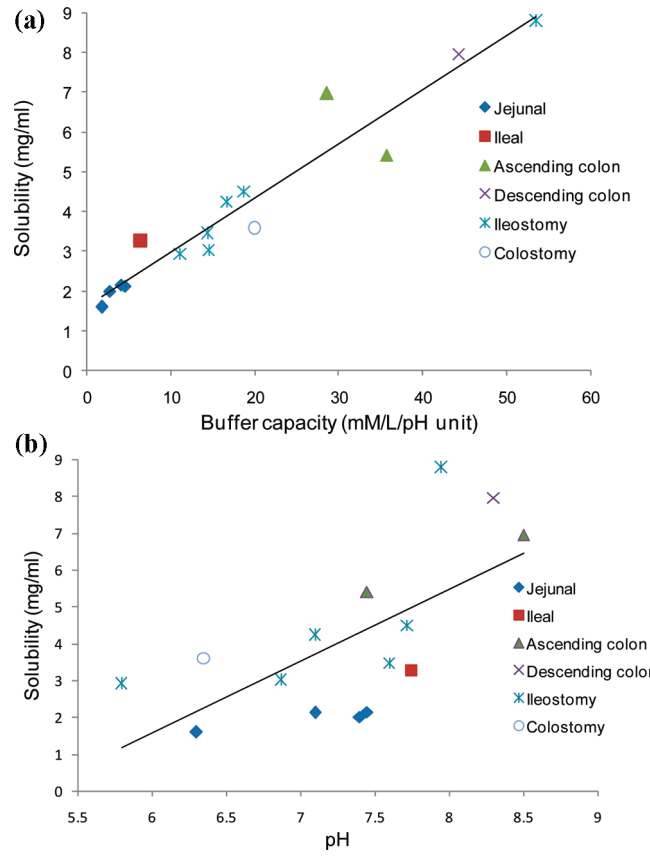

Figure 1. (a) Solubility of 5-ASA in human intestinal luminal and stoma (ileostomy and colostomy) fluids as a function of buffer capacity. (b) Solubility of 5-ASA in human intestinal luminal and stoma (ileostomy and colostomy) fluids as a function of pH.

1 pH unit relative to the starting pH. The magnitude of this drop in pH is dependent on a multitude of factors including the amount of 5-ASA going into solution, the buffer species present and their concentration in the system. This drop in pH may also, however, contribute to influencing the saturation solubility of 5-ASA. The difficulty in predicting drug solubility based on media starting pH alone has been described by Bergstrom et al.[23] They show that the Henderson−Hasselbalch (HH) relationship will only give rough estimates of the pH-dependent solubility of drugs in buffer media. If the compounds display high intrinsic solubility, they are likely to form salts with the buffer counterions. The large impact of the type and amount of counterion on the common ion effect has been reported. Streng et al.[24] illustrated the effect of different acids on the solubility of a weak base. GI luminal fluids contain a vast array of salts and buffer components that are further complicated in the fed state, which may "salt in" 5-ASA and therefore impact on its

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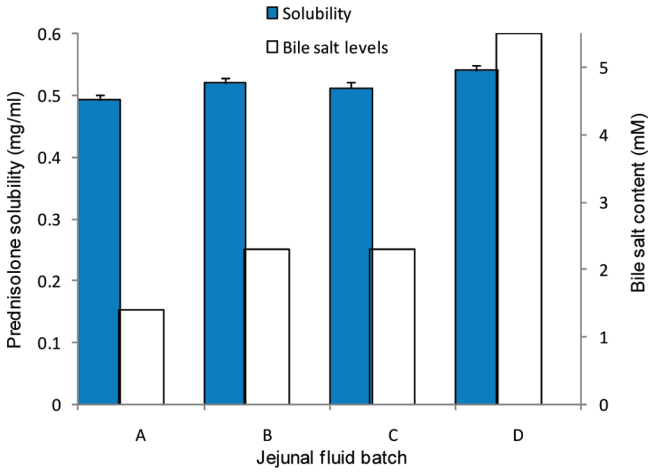

Figure 2. Prednisolone solubility (mean + SD) and bile salt content in different batches of human jejunal fluid.

solubility. These ions and buffer components vary in their presence and concentrations over the entire GI region.[2,18]

4.4. Influence of Bile Salts on Solubility of 5-Aminosalicylic Acid. 5-ASA solubility in pH 6.8 phosphate buffer (with no intestinal surfactants) was measured to be 2.7 ± 0.02 mg/mL and did not significantly change in the presence of the three different concentrations of intestinal surfactants. This lack of solubilization of 5-ASA is likely to be attributable to its hydrophilic nature and repulsion between its anionic charge and that of the mean surfactant at near neutral pH.[25]

4.5. Solubility of Prednisolone in Different Media. The mean solubility of prednisolone in jejunal fluids was measured to be 0.515 mg/mL (±0.021). Interestingly, despite substantial variations in the bile salt levels of the various batches of jejunal fluids, no significant differences in prednisolone solubility were observed (Figure 2). Bile salt content ranged from 1.4 to 5.5 mM in the different batches of jejunal fluid analyzed. Moreover, there were no significant differences in prednisolone solubility measured in jejunal, colonic and ileostomy fluids (Kruskal−Wallis nonparametric analysis, $p < 0.05$) (Table 2). Prednisolone solubility in colonic and ileostomy fluids was found to be 0.499 (±0.077) and 0.471 (±0.082) mg/mL respectively. The solubility attained in ileal fluids (0.462 mg/mL) is similar to these values. In healthy subjects, bile undergoes enterohepatic recycling through absorption from the terminal ileum. Drug solubilization in micelles correlates to a compound's lipohilicity;[3] this may explain the minimal variation of prednisolone solubility in fluids from different regions of the GI tract. Interestingly, however, significant changes are observed in buffer media with prednisolone solubility increasing by 14.4% and 31.9% in media with 3 mM and 7.5 mM of NaTC respectively in comparison

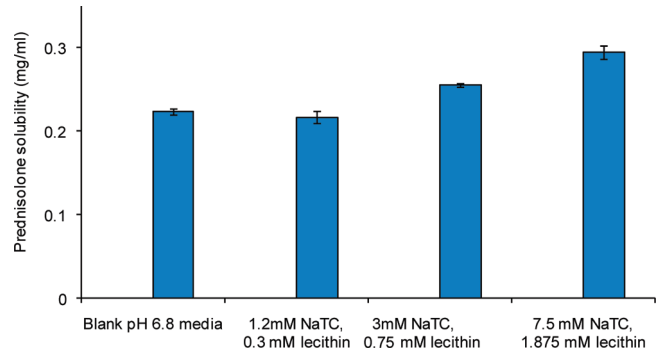

Figure 3. Solubility of prednisolone (mean ± SD) in pH 6.8 media with different concentrations of the surfactants sodium taurocholate (NaTC) and lecithin.

to blank media (ANOVA, $p < 0.05$) (Figure 3). In a previous study by Pedersen et al.[26] a similar finding was observed whereby hydrocortisone solubility in mid-jejunal fluids was not found to correlate to bile salt content; however, a correlation was seen in simulated intestinal fluids. It may be that in GI fluids other more important intraluminal parameters come into play in determining drug solubility such as surface tension, protein and lipid levels.

5. Conclusions

Buffer capacity increases down the gastrointestinal (GI) tract due to the complex ion exchange mechanisms that take place. The solubility of 5-ASA was found to significantly change down the GI tract; with buffer capacity being the most important determinant of its solubility. The solubility of prednisolone, however, was not found to change in different regions of the GI tract. For drugs delivered as modified release formulations it is important to consider solubility in different regions of the gut as significant differences can arise which will ultimately influence drug bioavailability. Stoma fluids provide useful information on drug solubility and are more reflective of *in vivo* than compendia buffers that are typically used. However, due to their variability and the multitude of factors they are influenced by, they are not fully representative of GI luminal fluids.

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