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Article

Impact of Permeation Enhancers on the Release of Insulin from Tablets in Biorelevant Media

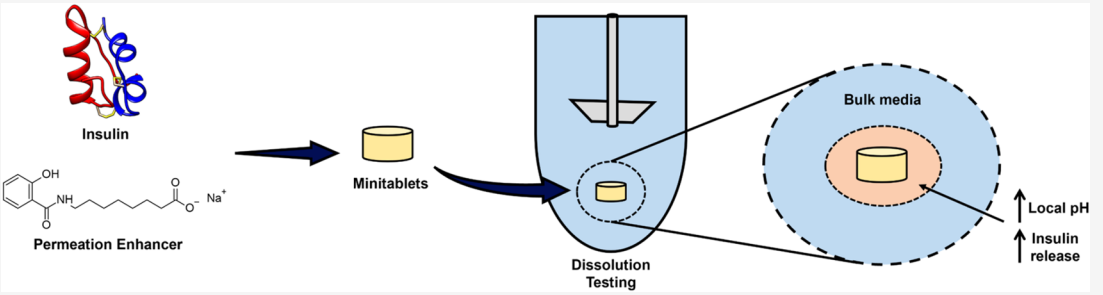
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ABSTRACT: The use of chemical permeation enhancers (PEs) to improve the permeation of peptides across gastric and intestinal epithelia has proven an effective strategy in the development of oral dosage forms of peptides. However, there remains a poor understanding of how the presence of PEs impacts the dissolution characteristics of oral formulations containing peptides, nor is it known how the complex composition of biological media can influence their behavior *in vivo*. This investigation sought to examine the effect of two widely studied PEs, sodium caprate (C10) and salcaprozate sodium (SNAC), on the release behavior of a model peptide, insulin, from minitablets in a variety of biorelevant media. First, the equilibrium solubilities of insulin, C10, and SNAC were determined in simulated gastric and intestinal media. Insulin, C10, and SNAC all displayed pH-dependent solubility across a physiologically relevant range of pH conditions. Moreover, at high concentrations, C10 was found to overwhelm the buffer capacity of the simulated media, increasing the pH of fasted state simulated intestinal fluid (FaSSIF) from 6.5 to 9.0, fed state simulated intestinal fluid (FeSSIF) from pH 5.0 to 8.8 and fasted state simulated gastric fluid (FaSSGF) from pH 1.6 to 9.2. Similarly, SNAC caused an increase in the pH of FaSSIF from 6.5 to 7.9, FeSSIF from pH 5.0 to 7.7, and FaSSGF from pH 1.6 to 7.6. Relative to in simulated intestinal media, the solubility of insulin was found to increase significantly in media at pH representative of saturated C10 and SNAC solutions, increasing from 0.1 mg/mL in blank FaSSIF to 14.0 mg/mL in phosphate buffer at pH 7.6 and to 23.7 mg/mL in phosphate buffer at pH 9.2, suggesting that the presence of C10 and SNAC at high concentrations could have a considerable favorable impact on insulin solubility. Furthermore, the release profiles of insulin from minitablets containing C10 and SNAC were investigated in each of the biorelevant media and compared with the release profiles of insulin from blank minitablets in the absence of PEs. Insulin release from the blank minitablets was found to be media dependent, following an apparent solubility trend. Complete release of insulin was observed in simulated gastric media; however, only between 67 and 82% release was observed in the simulated intestinal media. On the other hand, on the addition of C10 and SNAC to the formulation, greater than 90% release was observed across all media investigated. This difference in release behavior was determined to be caused by an increase in pH at the surface of the minitablets due to the presence of high local concentrations of C10 and SNAC, respectively, as confirmed by a change in color of a universal indicator solution. These findings offer a key insight into the influence that C10 and SNAC have on the dissolution characteristics of insulin from an oral dosage form in a variety of simulated gastric and intestinal media.

KEYWORDS: *salcaprozate sodium, fasted state simulated intestinal fluid, permeation enhancers, gastrointestinal tract, permeation enhancers*

1. INTRODUCTION

Since their advent in the 1920s, therapeutic peptides have received considerable attention as potential alternatives to traditional small-molecule therapies in the treatment of oncological, metabolic, and genetic diseases, among others.[1] The structures of peptides are unique and often complex, conferring them with a number of desirable properties, such as

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high target selectivity, high potency, and low inherent toxicity. The preference for oral delivery of pharmaceuticals due to patient acceptance, convenience, and cost-effectiveness has driven extensive innovation in the design of oral delivery systems for peptide therapeutics. However, the structural complexity of peptides means that they are highly susceptible to degradation by digestive enzymes in the gastrointestinal tract (GIT), while their large size and hydrophilicity restricts permeation across the intestinal epithelium, thereby limiting oral bioavailability, typically to below 2%.[2] As a result, the majority of peptides are delivered parenterally via injectable dosage forms, and the widespread adoption of oral dosage forms for the delivery of hydrophilic peptides remains elusive.

A number of strategies have been adopted to improve the oral bioavailability of peptides, including the use of nanoparticles, hydrogels, or ionic liquids.[3−5] However, the clinical and commercial viability of these approaches are yet to be proven. One approach that has gained significant interest for its efficacy and simplicity is the addition of permeation enhancers (PEs) to formulations to improve peptide permeation across the gastric and intestinal epithelia, leading to the recent approval of oral dosage forms of semaglutide and octreotide.[6] There are several types of PEs, such as bile salts, medium chain fatty acids, and chelating agents, which can act on epithelia via a paracellular pathway by opening tight junctions or via a transcellular pathway by increasing the hydrophobicity of peptides or by disrupting the integrity of cell membranes. Two of the most widely studied PEs clinically include sodium caprate (C10) and salcaprozate sodium (SNAC), where SNAC has been used successfully in Rybelsus tablets for the oral delivery of semaglutide. While a considerable amount of research has been conducted on both of these PEs to date, focus has been primarily placed on understanding their efficacies and mechanisms of action.[7] However, there remains a lack of understanding of how the addition of these PEs to oral dosage forms, such as tablets, impacts the release characteristics of peptides from the dosage form, nor is it understood how the complex composition of the gastrointestinal milieu affects this behavior.

C10 and SNAC possess a number of physicochemical properties that are likely to impact the release properties of oral formulations in which they are present. First, at pH values at least 2 pH units above their respective pK_as (approximately 5 for both), C10 and SNAC exhibit high aqueous solubility due to extensive ionization, while at pH values below the pK_a, C10 and SNAC exist mainly in the poorly soluble, free acid form.[8,9] As a result, it is expected that the dissolution behaviors of C10 and SNAC will vary significantly in gastric and intestinal media. Additionally, it has been reported that at high concentrations, such as those observed at the surface of a tablet, SNAC causes an increase in pH of the surrounding environment.[10] However, it is unknown how this shift in pH is impacted by the composition of the surrounding media, nor how the elevated pH at the surface of the tablet impacts the release behavior of the peptide present in the dosage form. The solubilities of peptides vary depending on a number of environmental conditions, including pH, buffer type, and ionic strength.[11] In particular, peptide solubility is impacted by its isoelectric point (pI), the pH at which the peptide possesses a net neutral charge. At the pI, solubility is minimized, while at 1−2 pH units above and below the pI, peptide molecules possess net negative and net positive charges, respectively, causing solubility to increase.[12] Therefore, the solubility and consequently the release profile of a peptide from an oral dosage form may be highly dependent not only on the pH of the media present in the GIT, but also on pH changes which occur due to the presence of a PE such as SNAC or C10.

The purpose of this study was to investigate the impact of two different PEs, C10 and SNAC, on the release properties of minitablets in a variety of biorelevant media, including simulated gastric and intestinal fluids, using human insulin as a model peptide. First, the equilibrium solubilities of insulin, C10, and SNAC were determined in different biorelevant media, and the effects of high concentrations of C10 and SNAC on the pH of the media were evaluated. Insulin minitablets containing a high proportion of either C10 or SNAC were prepared by direct compression. The dissolution performance of the minitablets was assessed in each of the biorelevant media and compared to minitablets prepared in the absence of C10 and SNAC. To ascertain the impact of C10 and SNAC on the pH at the surface of the minitablets, a drop of universal indicator solution was applied to the C10, SNAC, and blank minitablets, and a color change was used to indicate differences in surface pH.

2. EXPERIMENTAL SECTION

2.1. Chemicals. The recombinant human insulin used in this investigation was procured from Merck KGaA (Germany). Two different permeation enhancers, C10 and SNAC, were investigated and acquired from Merck KGaA (Germany) and BOC Sciences (New York), respectively. Microcrystalline cellulose (MCC), Avicel PH102 grade, and polyvinylpyrrolidone (PVP K30), Kollidon 30 LP grade, used for tableting, were purchased from FMC Biopolymer (Ireland). Fasted state simulated intestinal fluid (FaSSIF), fed state simulated intestinal fluid (FeSSIF), and fasted state simulated gastric fluid (FaSSGF) were prepared using 3F powder procured from Biorelevant (United Kingdom). The compositions of the biorelevant media are given in Table 1.

Table 1. Compositions of FaSSIF, FeSSIF, and FaSSGF

components	FaSSIF	FeSSIF	FaSSGF
sodium taurocholate (NaTC)	3 mM	15 mM	0.08 mM
lecithin	0.75 mM	3.75 mM	0.02 mM
sodium dihydrogen phosphate (NaH_2PO_4)	29 mM	-	-
sodium chloride (NaCl)	106 mM	203 mM	34.2 mM
hydrochloric acid (HCl)	-	-	as necessary for pH adjustment
acetic acid (CH_3COOH)	-	144 mM	-
sodium hydroxide (NaOH)	10.5 mM	50.5 mM	-
pH	6.5	5.0	1.6
buffer capacity (Osm/L)	270	670	120

2.2. Solubility Study. The saturation solubilities of insulin, SNAC, and C10 were evaluated in FaSSIF, FeSSIF, FaSSGF, and their blank equivalents, that is, media in the absence of sodium taurocholate and lecithin. Excess amounts of insulin, C10, and SNAC were added individually to 1 mL of media in 2 mL Eppendorf tubes. Eppendorf tubes were shaken at 350 rpm for 24 h on a Thermomixer Comfort (Eppendorf, Germany) at 37 °C. Prior to analysis, samples were centrifuged using a Mikro 120 Centrifuge (Hettich Lab, Germany) at 18626 RCF for 2 min. All samples were prepared in triplicate.

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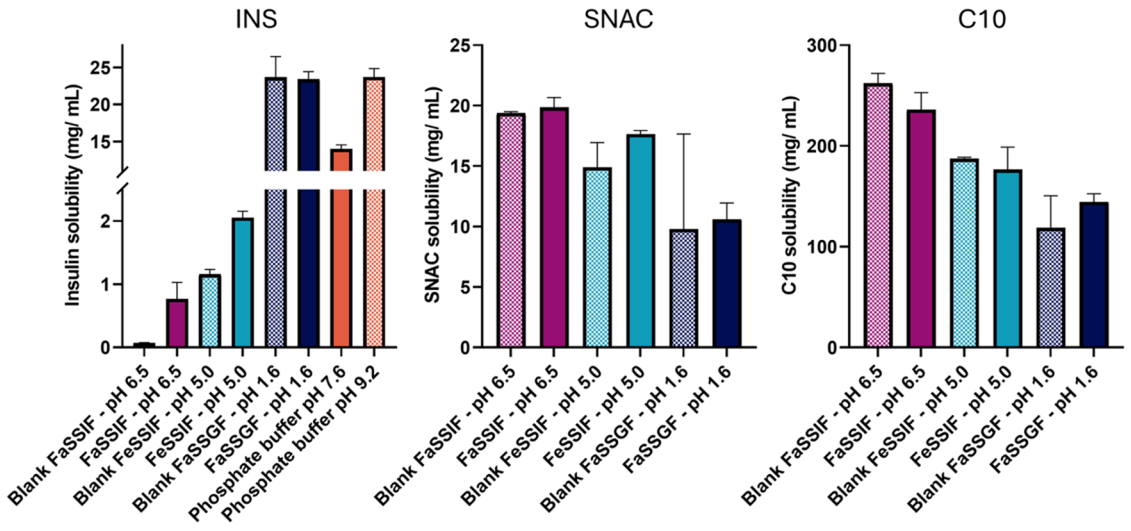


Figure 1. Saturation solubility of insulin, C10, and SNAC in FaSSIF, FeSSIF, FaSSGF, and their blank equivalents after shaking for 24 h at 350 rpm and 37 °C. Insulin saturation solubility was also investigated in 0.1 M phosphate buffer, isotonic with FaSSIF, at pH 7.6 and pH 9.2. Error bars represent standard deviation, $n = 3$.

2.3. Minitablet Preparation. Two different minitablet formulations were prepared in this investigation, composed of 72% PE (C10 or SNAC), 20% MCC, 5% PVP K30, and 3% insulin, all by weight. These formulations were designed to be similar to the commercial Rybelsus formulation in terms of PE content and excipient selection. Rybelsus consists of 75% w/w SNAC and variable amounts of semaglutide depending on the desired dose. Additionally, Rybelsus tablets contain MCC and PVP, although the specific proportions of these excipients are not disclosed.[13] A blank formulation was also prepared with 92% MCC, 5% PVP K30, and 3% insulin. The formulation components were blended in a pestle and mortar. Direct compression was performed manually using a Fette E1 tablet press with 4 mm tooling (I Holland−United Kingdom), with tablet weights of 22 ± 3 mg and tablet hardness values >1 MPa achieved for all formulations.

2.4. Dissolution Testing. Dissolution was performed using a Distek 2100B system (Distek, New Jersey) fitted with 100 mL vessels and paddles (Dissolution Accessories, The Netherlands). A total dissolution volume of 40 mL and a paddle speed of 75 rpm were used. 350 μL samples were taken over time intervals of 1, 5, 10, 15, 30, 45, 60, and 120 min, with an equivalent volume of medium being replaced after sampling. Release rates of insulin from all minitablets were assessed in FaSSIF, FeSSIF, FaSSGF, and their blank equivalents, while the blank minitablets were also tested in 0.1 M phosphate buffer, pH 7.6, isotonic with FaSSIF. Prior to analysis, samples were centrifuged using a Mikro 120 Centrifuge (Hettich Lab, Germany) at 18626 RCF for 2 min. All samples were prepared in triplicate.

2.5. Quantitation. Quantitation was performed using an AdvanceBio SEC 130Å, 2.7 μm, 7.8mm × 300 mm (Agilent Technologies, California) column. An isocratic elution method was used, where the mobile phase consisted of L-arginine (1 g/L), acetonitrile (ACN), and 0.15 M phosphate buffer (65/20/15%, all given as % v/v), adjusted to pH 7.5 with 1 M hydrochloric acid (HCl). An injection volume of 10 μL and detection wavelengths of 210 and 280 nm were used. The total runtime was 15 min. All samples were analyzed in triplicate. OriginPro 2022b and GraphPad Prism 8.4.3 were used for data analysis and graphical representation of results.

2.6. pH Measurements. The pH of saturated solutions of insulin, C10, and SNAC in all media investigated was measured using a pH probe. Subsequently, the surface pH of the minitablets was assessed by adding a drop of universal indicator solution (Merck, Germany) to each of the blank, C10, and SNAC minitablets, and images were captured using a Canon EOS 1500D digital camera (Canon, Japan).

3. RESULTS

3.1. Solubility Study. As the pH and composition of gastric and intestinal media vary widely, it is important to understand the solubility differences of insulin, C10, and SNAC in biorelevant media to determine how their behavior may be affected in different regions of the GIT. As such, in this investigation, the saturation solubility of each solid was determined in FaSSIF, FeSSIF, FaSSGF, and their blank equivalents, *i.e.*, in the absence of sodium taurocholate and lecithin. The solubility values are given in Figure 1.

Insulin exhibited the highest solubility in gastric media, independent of the presence of sodium taurocholate or lecithin. The lowest solubility was observed in blank FaSSIF at 0.1 mg/mL, while this was observed to increase by a factor of 8 to 0.8 mg/mL in FaSSIF, suggesting that the solubility of insulin was enhanced by the presence of sodium taurocholate and lecithin in this medium. Similarly, the solubility of insulin approximately doubled from 1.2 mg/mL in blank FeSSIF to 2.1 mg/mL in FeSSIF, indicating that the presence of sodium taurocholate and lecithin also had an impact on insulin solubility in this medium. It is interesting to note that according to proximity to the pI of insulin, which is at approximately pH 5.3, the solubility of insulin was expected to be highest in gastric media, which was indeed observed.[14] However, it would also be expected that insulin solubility would be lower in FeSSIF (pH 5.0) than in FaSSIF (pH 6.5), but the converse was observed. Given that FaSSIF is composed of a NaH_2PO_4−NaOH buffer system, while FeSSIF is buffered by CH_3COOH−NaOH, it is likely that the unexpected behavior observed was due to a specific ion effect, as NaH_2PO_4 has been observed to have a stronger salting-out effect on proteins than CH_3COOH.[15] Additionally, solubility

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may have been impacted by the NaCl concentration, which is twice as high in FeSSIF as compared to FaSSIF.

The solubilities of C10 and SNAC were also observed to be pH dependent, although the presence of sodium taurocholate and lecithin did not appear to impact the observed solubility. This suggests that pH dominates PE solubility, rather than the mixed micelles present in the simulated media. Solubilities of both were highest in FaSSIF, which has a pH above the respective pK_as of C10 and SNAC, approximately 5 for each, while at very low pH in gastric media, the solubilities were lowest and more variable, particularly in the case of SNAC. Interestingly, in the presence of excess PE material, there was a significant increase in the pH of all media investigated, with C10 increasing the pH to between 8.8 and 9.2, while SNAC caused an increase in pH to between 7.6 and 7.9. Insulin, on the other hand, did not appear to impact media pH. This can be seen clearly in Figure 2, which shows the pH of solutions

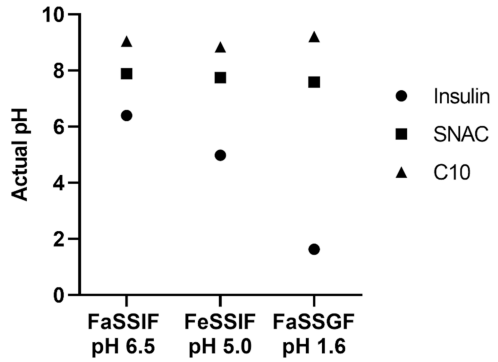


Figure 2. pH of saturated solutions of insulin, C10, and SNAC in FaSSIF, FeSSIF, and FaSSGF after shaking for 24 h at 350 rpm and 37 °C. Error bars represent standard deviation, $n = 3$.

after the addition of excess insulin, C10, and SNAC material. Similar pH values were obtained in both the biorelevant and blank media, and as such, only the pH values of the biorelevant media are shown in Figure 2. These data confirm the previous reports that SNAC elevated the pH of simulated gastric media from acidic to neutral, while also confirming that C10 exhibits similar behavior, although C10 caused a larger increase in pH than did SNAC.[10] Additionally, these results suggested that the ability of C10 and SNAC to alter pH was independent of the composition and initial pH of the media investigated.

To determine if the shift in pH caused by C10 and SNAC influenced the solubility of insulin, excess insulin material was added to 0.1 M phosphate buffer, isotonic with FaSSIF, at pH 7.6 and pH 9.2 to mimic the pH of solutions containing excess SNAC and C10 material, respectively. While outside its buffering range, 0.1 M phosphate buffer was confirmed to be stable at pH 9.2 for at least 48 h. The saturation solubilities at each pH were recorded and graphed in Figure 3. As expected, it appeared that at increasing distance from the pI, represented by a dashed line at pH 5.3 in Figure 3, the solubility of insulin was increased. In comparison to the lowest solubility observed in blank FaSSIF, a 140-fold increase in solubility was observed at pH 7.6, while a 240-fold increase was observed at pH 9.2. Solubility at pH 9.2 was approximately twice that at pH 7.6 and was comparable to that observed in simulated gastric media.

Overall, pH was observed to have a profound impact on the solubility of insulin across all the media investigated, and while

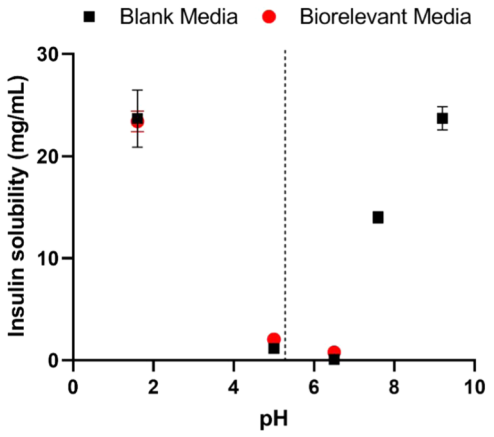


Figure 3. Solubility of insulin as a function of pH. The pI of insulin is represented by a dashed line at pH 5.3. Error bars represent standard deviation, $n = 3$.

the presence of sodium taurocholate and lecithin appeared to have an effect at pH 5.0 and pH 6.5, the proximity of the pH to the pI was the dominant factor affecting solubility. These results suggested that alterations in pH due to the presence of high concentrations of C10 and SNAC may greatly affect the solubility, and therefore, release of insulin in gastric and intestinal media. As such, the release rates of insulin from minitablets containing C10 and SNAC in biorelevant media were investigated.

3.2. Dissolution Testing. To determine the impact of C10 and SNAC on the release rates of insulin, minitablets were prepared and dissolution testing was carried out in a variety of blank and biorelevant media described previously. Minitablets were produced via direct compression using a single-punch tablet press. The formulations were composed of 72% SNAC or C10, 20% MCC, 5% PVP K30, and 3% insulin. Blank minitablets were also prepared in the absence of PEs and were composed of 92% MCC, 5% PVP K30, and 3% insulin.

3.2.1. Blank Minitablets. Dissolution testing was first performed on blank insulin minitablets to determine if media composition had an impact on the release profiles of insulin in the absence of PEs, and results are shown in Figure 4.

Insulin appeared to exhibit solubility-dependent release behavior in both the blank and biorelevant media. Complete release was observed in FaSSGF and blank FaSSGF after 30 min, and while maximum release was also observed after approximately 30 min in FaSSIF, FeSSIF, and their respective blank equivalents, complete release was not achieved in these media. Given that the maximum possible concentrations reached during dissolution were far lower than the saturation solubility of insulin in each of the media, the reason for the incomplete release in the simulated intestinal media was unclear. During dissolution, undissolved material was observed to settle at the bottom of the vessels, and it was suspected that incomplete disintegration of the minitablets may have restricted insulin release. This phenomenon, known as coning, is a well-known artifact of dissolution involving insoluble excipients such as MCC.[16] An attempt to increase the amount of insulin released in blank FaSSIF was performed by increasing the paddle rotation speed until total release was observed. This is shown in Figure 5.

As expected, increasing the paddle speed caused an increase in the total amount of insulin released from the blank minitablets, although the time taken to achieve maximum

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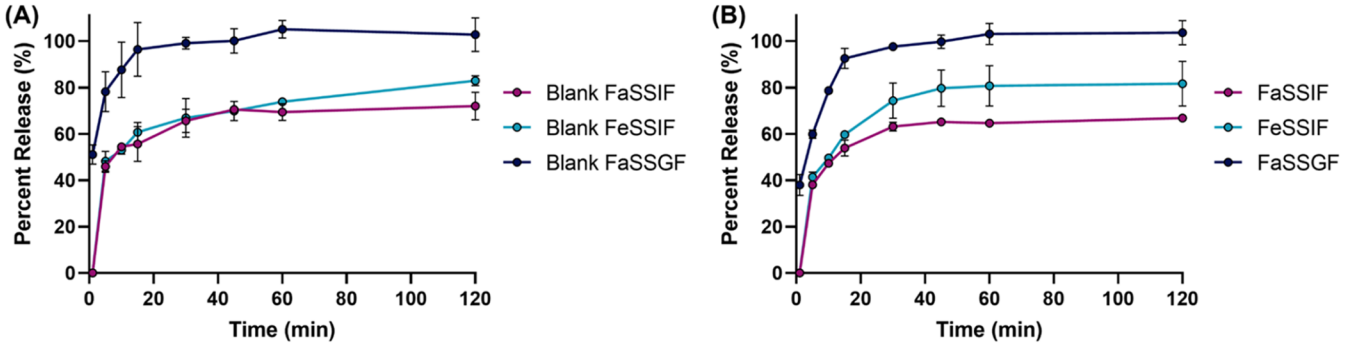


Figure 4. Release profiles of insulin from minitablets composed of 92% MCC, 5% PVP K30, and 3% insulin in (A) blank FaSSIF (purple), blank FeSSIF (light blue), and blank FaSSGF (dark blue), and (B) FaSSIF (purple), FeSSIF (light blue), and FaSSGF (dark blue). Error bars represent standard deviation, $n = 3$.

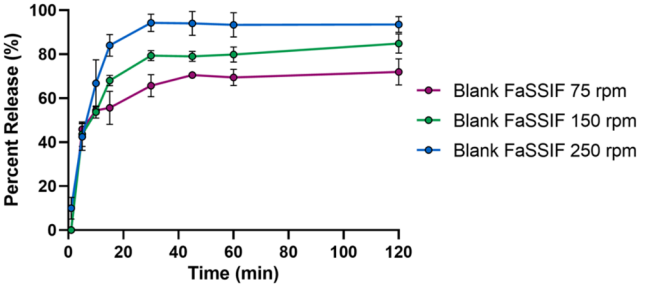


Figure 5. Release profiles of insulin from blank minitablets in blank FaSSIF at paddle rotation speeds of 75 rpm (purple), 150 rpm (green), and 250 rpm (blue). Error bars represent standard deviation, $n = 3$.

release remained at approximately 30 min for all paddle speeds. While increasing the paddle speed from 75 to 150 rpm caused a notable increase in total insulin released from 72 to 85%, a very high paddle speed of 250 rpm was required to achieve greater than 90% release. These results suggested that at low rotation speeds, instead of being dispersed in the medium, insoluble MCC powder instead settled at the bottom of the dissolution vessels and may have trapped insulin above its saturation solubility, thereby preventing its dissolution into the bulk medium. To test this hypothesis further, the release profiles of insulin from minitablets in 0.1 M phosphate buffer, pH 7.6, were assessed and are shown in Figure 6.

Increasing the pH of the dissolution medium to 7.6 was found to increase the release rate and the total amount of insulin released in comparison to blank FaSSIF, with greater than 90% release being achieved. Given that the solubility of

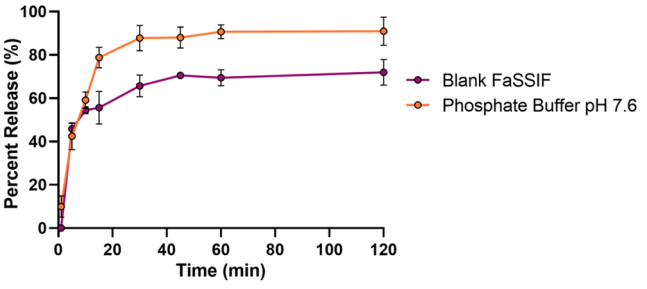


Figure 6. Insulin release from blank minitablets in blank FaSSIF (purple) and 0.1 M phosphate buffer, pH 7.6 (orange). Error bars represent standard deviation, $n = 3$.

insulin was found to be approximately 140-fold higher in phosphate buffer, pH 7.6, than in blank FaSSIF, as shown in Figure 3, it is clear that solubility played an important role in the release behavior of the blank minitablets. This is further supported by the complete release of insulin observed in the simulated gastric media in Figure 4A,B and offers a plausible explanation for the limited release found in simulated intestinal media.

3.2.2. Minitablets Containing Permeation Enhancers. To determine the impact of PEs on the release of insulin from the minitablets, dissolution testing was performed on the minitablets containing C10 and SNAC and the insulin release profiles from each are given in Figure 7.

As seen in Figure 7A,B, the dissolution media did not appear to affect the release of insulin from the C10-containing minitablets, regardless of pH or composition, where near-complete release was observed across all of the media investigated. Similarly, the release of insulin from the SNAC-containing minitablets was observed to be independent of the dissolution media, as shown in Figure 7C,D, and maximum release from both the C10- and SNAC-containing minitablets was observed to occur after approximately 30 min in all media. This is in direct contrast with the blank minitablets, which displayed an apparent solubility-dependent insulin release. Therefore, it is clear that the presence of C10 and SNAC had a significant impact on the release of insulin from the minitablets investigated.

The release of C10 and SNAC from the minitablets was also monitored, and the release profiles are given in Figure 8. C10 was observed to exhibit rapid and complete release in the simulated intestinal media after 15 min; however, no C10 was detectable from the dissolution samples in blank FaSSGF and FaSSGF. On visual inspection, the vessels appeared turbid during dissolution in the simulated gastric media, while in the simulated intestinal media they appeared clear. It was suspected that at low pH, C10 was converted to the un-ionized, free acid form, which is known to form an oily precipitate.[17] This precipitate may have been removed during centrifugation and was not detectable. SNAC was observed to display similar release behavior, with maximum release achieved after approximately 15 min and minimal release observed in the simulated gastric media. However, unlike C10, complete release of SNAC was not achieved in any of the media investigated. As complete release of insulin was observed from all of the samples, it is unlikely that poor disintegration prevented the release of SNAC in this case. Instead, it is possible that SNAC also formed a second phase in

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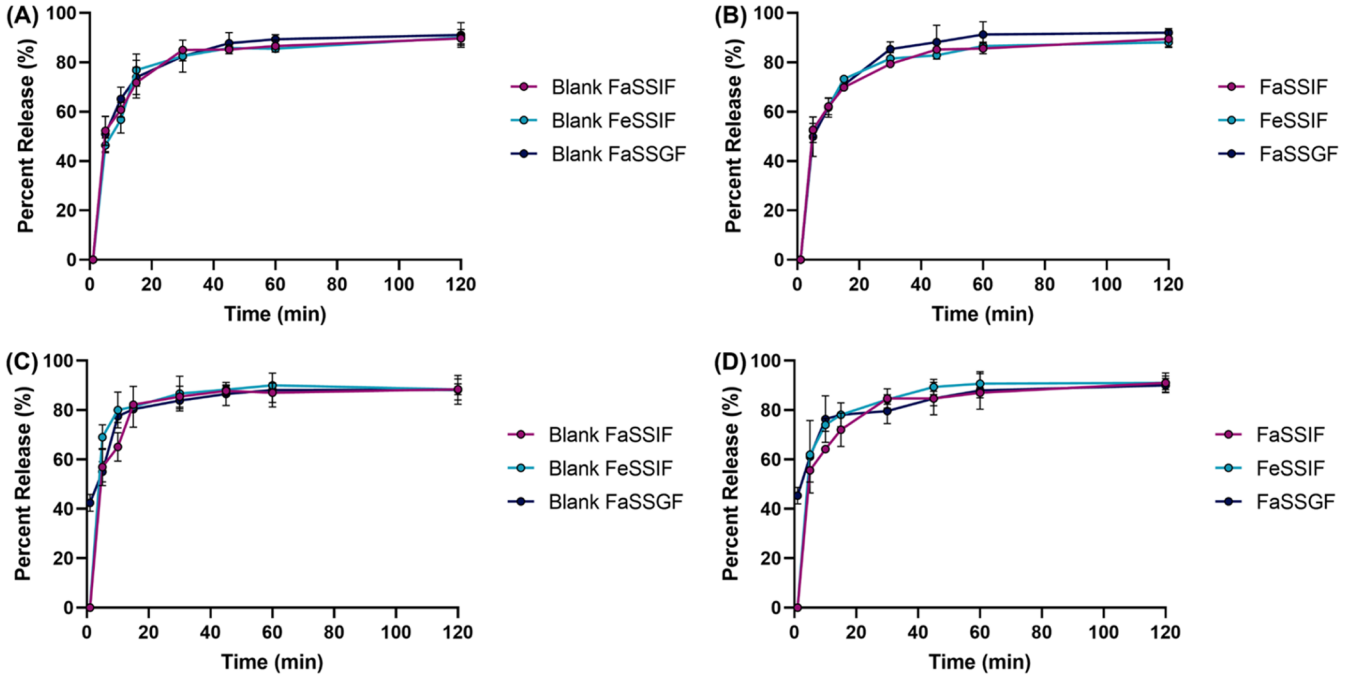


Figure 7. Release profiles of insulin from minitablets composed of 72% C10, 20% MCC, 5% PVP K30, and 3% insulin in (A) blank FaSSIF (purple), blank FeSSIF (light blue), and blank FaSSGF (dark blue), and (B) FaSSIF (purple), FeSSIF (light blue), and FaSSGF (dark blue). The release profiles of insulin from minitablets composed of 72% SNAC, 20% MCC, 5% PVP K30, and 3% insulin are also given in (C) blank FaSSIF (purple), blank FeSSIF (light blue), and blank FaSSGF (dark blue), and (D) FaSSIF (purple), FeSSIF (light blue), and FaSSGF (dark blue). Error bars represent standard deviation, $n = 3$.

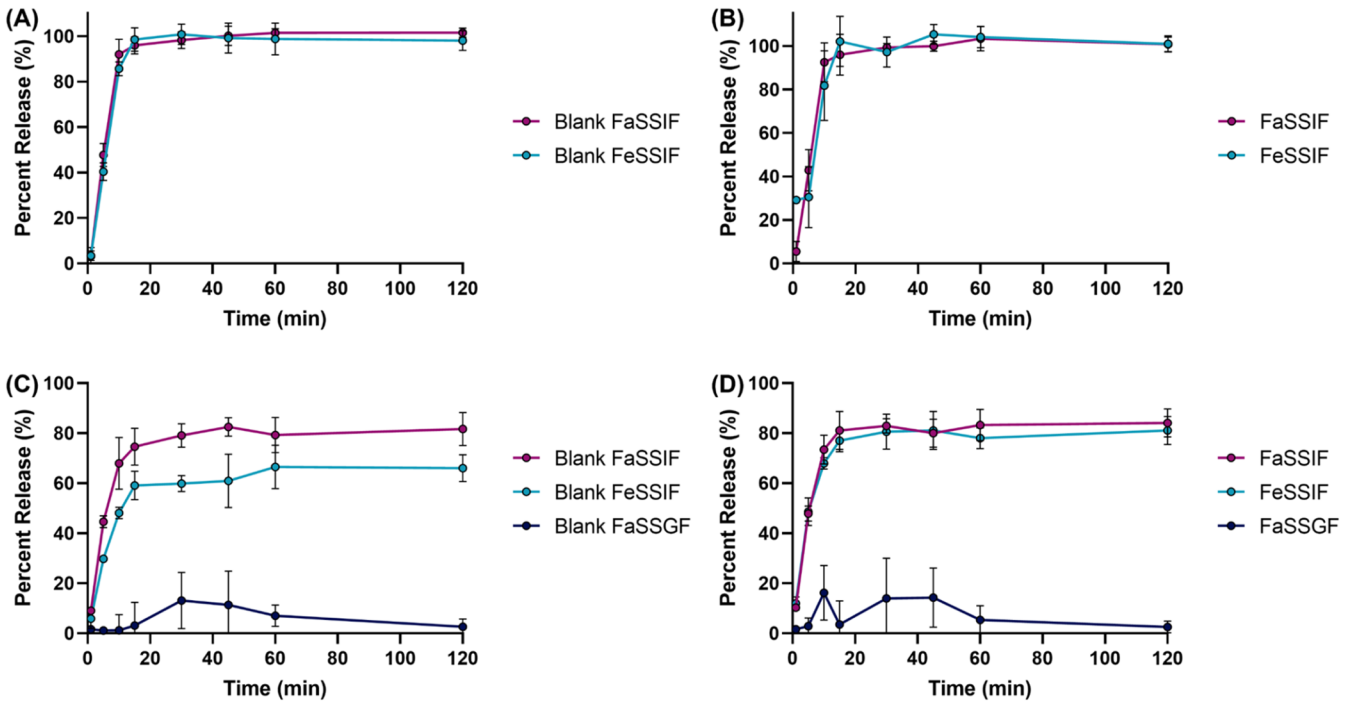


Figure 8. C10 release profiles from minitablets composed of 72% C10, 20% MCC, 5% PVP K30, and 3% insulin in (A) blank FaSSIF (purple) and blank FeSSIF (light blue) and (B) FaSSIF (purple) and FeSSIF (light blue). The release profiles of SNAC from minitablets composed of 72% SNAC, 20% MCC, 5% PVP K30, and 3% insulin are also given in (C) blank FaSSIF (purple), blank FeSSIF (light blue), and blank FaSSGF (dark blue), and (D) FaSSIF (purple), FeSSIF (light blue), and FaSSGF (dark blue). Error bars represent standard deviation, $n = 3$.

the dissolution vessels, which was removed during centrifugation and not detected. To test this, after the final time point was sampled, at the end of dissolution 1 M NaOH was added to each of the vessels containing FaSSIF, FeSSIF and FaSSGF to adjust the pH to above 10, and the amount of C10 and SNAC recovered is given in Figure 9. Complete recovery of C10 and SNAC was achieved in all of the biorelevant media on increasing the pH. In particular, the appearance of the vessels

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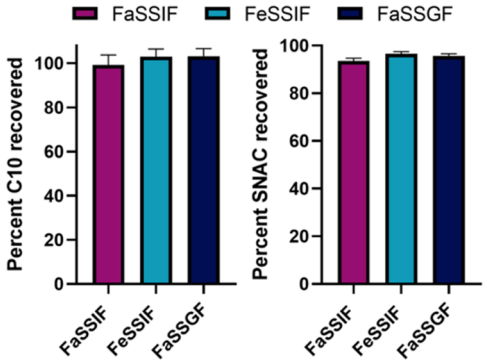


Figure 9. Percent C10 and SNAC recovered after dissolution on adjustment of pH of FaSSIF (purple), FeSSIF (light blue), and FaSSGF (dark blue) to basic. Error bars represent standard deviation, $n = 3$.

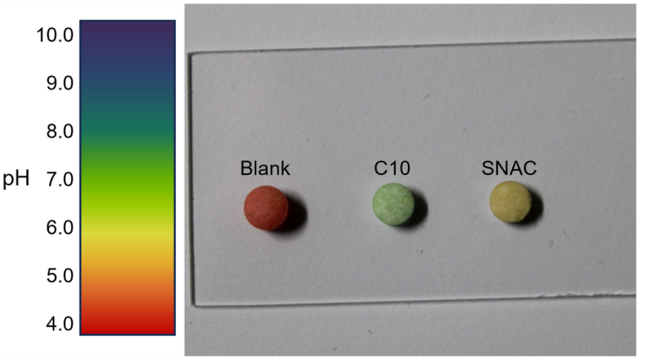


Figure 10. Representative image of blank, C10, and SNAC minitablets after the addition of a single drop of universal indicator solution. Images were captured using a Canon EOS 1500D digital camera. A pH-color scale is also given.

containing the C10 and SNAC minitablets in the simulated gastric media changed from turbid to clear, suggesting that the second phase formed was disrupted at the elevated pH, enabling complete recovery to be achieved. Similarly, the total recovery of SNAC observed on elevating the pH of FaSSIF and FeSSIF is suspected to be due to the disruption of colloidal self-assemblies or precipitated material.

3.2.3. Surface pH Measurements. The differences in insulin release behavior observed in the presence of the PEs in comparison to the blank minitablets were hypothesized to be due to high concentrations of C10 or SNAC present at the surfaces of the minitablets at the onset of dissolution. This would result in the actual pH experienced by insulin in these minitablets being higher than the pH of the surrounding dissolution media, potentially reaching as high as pH 9.2 in the presence of C10 and pH 7.9 in the presence of SNAC. Although it was previously confirmed in Figure 2 that C10 and SNAC solutions altered the pH of biorelevant media during solubility measurements, there was no direct evidence for this occurring during dissolution, as measurement of the bulk media before and after dissolution showed no detectable change in pH. As such, differences in pH at the surfaces of the blank, C10-, and SNAC-containing minitablets were investigated. To do so, a single drop of universal indicator solution was applied to each of the minitablets, and the color change was captured using a digital camera, with results shown in Figure 10.

On application of the universal indicator solution, which is acidic and appears red in color, to the blank minitablets, no color change was observed. On the other hand, the C10 minitablets appeared dark green after the addition of the indicator solution, while the SNAC minitablets appeared pale green. This suggested that the pH values at the surfaces of the C10 and SNAC minitablets were close to pH 8 and pH 7, respectively, much higher than that of the blank minitablets. It is clear, therefore, that the presence of C10 and SNAC considerably altered the surface pH of the minitablets, and this likely accounts for the notable differences in release characteristics of the blank minitablets compared to the C10- and SNAC-containing minitablets.

4. DISCUSSION

PEs have garnered considerable attention from both industry and academia for their potential to improve the oral bioavailability of peptide therapeutics, with the approval of Rybelsus in 2019 being a breakthrough success for this approach. Although their applicability is limited to peptides with a favorable therapeutic index, long half-life and high potency, the use of PEs in oral dosage forms has proven a simple, yet effective strategy to improve the absorption of peptides from the GIT and has advanced the field of oral peptide delivery further than any other approach to date.[9] Popular PEs, such as C10 and SNAC, have been studied extensively, and considerable effort has been placed on elucidating their mechanisms of action, efficacies, and safety profiles *in vivo*.[18−21] However, little focus has been placed on understanding the formulation aspect of this approach, and there remains a dearth of information available in the literature on the release kinetics of PEs and peptides from oral dosage forms and how this behavior is further influenced by the various components of gastric and intestinal media.

A number of important considerations have been identified as vital to obtain optimal absorption during codelivery of peptides and PEs to the GIT. First, it is important that there is temporal synchronicity in the release of the peptide and PE from the dosage form. Several studies have highlighted that the efficacy of a PE *in vivo* is dependent on the duration for which the PE and peptide are colocalized at the gastric and intestinal epithelia above a particular concentration.[22−24] As such, for PEs to exert an enhancing effect, a threshold concentration must be achieved at the site of action. Thereafter, there is a short time frame within which the peptide must also be present at the site of action prior to absorption and/or dilution of the PE in the GI media or transit of the dosage form away from the site of action. Another factor to be considered is the composition of biological media.[25] As described above, the efficacy of a PE is highly dependent on its concentration. Therefore, sequestration of PEs into mixed micellular or vesicular structures with bile salts or phospholipids present in the intestinal fluids may unfavorably alter their activities. Indeed, in an investigation by Roos et al. it was observed that the enhancement action of a surfactant-based PE, sodium dodecyl sulfate (SDS), was reduced in FeSSIF relative to FaSSIF, where it was hypothesized that the greater abundance of micellular structures present in FeSSIF may have reduced the amount of SDS available to interact with the epithelium in the rat single-pass intestinal perfusion model used.[26] On the contrary, in an investigation by Berg et al., the permeation-enhancing effect of C10 was found to be unaffected by the presence of bile salts and phospholipids present in simulated

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intestinal media.[27] Media composition can also have a profound impact on the properties of peptides. For example, peptides can exist in multiple charge states, both positive and negative, depending on the pH of the environment, and as previously noted in the Introduction, the solubility of a peptide is inherently connected to the number of charges it possesses. However, the charge of a peptide may also affect its absorption across the epithelial wall. As tight junctions (TJs) exhibit charge-dependent permeability, peptide charge may influence paracellular transport, where positively charged molecules tend to pass through TJs more easily than do negatively charged molecules.[28,29] Further, diffusion of peptides through the mucus layer has been found to be charge dependent.[30] In addition, it has been reported that two water-soluble peptides, octreotide and desmopressin (to a lesser extent) interacted with micelles in biorelevant media, leading to reduced flux across a membrane.[31] Based on the increase in insulin solubility in both FaSSIF and FeSSIF, insulin also appears to interact with micellar species. The nature of these interactions is unclear at this time. It is clear, therefore, that the release kinetics of the dosage form and the media into which the PEs and peptides are released are critical to maximize peptide bioavailability.

The current investigation sought to bridge this gap in knowledge by investigating the *in vitro* release characteristics of minitablets containing two different PEs, C10 and SNAC, in a number of biorelevant media, using insulin as a model peptide. C10 and SNAC were observed to exert a profound influence over the release of insulin from the minitablets. In the absence of C10 or SNAC, the release of insulin from blank minitablets was found to be media dependent. Complete insulin release was achieved in FaSSGF and blank FaSSGF, however, limited release of insulin was observed to occur in FaSSIF, FeSSIF and their blank equivalents. As shown in Figure 3, the solubility of insulin was highly dependent on medium pH, where the highest solubility was observed at pH 1.6 and pH 9.2, while close to the pI at pH 5.3, solubility was lowest. These results suggested that insulin release from the blank minitablets in the simulated intestinal media was solubility-limited, and this was further confirmed by the increase in insulin release rate in phosphate buffer at pH 7.6 seen in Figure 6. In contrast, complete release of insulin was observed in all media from minitablets containing C10 and SNAC.

There is compelling evidence to suggest that the difference in release behavior noted in the presence of the PEs was the result of a local increase in pH at the surface of the minitablets due to high concentrations of C10 and SNAC at the solid-solution interface. First, in Figure 2 it was observed that the pH of all simulated gastric and intestinal media investigated were elevated in the presence of excess C10 and SNAC material, where C10 caused an increase in pH to between pH 8.8 and 9.2 and SNAC caused an increase to between pH 7.6 and 7.9. SNAC has been previously reported to alter the pH of simulated gastric media; however, similar investigations have not been performed in simulated intestinal media, nor have the effects of C10 on the pH of biorelevant media been studied elsewhere.[10] Although there was no detectable change in the pH of the bulk media after dissolution of the minitablets, the use of a universal indicator solution provided visual confirmation that the surface of the C10 minitablets had an approximate pH of 8 and the surface of the SNAC minitablets had an approximate pH of 7. No color change was observed to occur at the surface of the blank minitablets, as seen in Figure 10. In comparison to the blank minitablets, the pH at the surface of the minitablets containing C10 and SNAC was higher than that of the surrounding media, and this elevated pH likely caused a considerable increase in the saturation solubility of insulin above that observed in the simulated intestinal media, allowing for complete release to be achieved. If correct, this hypothesis proposes that for tablets containing large proportions of C10 or SNAC, the pH experienced by insulin during the initial stage of dissolution would be independent of the dissolution medium and instead its solubility and, therefore, release would be a function of the elevated pH caused by high local concentrations of C10 and SNAC. While the current study has focused on the dissolution of insulin, the above findings may also provide crucial insights into the release of other therapeutic peptides, such as glucagon-like peptide-1 (GLP-1) analogues, from oral dosage forms in which PEs are present. Depending on their composition and size, GLP-1 analogues, such as semaglutide and exenatide, typically possess a pI in the range of 4.0−7.0, and, therefore, exhibit low aqueous solubility at the pH of intestinal fluid.[32−34] This suggests that, similarly to insulin, an increase in pH driven by the presence of C10 or SNAC would have an impact on their solubilities and, therefore, release behavior. However, given that PEs have previously been observed to form peptide-specific noncovalent and hydrophobic ion-pairing interactions, which can reduce the aqueous solubility of peptides, it is not clear without further studies if the behavior observed in this investigation will translate to other clinically relevant peptides.[35,10]

The dissolution of C10 and SNAC from the minitablets was observed to be rapid in simulated intestinal media, with maximum release observed after 15 min. Minimal release of C10 and SNAC was observed in simulated gastric media, and this was suspected to be due to the formation of a second phase, which was removed before analysis.[17] Similarly, the incomplete release of SNAC in simulated intestinal media was proposed to be due to the formation of large colloidal self-assemblies/precipitates in solution, which were likely removed during centrifugation and not detected. On addition of 1 M NaOH and elevation of the media pH to above 10, complete recovery of C10 and SNAC was achieved, suggesting disruption of the vesicular structures/precipitates, which enabled detection. It is proposed, therefore, that rapid and complete release of the PEs did indeed occur in each of the biorelevant media. On the other hand, although it was apparent that the PEs were not freely available in their monomeric/micellar form, which may restrict their efficacies, the release of insulin from the minitablets was not affected by the dissolution media, indicating that insulin release was purely a function of the elevated pH at the minitablet surface during the initial stage of dissolution. Overall, insulin release was, however, found to be slower than that of C10 and SNAC, with maximum concentration being reached after 30 min in all media, rather than in 10 or 20 min as observed for C10 and SNAC, respectively. From a delivery perspective, the staggered release of insulin and PEs from the minitablets in this investigation would likely result in incongruous presentation at the epithelial walls, leading to suboptimal enhancement of insulin permeability.

It is clear from the above discussion that further work is required to investigate the release mechanisms of peptides from oral dosage forms containing C10 and SNAC, and an understanding of the impact of biologically relevant fluids on

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release may prove essential for successful dosage form development. A particular focus of research in this area should be placed on improving the corelease of peptides and PEs, for example, in this case by slowing the dissolution rate of C10 or SNAC or increasing the dissolution rate of insulin, as this may prove beneficial for achieving optimal oral peptide bioavailability. Additionally, an understanding of how the association state of PEs in different biorelevant media impacts their efficacies will be vital for optimizing the design of oral dosage forms, while also informing the need for site-specific delivery.

5. CONCLUSIONS

The use of PEs has become the predominant method of improving the oral bioavailability of peptides. However, while the therapeutic efficacies and safeties of PEs have been well characterized, very little attention has been given to understanding how the addition of PEs impacts the release behavior of peptides from oral dosage forms or how the composition and pH of the surrounding media further influence this. As such, this investigation of the impact of two widely employed PEs, C10 and SNAC, on the release of insulin from minitablets in a variety of biorelevant media revealed potentially noteworthy observations. First, high concentrations of C10 were found to cause an increase in pH to between 8.8 and 9.2, while SNAC was found to elevate the pH to between 7.6 and 7.9, independent of the composition or initial pH of the media. Furthermore, the solubility of insulin was found to increase significantly at pH 7.6 and pH 9.2, representative of saturated solutions of SNAC and C10, respectively, relative to simulated intestinal fluid, suggesting that high local concentrations of C10 and SNAC could have a notable impact on insulin solubility. Second, while minitablets without a PE exhibited medium-dependent insulin release, release of insulin from the C10- and SNAC-containing minitablets was found to be independent of the dissolution media. This difference in release behavior was determined to be the result of an increase in pH at the surface of the minitablets containing C10 and SNAC relative to the blank minitablets, caused by high concentrations of C10 and SNAC, respectively, at the solid-solution interface. Interestingly, while C10 and SNAC did have an impact on the total amount of insulin released from the minitablets, insulin release was found to be slower than that of C10 and SNAC, suggesting a staggered release of insulin and the PEs from the minitablets. Overall, it is clear that the presence of C10 and SNAC had a profound influence on the release characteristics of insulin from direct compression minitablets, although further work is required to understand and optimize the corelease of peptides and PEs from oral dosage forms in order to achieve substantial improvements in bioavailability.

■ ASSOCIATED CONTENT

Data Availability Statement

Raw data were generated at the Department of Industrial and Molecular Pharmaceutics, Purdue University, United States, and the School of Pharmacy, University College Cork, Ireland. Derived data supporting the findings of this study are available from the corresponding author on request.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ACN, acetonitrile; CH_3COOH, acetic acid; C10, sodium decanoate; FaSSIF, fasted state simulated intestinal fluid; FaSSGF, fasted state simulated gastric fluid; FeSSIF, fed state simulated intestinal fluid; GIT, gastrointestinal tract; HCl, hydrochloric acid; MCC, microcrystalline cellulose; NaCl, sodium chloride; NaH_2PO_4, sodium dihydrogen phosphate; NaOH, sodium hydroxide; NaTC, sodium taurocholate; PE, permeation enhancer; pI, isoelectric point; PVP, poly(vinylpyrrolidone); SDS, sodium dodecyl sulfate; SNAC, salcaprozate sodium; TJ, tight junction

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