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Research article

Quantitative analyses to estimate the bioaccessibility of a hydrolytically degradable cationic flocculant

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HIGHLIGHTS

- ^{1}H-NMR combined with HPLC are used to quantify poly(PLA_4ChMA) bioaccessibility.
- Poly(PLA_4ChMA) is 100% bioaccessible in intestinal but not in gastric fluids.
- Degradation products lactic acid and choline chloride are solubilized in GI tract.
- Molar ratio of lactic acid to choline chloride remains constant throughout GI tract.
- Degraded poly(PLA_4ChMA) does not degrade further in simulated human GI tract.

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ABSTRACT

Poly(lactic acid) choline iodide ester methacrylate, poly(PLA_4ChMA), is a cationic degradable polymer that can flocculate particles and dewater oil sands from tailings ponds. This novel material has yet to be characterized in terms of environmental and human health. If ingested, this substance may become bioaccessible. The bioaccessibility (bioaccessible fraction) of an ingested contaminant is a measure of the portion of an ingested dose that solubilizes and may be available for systemic absorption. In the present study, the partially degraded flocculant and its degradation products, modelled using lactic acid and choline chloride, were subjected to a modified physiologically based extraction test (PBET). Bioaccessible fractions were estimated by proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and by high-performance liquid chromatography (HPLC). The measured bioaccessibility of lactic acid in gastric solution containing choline chloride is ~100% but slightly dropped to 94% in intestinal solution at a solid-to-liquid ratio of 1:200. The partially degraded poly(PLA_4ChMA) did not degrade further during the PBET and is not solubilized (i.e., 0% bioaccessibility) in the gastric phase but is fully solubilized (i.e., 100% bioaccessibility) in the intestinal phase. At the end of PBET intestinal digestion, the molar ratio of lactic acid to choline chloride in the presence of degraded poly(PLA_4ChMA) was 2, approximately the same as in the initial solution. Thus, lactic acid and choline chloride are solubilized to the same extents in both gastric and intestinal solutions. Results suggest that HPLC can be used to directly estimate the bioaccessibility of lactic acid, whereas ^{1}H - NMR may be used to indirectly quantify the bioaccessibility of both lactic acid and choline chloride by determining their molar ratio in PBET extracts. In future works, these findings may be applied to the estimation of risks from exposure to poly(PLA_4ChMA) as well as to the remediation of contaminants flocculated by poly(PLA_4ChMA) in tailings ponds and in other wastewaters.

1. Introduction

Bitumen extraction from oil sands produces oil and fine clay particles mixed in process water that is stored in tailings ponds [1, 2, 3]. Current particle settling methods rely on a polyacrylamide (PAM) flocculant with limited dewatering capabilities [3, 4]. A novel hydrolytically degradable polymer has been developed by the Hutchinson research group at Queen's University [3, 4]. Poly(lactic acid) choline iodide ester methacrylate, poly(PLA_4ChMA), can flocculate mature fine tailings (MFT) and dewater the consolidated MFT [3]. The cationic functionality of the polymer repeat unit promotes the consolidation of oil sands tailings and becomes hydrophobic over time in response to partial hydrolytic

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degradation releasing choline iodide and lactic acid, thus enhancing the dewatering of flocculated sediments [3, 5]. Ratios of lactic acid to choline iodide have previously been determined by proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) after degradation of poly(PLA_4ChMA) [5]. ^{1}H-NMR was also used to characterize partially degraded poly(PLA_4ChMA) [5]. Yu et al. used high-performance liquid chromatography (HPLC) to determine the concentrations of lactic acid, lactyl lactate (a linear dimer form of lactic acid), lactide (a cyclic dimer), and linear trimer compounds in supernatant solution [6]. In parallel, also using HPLC, Morbidelli's research team measured the relative amounts of lactic acid oligomer degradation products to obtain kinetic degradation rate constants for poly(lactic acid) - based compounds under various temperature and pH conditions [7, 8, 9].

According to toxicity assays conducted on human umbilical vein endothelial cells, PLA-based compounds degrade to water-soluble products that are considered nontoxic, based on 93–95% cell-survival rates [10]. Therefore, PLA-based compounds could potentially be used as flocculants in wastewater treatment. However, no hazard and toxicity studies have been conducted to evaluate the effects of poly(PLA_4ChMA) on living organisms to date. Similar compounds, such as cationic polyelectrolytes, are generally toxic to fathead minnows due to clogging of gill surfaces that result in suffocation [11, 12, 13]. Upon adsorption of polyelectrolytes on biological membranes of aquatic organisms, electrostatic interactions disrupt cell integrity (i.e., chemical exchanges across the membrane) [14]. These findings provide insights into the potential toxicity of cationic degradable flocculants and degradation products, and how they may affect human via ingestion. At this time, the human-health risks of introducing poly(PLA_4ChMA) into MFT and to the environment are unknown.

For the case of a human exposed to a contaminant through incidental ingestion, bioaccessibility is a measure of the solubilized fraction of ingested contaminant material that is available for absorption through epithelial tissue [15, 16]. A physiologically based extraction test (PBET) that simulates gastrointestinal (GI) fluids in a fasted state [16] was previously applied to determine the bioaccessibility of inorganic contaminants [15, 17]. Bioaccessibility measurements are relatively recent, and the methods currently available have only been validated against in-vivo data for inorganic contaminants such as arsenic, cadmium, and lead [18]. Among these, Bruce et al. subjected various mine waste samples to PBET, and the results indicated a correlation between the bioaccessibility of arsenic and lead contaminants and the corresponding bioavailability in rats and cattle [17]. Such a correlation between in-vitro bioaccessibility and in-vivo data is required for a method to be approved by regulatory agencies [19]. A key parameter, the solid-to-liquid ratio (S:L), does not affect the bioaccessibility of inorganic compounds (such as arsenic) of gold mining district samples for the S:L range of 1:5000 to 1:100 [15]. The bioaccessibility of most inorganic contaminants tends to have good within-laboratory repeatability (<15%) after PBET using the similar parameter, at an S:L of 1:100 [18].

However, there are currently no validated bioaccessibility methods for organic contaminants [20] such as poly(PLA_4ChMA)and its degradation products. The present study therefore requires the development of a suitable bioaccessibility method and chemical analysis to quantify solubilized degraded poly(PLA_4ChMA), lactic acid, and choline chloride (as a model for choline iodide) in GI fluids after the long-term degradation of poly(PLA_4ChMA). These bioaccessibility tests are intended to represent potential exposure by incidental ingestion of degraded poly(PLA_4ChMA) compounds in the environment. Both ^{1}H-NMR and HPLC are explored as methods to measure lactic acid and choline chloride in solution, with ^{1}H-NMR and gravimetry used to quantify the bioaccessibility of the partially degraded poly(PLA_4ChMA) flocculant. The findings from this present study are important because they establish a quantification method and a reference point for the pure compound. A full quantification is necessary before further investigation can be carried out to include interactions with real soil or sediment samples contaminated with poly(PLA_4ChMA), and eventually with real contaminated soils and sediments to be remediated using poly(PLA_4ChMA).

2. Materials and methods

Sample materials were subjected to a modified PBET, and extracts were analyzed by HPLC. Relative molar amounts of lactic acid and choline chloride in solution were determined through ^{1}H-NMR spectroscopy. ^{1}H-NMR spectroscopy was also used to characterize the partially degraded poly(PLA_4ChMA) in PBET extracts.

2.1. Materials

Lactic acid (reagent grade, $\geq$ 85 wt.% in water) and choline chloride ((2 - hydroxyethyl) trimethyl ammonium chloride, reagent grade, $\geq$ 98%), both procured from Sigma-Aldrich (Oakville, Canada), were used as model compounds representing the poly(PLA_4ChMA) degradation products. Poly(PLA_4ChMA) was synthesized, then degraded and centrifuged to isolate the partially degraded poly(PLA_4ChMA) according to a procedure described by Russell et al. [5] with the following modifications: ultra-pure water (UPW, 18.2 MΩ cm, Milli-Q®) was used instead of deuterium oxide (D_2O), with an initial polymer concentration of ~4 wt.%. The portion of degraded polymer corresponds to an averaged weight fraction of ~0.3 of the original polymer in this study. This result matches previous results [5] and confirms the proposed mechanism shown in Figure 1. Based on the mechanism in Figure 1, the 2:1 M ratio of lactic acid to choline chloride is maintained as an important parameter for PBET of the mixture of both components.

Subscript m represents the number of repeat units in the polymer chain. Following partial degradation, the polymer contains on average two lactate groups attached to the hydrocarbon backbone. Choline is cleaved first, followed by sequential release of lactate units, resulting in approximately two cleaved molecules of lactic acid per repeat unit, as identified in previous work [5].

Simulated gastrointestinal (GI) fluids were prepared in UPW according to Meunier et al. [15] as described in Table A.1 (in Supplementary Materials, SM). Hydrochloric acid (HCl, ACS grade, 12.06 M) and sodium bicarbonate (Na_2CO_3, ACS grade, $\geq$ 99.7%) both procured from Fisher Scientific (Ottawa, Canada) were used to adjust aqueous pH as necessary. Deuterium oxide (D_2O, 99.9%, Sigma-Aldrich, Oakville, Canada) and deuterated dimethyl sulfoxide (DMSO-d_6, 99.5%, Cambridge Isotope Laboratory, Tewksbury, United States) were used as solvents for ^{1}H-NMR analyses. Lactic acid was quantified by HPLC using a mobile-phase solution prepared from UPW and phosphoric acid (H_3PO_4, HPLC grade, $\geq$ 85%, Fisher Scientific, Ottawa, Canada).

2.2. Physiologically based extraction test (PBET)

The PBET experimental procedure included the following steps: (1) preparation of gastric and intestinal solutions, (2) addition of partially degraded poly(PLA_4ChMA), lactic acid, and/or choline chloride to solutions, (3) gastric phase extraction, (4) intestinal phase extraction, and finally (5) centrifugation. A blank solution containing only GI fluids was included in each PBET series. Between ~0.25 g and 0.50 g of a compound (except for the blank solution) was measured into a 100-mL polyethylene (P) cup (STARPLEX Scientific Corporation) to formulate test solutions with solid-to-liquid ratios (S:L) between 1:200 and 1:100. These two S:L were selected as physiologically representative for the case of incidental ingestion of sample material in a toddler stomach [20]. The mass of all the components was measured using a Mettler Toledo MS204TS analytical balance (±0.1 mg). The gastric stock solution was acidified to a pH of 1.8 ± 0.05 using HCl and a pH probe (Mettler Toledo LE409). At the beginning of the test, 50 g of gastric solution was measured into each P-cup (normally three samples, two duplicates, and one blank). Solutions were heated to a temperature of 37 °C in a rotational shaker-incubator (Excella E24 Incubator Shaker Series). To simulate human digestion, gastric and intestinal PBET solutions were prepared according to the procedure described by Meunier et al. with the following modifications: an agitation rate of 225 rpm; mid-point temperature was recorded; and

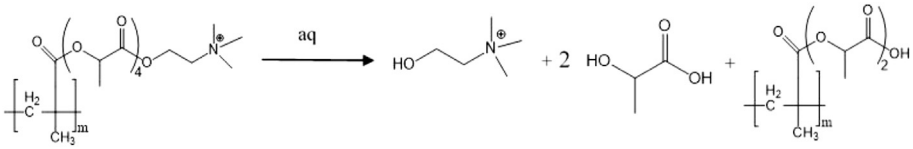

Figure 1. Proposed mechanism for partial hydrolytic degradation of poly(PLA_4ChMA) repeat units in H_2O.

the pH of the intestinal solution was set to 7.0 ± 0.05 [15]. At the end of gastric and intestinal phases, 5-mL or 10-mL aliquots of extract were transferred to centrifuge tubes and reserved for analysis. The PBET experimental runs are listed in Table 1.

2.3. Preparation of samples for analysis

Aliquots of selected gastric and intestinal extracts from PBET without the degraded polymer were centrifuged at 3154 g (4000 rpm) for 25 min (IEC Centra CL 3R, Thermo Electron Corporation) to separate supernatant from solids (pellets). The supernatant was removed via glass pipette and filtered (0.45 μm Millipore filter paper) prior to HPLC analysis. Extracts from PBET carried out with degraded polymer were centrifuged (1702 g (4000 rpm) for 25 min, Marathon 21000 (R) Fisher Scientific). Extracts from PBET of lactic acid and choline chloride mixture without degraded polymer (S:L of 1:100 and 1:200) were centrifuged using the same procedure.

To verify mass balance, supernatants and solids containing partially degraded polymer were transferred to pre-weighed Petri dishes and weighed prior to drying at 50 °C (Thermo Scientific OGS60 Heratherm oven). Dried gastric supernatant residues containing the partially degraded polymer only and those containing residues of degraded polymer mixed with lactic acid and choline chloride were redissolved in DMSO-d_6 prior to ^{1}H-NMR analysis. Dried intestinal residues did not dissolve well in DMSO-d_6 and were instead redissolved in D_2O. Blank gastric and intestinal PBET solutions were similarly dried and redissolved in DMSO-d_6 and D_2O, respectively. Because they contained no residue, intestinal supernatants were analyzed directly from PBET solutions. Therefore, to analyze PBET extract containing mixtures of lactic acid and choline chloride with and without partially degraded polymer, 10 wt.% of gastric or intestinal supernatant was diluted in 90 wt.% of D_2O.

2.4. ^{1}H-NMR analyses of poly(PLA_4ChMA)and degradation products

Dried residues and solids, redissolved in DMSO-d_6 or D_2O as appropriate, were analyzed by ^{1}H - NMR according to the procedure described by Russell et al. [5]. Aliquots of the lactic acid and choline chloride mixture (2:1 M ratio), prepared at S:L of 1:100 and 1:200 and in presence of the partially degraded polymer (at S:L of 1:200 only), were each analyzed in triplicate for quality assurance (reproducibility). A diagram of the PBET procedure and the subsequent analysis by ^{1}H - NMR is shown in Figure 2 for the mixture of partially degraded polymer, lactic acid, and choline chloride.

Gastric solution includes gastric supernatant and suspended solids. No pellet was recovered following centrifugation of intestinal extracts. 10 wt.% of each gastric solution, gastric supernatant, and intestinal supernatant was mixed with 90 wt.% D_2O prior to ^{1}H-NMR analysis. Aliquots of gastric and intestinal supernatant (~2.8 g) were dried prior to redissolution in DMSO-d_6 and D_2O, respectively, for ^{1}H - NMR analysis. The weight of combined dried gastric supernatant, gastric solids, and intestinal supernatant was recorded for gravimetric analysis.

Table 1. PBET experimental runs.

Compounds	Solid-to-liquid ratio (S:L)
Lactic acid[a]	1:100, 1:200
Lactic acid and choline chloride[b]	1:100, 1:200
Partially degraded polymer[c]	1:200
Partially degraded polymer, lactic acid, and choline chloride[d]	1:200[e]

[a] A measured amount was added to each P-cup to produce lactic acid with S:L of 1:100 and 1:200 (calculations included in SM Section A).

[b] Mixtures of lactic acid and choline chloride (2:1 M ratio) were added to solutions to produce samples with S:L of 1:100 and 1:200 (calculations included in SM Section A).

[c] Approximately 0.250 g of partially degraded polymer was added to the solution to produce S:L of 1:200.

[d] In these tests, ~40 wt.% of partially degraded polymer (~0.250 g) and ~60 wt.% of lactic acid and choline chloride (at 2:1 M ratio for a total of ~0.375 g) were used as representative proportions of polymer and degradation products [5].

[e] This S:L is based on the mass of partially degraded polymer.

2.5. Chromatography of lactic acid

Lactic acid was separated from PBET solution components and choline chloride by an Agilent 1260 HPLC system equipped with a diode-array detector and a high-sensitivity 60-mm flow cell. The injection volume of each sample was 10 μL; the operating temperature of the instrument was set at 37 °C, and the flow rate of the mobile phase (UPW with 20 mM H_3PO_4) was set at 0.425 mL min^{-1}. These operating conditions were set based on findings from YMC CO., Limited (Kyoto, Japan) for the analysis of highly polar compounds including lactic acid aqueous solutions [21]. Elution times varied from 8 to 18 min. Elution times were greater for the more complex components of PBET solution, such as porcine pepsin, bile salts, and pancreatin. Quantitative analyses were carried out by normal-phase chromatography on a YMC-Triart C18 column (150 × 3.00 mm as inner diameter, with 3 μm particle size). Lactic acid was detected by ultraviolet (UV) at 220 nm and 214 nm (two replicates in each case) and quantified using a calibration curve (details in SM Section B). The calibration curve was established from solutions of lactic acid in UPW at concentrations varying from 1.50×10^{-2} to 1.13 ×

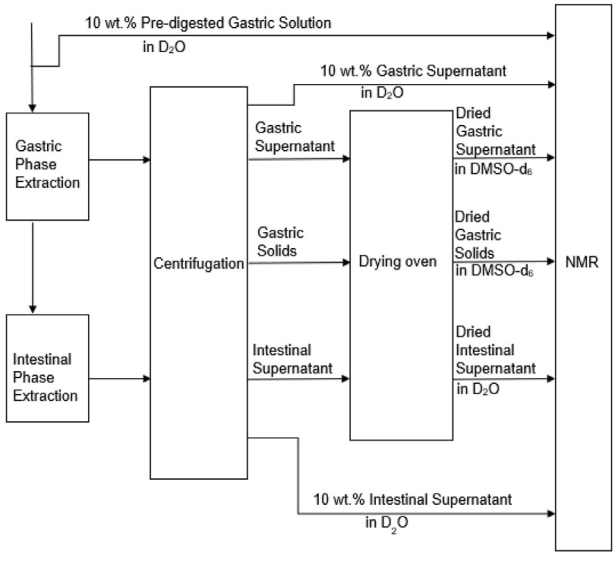

Figure 2. PBET procedure for mixtures of partially degraded polymer, lactic acid, and choline chloride followed by centrifugation and ^{1}H-NMR analysis.

10^{-1} mol·L^{-1}. Results were averaged to obtain the bioaccessibility of lactic acid in gastric and intestinal phases. Percent bioaccessibility was calculated as the ratio of lactic acid concentration in digested PBET supernatant to the initial lactic acid concentration in the GI solution multiplied by 100%.

2.6. Statistical analysis of molar ratio in solution

The molar ratio of model degradation products in gastric and intestinal solutions was measured by ^{1}H-NMR for three replicates at S:L of 1:200 in the presence of the partially degraded polymer and at S:L of 1:100 in absence of the partially degraded polymer. The averaged and standard deviation of molar ratios were determined from three replicates. These values were then subjected to analysis of variance (ANOVA) statistical tests (ANOVA: Single Factor package [22]). The quality assurance (reproducibility) in the analysis of replicates by ^{1}H-NMR was also evaluated by ANOVA. Experimental error and relative percent differences were calculated to estimate variations in bioaccessibility results.

3. Results and discussion

Lactic acid and choline chloride were selected as model compounds representing poly(PLA_4ChMA) polymer degradation products. Amorphous poly(PLA_4ChMA) forms a nanosuspension in aqueous solution, with the particle morphology changing to a more open structure (i.e., increased particle size and internal surface area) during degradation due to cleavage of the charged choline groups [3, 4]. However, the solubility of the partially degraded polymer in GI fluids is not known. Thus, samples of partially degraded polymer and model compounds were subjected to a series of PBET for two S:L ratios, and extracts were examined by ^{1}H-NMR and HPLC to identify analytical methods suitable to quantify the bioaccessibility of the polymer degradation products.

3.1. ^{1}H-NMR spectra of partially degraded polymer and degradation products

The ^{1}H-NMR spectra of a blank intestinal PBET solution and intestinal supernatant containing partially degraded polymer are shown in Figure 3(A),(B), respectively. Proton peaks corresponding to components of the intestinal solution are visible in both spectra (bile salts and of pancreatin spectra are presented in Figure C.1 of SM Section C). Specific peaks corresponding to protons in the partially degraded polymer are circled in dark blue, light blue, and brown (Figure 3(B)). Their presence confirms water-solubility and indicates that the partially degraded polymer is bioaccessible. The structure of the partially degraded polymer is identified by the relative area under the peak for the CH protons from the interior lactate units and from the chain end of the repeat units. The unity relative areas (1.00) under the peaks circled in dark blue and light blue confirm that the average number of lactate units attached to the backbone of the partially degraded poly(PLA_4ChMA) is two, as previously reported [5]. Thus, the structure of the partially degraded polymer following digestion by PBET remains unchanged (details in SM Section C). This result suggests that, although fully dissolved in the intestinal phase, the polymer does not undergo further degradation during simulated GI digestion (i.e., 1 h of gastric phase followed by 4 h of intestinal phase in PBET). Thus, it can be concluded that, in GI fluids, the partially degraded polymer would not release further degradation compounds, which would otherwise affect the estimated bioaccessibility of lactic acid and choline chloride. This result is confirmed by gravimetric analysis: the recovered mass of partially degraded polymer in the intestinal supernatant after digestion was 0.254 g, which is approximately same as the original mass (0.250 g) added to the GI solution before PBET (1.6% difference).

In contrast to the 100% bioaccessibility of the partially degraded polymer found in the PBET intestinal fluid, the analysis of gastric phase extracts indicates that the partially degraded polymer is not solubilized (details in SM Section C and Figure C.3). The recovered mass of partially degraded polymer in the gastric solids after digestion was 0.218 g, which is lower than the original mass of 0.250 g (12.8% difference). Mass loss may be attributed to incomplete recovery of the partially degraded polymer because particles <0.37 μm in diameter may remain in solution following centrifugation (calculation in SM Section A).

Based on the present results, ^{1}H-NMR is an effective technique to characterize partially degraded poly(PLA_4CHMA) in PBET extracts, which is consistent with previous results obtained for the analysis of partially degraded poly(PLA_4ChMA) in DMSO-d_6 [5] and for similar cationic flocculants in D_2O [4, 23]. Even though a complete quantification cannot be performed by ^{1}H-NMR analysis due to complex spectral

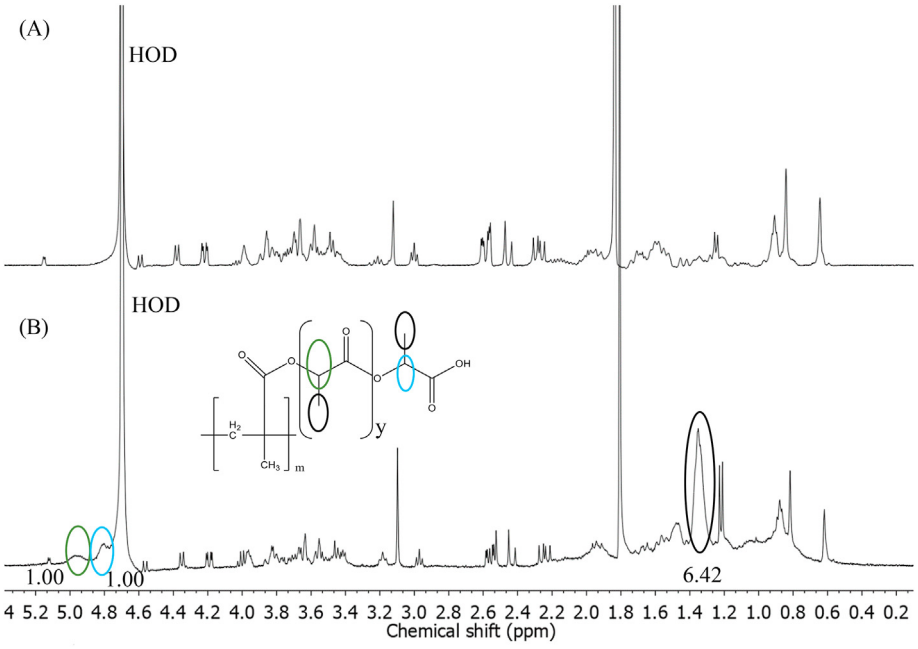

Figure 3. ^{1}H-NMR spectra of a blank intestinal solution (A) and partially degraded poly(PLA_4ChMA) polymer (S:L of 1:200) in intestinal supernatant residue (B) dissolved in D_2O.

signatures of PBET components (e.g., porcine pepsin, bile salts, and pancreatin), this technique may be applied to the investigation of polymer degradation products, including the ratio of the two bioaccessible compounds of interest, i.e., lactic acid and choline chloride.

Spectrum A can be subtracted from spectrum B to reveal the signature of the polymer. f_1 is the chemical shift relative to tetramethylsilane. The D_2O (HOD) peak is located at ~4.7 ppm. Areas under each relevant peak are relative to the peak circled in dark green (-CH group of the polymer lactate unit). The structure of the partially degraded polymer is verified by relative areas under the circled proton peaks. Subscript y represents the number of internal lactate unit (1–3). Peaks are observed for the partially degraded polymer in the intestinal supernatant, and quantification based on areas under the peaks suggests that the partially degraded polymer is fully solubilized in the intestinal phase of PBET.

The ^{1}H-NMR spectra of a lactic acid and choline chloride mixture in gastric and intestinal supernatants are shown in Figure 4(A),(B), respectively. The structure of choline chloride is identified by the areas under proton peaks at 3.09, 3.41, and 3.95 ppm. One peak is observed at approximately the same chemical shift (1.26 ± 0.05 ppm) as the proton peak corresponding to the methyl group of lactic acid in both spectra. These spectra are nearly identical to those of lactic acid and choline species in D_2O [4, 5, 23, 24, 25], which confirms that degradation products are solubilized in both gastric and intestinal PBET phases and hence are bioaccessible. The molar ratio of lactic acid to choline chloride cannot be determined by integration of the area under the lactic acid C–H peak circled in dark blue due to presence of water in the sample, which widens the solvent peak (HOD). Instead, this ratio is based on the area under the CH_3 lactic acid peak circled in brown relative to the choline chloride CH_2 peak circled in yellow. The molar ratios of lactic acid to choline chloride are 1.55 and 1.36 in the digested gastric and intestinal supernatant, respectively. These values are approximately the same as the molar ratios of 1.55 and 1.41 obtained from the analysis of initial PBET gastric and intestinal solutions, respectively (details in SM Section C). This ratio is lower than 2 for all samples due to the conversion of some of the lactic acid to lactyl lactate in the lactic acid solution (supplied by Sigma-Aldrich), as previously reported [5]. These results indicate that lactic acid and choline chloride ratios are maintained throughout GI digestion. This conclusion is also consistent with the fact that a negligible mass of solids was recovered after centrifugation of the gastric and intestinal PBET extracts following digestion of lactic acid and choline chloride. Thus, ^{1}H-NMR may be used to indirectly quantify the bioaccessibility of lactic acid and choline chloride by determining their molar ratio in PBET extracts. This finding is consistent with the previously reported quantification of lactic acid and choline iodide in D_2O obtained by ^{1}H-NMR analysis following the long-term degradation of poly(PLA_4ChMA) [5] and parallels the release of ω-hydroxy polycaproic acid oligomers and choline iodide, also measured by ^{1}H-NMR analysis, by the partial degradation of the similar flocculant (poly(caprolactone) choline iodide ester methacrylate, poly(PCL_3ChMA)) [4]. Although ^{1}H-NMR analysis may be applied to estimate some of the quantities of interest, this is only one of several analytical techniques investigated to characterize the partially degraded polymer in PBET extracts.

f_1 is the chemical shift relatives to tetramethylsilane. Structures of lactic acid and choline chloride are shown in left and right sides, respectively. Numbers below each peak represent the area under a peak relative to the peak circled in yellow. Peaks circled in light green, yellow, and red correspond to protons in choline chloride. Peaks circled in dark gold and purple correspond to protons in lactic acid. Peaks are observed for lactic acid and choline chloride in both supernatants, and quantification based on areas under the peaks suggests that these compounds are fully solubilized in both gastric and intestinal phases of PBET.

A similar ^{1}H-NMR analysis was applied to the mixture of partially degraded polymer, lactic acid, and choline chloride in intestinal supernatants. The ^{1}H-NMR spectra of a blank intestinal solution and intestinal supernatant containing a mixture of partially degraded polymer, lactic acid, and choline chloride are shown in Figure 5(A),(B), respectively. Peaks are located at the same chemical shift in Figure 5(B) as those in Figures 3(B) and 4(B). This observation confirms water-solubility of the mixture and indicates that the partially degraded polymer is bioaccessible even in the presence of lactic acid and choline chloride. The relative areas under peaks in Figure 5(B) are approximately the same as those in Figure 3(B) for the protons in the partially degraded polymer. This result indicates that the polymer does not undergo further degradation during simulated GI digestion in presence of lactic acid and choline chloride. The measured molar ratio of lactic acid to choline chloride in Figure 5(B) is 2.01, which is higher than the initial ratio of lactic acid to choline chloride of 1.40 prior to PBET (details in SM Section D). This increased ratio in the intestinal supernatant can be attributed to

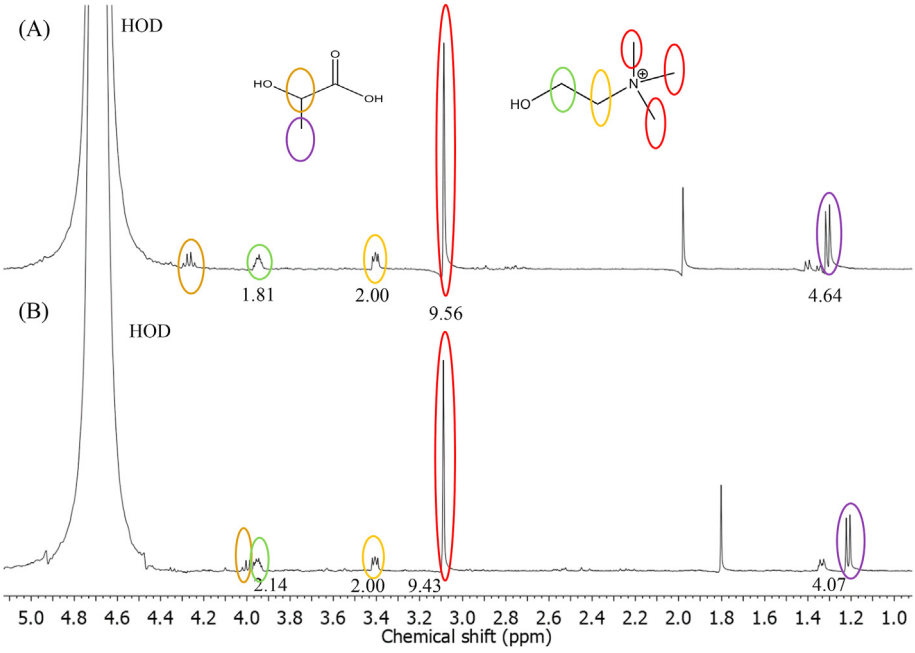

Figure 4. ^{1}H-NMR spectra of the lactic acid and choline chloride mixture (S:L of 1:200) gastric supernatant (A) and intestinal supernatant (B).

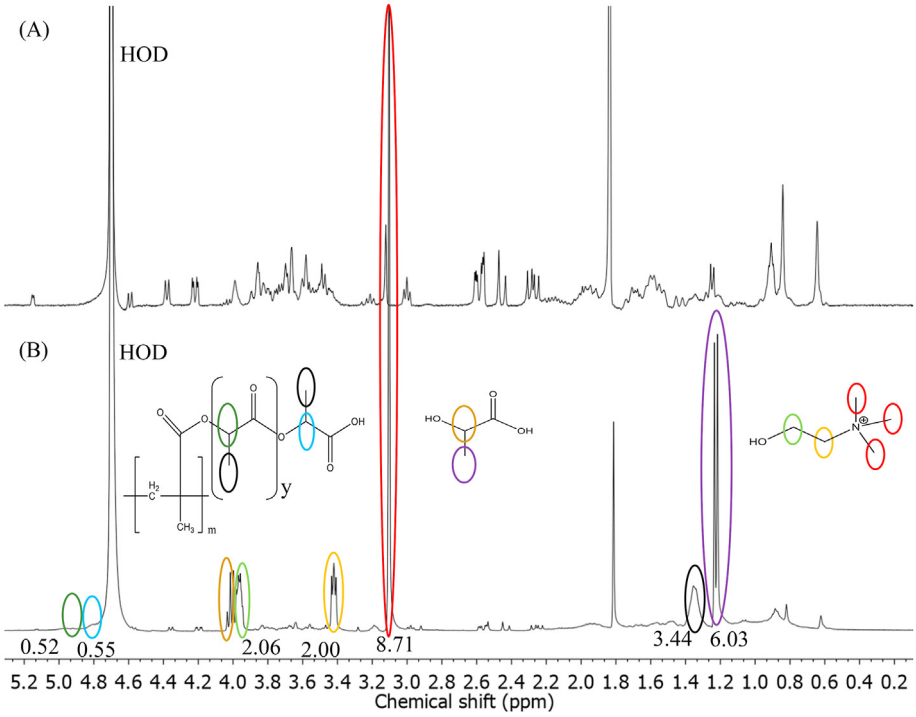

Figure 5. ^{1}H-NMR spectra of a blank intestinal solution (A) and a mixture of partially degraded poly(PLA$_4$ChMA) polymer (S:L of 1:200), lactic acid, and choline chloride in intestinal supernatant residue (B) dissolved in D_2O.

the hydrolysis of lactyl lactate to produce two molecules of lactic acid [5, 6, 7] during the PBET. These results suggest that the molar ratio of acidic species (lactyl lactate and lactic acid) to choline chloride is not altered during the PBET, and hence both lactic acid and choline chloride are fully solubilized in the intestinal supernatant in presence of the partially degraded polymer. The gravimetric analysis supports this finding, as the recovered mass of partially degraded polymer, lactic acid, and choline chloride mixture in the intestinal supernatant after digestion was 0.616 g, which is approximately same as the original mass (0.622 g) added to the GI solution (details in SM Section D) before PBET (0.96% difference). Hence, the bioaccessibility of each compound in the mixture is ~100%. The molar ratio of lactic acid to choline chloride obtained from ^{1}H-NMR does not change throughout the PBET in presence of partially degraded polymer based on analysis of variance (ANOVA, e.g. $F(2,5) = 3.30$, $p = 0.122$; details in SM Section E). These results suggest that the presence of polymer would not affect the bioaccessibility of lactic acid and choline chloride in the GI fluid or vice versa. Similar results were obtained from the analysis of PBET gastric-phase extracts containing the partially degraded polymer and model degradation products mixture (details in SM Section C and D). In all gastric-phase extracts, the polymer was recovered along with solids, whereas lactic acid and choline chloride remained in solution. Based on ^{1}H-NMR analyses, this technique can be used to quantify all components of poly(PLA$_4$ChMA), including its degradation products [5], in both gastric and intestinal PBET solutions.

f_1 is the chemical shift relatives to tetramethylsilane. The D_2O (HOD) peak is located at ~4.7 ppm. Structures of partially degraded poly(-PLA$_4$ChMA) polymer, lactic acid, and choline chloride are shown from left to right. Subscript y represents the number of internal lactate unit (1–3). Numbers below each peak represent the area under a peak relative to the peak circled in yellow. Peaks circled in dark green, light blue, and black correspond to protons in the polymer. Peaks circled with dark gold and purple correspond to protons in lactic acid. Peaks circled with light green, yellow, and red correspond to protons in choline chloride. Peaks are observed for the mixture of partially degraded polymer, lactic acid, and choline chloride in intestinal supernatant, and quantification based on areas under the peaks suggests that these compounds are fully solubilized in the intestinal phase; however, the partially degraded polymer does not undergo further degradation during PBET.

3.2. Detection of lactic acid by HPLC

Supernatant from gastric and intestinal phase PBET extracts were analyzed by HPLC, equipped with a UV detector, to estimate the bioaccessibility of lactic acid. A calibration curve could not be established for HPLC/UV analysis of choline chloride, as this substance absorbs UV light poorly. However, HPLC analysis provides useful information that complements the ^{1}H-NMR analysis described in Section 3.1. In the analysis of gastric supernatants, the retention time of lactic acid was ~3.5 min, whereas it took only ~1.75 min for intestinal supernatants. This phenomenon can be attributed to presence of bile salts and pancreatin components in the intestinal solution. These components can sorb to the stationary phase of the column thereby occupying sorption sites, which results in reduced retention time of lactic acid. Results were similar for all measurements (taken at 214 and 220 nm, as described in Section 2.5) of gastric supernatant samples. Therefore, reliable HPLC measurements for gastric extracts may be taken at either wavelength. Conversely, measurements of intestinal supernatant samples taken at 214 nm were not reliable, and only those taken at 220 nm were retained for analysis. For eight gastric supernatant samples, the results of four measurements each were averaged to obtain the percent bioaccessibility shown in Figure 6. For three intestinal supernatant samples, two measurements each were averaged to estimate lactic acid bioaccessibility, and a single HPLC measurement at 220 nm was used to estimate bioaccessibility from a mixture of lactic acid and choline chloride.

Percent bioaccessibility of lactic acid is shown in Figure 6 for gastric and intestinal PBET phases at S:L of 1:200 and for cases where lactic acid was tested alone and in the presence of choline chloride. Overall, combining the results obtained for both cases, the elevated bioaccessibility in gastric supernatant (average 96.6%) and intestinal supernatant (93.1%) suggests that lactic acid is readily solubilized in both GI phases. When considering lactic acid alone, the measured bioaccessibility is also similar in both gastric (average 90.8%) and intestinal

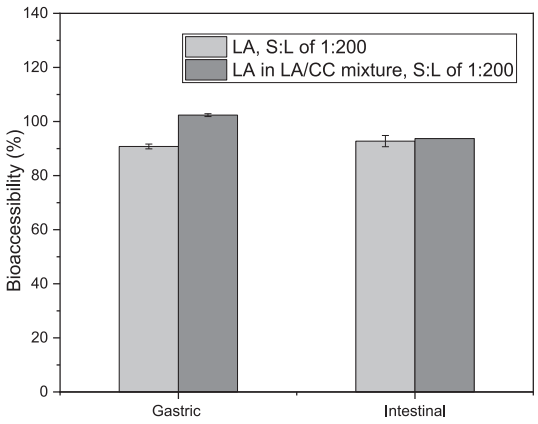

Figure 6. Bioaccessibility of lactic acid (LA) in gastric supernatant and intestinal supernatant in the presence and absence of choline chloride (CC) (S:L of 1:200). The bioaccessibility of lactic acid is the averaged value obtained from 220 and 214 nm cases (the number of replicates of 2 in each case). Error bars represent the standard deviation of averaged bioaccessibility value. The maximum standard deviation is 2.1% for all samples, which suggests that the HPLC gives a repeatable measurement of lactic acid bioaccessibility in gastric and intestinal supernatants.

phases (92.8%). These results suggest that the bioaccessibility of lactic acid does not change throughout the PBET, and that the presence of choline chloride does not affect the bioaccessibility of lactic acid. These results are also consistent with the molar ratios of lactic acid to choline chloride, which remain constant throughout the PBET solutions (as measured by ^{1}H-NMR, see Figure 4). Furthermore, these results are consistent with the gravimetric analysis of GI solutions containing lactic acid and choline chloride in the presence of partially degraded polymer as discussed in Section 3.1. Similar bioaccessibility results were obtained for lactic acid and choline chloride mixtures at S:L of 1:100 in both gastric and intestinal phases (details in SM Section F). Based on these consistent and repeatable results, the HPLC method suggested by YMC CO., Limited [21] is considered effective to analyze lactic acid in gastric and intestinal aqueous supernatants. This finding is also consistent with previous studies that relied on the same HPLC technique to analyze lactic acid and similar compounds [6, 7, 8, 9, 26, 27].

Overall, results indicate that degraded poly(PLA_4ChMA) and its degradation products are nearly 100% bioaccessible. Given this high solubility, environmental implications must be considered before these compounds are released in the environment.

3.3. Environmental implication

The present study offers a full characterization and quantification of poly(PLA_4ChMA) in simulated GI fluids based on pure compounds. The results obtained in this study will be applied to future investigations that will include interactions with pristine soil and sediment samples mixed with poly(PLA_4ChMA), and eventually with real contaminated soils and sediments to be remediated using poly(PLA_4ChMA). Based on ^{1}H-NMR analysis, poly(PLA_4ChMA) is fully bioaccessible, which means that the total amount ingested may reach systemic circulation. To date, no toxicological studies have quantified the potential risks associated with uptake of poly(PLA_4ChMA). Nevertheless, because this compound is fully solubilized in the GI tract, it could release any contaminant that was sorbed and transported, and which may constitute risk. Therefore, characterization based on the measurement of bioaccessibility of the pure poly(PLA_4ChMA) is important because the effects of this exposure may be different and separate from the health risks associated with other contaminants that may sorb to this flocculant. A conservative estimate for the bioaccessibility of poly(PLA_4ChMA) must be set at 100% based on the combination of ^{1}H-NMR, HPLC, and gravimetric analyses. Thus, the influence of the presence of poly(PLA_4ChMA) on the bioaccessibility of other contaminants in soils and sediments is an important part of risk assessment.

4. Conclusions

The bioaccessibility of partially degraded poly(PLA_4ChMA) and its degradation products in simulated human PBET gastric and intestinal solutions was estimated following analysis by ^{1}H - NMR and HPLC. Partially degraded poly(PLA_4ChMA) exhibited low solubility in gastric phase, and is essentially not bioaccessible. However, this material exhibited high solubility in intestinal phase and is essentially fully bioaccessible. Conversely, the model degradation products of lactic acid and choline chloride solubilize to the same extent in both gastric and intestinal phases. The molar ratio of lactic acid to choline chloride remains approximately the same throughout PBET digestion. The bioaccessibility of lactic acid is above 90% for both gastric and intestinal phases of PBET. The bioaccessibility of choline chloride cannot be estimated using HPLC due to poor absorption of UV light by choline chloride. However, the ^{1}H-NMR analysis supports the conclusion that the ratio of choline chloride to lactic acid does not change in both gastric and intestinal supernatants, indicating the two degradation products have similar bioaccessibilities. The findings of the present work may be applied to the estimation of potential risks from exposure to poly(PLA_4ChMA) as well as to contaminants flocculated by poly(PLA_4ChMA) in tailings ponds and in other wastewater treatment applications. With a baseline established on the pure compounds examined in this study, the next step will be to carry out bioaccessibility tests involving poly(PLA_4ChMA) and clean soils and sediments, then poly(PLA_4ChMA) and contaminated soils and sediments. In future studies, the PBET method should be modified to also study the interactions between PBET solution components and polymer degradation products of interest. Instead of a PBET in fasted state, food particulates, such as milk powder, should be introduced to the PBET in future work because of their potential interaction with polymer degradation products. Additional in-vitro and in-vivo tests should also be conducted to determine the fraction of soluble degradation products that may be absorbed into the circulatory system. The PBET method used in this work can be applied to determine the bioaccessibility of other flocculants. The analytical methods (i.e., ^{1}H-NMR and HPLC) used in this study can be applied to other biodegradable polymeric materials, including situations where these compounds are mixed with complex organic matrices (e.g., oil sands tailings ponds, wastewater, and sewage sludge) [28, 29].

Declarations

Author contribution statement

Derek A. Russell: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Robin A. Hutchinson & Louise Meunier: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

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