SÍNTESE E PROPRIEDADES DE PROTEÇÃO DOS COMPÓSITOS DE PECTINA /
MONTMORILONITA CONTRA A ENTEROCOLITE INDUZIDA POR ASPIRINA

SYNTHESIS AND PROTECTIVE PROPERTIES OF PECTIN/MONTMORILLONITE
COMPOSITES AGAINST ASPIRIN-INDUCED ENTEROCOLITIS

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RESUMO

O uso prolongado de aspirina para prevenção de eventos cardiovasculares é limitado por seus efeitos adversos gastrointestinais e, portanto, pacientes com alto risco devem receber agentes gastroprotetores. No entanto, os medicamentos gastroprotetores também apresentam alguns efeitos adversos que exigem a busca de alternativas seguras e eficazes. Os compósitos que combinam as propriedades de intumescimento das argilas e a atividade biológica dos polissacarídeos parecem ser candidatos promissores à proteção do trato gastrointestinal. O objetivo deste estudo foi caracterizar compósitos de pectina / montmorilonita e avaliar seu efeito protetor no trato gastrointestinal de ratos em uso de aspirina por um longo período. Uma série de compósitos à base de montmorilonita (MMT) e pectina com baixa esterificação (Pec) foi sintetizada usando o método de adsorção-precipitação. As razões de peso de polissacarídeo para argila foram 1:19, 1: 9 e 1: 4. Os compósitos foram caracterizados por Análise Termogravimétrica (TGA), Espectroscopia no Infravermelho (IR), Microscopia Eletrônica de Varredura (MEV) e Difração de Raios-X (DRX). A pectina foi completamente fixada em MMT e o teor de polissacarídeos nos compósitos foi de aproximadamente 5, 10 e 20% em peso, respectivamente. O deslocamento da banda de absorção do grupo C = O da pectina indicou a interação do polissacarídeo com a argila, confirmando a imobilização efetiva do Pec no MMT. A modificação com pectina alterou a morfologia e a estrutura do MMT devido ao revestimento superficial e à intercalação no espaço entre camadas. Os compósitos incharam em água acidificada (pH = 2,0) e a capacidade de intumescimento foi maior para comparar com o MMT não modificado. A capacidade de sorção dos compósitos Pec / MMT em relação ao ácido acetilsalicílico (ASA) diminuiu de 6,8 para 1,0 mg g⁻¹ com o aumento do conteúdo de pectina de 5 para 20% em peso. Os compósitos híbridos promoveram proteção do trato gastrointestinal de ratos, aos quais foi administrado ASA com Pec / MMTs por um período de 16 dias. As propriedades protetoras do Pec / MMT foram aprimoradas com o aumento do conteúdo de pectina de 5 a 20% em peso.

Palavras-chave: *pectina, MMT, aspirina, doença intestinal, propriedades protetoras.*

ABSTRACT

Long term use of aspirin for prevention of cardiovascular events is limited by its gastrointestinal adverse effects, and, therefore, patients at high risk should receive gastroprotective agents. However, gastroprotective drugs also have a few adverse effects that require searching for safe and effective alternatives. The composites combining the swelling properties of clays and the biological activity of polysaccharides seem to be promising candidates for gastrointestinal protection. This study aimed to characterize pectin/montmorillonite composites and evaluate their protective effect on the gastrointestinal tract of rats taking aspirin over a long period. A series of composites based on montmorillonite (MMT) and low esterified pectin (Pec) was synthesized using the adsorption-precipitation method. The polysaccharide to clay weight ratios were 1:19, 1:9, and 1:4. The composites were characterized using Thermogravimetric Analysis (TGA), Infrared Spectroscopy (IR), Scanning Electron Microscopy (SEM), and X-Ray Diffraction (XRD). Pectin was completely fixed on MMT, and the polysaccharide content in the composites was approximately 5, 10, and 20 wt%, respectively. The shifting of the absorption band

of the C=O group of the pectin indicated the interaction of the polysaccharide with the clay, confirming effective immobilization of Pec on MMT. Modification with pectin changed the morphology and structure of the MMT due to the surface coating and intercalation into the interlayer space. The composites swelled in acidified water (pH = 2.0), and their swelling ability was higher to compare with unmodified MMT. The sorption capacity of Pec/MMT composites towards acetylsalicylic acid (ASA) was decreased from 6.8 to 1.0 mg g⁻¹ with increasing of pectin content from 5 to 20 wt%. The hybrid composites promoted the protection of the gastrointestinal tract of rats, which were administered ASA with Pec/MMTs for 16 days. Protective properties of the Pec/MMT have been improved with increasing pectin content from 5 to 20 wt%.

Keywords: pectin, MMT, aspirin, intestinal disease, protective properties.

1. INTRODUCTION:

Acetylsalicylic acid (ASA, aspirin) is now a commonly used drug for the prevention of cerebrovascular and cardiovascular diseases (CVD) (Iijima, 2016; Ali & Sharma, 2018). However, daily aspirin therapy, even at a low dose, can damage the gastrointestinal tract (GI), causing Crohn's disease and ulcerative colitis (Moninuola *et al.*, 2018; Bjarnason *et al.*, 2018). This may lead patients to discontinue therapy, increasing their CVD risk (Lavie *et al.*, 2017). Co-administration of proton pump inhibitors (PPIs) in patients taking aspirin reduces the risk of aspirin-induced upper GI adverse events (Rodriguez *et al.*, 2020). However, PPIs also have adverse effects such as allergic reaction, diarrhea, changed gut microbiome, fundic gland polyp, drug interaction in the gut, etc. (Kinoshita *et al.*, 2018; Fossmark *et al.*, 2019). This requires a search for safe and effective alternatives to PPIs.

The composites combining the properties of clays and polysaccharides seem to be promising candidates for GI protection. The mineral component of such composites (clay) can act as antacid reducing the gastric acidity through adsorption of protons and release of non-toxic ions such as Mg²⁺ and Al³⁺ (Massaro *et al.*, 2018). The ability of natural clays to adhesion on the gastrointestinal mucosa surface, causing increase barrier thickness, is also of interest (Massaro *et al.*, 2018; Ali *et al.*, 2017). Modification of the layered silicates with polysaccharides can improve their mucoadhesive properties (Sharma *et al.*, 2018). Besides, non-starch polysaccharides provide many benefits for intestinal bowel disease treatment, including tissue healing, anti-oxidation, reduction of toxins adsorption, modulate gut microbiota, etc. (Nie *et al.*, 2017). Of note is that certain gut bacteria ferment the polysaccharides to produce short-chain fatty acids (SCFAs), which maintain the epithelial barrier function and protect against colitis (Zhang *et al.*, 2018). Biocompatible and non-toxic polysaccharide/clay composites are widely used for biomedical applications as drug

delivery systems, tissue engineering scaffolds, wound dressings and detoxifying agents (Wu *et al.*, 2019; Liu *et al.*, 2017). However, such composites have practically not been studied on alleviating ailments caused by the administration of nonsteroidal anti-inflammatory drugs (Dragan & Dinu, 2020; Meirelles & Raffin, 2017).

Long-term co-administration of pectin/montmorillonite intestinal adsorbent with aspirin in rats has previously been shown to possess a positive effect on their blood biochemical parameters (Kapyseva *et al.*, 2019). Therefore, this study aimed to characterize pectin/montmorillonite composites synthesized using a simple adsorption-precipitation method and evaluate their protective effect on the gastrointestinal tract of rats taking aspirin over a long period.

2. MATERIALS AND METHODS:

2.1. Materials

Montmorillonite (MMT), from the Tagansky deposit (Kazakhstan) produced by Sorbent (Ust-Kamenogorsk, Kazakhstan), and ethanol (96.3%), purchased from Talgar Spirt (Kazakhstan), was used without additional purification. Acetylsalicylic acid, obtained in tablet form from the Borisov Plant of Medical Preparations (Belarus), was separated from the other ingredients using recrystallization with ethanol as the solvent.

2.2. Extraction and characterization of pectin

Extraction of the pectin (Pec) from ripe Golden Delicious apple pomace (5 g) was carried out using water acidified with citric acid (pH = 2.0) at 80 °C for 4 hours according to the procedure described in (Devi *et al.*, 2014). The pectin yield (Y_{Pec}) was calculated using the following equation (Eq. 1).

$$Y_{Pec}(\%) = \frac{p}{B_i} \times 100 \quad (\text{Eq. 1})$$

Where, p is the amount of extracted pectin (0.38 g), and Bi is the initial amount of apple pomace (5 g).

Characterization of the resulting pectin included determination of equivalent weight (EW), methoxyl content (MeO), total anhydrouronic acid content (AUA), and degree of esterification (DE) (Khamsucharit *et al.*, 2017). Pectin (0.1 g), ethanol (1 mL), sodium chloride (0.2 g) and carbon dioxide-free distilled water (20 mL) were dissolved and titrated against standard 0.01M NaOH until the mixture adjusted to pH 7.5-8.0. The pH values were determined using an ANION-4100 pH meter containing a glass electrode. The volume of NaOH consumed (V_1 , mL) was used for determination of equivalent weight (EW), using the equation (Eq. 2).

$$EW \text{ (g mol}^{-1}\text{)} = \frac{Wt \times 1000}{V_1 \times N} \quad (\text{Eq. 2})$$

Where, Wt is weight of pectin (0.1 g), V_1 is the volume of NaOH consumed for titration (20.6 mL) of free acid groups, and N is normality of NaOH (0.01 M).

The neutral solution was collected after determination of equivalent weight, and 10 mL of sodium hydroxide (0.01 M) was added. The mixed solution was stirred thoroughly and kept at room temperature for 30 min, after which 10 mL of 0.01 M hydrochloric acid was added and titrated against 0.01 M NaOH to the same endpoint as in the previous equivalent weight titration.

Methoxyl content (MeO) was calculated using the following equation (Eq. 3).

$$\text{MeO}(\%) = \frac{V_2 \times N \times 31}{Wt \times 1000} \times 100 \quad (\text{Eq. 3})$$

Where, V_2 is volume of NaOH consumed for titration (9.5 mL) of de-esterified acid groups, Wt is weight of pectin (0.1 g), N is normality of NaOH (0.01 M), and 31 is the molecular weight of the methoxyl group.

Total anhydrouronic acid content (AUA) of pectin was obtained using the equation (Eq. 4).

$$\text{AUA}(\%) = \frac{176 \times 0.01 \times (V_1 + V_2) \times 100}{Wt \times 1000} \quad (\text{Eq. 4})$$

Where, 176 is the molecular weight of 1 unit of the polygalacturonic acid, V_1 (20.6 mL) is the volume of NaOH from equivalent weight determination, V_2 (9.5 mL) is the volume of NaOH

from methoxyl content determination, and Wt is the weight of the sample (0.1 g).

The degree of esterification (DE) of pectin was measured on the basis methoxyl and AUA content and was calculated using the following equation (Eq. 5).

$$\text{DE}(\%) = \frac{176 \times \text{MeO}}{31 \times \text{AUA}} \times 100 \quad (\text{Eq. 5})$$

Where, MeO is methoxyl content (%), AUA is anhydrouronic acid content (%), 176 is the molecular weight of 1 unit of the polygalacturonic acid, and 31 is the molecular weight of the methoxyl group.

2.3. Synthesis of Pec/MMT composites

Pec/MMT composites with different pectin content were synthesized as follows. Pectin (0.05, 0.11 and 0.25 g) was dissolved in water (35 mL) at 60 °C and then added to a well-dispersed MMT suspension obtained by the intensive magnetic stirring of 1 g clay in 15 mL of water. Pec:MMT weight ratios were 1:19, 1:9, and 1:4. The mixtures were stirred continuously at ambient temperature for 2 h. Then, the composites were precipitated with ethanol and dried in a weak vacuum at 60°C.

2.4. Characterization of Pec/MMT composites

Thermogravimetric analysis (TGA) of samples was carried out in a nitrogen atmosphere at a temperature of 30 to 1000 °C, using a Labsys Evo analyzer from Setaram (France) at a heating rate of 10 °C per minute.

X-ray diffraction (XRD) patterns were obtained on a DRON-4-0.7 diffractometer from Bourevestnik (Russia), with a Co Ka radiation at a wavelength of 0.179 nm. XRD mounts for each sample were prepared by pipetting a small amount of a water-dispersed sample onto an XRD slide and drying in ambient air at room temperature for 3 h. To ensure the comparability of the XRD measurements of the various samples, the XRD mounts were dried at the same temperature and during the same period for all samples, with the XRD measurements taken shortly afterward.

IR spectra were obtained using a Nicolet iS5 from Thermo Scientific (USA), with a resolution of 3 cm^{-1} in the 4000–350 cm^{-1} region. Pellets for infrared analysis were obtained by grinding a mixture of 1 mg sample with 100 mg dry KBr, followed by pressing the mixture into a mold.

Morphology and structure of MMT and Pec/MMT composites were studied using a

scanning electron microscope, JEOL JSM-6610 LV (Japan).

2.5. Evaluation of sorption properties

The sorption properties of the composites were studied using the following procedure: 10 mL of water were added to 0.1 g of the sorbent and mixed until a homogeneous suspension had been formed. The mixture obtained was thermostated at 36 °C in a water bath. A 10 mL hydrochloric acid solution (0.01 N) was added to 20 mL acetylsalicylic acid solution (0.53 g L⁻¹). The resulting ASA solution with a pH of 2.0 was heated to 36 °C and added over a stirred sorbent suspension. Adsorption was carried out for 30 minutes under static conditions. Then an aliquot of the supernatant solution (10 mL) was diluted in 20 mL of ethanol and analyzed using an SF-2000 spectrophotometer (OKB Spectr, Russia), at a 275 nm wavelength (Popova *et al.*, 2016). The amount of ASA adsorbed was determined by change in concentration of supernatant solution before and after sorption.

2.6. Histological studies

The protective effect of Pec/MMT composites against aspirin-induced damage to the small intestine was studied in albino Wistar rats (SPF category). The studies were approved by Local Ethical Committee, S Asfendiyarov Kazakh National Medical University, Registrations No 3, and 4. In this study, 50 albino rats (220±10 g) were equally divided into five groups. Oral delivery of acetylsalicylic acid (20 mg) and sorbents (6.3 mg) was performed daily for 16 days. The control group of rats was kept in equivalent laboratory conditions and received the same diet as the experimental animals. Histological studies were carried out on small intestine tissue samples, which were fixed in 10% neutral formaldehyde and routinely processed (Korzhevsky & Gilyarov, 2010). Approximately 5µm thick serial sections were cut and stained with hematoxylin and eosin (H&E). The photos were recorded through an Axiostar plus microscope from Carl Zeiss (Germany).

3. RESULTS AND DISCUSSION

3.1. Characterization of pectin

The yield of pectin extracted from the ripe Golden Delicious apple pomace was found to be 7.6%, which is consistent with data from prior studies (Perussello *et al.*, 2017). The equivalent weight (EW), methoxyl content (MeO), total anhydrouronic acid content (AUA), and degree of

esterification (DE) of the resulting sample of pectin were found to be 485.4 g mol⁻¹, 2.9%, 53.0%, and 31.1%, respectively. These values are comparable with parameters of apple peel pectin (EW = 652.5 g mol⁻¹, MeO = 3.7%, AUA = 62.8% and DE = 33.4%), as previously reported (Virk and Sogi, 2004).

3.2. Characterization of Pec/MMT composites

Pectin extracted from apple pomace was used for the preparation of Pec/MMT composites. The composites were obtained by adsorption of the polysaccharide onto a clay mineral (Pec:MMT weight ratios were 1:19, 1:9 and 1:4), followed by precipitation of the composites with ethanol, filtration and drying. The treatment with ethanol promotes the aggregation of well-dispersed polymer-inorganic composites and greatly facilitates the filtration process. The filtrate was clear, and visible traces of pectin molecules and MMT particles were not observed.

According to previous results (Talgatov *et al.*, 2019), the mixing of commercial pectin solution and natural montmorillonite suspension led to quantity adsorption of the polysaccharide on clay mineral (more than 90%). Therefore, taking into account the treatment with ethanol, the content of pectin in the Pec/MMT composites may be assumed to be 5, 10 and 20 wt.%.

To confirm this, the composites obtained, with Pec:MMT weight ratios of 1:9 and 1:4, were studied using thermogravimetric analysis (Figure 1).

Figure 1. TGA of pectin (a), MMT (b) and Pec/MMT composites (c, d)

Figure 1 shows the weight loss curves of MMT, pectin, and Pec/MMT composites, which demonstrate multiphase degradation behaviors.

The thermal degradation of pectin occurred in two stages. Below 200 °C, there is an initial weight loss of 10.3%, which corresponds with the evaporation of bound water in the hydrophilic polymer and the removal of other small molecules (Ahn *et al.*, 2017). The principal weight loss (50.4%) occurs in the range from 200 to 500 °C, corresponding to the decomposition of the polymer. The residual weight of pectin at 500 °C was 39.3%. This result is not far from previously reported values (Ahn *et al.*, 2017; Minhas *et al.*, 2018), where the residual weight of commercial pectin samples was around 32-34% at 500°C. MMT also shows two stages of thermal degradation. The first stage observed below 200 °C, with a weight loss of 7.1%, was related to the evaporation of water molecules adsorbed in pores and between the silicate layers (Alshabanat *et al.*, 2013). The second thermal degradation stage of MMT, observed in the range from 500 to 750 °C, with weight loss 5.1%, can be explained by the elimination of the –OH group from the [AlO₆] octahedral sheet of MMT (Yi *et al.*, 2017). These dehydration stages are observed for both Pec/MMT samples. The significant difference between the thermogram of the unmodified MMT and that of the Pec/MMT composites is that the organic constituent in the composites decomposes in the range from 200 to 500 °C (Al-Shemmar *et al.*, 2013). The weight loss in this range was found to be 7.4% and 11.2 % for composites with Pec:MMT weight ratios of 1:9 and 1:4, respectively. This suggests that the polymer was fixed entirely on the clay, as pectin lost only 50% of its weight in this temperature range.

The purity of pectin extracted from apple pomace and its interaction with MMT was evaluated using FTIR analysis, and the results are shown in Figure 2.

The spectrum of the pectin extracted (Figure 2a) showed a good match with the spectra of commercial pectin (Kumar & Chauhan, 2010; Luo *et al.*, 2019). The characteristic bands at 3432, 2939, 1749, and 1625 cm⁻¹ correspond to the stretching vibrations of –OH, –CH, C=O of ester, and C=O of acid groups in the pectin molecule, respectively (Sumathra *et al.*, 2017). The absorbance is lower at 1749 cm⁻¹ than at 1625 cm⁻¹, confirming a low degree of esterification of the pectin. The bands in the wavelength range of 1200-1500 cm⁻¹ correspond to the stretching and bending vibrations of –C-OH, –C-H and –O-H (Thompson, 2018). The wide band in the area from 950 to 1200 cm⁻¹ are considered to be the “finger print” region for carbohydrates, because it helps in the identification of major chemical groups (–C-O, C-O-C and C-C) in polysaccharides (Urias-Orona

et al., 2010). The spectrum showed in Figure 2b is typical for MMT (Dankova *et al.*, 2014). The absorption band at 3627 cm⁻¹ is due to the stretching vibrations of structural OH groups of the MMT. The characteristic bands at 1043, 915, 525 and 471 cm⁻¹ correspond to the stretching vibrations of Si–O, the bending vibrations of AlAlOH, and the bending vibrations of Al–O–Si and Si–O–Si, respectively. Water in montmorillonite resulted in broadband that peaked at 3429 cm⁻¹, corresponding with the H₂O-stretching vibrations. The shoulder close to 3240 cm⁻¹ is due to an overtone of the bending vibration of water observed at 1632 cm⁻¹ (Dankova *et al.*, 2014). The spectrum of the Pec/MMT composite was similar to unmodified MMT (Figure 2c). However, the appearance of bands at 2931, 1745, and 1200-1500 cm⁻¹ confirmed the presence of pectin. The shifting of the absorption band of the C=O group, compared to the same group in pectin, indicated the interaction of the polysaccharide with montmorillonite. A slight shift of some bands characteristic of Al–O and Si–O was also observed (Table 1).

Figure 2. IR spectra of pectin (a), MMT (b) and Pec/MMT composite (c)

The anisometric shape of the initial MMT was observed using scanning electron microscopy. A clay mineral consists of microaggregates with poorly traced boundaries (Figure 3a) (Kasprzhitsky *et al.*, 2013). Modification of MMT with pectin leads to a change in surface morphology (Figure 3b). The surface of the composite is more uniform, perhaps, due to the formation of a polymer film.

The results of the XRD study of MMT and Pec/MMT composites are shown in Table 2. The 001 reflection peaks at $2\theta = 7.0^\circ$, 7.2° and 7.5° , corresponding to the basal spacing of calcium ($d_{001} = 14.6 \text{ \AA}$), magnesium ($d_{001} = 14.3 \text{ \AA}$) and sodium ($d_{001} = 13.6 \text{ \AA}$) forms of montmorillonite, were observed in the unmodified MMT (Fedorenko *et al.*, 2010). The appearance of a new 001 reflection peak at $2\theta = 6.6\text{--}6.7^\circ$ ($d_{001} = 15.4\text{--}15.6 \text{ \AA}$) detected on the XRD patterns of Pec/MMT composites indicates the intercalation of pectin into the interlayer space of layered silicate (Table 2) (Mittal, 2009). The same results have been reported for MMT modified with commercial pectin, where the polysaccharide contributed to changing the surface and structure properties of the clay mineral, due to coating and intercalation, respectively (Talgatov *et al.*, 2016).

3.3. Sorption and swelling properties of Pec/MMT composites

Aspirin is rapidly absorbed from the stomach and upper small intestine, reaching maximum drug concentration in plasma through 15–30 minutes after oral administration (Kanani *et al.*, 2015). Therefore, the sorption process was carried out over 30 minutes in an environment simulating the stomach (36°C and $\text{pH} = 2.0$) (Lu *et al.*, 2010). The sorption properties of Pec/MMT composites were evaluated using the residual concentration of ASA in the supernatant, and compared with those of unmodified MMT. Results of ASA sorption on MMT and Pec/MMT composites are presented in Table 3.

According to the analysis of the supernatant solution using spectrophotometry, the sorption capacity of MMT increased from 3.1 mg g^{-1} to 6.8 mg g^{-1} after modification with 5 wt.% of pectin. However, further, increase in pectin content to 20 wt.% led to a decrease in the sorption capacity of the hybrid composites to 1.0 mg g^{-1} . The capacity of the sorbents towards ASA was correlated with their swelling ability (Figure 4).

Of note is that the Pec/MMT particles demonstrate an excellent dispersibility in neutral medium and swells only in acidified water.

Improving the swelling ability of hybrid composites occurs probably due to the interaction of pectin macromolecules with cations (Mg^{2+} , Ca^{2+} , Al^{3+}) released from clay at low pH (Massaro *et al.*, 2018) and cross-linking of Pec/MMT particles (Gawkowska *et al.*, 2018) with the formation of gel-like structure. This is of interest because polysaccharide hydrogels are considered as an alternative to other gastroprotective drugs (Bor *et al.*, 2019).

Figure 4. Swelling of MMT and Pec/MMT composites in acidified water

3.4. Protective properties of Pec/MMT composites

The protective effect of the hybrid composites obtained was evaluated by observing changes in the morphological characteristics of the small intestine of the rats (3 groups), which were administered ASA (20 mg) with Pec/MMT composites (6.3 mg), in comparison with those of control rats and group of rats taking only ASA. A high dose of aspirin ($90 \text{ mg kg}^{-1} \text{ d}^{-1}$) was taken to cause GI adverse events. The results of the histological studies are presented in Figure 5.

The photomicrograph of the small intestine of control rats showed a normal histological structure (Figure 5a). Oral administration of a high dose of ASA to rats for 16 days led to acute small intestinal mucosal injury, corresponding to the development of enterocolitis in test animals (Figure 5b). The mucosal injury scores of groups taking aspirin with Pec/MMT composites (Figure 5c,d,e) were decreased compared with those of rats taking only ASA (Figure 5b). Maximum protective effect demonstrated the composite with a 20 wt.% pectin content. In this case, a normal histological structure of the intestinal mucosa with minor areas of swelling and dystrophy of the parenchyma of the glands was observed (Figure 5e). ASA adsorption on Pec/MMT insufficiently

contributes to the protection of the gastrointestinal tract (Table 3). Therefore, improvement of the protective properties of Pec/MMT composites by increasing the polymer content can be explained by the biological activity of pectin, leading to wound healing (Minzanova *et al.*, 2018). The effect of polymer content on mucoadhesive properties of hybrid composite is also possible (Sharma *et al.*, 2018).

4. CONCLUSIONS:

This paper presents a simple and effective method for the synthesis of Pec/MMT composites with a given biopolymer content. According to data derived from physical and chemical studies, the polysaccharide not only coated the surface of MMT, but was also intercalated into the interlayer space of the natural clay mineral.

The resulting Pec/MMT composites showed insufficient sorption capacity towards ASA molecules (1.0-6.8 mg g⁻¹) to protect the gastrointestinal tract. However, regeneration of the small intestine of the experimental rats, which were administered high doses of aspirin along with the composites for 16 days, was observed. Optimal normalization of the morphological parameters of the small intestine was observed in rats treated with the Pec/MMT composite containing 20wt.% pectin. The protective properties of the composites were improved by increasing the pectin content, probably due to the biological activity of the polysaccharide.

It should be noted that the composites swelled in acidified water (pH = 2.0), and their swelling ability was higher to compare with unmodified MMT. This can be caused by the cross-linking of Pec/MMT particles due to the interaction of pectin with cations released from the clay, apparently requiring a separate investigation.

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Figure 3. SEM images of MMT (a) and 5wt.%Pec/MMT composite (b)

Figure 5. The histological structure of the small intestine (H&E 200x) of control rats (a), rats taking only ASA (b), rats taking ASA and treated with 5wt. % Pec/MMT (c), 10%wt. Pec/MMT (d) and 20wt.% Pec/MMT (e) composites

Table 1. The results of IR spectroscopy study of pectin, MMT and Pec/MMT composite

Sample	νOH	νCH	$\nu(\text{C=O})_{\text{E}}$ νCOOH	δCH δOH	$\nu\text{C-O-C}$ $\nu\text{C-C}$ $\nu\text{C-O}$	$\nu\text{Si-O}$ δAlAlOH $\delta\text{Si-O-Si}$ $\delta\text{Al-O-Al}$
Pectin	3432	2939 2900	1749 1625	1443 1330 1238	1151 1076 1018	-
MMT	3627 3429 3242	-	-	-	-	1043 915 525 471
Pec/MMT	3626 3430 3256	2931 2853	1745	1442 1329	Overlapped by the MMT	1039 917 527 472

Table 2. The results of XRD study of MMT and Pec/MMT composites

Peak number	Position, 2 θ degree	d_{001} , Å	Peak area, %
MMT			
1	7.0	14.6	49.3
2	7.2	14.3	36.7
3	7.5	13.6	39.3
5wt.% Pec/MMT			
1	6.7	15.4	100.0
2	7.2	14.3	16.3
3	7.5	13.6	45.0
10wt.% Pec/MMT			
1	6.6	15.6	46.5
2	7.5	13.6	46.9

Table 3. Sorption of ASA on Pec/MMT composites

Pec content in the composite, wt. %	ASA concentration after sorption, mg L ⁻¹	Amount of ASA adsorbed, mg	Sorb. capacity, mg g ⁻¹
0	255.7	0.31	3.1
5	246.4	0.68	6.8
10	246.9	0.66	6.6
20	261.0	0.10	1.0

Note. Process conditions: temperature – 36 °C; pH = 2.0; sorbent loading – 0.1 g; solution volume – 40 mL; initial ASA concentration – 263.5 mg L⁻¹; no mixing