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Hydroxyethyl cellulose functionalised with maleimide groups as a new excipient with enhanced mucoadhesive properties

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ABSTRACT

Hydroxyethylcellulose (HEC) is a non-ionic water-soluble polymer with poor mucoadhesive properties. The mucoadhesive properties of hydroxyethylcellulose can be improved by modifying it through conjugation with molecules containing maleimide groups. Maleimide groups interact with the thiol groups present in cysteine domains in the mucin via Michael addition reaction under physiological conditions to form a strong mucoadhesive bond. This will prolong the residence time of a dosage form containing this modified polymer and drug on mucosal surfaces. In this study HEC was modified by reaction with 4-bromophenyl maleimide in varying molar ratios and the successful synthesis was confirmed using ^{1}H NMR and FTIR spectroscopies. The safety of the newly synthesised polymer derivatives was assessed with *in vivo* planaria assays and *in vitro* MTT assay utilising Caco-2 cell line. The synthesized maleimide-functionalised HEC solutions were sprayed onto blank tablets to develop a model dosage form. The physical properties and mucoadhesive behavior of these tablets were evaluated using a tensile test with sheep buccal mucosa. The maleimide-functionalised HEC exhibited superior mucoadhesive properties compared to unmodified HEC.

1. Introduction

In modern medicine, excipients play an important role in pharmaceutical drug formulation. US Food and Drug Administration defines excipients as inactive or inert ingredients or substances intentionally added to a drug that is not part of the active substance (Saluja and Sekhon, 2013). Pharmaceutical excipients are typically included in larger quantities in dosage forms and can account for up to 90% of medicinal products (van der Merwe et al., 2020). The excipients with added functionalities or 'multifunctional excipients' are subject of recent interest among pharmaceutical manufacturers. The added functionality can be achieved by developing a new excipient by chemical modification, co-processing existing excipients or synthesis of novel materials (Kolter and Guth, 2016).

Here, we are interested to develop a new excipient from non-ionic hydroxyethylcellulose (HEC) which is widely used in food and drug formulations as a thickening agent (Stolz et al., 2021). Enhanced mucoadhesive characteristics is one of the desirable properties to improve in HEC. Mucoadhesion is the ability of materials to adhere to mucosal membranes in the human body and it provides an improved retention on the tissue allowing more efficient absorption of drug molecules (Khutoryanskiy, 2011). Non-ionic polymers often have poorer mucoadhesive properties compared to polyelectrolytes (Brannigan and Khutoryanskiy, 2019; Khutoryanskiy, 2011).

Enhancement of mucoadhesive properties of non-ionic polymers can be achieved by their chemical modification, which involves the introduction of new functional groups. This enhancement can be accomplished by incorporating functional groups of an ionic nature, thereby transforming a non-ionic polymer into a polyelectrolyte. Alternatively, a non-ionic polymer can be functionalised with groups capable of forming covalent bonds with mucin under physiological conditions. In this case, the mucoadhesive polymer is classified as a mucoadhesive of the second generation. Several approaches are known to make mucoadhesive polymers of the second generation. These include introduction of free thiols (Bernkop-Schnürch, 2005), phenylboronic acid (Prosperi-Porta et al, 2016), catechols (Kim et al, 2015), acryloyls (Shitrit and Bianco-

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Peled, 2017), methacryloyls (Buang et al, 2022), aldehydes (Brotherton et al, 2022), maleimides (Tonglairoum et al., 2016) and some other groups (Brannigan and Khutoryanskiy, 2019). Introduction of thiol groups into water-soluble polymers has become most widely explored strategy to enhance mucoadhesive properties with over 450 papers published and recent translation of this approach into commercial products such as Lacrimera eye drops (Brannigan and Khutoryanskiy, 2019). However, thiolated polymers have limitations such as stability issues caused by their easy oxidation. Therefore, the development of alternatives to thiolation is of great interest.

Several attempts to improve mucoadhesive properties of HEC have been reported previously. Bernkop-Schnurch research group reported the synthesis of betaine-modified HEC (Efiana et al., 2023) and S-protected thiolated HEC (Leonaviciute et al., 2016). More recently, we also reported modification of HEC by reaction with glycidol methacrylate that resulted in improved mucoadhesive properties (Buang et al., 2022).

Our group previously pioneered the use of materials with maleimide groups in the design of dosage forms with enhanced mucoadhesive properties. These include the design of hydrophilic nanogels (Tonglairoum et al., 2016), liposomes (Kaldybekov et al., 2018; Moiseev et al, 2022) and nanoparticles (Kaldybekov et al., 2019) functionalised with maleimide groups. Other research groups have also picked up this idea and developed maleimide-functionalised alginate (Shtenberg et al., 2017), chitosan (Sahatsapan et al., 2022, Sahatsapan et al., 2018) and carboxymethylcellulose (Pornpitchanarong et al., 2022). Maleimide groups are expected to show better stability to oxidation compared to thiols.

HEC is much less reactive than chitosan or carboxymethylcellulose because it contains OH groups for potential conjugation with functional molecules. Nevertheless, in this study we developed a new method for introducing maleimide groups into this polymer by reacting HEC with N-(4-bromophenyl)maleimide. The advantage of the new synthetic approach reported in this study is the preservation of the non-ionic nature of HEC derivatives, which may provide better compatibility with charged drug molecules. The resulting HEC derivatives were fully characterized using FTIR and ^{1}H NMR spectroscopies and elemental analysis. The toxicological properties of these derivatives were assessed using *in vivo* assays with planaria and MTT assay in Caco-2 cell line. Model tablets were prepared and coated with maleimide-functionalised HEC for subsequent assessment of their mucoadhesive properties using a tensile test.

2. Materials and methods

2.1. Materials

HEC 720,000 Da, chitosan low molecular weight (chitosan$_{LMW}$, 50,000–190,000 Da), chitosan medium molecular weight (chitosan$_{MMW}$, 190,000–310,000 Da), chitosan high molecular weight (chitosan$_{HMW}$, 310,000–375,000 Da), triethylamine (TEA), N-(4-bromophenyl)maleimide (BPM), hydrochloric acid, benzalkonium chloride (BAC) and sodium hydroxide were obtained from Sigma Aldrich Co., Ltd., Gillingham, UK. N,N-dimethylformamide (DMF) was provided by SLS Supplies Ltd., Nottingham, UK.

The cell viability assay utilised the following cell culture materials: DMEM High Glucose (Capricorn Scientific GMbH, Germany), foetal calf serum (GE Healthcare Life Sciences, Chicago, IL, USA), penicillin/streptomycin (Nacalai Tesque Inc., Kyoto, Japan), CellTiter 96 Aqueous MTS reagent powder (Promega Corporation, Wisconsin, USA), and phenazine methosulfate (Thermo Fischer Scientific UK Ltd., Leicestershire, UK). The Caco-2 cells were generously donated by Faculty of Pharmacy, UiTM Puncak Alam (Shah Alam, Selangor, Malaysia). The fresh sheep buccal tissues were obtained from PC Turner Abattoir (Farnborough, Hampshire, UK).

2.2. Synthesis and characterization of HEC derivatives

HEC derivatives (HECMAL) were synthesised in three different molar ratios of [HEC]:[BPM] = [1]:[1] (HECMAL$_{low}$), [1]:[2] (HECMAL$_{medium}$) and [1]:[3] (HECMAL$_{high}$). Briefly, BPM (857 mg for HECMAL$_{low}$, 1715 mg for HECMAL$_{medium}$ and 2572 mg for HECMAL$_{high}$) was dissolved in 50 mL of DMF. Subsequently, TEA (473 µL) was added, and the mixtures were stirred for 30 min at 0 °C. Then, 50 mL of 1% w/v HEC solution (prepared in deionised water) was added dropwise in these mixtures. The mixtures were then constantly stirred at 25 °C for 24 h. The resulting solutions were added to excess of cold ethanol and centrifuged at 10000 rpm (10 min) for three times. The precipitates from solution were purified by dialysis against deionised water over 72 h using a cellulose membrane with molecular weight cut off 12–14 kDa (8 changes of water). Finally, the product was recovered after 3–4 days by freeze-drying using Heto PowerDry LL3000 Freeze Dryer (Thermo Fischer Scientific UK Ltd, Loughborough, UK).

The ^{1}H NMR spectra of polymers were recorded using a Bruker Nanobay 400 MHz two-channel NMR instrument (Bruker UK Ltd., Coventry, UK). NMR tubes with a 5 mm internal diameter were used to record the spectra for HEC and HECMAL solutions (20 mg/mL) prepared in D_2O. The NMR spectra were analyzed using MestReNova (Mnova) Version 6.0.2–5475. FTIR spectra of HEC and HECMAL were recorded using a Spectrum 100 FTIR Spectrophotometer (Perkin–Elmer UK Ltd., Buckinghampshire, UK) from 4000 to 650 cm^{-1}, with a resolution of 4 cm^{-1}, and accumulation of 16 scans. The data obtained were analysed using Spectrum One software.

2.3. Quantification of maleimide

The degree of substitution (DS) was determined for HECMAL polymers, where all peak integrations were normalized to the peak at 3.0–5.0 ppm, which corresponds to HEC. The DS was calculated as the ratio between the integral at 6.93 ppm divided by 2 and the sum of the integral at 3.0–5.0 ppm divided by 16 and the integral at 6.93 ppm divided by 2 as shown in Eq. (1). Values of 2 and 16 used to divide the peak integrations represent the protons on the maleimide and the HEC, respectively.

$$DS = \frac{\text{IH maleimide}/2}{\text{IH HEC}/16 + \text{IH maleimide}/2} \quad (1)$$

Subsequently, the degree of substitution (DS) of the amino moieties was calculated from the elemental composition according to Eq. (2).

$$DS = \frac{M_{AGU}.\ N\%}{MN.100 - M_{SG}.N\%} \quad (2)$$

M_{AGU} is the molar mass of anhydroglucose repeating unit, N% is the nitrogen content determined by elemental analysis, MN is the molar mass of nitrogen, M_{SG} is the molar mass of the substituent group (Liesiene and Kazlauske, 2013; Zarth et al., 2011).

2.4. In vitro toxicity

2.4.1. Cells

Caco-2 cells were utilised to assess the cytotoxicity of each polymer. The cells were grown in DMEM high glucose containing 10% (v/v) foetal calf serum and 1% (v/v) penicillin/streptomycin. The cells were kept in an incubator at 37 °C with 5 % CO_2 in the air and 100% relative humidity. The cells were seeded at a density of 1×10^4 cells per well in 96-well plates.

2.4.2. Cell viability assay

The cells were subsequently exposed to varying concentrations of the compounds for 24 h (1.0%, 0.50%, 0.25%, 0.10%, and 0.05% w/v). The untreated cells in the negative control group were found to be 100 %

viable. At the end of each treatment, the growth medium was replaced with fresh portion. Each well received 20 μL of a 5 mg/mL MTT solution (in the dark). The cells were incubated for an additional 4 h at 37 °C in a humidified 5% CO_2 incubator. DMSO was added, thoroughly mixed, and incubated for another 10 min. The absorbance was measured at 540 nm using an Infinite 200 PRO microplate reader (Tecan Group Ltd., Switzerland).

2.5. In vivo toxicity

2.5.1. Acute toxicity assay

Schmidtea mediterranea planaria were kindly provided by Dr Jordi Solana (Oxford Brookes University). The worms were maintained in artificial pond water (APW) at room temperature in the dark. They were fed chicken liver once per week, and APW was replaced on a weekly basis. APW was prepared as a mixture of the following salts: 5 M NaCl, 1 M $CaCl_2$, 1 M $MgSO_4$, 1 M $MgCl_2$ and 1 M KCl in ultra-pure water. Planaria (1.0–1.5 cm long) were placed in 24 wells of plate culture (one in each well), and 2 mL of HECMAL or HEC (1.0%, 0.50%, 0.25%, 0.10%, and 0.05% w/v) solutions were added. 1% w/v BAC solution was used as a positive control. APW was used to dissolve all the test compounds. Acute toxicity tests were run for 24, 48, and 72 h and the number of live/dead planaria was recorded. Planaria that did not move after agitation were considered dead.

2.5.2. Fluorescence intensity test

The experiment was slightly modified from the procedure previously developed by our group (Shah et al., 2020). Following a 24-hour treatment with 1% of the test substances (unmodified HEC and modified HECMAL polymers), planaria were exposed to a 0.1% (w/v) sodium fluorescein solution in APW for 1 min. The excess fluorescein solution was then removed from planaria by immersing them in APW for 15 min. Each worm was placed on a microscopy glass slide and then immobilised with a few drops of a 2.0 % w/v agarose solution. The microscopy glass slide was placed on level surface of ice flakes (−0.5 to −0.8 °C) until the gel hardened. Fluorescence images of the worms were captured using a Leica MZ10F stereomicroscope (Leica Microsystems, UK) fitted with a DFC3000G digital camera set at 2.0× magnification, 160 ms exposure time, and gamma 0.7.

2.6. Preparation of blank tablets

The blank tablets were prepared by mixing 400 g hydroxypropyl cellulose (HPMC), 400 g microcrystalline cellulose (MCC), and 190 g barium sulfate (BS) in a TURBULA® powder blender mixer/3d shaker mixer (Willy A. Bachofen Maschinenfabrik, Germany) for 10 min, following addition of 10 g magnesium stearate (MS) for another 2 min. The powder mixtures were dispensed into a hopper above the tablet compression machine Riva Minipress MII (Riva GB Ltd, Aldershot UK). The mixture was compacted with single die set at an automatic mode with the speed of production at 40 tablets/min. The average tablet weight, thickness, diameter and hardness were determined for 20 tablets in every batch. Tablet hardness was assessed using a tablet hardness tester (Copley Scientific Limited, Nottingham UK).

2.7. Preparation of spray coated tablets

Solutions of 0.1% (w/v) of HEC, $HECMAL_{low}$, $HECMAL_{medium}$ and $HECMAL_{high}$ were prepared by dispersing the polymers in deionised water, while $chitosan_{low}$, chitosan $_{medium}$ and $chitosan_{high}$ were dissolved in 0.1 M HCl. 2.5 mg/mL of sodium fluoresceine was added to these solutions before being spray-coated onto blank model tablets using Mini Coater Drier (MCD-2) equipment from Caleva (Dorset, UK). The equipment setting for the experiment was consistent with the agitator at 55 %, fan at 9.5 m/sec, temperature at 40 °C, and pump at 4 rpm. The thickness of the tablets coating was evaluated using fluorescence microscopy and subsequent image analysis with Image J software.

2.8. Ex vivo mucoadhesion

The TA-XT Plus Texture Analyser (Stable Micro Systems Ltd, UK) with a 5 kg load cell was used to study the mucoadhesive properties of all formulations. Sheep buccal tissue was cut into squares and placed in between a cylindrical device and the top cover. The cover had a circular opening of 20 mm in diameter. The mucosal surface of the tissue was exposed through this opening. The tissues were kept at 37 °C using a water bath.

Each tablet was attached to the aluminium probe (12 mm in diameter) using a sticky adhesive tape. Then the probe was lowered, and the table was brought in contact with a mucosal tissue. The following test parameters were used: pre-speed test 0.5 mm/s; test speed 0.5 mm/s; post-speed test 1 mm/s; applied force 0.5 N; contact time 60 s; trigger type auto; trigger force auto; and return distance 20 mm.

2.9. Statistical evaluation

A two tailed Student *t*-test with 95% confidence interval as the minimal level of significance was employed as the statistical tool to evaluate the data.

3. Results and discussions

Maleimide-functionalised HEC was synthesized by reacting HEC with N-(4-bromophenyl)maleimide according to the reaction scheme shown in Fig. 1. This reaction was catalyzed by addition of TEA as a base and was conducted in an aqueous solution. Three different derivatives were synthesized with different molar ratios of HEC to BPM.

3.1. ^1H NMR spectra

The ^1H NMR spectra of unmodified HEC and HECMAL samples recorded in D_2O are shown in Fig. 2. The spectral data confirmed that the synthesis of HECMAL was successful with the presence of signals from the protons that belong to maleimide moieties at 6.93 ppm. The spectrum of BPM recorded in DMSO-d_6 can be found in Fig. S1 in Supporting information. The signals of the aromatic group are detected in the spectra at 7.65–7.63 ppm, 7.51–7.49 ppm, 7.35–7.33 ppm and 7.20–7.18 ppm. We also found additional signals at 6.50–6.30 ppm. According to Morrison et al. (2019) there is likely an opening of some of maleimide rings in the resulting product with the presence of water that contributes to additional signals in the spectra (Morrison et al., 2019). This is in agreement with Barradas et al. (1976), who reported that maleimide ring may undergo hydrolysis in alkaline media. Thus, in this reaction, there could be a product with both intact and ring-opened maleimide groups as shown in Fig. 2. The other remaining signals are attributed to the backbone HEC including the peaks at 1.21 ppm and 1.83 ppm with unidentified structure similarly found and reported in D'Avino et al.(2022) and Ray et al. (2018).

3.2. Fourier-transformed infrared (FTIR) spectroscopy

FTIR spectroscopy was used for further evaluation of the chemical structures before and after the functionalisation of HEC. Fig. 3 shows the FTIR spectra of both unmodified HEC and HECMAL derivatives. There are two new bands appear at around 1506 cm^{-1} and 1377 cm^{-1} in the spectra of HECMAL derivatives. These bands confirm the successful introduction of maleimide group into HEC as they represent benzene ring (1506 cm^{-1}) and C–N stretching of maleimide groups (1377 cm^{-1}). Additionally, C=O band appeared at 1703 cm^{-1} is attributed to maleimide ring. The intensity of these bands increased in the spectra with increase in [BPM]/[HEC] molar ratio.

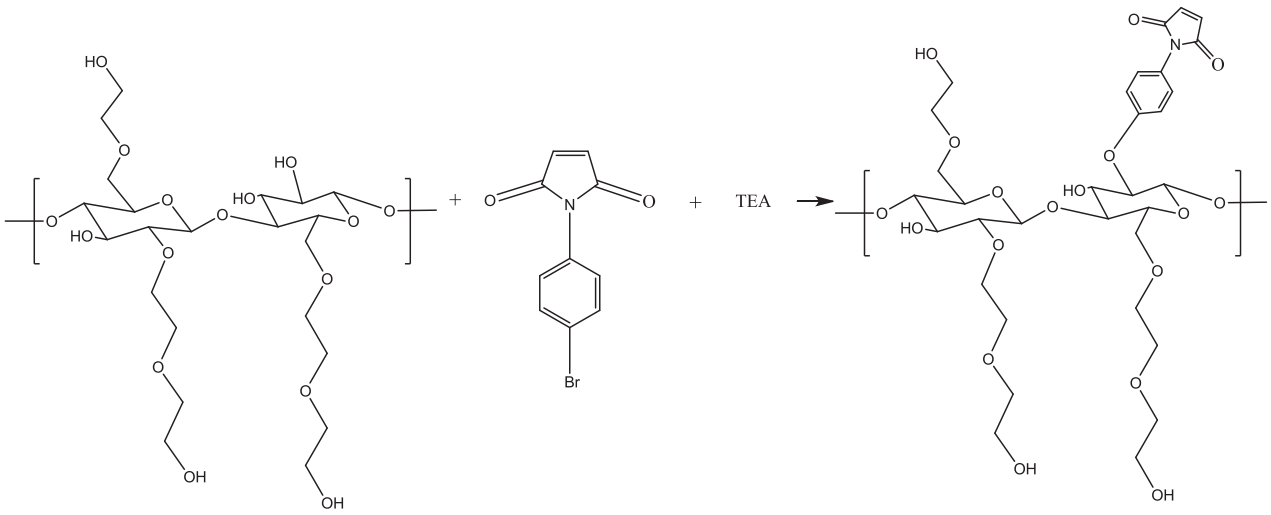


Fig. 1. Reaction of HEC with N-(4-bromophenyl)maleimide.

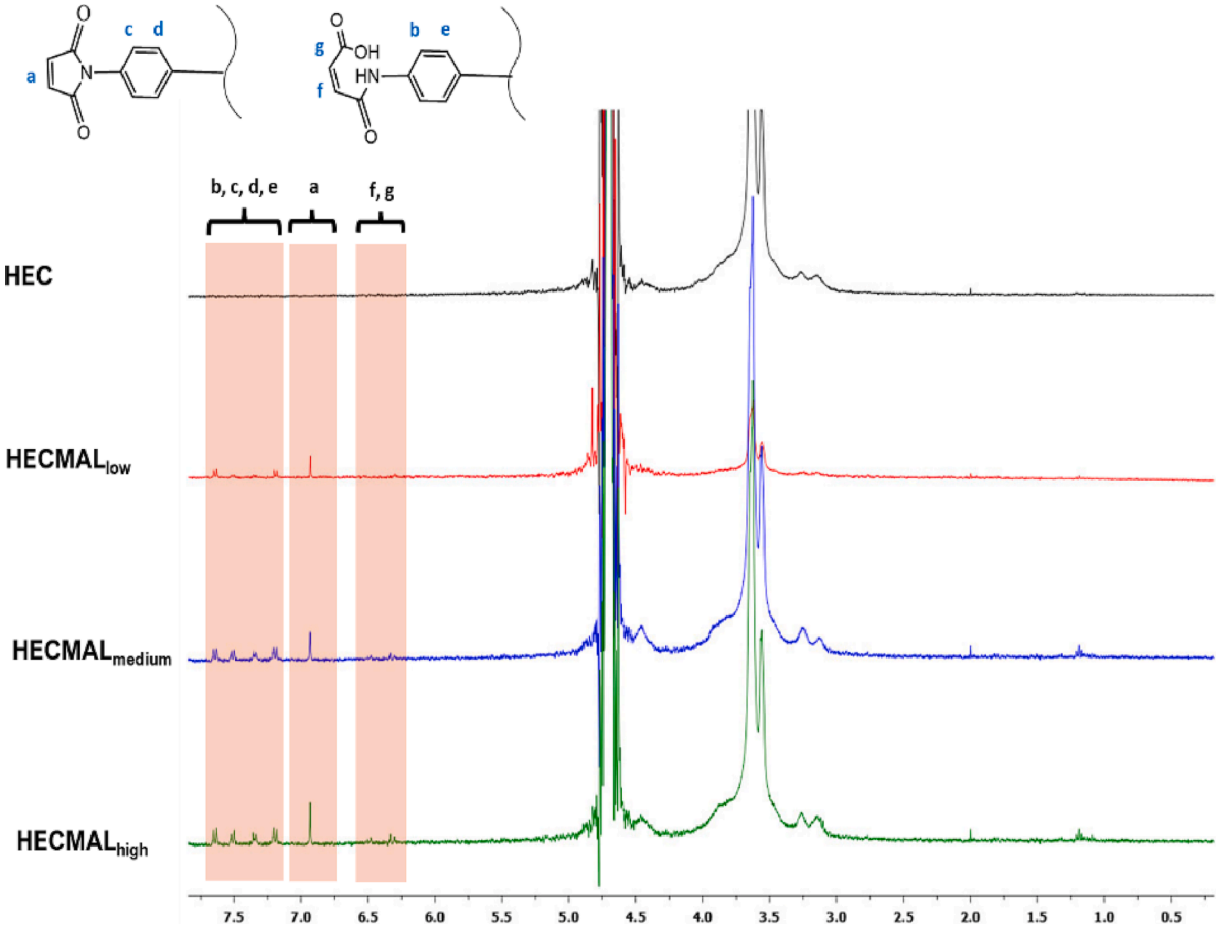


Fig. 2. ^{1}H NMR spectra of unmodified HEC and HECMAL derivatives recorded in D_2O.

3.3. Quantification of maleimide groups in HECMAL

The values of DS for HECMAL derivatives determined using two independent methods (NMR and elemental analysis) are summarized in Table 1. The DS values are in good agreement with the compositions of the reaction mixtures and as expected show an increase from HECMAL$_{low}$ to HECMAL$_{medium}$ to HECMAL$_{high}$. The results from elemental analysis revealed that the nitrogen content of the modified HECMAL polymers increased as the molar ratio of BPM to HEC increased. The higher the molar ratio, the greater the nitrogen content in the polymer, which is related to the presence of maleimide groups. The nitrogen content ranges from 0.43% to 1.35%, with the estimated DS increases from 0.07 to 0.22. Comparable data on DS values were also calculated from ^{1}H NMR with the estimate DS increases from 0.08 to 0.23. The summary of the calculation is shown in Tables S1–S4 (Supplementary information).

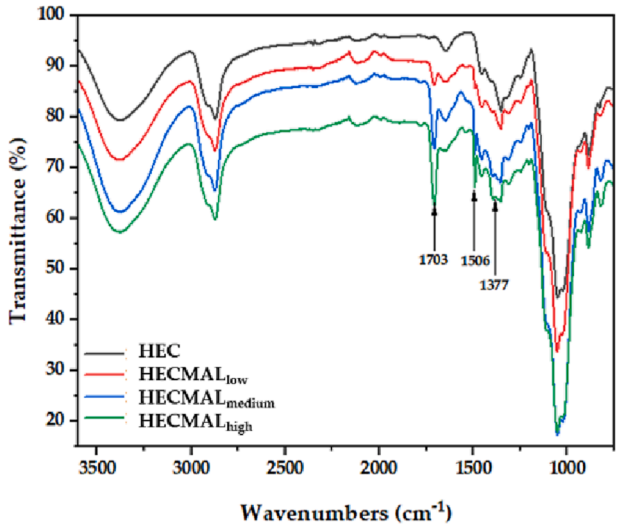


Fig. 3. FTIR spectra of unmodified HEC, $HECMAL_{low}$, $HECMAL_{medium}$ and $HECMAL_{high}$.

Table 1
Percentage of nitrogen content and DS of HEC and HECMAL.

	N%	[1]DS_{EA}	[2]DS_{NMR}
HEC	0.00	0.00	0.00
$HECMAL_{low}$	0.40 ± 0.05	0.07	0.08
$HECMAL_{medium}$	0.62 ± 0.01	0.11	0.13
$HECMAL_{high}$	1.22 ± 0.19	0.22	0.23

[1] DS_{EA} calculated from elemental analysis (equation S1).
[2] DS_{NMR} calculated from ^{1}H NMR (equation S2).

3.4. Toxicity evaluation in planaria

HEC has a well-established safety profile and is commonly used as a polymeric excipient for mucosal applications, for example, in ocular (Mohamed et al., 2022) and vaginal drug delivery (Hiorth et al., 2014). However, when a pharmaceutically acceptable polymer is modified chemically, its toxicological properties should be extensively evaluated before it can be introduced as a new pharmaceutical excipient. An initial toxicological evaluation of new chemicals can be conducted using invertebrate models such as drosophila, brine shrimp, slugs (Lenoir et al., 2013, 2011; Rand et al., 2014; Sasidharan et al., 2010). Previously, we also proposed to use planaria as a rapid and cheap prescreening tool for potential skin irritants (Shah et al., 2020) and more recently this model was used to evaluate HEC and methacryloylated HEC for mucosal delivery (Buang et al., 2022). In this study we evaluated the toxicological properties of HEC and HECMAL using planaria *in vivo* assays.

An acute toxicity of live-dead assay was conducted with planaria following 24 h exposure to 1% v/w polymer solutions. No mortality was observed in all tested polymers. All planarian worms were alive and a few were seen adhering to the side of the walls of the well plates. Ireland et al reported that *S. mediterranea* species have a preference to attach to the side of the wall and have less motility (Ireland et al., 2020).

An additional evaluation of the polymer effects on planaria was conducted using fluorescent assay, which determines the effect of chemicals on planarian body wall integrity and barrier function. When planaria are exposed to irritant chemicals this causes a damage in their epithelia and reduces their barrier function with respect to small molecules. When planaria are subsequently exposed to a solution of sodium fluorescein, this dye penetrates their body and the extent of penetration can then be assessed using fluorescence microscopy to provide quantitative information (Shah et al., 2020). The results of the fluorescent assay following the worms exposure to HEC and HECMAL solutions are presented in Fig. 4. An exposure of planaria to HECMAL solutions for 24 h did not reveal any statistically significant reduction in the epithelial barrier function compared to HEC ($P > 0.05$). This result indicates that the polymers bearing maleimide functional groups do not exhibit any increase in their irritation properties compared to parent HEC. However, when planaria were exposed to strongly irritant 1% w/v BAC solutions as a positive control for just 1 h, statistically significant increase in the fluorescence intensity was observed due to enhanced penetration of sodium fluorescein into their body.

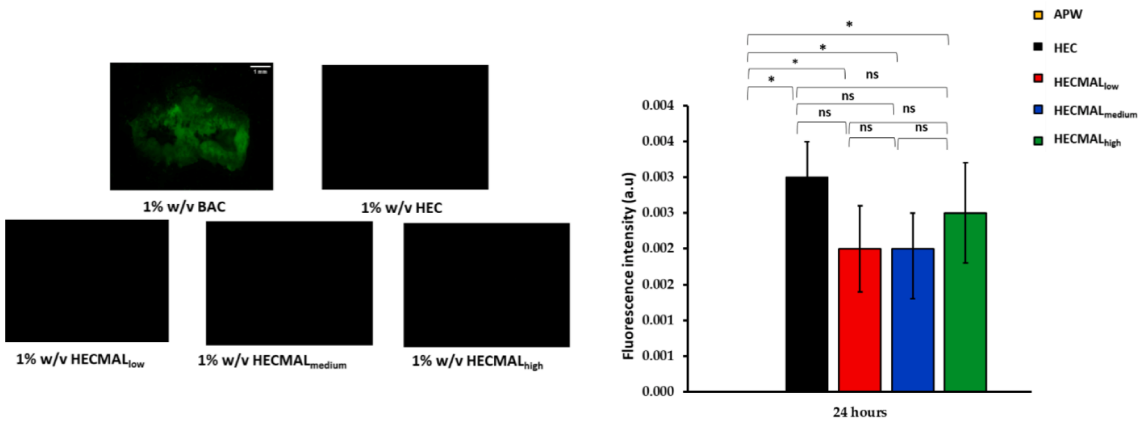
3.5. In vitro MTT toxicity test

The MTT test was performed to assess the levels of viable cells following their 24 h exposure to solutions of HEC and HECMAL at various concentrations (0.05, 0.1, 0.25, 0.5 and 1 % w/v). Caco-2 cell line was chosen for these experiments because this originates from a mucosal tissue (human colon) and is often used for toxicological assessment of pharmaceutical materials for transmucosal drug delivery applications (Jelkmann et al., 2020; Prüfert et al., 2020). Fig. 5 presents the data on cell viability in the presence of HEC and HECMAL. Exposure of Caco-2 cells to all polymer samples tested did not result in a dramatic reduction in their viability in the studied concentration range (0 – 1 % w/v); the levels of cell viability remained high (>66.57 %) even at the highest polymer concentration (1 % w/v). As expected, an increase in the concentration of polymers in solutions resulted in some reduction in the cell viability; however, the viability data generated for 1 % w/v HEC in the present work (67 ± 5 %) are somehow lower than the results reported by Leonaviciute et al. (2016) (~96 %) (Leonaviciute et al., 2016). This deviation from the literature data may be related to the difference in the molecular weights of HEC used in our study (HEC MW 720,000 Da) and in Leonaviciute et al. (2016) study (HEC MW 250,000 Da). There was no statistically significant difference between the cell viabilities observed for all polymer samples in the concentration range of 0.05–0.5 %. The results generated for HECMAL samples indicate that cell viabilities are above 80% when compared to the unmodified control cells. These results are also in agreement with the study of Pornpitchanarong et al (2022), who reported non-toxic nature of carboxymethylcellulose functionalised with maleimide groups with using HGF-1 cells The results of the study of HECMAL in cell culture are in good agreement with the findings from the experiments with planaria. It can be concluded that the newly synthesised HECMAL is non-toxic and suitable for further development in pharmaceutical applications.

3.6. Design of model tablets coated with different polymers

Blank round tablet formulations were designed as a model dosage form for their subsequent coating with HEC and HECMAL. It is more reasonable to coat tablets with polymers that possess improved mucoadhesive properties, rather than manufacturing tablets using direct compression of these materials. The mucoadhesive polymer present within the tablet bulk does not significantly contribute to the enhancement of mucoadhesive properties, as it primarily acts on the tablet's surface.

The blank tablets were prepared by mixing hydroxypropyl cellulose, microcrystalline cellulose, barium sulfate as bulking agents and binders, with addition of magnesium stearate as a lubricating agent and subsequent compression of powder mixtures. This specific composition was chosen to prepare tablets that will not exhibit any quick disintegration or swelling in an aqueous medium. These tablets were subsequently spray coated with 0.1 % w/v HEC or HECMAL or chitosans of different weights mixed with sodium fluorescein using spray coating. Sodium fluorescein was used in this case to facilitate evaluation of the efficiency of their surface coating with mucoadhesive polymers. Chitosans of different molecular weights (low, medium and high) were used as a positive control due to well documented excellent mucoadhesive



(a) (b)

Fig. 4. Fluorescence intensity test using planaria: (a) exemplar fluorescent images (scale bar is 1 mm) and (b) fluorescence intensity values calculated using analysis of fluorescent images, following 24 h exposure of planaria to 1 % w/v solutions of HEC, $HECMAL_{low}$, $HECMAL_{medium}$, $HECMAL_{high}$ and 1 h exposure to 1 % w/v BAC, with subsequent immersion of the worms in 0.1% w/v sodium fluorescein. Each experiment was performed using 3 different worms (n = 3) and mean fluorescence intensity values ± standard deviations were calculated. *Statistical significance was determined using *t*-test and significant differences are shown with * when $p < 0.05$.

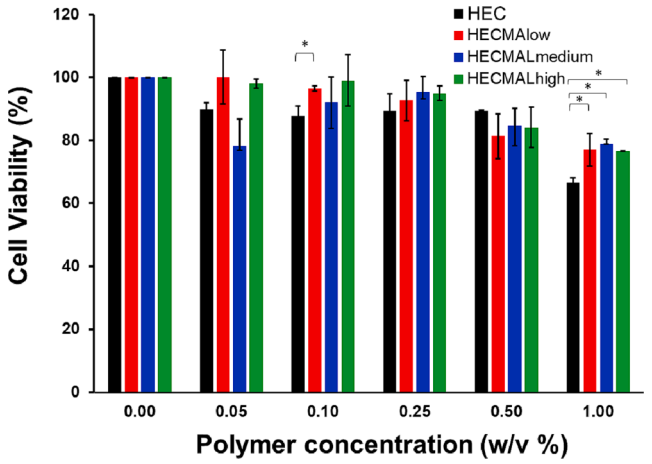


Fig. 5. Caco-2 cell viability evaluated using MTT assay following their exposure to HEC and HECMAL samples for 24 h. Data show the mean ± standard deviations (n = 3) *Statistical significance was determined using *t*-test and $p < 0.05$ was statistically significant.

properties of this polysaccharide (Brannigan and Khutoryanskiy, 2019; Khutoryanskiy, 2011).

The average weight of these blank model tablets was 102 ± 1 mg, their diameter was 6.00 ± 0.05 mm and their thickness was 3.00 ± 0.04 mm. The hardness of the tablets was found to be 140 ± 1 N. Fig. 6 shows the fluorescent microscopy images of these model tablets. Image analysis helped to establish that in all cases the tablets were fully and homogeneously coated. However, slightly different polymer thicknesses were observed despite the use of the same spray coating amount and time. Tablets coated with HEC displayed the thickest coating (0.33 ± 0.01 mm), followed by HECMAL at 0.15 ± 0.01 mm and chitosan at 0.11 ± 0.01 mm.

3.7. Mucoadhesive properties of tablets coated with different polymers

Mucoadhesive properties of the tablets coated with different polymers were studied with respect to freshly excised sheep buccal mucosa using a tensile test *ex vivo* (Khutoryanskiy, 2011)]. This test involves the placement of each tablet in contact with mucosal tissue with its subsequent withdrawal and recording of detachment profiles. Usually, this experiment provides two important characteristics of mucoadhesion through the analysis of each detachment profile: the maximal force observed on the detachment profile is called maximal force of detachment, and the area under the curve provides the total work of adhesion.

Fig. 7 shows both the values of maximal detachment force (F_{det}) and total work of adhesion (W_{adh}) calculated from these tensile test experiments. The tablets coated with high molecular weight chitosan exhibited superior mucoadhesive properties compared to all other tablets (F_{det} = 0.077 ± 0.002 N and W_{adh} = 0.162 ± 0.010 N·mm). This was expected as chitosan has well documented excellent mucoadhesive characteristics (Khutoryanskiy, 2011) and increase in the polymer molecular weight typically results in improvements in its mucoadhesive performance (Leitner et al., 2003). Indeed, tablets coated with medium and low molecular weight chitosan exhibited substantially lower F_{det} and W_{adh} values. This decrease in mucoadhesive performance could be related to poorer ability of lower molecular weight macromolecules to form entanglements with biomacromolecules of mucins.

Tablets coated with unmodified HEC exhibited the poorest mucoadhesive properties (F_{det} = 0.024 ± 0.004 N and W_{adh} = 0.036 ±

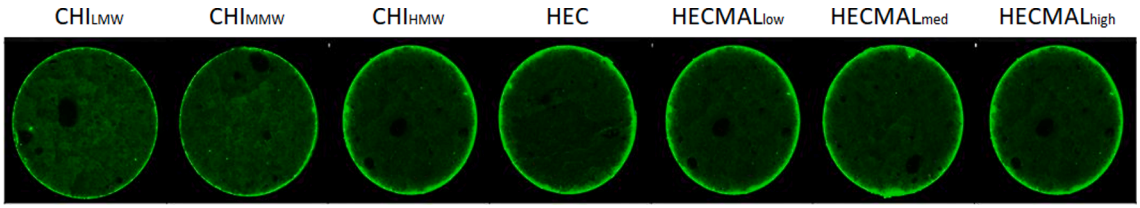


Fig. 6. Fluorescent images of tablets spray coated with various polymer solutions containing 0.1 % sodium fluorescein.

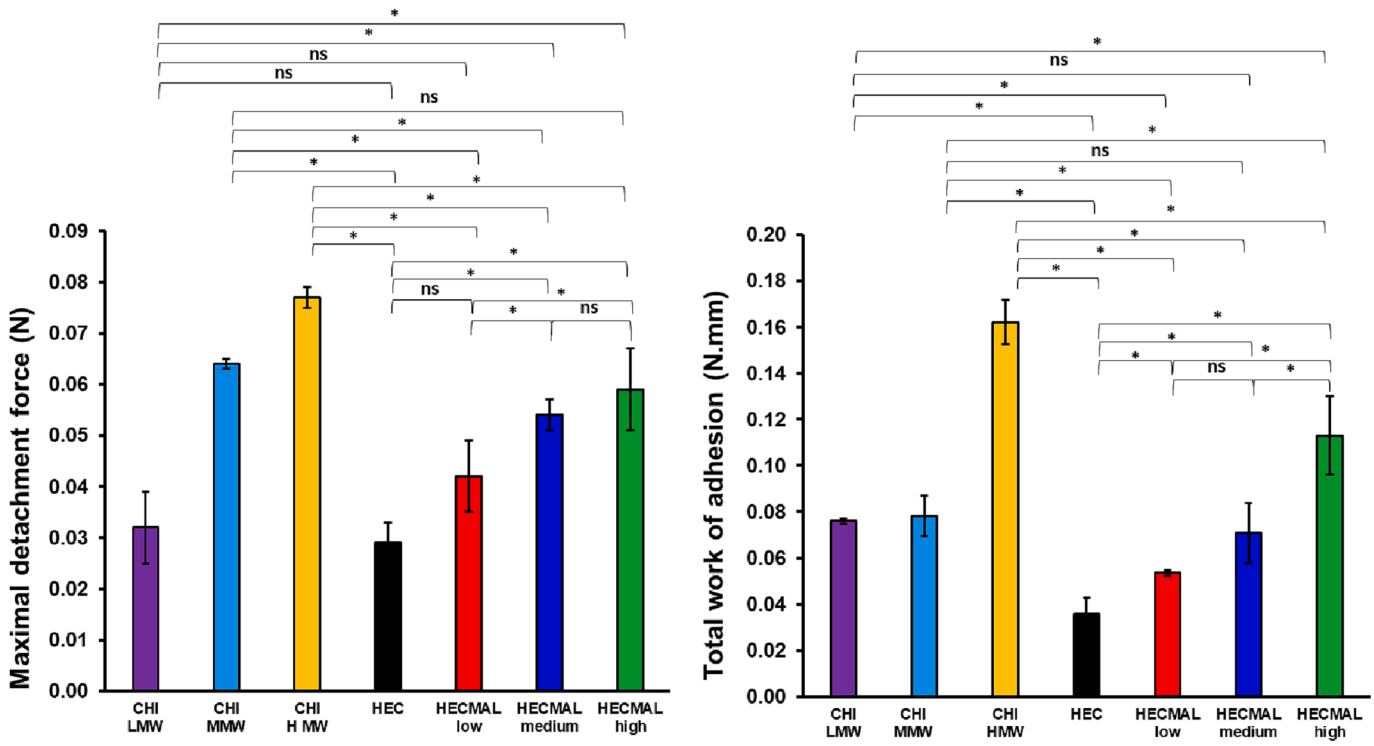


Fig. 7. Mucoadhesive characteristics of tablets coated with different polymers: (a) Maximal detachment force and (b) Total work of adhesion. Data show the mean values ± standard deviations (n = 3). *Statistically significant difference when $p < 0.05$.

0.007 N·mm). This was also expected because HEC is a non-ionic polymer (Khutoryanskiy, 2011). The introduction of maleimide groups into HEC structure improves the mucoadhesive properties of the tablets coated with HECMAL dramatically and this property correlates very well with the degree of polymer substitution (DS). Tablets coated with HECMAL samples with greater DS exhibit superior mucoadhesive properties. This is related to the ability of maleimide groups to form covalent linkages with thiol groups present in the mucins via thiol-ene click Michael addition reactions happening under physiologically relevant conditions (Gunnoo and Madder, 2016; Ravasco et al., 2019). It is important to note that formation of covalent bonds between maleimide and thiol groups is a quick process that allows achieving improved mucoadhesion within a reasonable period following tablet administration on the mucosal surface.

Although there is a substantial improvement in mucoadhesive properties of tablets coated with HECMAL polymers, the values of F_{det} and W_{adh} for all HECMAL polymers are still lower than similar characteristics recorded for the tablets coated with high molecular weight chitosan. Perhaps the cationic nature of chitosan as well as its high molecular weight for $chitosan_{HMW}$ provide it superior performance (Khutoryanskiy, 2011).

4. Conclusions

This study reveals that modification of HEC with maleimide moieties results in formation of polymers with improved mucoadhesive properties. The properties of these polymers can be tailored by varying the molar ratio of N-(4-bromophenyl)maleimide to HEC in the reaction mixture. HEC with greater concentration of maleimide groups exhibits superior mucoadhesive properties. The introduction of maleimide groups into these polymers is not detrimental for their toxicological characteristics as evaluated using *in vivo* planaria model and *in vitro* cell culture assay in Caco-2 cells. HECMAL polymers can be considered as a new excipient for formulation of mucoadhesive dosage forms for transmucosal drug delivery. In this work we have demonstrated the use of these polymers to enhance the mucoadhesive properties of tablets through their surface coating. However, HECMAL can also find applications in other areas of transmucosal drug delivery, for example, when formulated as gels, films and nanoparticles.

CRediT authorship contribution statement

Fhataheya Buang: Investigation, Methodology, Formal analysis, Writing – original draft. **Manfei Fu:** Investigation. **Afroditi Chatzifragkou:** Supervision, Resources, Writing – review & editing. **Mohd Cairul Iqbal Mohd Amin:** Resources. **Vitaliy V. Khutoryanskiy:** Conceptualization, Supervision, Project administration, Resources, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpharm.2023.123113.

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