Lubricating performance of polymer-coated liposomes

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Abbreviations

HM, hydrophobically modified; HEC, hydroxyethyl cellulose; EHEC, ethyl hydroxyethyl cellulose; PosLip, positively charged liposomes; NegLip, negativerly charged liposomes; NeuLip, neutrally charged liposomes; Alg-sol, alginate solution; Chit-sol, chitosan solution; HM-EHEC-sol, HM-EHEC solution; HM-HEC-sol, HM-HEC solution; AlgcLip, alginate coated liposomes; ChitcLip, chitosan coated liposomes; HM-EHECcLip, HM-EHEC coated liposomes; HM-HECcLip, HM-HEC coated liposomes.

Highlights

- All positively charged solutions and liposomes had low friction coefficients
- Neutral and negatively charged solutions and liposomes did not reduce friction
- Chitosan-coated liposome showed lower friction force than the individual components
- Lubrication was controlled seemingly by surface charge interactions

Abstract

Dry mouth is a troublesome condition linked to lubrication failure and leads to other diseases such as fungal infections and wounds in the oral cavity. There are many commercial salivary substitutes in the market, but none with a long-lasting lubrication effect. Polymer-coated liposomes can be an interesting formulation strategy for retrieving the symptoms of dry mouth by mimicking the micelles of saliva. In the present study, polymer coated-liposomes were prepared by the conventional thin film method and subsequently coated with three different polymers with different charge densities; alginate, chitosan and hydrophobically modified ethyl hydroxyethyl cellulose (HM-EHEC). The prepared polymer-coated liposomes were studied concerning their lubricating properties using a ball-on-disc tribometer at 2 N load at 37 ºC, and their flow behaviours were also measured. Solutions of the pure polymers and dispersions of the uncoated liposomes were also studied to investigate any contributions from the individual components. A commercial dry mouth product based on HEC (hydroxyethyl cellulose) and glycerol was also included. The formulations were measured as soon as possible after preparation and some of them after more than 4 weeks. Results demonstrated that all the positively-charged formulations (chitosan, positive liposomes and chitosan-coated liposomes) had superior lubricating properties with friction coefficients ($\mu < 0.1$) at orally relevant speeds (50 mm s^{-1}) as compared to the neutral or negatively-charged systems. At boundary lubrication conditions (3 mm s^{-1}), the chitosan-coated liposomes obtained an even lower friction force than the individual components, thus indicating a synergistic effect between the polymer and the liposome.

Key words

Dry mouth, tribology, polymers, liposomes, polymer-coated liposomes, lubrication

1 Introduction

Dry mouth is a common but often overlooked condition and is due to systemic diseases such as Sjøgren's syndrome, radiation towards the neck and the head region and last but not least multi-medication [1]. The condition might seem trivial but could lead to several serious effects such as wounds, fungal infections, dental caries and erosion of the teeth [2]. Also, dry mouth leads to trouble with eating and swallowing and a considerably reduced quality of life [3]. Renewed research interests in more effective dry mouth therapies seem to be driven by a foreseen increase in the elderly population, with chronic diseases and polypharmacy that in turn commonly induce mouth dryness. The number of elderly in the population is thought to be doubled just 30 years ahead [4] and ageing is often associated with a decrement in the quality and quantity of saliva [5].

Saliva is important for maintaining good oral health and also for relieving the symptoms of dry mouth. Human saliva is composed of about 99% water. The rest include organic components, such as 0.3% protein, enzymes (α-amylase, lysozyme, lingual lipase *etc.*), antibacterial compounds and inorganic salts [6, 7]. Different proteins such as MG2, secretory IgA, glycosylated proline rich proteins, lactoferrin and amylase form the salivary micelles, and in combination with the glycoprotein mucin the acquired enamel pellicle is formed [8]. It is recently acknowledged that salivary pellicle is an electrostatic self-assembly of mucins and small molecular positively-charged proteins such as lactoferrin, where the positively-charged species act as "molecular glue" between mucin–mucin and mucin–mucosal and enamel surfaces, aiding boundary lubrication [9].The thickness of the pellicle layer could be as high as 1 μm and is fully formed in vivo after 1-2 hours [10].The acquired enamel pellicle is important for protecting and lubricating the teeth [11, 12]. Especially the enamel gets vulnerable towards erosion if the teeth lack the pellicle, even though the pellicle cannot fully protect against demineralization of the enamel [13].

Commercially, there are many products to be administered locally to the oral cavity intended to relieve the symptoms of dry mouth, but there seem to be a lack in their efficiency, and there is no product with long-term effect on mouth moistness and lubrication [14, 15]. The products on the market formulated as mouth rinses/gels and solutions can mainly be categorized in two groups; products based on hydrophilic polymers such as carboxymethyl cellulose, hydroxyethyl cellulose and xanthan gum and products based on animal mucin. The latter being less used, due to the concerns of transmitting spongiform encephalopathy [16]. The rationale behind the use of hydrophilic polymers is probably the possibility of the polymers to adsorb large amounts of water with a potential of being released when entering the oral cavity. The lack in their efficacy may be related to the challenge of delivering a formulation to the oral cavity, namely the short retention time [17] and do not offer any lubrication performance largely related to limited surface properties. Also, these products are developed to give moisture to the oral cavity and few of them are designed to protect the teeth from erosion, which rely on the acquired enamel pellicle. The ideal dry mouth product should be able to hydrate the oral cavity and give a long-term effect *via* reducing boundary friction and accelerating the onset of mixed boundary lubrication regime. In addition, it would be advantageous if the product also could lubricate and protect the teeth by mimicking the salivary pellicle.

Due to the lack of effective commercial therapies addressing dry mouth, there are many colloidal strategies that have been attempted to address dry mouth conditions such as microgels [18], liposomes [19], *etc*. A new approach to improve enamel protection and lubrication can be to develop polymer-coated liposomes. Liposomes are spherical entities with a double layer of lipids. The size of the liposomes is around 100-200 nm. The liposomes can be charged by including positive or negative lipids. Also, the surface of the liposomes can be modified by coating them

with a biopolymer [20, 21]. The coating process is based on electrostatic deposition of the charged liposomes with a biopolymer of opposite charge. The coating process is delicate, and in order to prepare stable coated polymers finding the correct ratio between the liposomes and the coating polymer is crucial [22]. Liposomes without charge, neutral liposomes can be coated with a hydrophobically modified polymer for instance HM-EHEC or HM-HEC [23]. The coating mechanism is probably based on HM-chains intruding into the liposome membrane and by such being anchored to the surface [24].

The polymer-coated liposomes could possibly mimic the acquired enamel pellicle due to the liposomes resembling the micelles of saliva and the loosely polymer layer around the liposomes mimicking the loosely mucin layer connected to the pellicle and also mimicking the electrostatic self-assembly found in real human saliva [9].

In previous studies, we have investigated the adhesive properties of the polymer-coated liposomes towards hydroxyapatite, a model material of the teeth surface [20]. Also the mucoadhesive properties of the formulations towards a mucus-secreting cell line HT29-MTX has been conducted [25]. The water adsorption properties of the polymer-coated liposomes by the use of dynamic water sorption measurements (DVS) have also been studied [26]. These studies revealed that the adhesive properties were in the first instance dependent on the charge of the particle implying more adhesiveness for the positively charged formulations but also the alginate coated liposomes showed some adhesive properties. When the water adsorption properties of different polymers, liposomes and polymer-coated liposomes were investigated, the results showed that the polymer-coated liposomes adsorbed most water. This was probably related to a synergistic effect of both components being able to adsorb water, and was not connected to the charge of the components. Although the adhesive properties have been well-studied, rare attention has been given to the

lubrication aspects of polymer-coated liposomes. From literature, it is evident that biopolymers dispersed in bulk aqueous phase, such as protein (*e.g.* microgel), glycoproteins (*e.g.* mucin, lubricin) and polysaccharides (*e.g.* xanthan gum, pectin, carrageenan, chitosan) show interesting aqueous lubrication efficiency, which could be attributed to both adsorbed film formation by the polymer at the surface and/or the viscosity of the polymer solution [27-33]. Interestingly, polymers, such as chitosan have also shown synergistic effects on the lubrication efficiency of mucin *via* electrostatic binding reducing the coefficient of friction to ~0.01 *i.e.* almost 2-orders of magnitude lower than water [34]. The aim of this study was to investigate the lubricating properties of positively, negatively and neutrally charged polymer-coated liposomes. All samples were measured few days after preparation and some of them after 4 weeks or more. The viscosity of the samples was also monitored. As reference, all the individual components i.e. solutions of the polymers and uncoated liposomes were investigated, as well as a commercial dry mouth product.

2 Materials and methods

2.1 Materials

2.1.1 Lipids

Phosphatidylcholine from soybean lecithin, Soya-PC, MW = 787 Da, > 98% phosphatidylcholine, was a gift from Lipoid GmbH (Ludwigshafen, Germany). The cationic lipid dioleoyl trimethylammoniumpropane, DOTAP, and anionic phosphatidylglycerol, Egg-PG, were purchased from Avanti Polar Lipids, Inc. (Alabaster, USA).

2.1.2 Polymers

Alginate, Protanal LF 10/60, was a gift from FMC Biopolymer (Sandvika, Norway). The M_w of alginate was 147 000 D [35], the G content was 65-75% and the M content was 25-35% given by

149 the manufacturer. Chitosan, Protasan UP CL 213, was purchased from Novamatrix, Norway.
150 This was a hydrochloride salt with a M_w of 307 000 D and a DDA of 75-90% given by the
151 manufacturer. The HM-EHEC was a gift from AkzoNobel Chemicals AS, Sweden. The M_w of
152 this polymer was 250 000 D [26]. Biotene® mouth wash was the commercial product
153 investigated. The most important components of this mouth wash were hydroxyethyl cellulose
154 (HEC) and glycerol. The alginate was purified by centrifugation, dialyzing and freeze-drying
155 before use as previously described [35]. All other constituents were used as received.

156 2.2 Methods

157 2.2.1 Preparation of liposomes

158 Liposomes were prepared by the thin film method described elsewhere [21]. Briefly, the selected
159 lipids were dissolved in chloroform and the organic phase were evaporated by a rotavapor at 40
160 ºC. The thin film prepared were freeze dried (AlphaCrist Freeze Drier) overnight to remove any
161 organic residues and hydrated with 5 mM phosphate buffer pH 6.8 the subsequent day. To
162 downsize the liposomes, the hydrated film was extruded with a Lipex extruder (Lipex
163 Biomembranes Inc., Vancouver, Canada) ten times using a two stacked 200 nm polycarbonate
164 membrane (Nucleopore®, Costar Corp., Cambridge, USA).

165 The liposomes were composed of 90 mol % Soy PC and either 10 mol % EggPG or DOPTAP to
166 give negatively or positively charged liposomes, respectively. For the neutral liposomes 100
167 mol % SoyPC was used.

168 2.2.2 Preparation of polymer-coated liposomes

Polymer-coated liposomes were prepared by a method described previously [21]. In short, the polymers were dissolved in phosphate buffer pH 6.8 with a concentration of 0.125 % (w/v). The polymer solutions were stirred overnight and subsequent filtered through a 2 μm polycarbonate membrane (Nucleopore®, Costar Corp., Cambridge, USA). The liposomes were coated by adding them under magnetic stirring to the polymer solutions in a 1:4 ratio by the help of a Watson Marlow peristaltic pump. This gave a final concentration of polymer of 0.1% (w/v) and 0.6 mM lipids.

2.2.3 Viscosity measurements

The viscosity of different polymer-coated liposomes, the uncoated liposomes and the polymer solutions was measured on a Paar-Physica MCR 301 (Anton Paar, Austria rotational rheometer with a cone-and-plate geometry, cone angle of 1° and diameter 75 mm) at a controlled shear rate. Samples were measured at 37 °C with an equilibration time of 5 min at shear rates from 0.1 – 100 1/s. Three replicates were measured of each sample.

2.2.4 Tribology measurements

The lubricating properties of the different polymer-coated liposomes, the uncoated liposomes and the polymer solutions were measured with a Mini Traction Machine (MTM2, PCS Instruments London, UK) with the use of a smooth stainless steel ball (AISI 440, Ø 19 mm)-on-disc (Ø 46 mm). The surface roughness (R_a) of the tribopairs was < 50 nm. The sample was loaded into the pot and the ball was lowered onto the disc. The pot was then covered with a lid to avoid any evaporation.

The sliding speed was increased from 1 mm/s (low) to 250 mm/s (high) and then decreased from high-to-low speed to measure friction force to obtain the friction curve. Only the measurements

from high to low speed were reported due to negligible hysteresis effects. The experiments were carried out at a load of 2 N, fixed temperature of 37 °C to mimic oral temperature and in mixed sliding–rolling conditions with a fixed slide–roll ratio (SRR) of 50%. The entrainment speed was calculated by equation 1:

$$\bar{U} = ½ (U' + U'') \quad (1)$$

where $\bar{U}$ is the entrainment speed, U' is the rolling speed of the ball and U'' is the sliding speed of the disc, all measured with the unit mm/s.

Each sample was measured in triplicate and the procedure of going from low to high speed and from high to low speed was run in a loop of two-three and reported as mean and standard deviation of these six readings.

2.2.5 Experimental design

The focus of the study was to investigate the lubricating properties of polymer coated liposomes. Since the polymer-coated liposomes are composed of different constituents, i.e., liposomes and polymers, it was interesting to also study the lubricating effect of the individual components. Ten different formulations were investigated. These comprised positive, negative and neutral liposomes (0.6 mM); 0.1 wt % alginate, 0.1 wt % chitosan and 0.1 wt % HM-EHEC all dissolved in phosphate buffer pH 6.8; alginate coated liposomes, chitosan coated liposomes and HM-EHEC coated liposomes (all 0.6 mM lipids and 0.1 wt% of the respective polymer). Also, a commercial dry mouth product based on HEC was included in the study. The samples were measured one week after preparation and the polymer coated liposomes were also measured four weeks or four months after preparation.

All formulations from the lubrication experiments were investigated for their rheological behavoiur except for the formulations with HM-EHEC, which were replaced by HM-HEC.

2.2.6 Statistics

To investigate statistical significance a one-way ANOVA ($\alpha<0.05$) was performed followed by the Tukey's pairwise comparison with a 95% Confidence interval. Minitab was used for calculating the statistics.

3 Results

3.1 The viscosity and lubrication property of the uncoated liposomes, the solutions of polymers and the polymer-coated liposomes

The viscosity of the formulations was measured. All formulations showed high standard deviations at low shear rates from 0.1-1 s^{-1}. In the area between 1-100 s^{-1} all the formulations, irrespective of the composition either being pure polymer solutions, liposomes or polymer-coated liposomes, had low and almost similar viscosity ending up in a plateu. There was one exeption namely the alginate coated liposomes that had higher viscosity and not ending up in a plateau at the investigated shear rates (Fig. 1).

The formulations were analyzed with respect to their coefficient of friction (μ) as a function of the entrainment speed using smooth steel tribopairs. As can be observed from Fig. 2a, the friction curves were difficult to distinguish for the negative and the neutral liposomes. More importantly, both negative and neutral liposomes resulted in much higher μ values($\mu \sim 1.0$) irrespective of the entrainment speed from 1 to 250 mm/s, showing mainly plateau boundary regime. Such high interfacial friction might suggest that these liposomes were squeezed out of the tribo-contacts. In stark contrast, positively-charged liposomes had a significantly low boundary μ (Fig. 2a). As

opposed to the friction curves of neutral and negative liposomes, clear plateau boundary ($\bar{U} \leqslant 3$ mm/s) and mixed lubrication regime ($3 < \bar{U} \leqslant 50$ mm/s) could be clearly identified in the case of positive liposomes. In the mixed regime, μ was one-order of magnitude lower in case of positively charged liposomes as compared to that of negatively charged and neutral counterparts. At a first glance, lubrication properties appeared to be charge-dependent.

Shifting focal point to the polymer solutions (*i.e.* without liposomes) (Fig. 2b), the friction data approached a plateau which was rather extended ($\bar{U} \leqslant 10$ mm/s for chitosan, $\bar{U} \leqslant 50$ mm/s for alginate, HM-EHEC and the commercial product), which is indicative of the boundary lubrication regime. The hydrodynamic pressure by the polymers appeared not to be enough to prevent contact between asperities of the surfaces in the measured range of entrainment speeds. All the polymer solutions showed a decreasing trend for the μ values to reach a minimum; latter is indicative of the mixed lubrication regime ($10 < \bar{U} \leqslant 250$ mm/s for chitosan, $50 < \bar{U} \leqslant 250$ mm/s for alginate, HM-EHEC and the commercial product) without the appearance of any hydrodynamic regime. Interestingly, even the commercial product composed of HEC and glycerol showed the same behavior as most of the aqueous polymers, chitosan being an exception. Chitosan was identified as the most lubricating polymer studied, irrespective of the lubrication regimes. In fact, as indicated above, the three other polymer solutions *i.e.* alginate, HM-EHEC and the commercial product had much higher and extended boundary lubrication regime as compared to that of the positively charged chitosan. This largely mirrors the results from the naked liposomes (Fig. 2 a) where the lubrication efficiency seems to be dependent on the electrostatic charge.

The polymer-coated liposomes (Fig 2c) interestingly showed similar behavior to the uncoated liposomes (Fig. 2a) showing almost no entrainment dependency and consequently high boundary

257 friction, except for the liposomes coated by chitosan. The chitosan-coated liposomes showed a
258 much lower μ as compared to that of HM-EMEC- or alginate-coated liposomes, irrespective of
259 the entrainment speeds.
260 3.2 Friction force of the formulations at low and high entrainment speeds
261 The friction force was investigated closer for the different formulations at low (3 mm/s) and high
262 (50 mm/s) entrainment speeds (Fig. 3). An ANOVA was conducted followed by the Tukey's
263 comparison test with a confidence interval of 95%. The p-value of the ANOVA was less than
264 0.0001 for both entrainment speeds investigated, and the grouping after the Tukey's test of the
265 different formulations implying statistical significance (confidence interval of 95%) can be found
266 in Fig. 3. At low entrainment speed (Fig. 3a) the alginate-coated liposomes had a high friction
267 force (more than 1.2 N). The alginate-coated liposomes are composed of positively charged
268 liposomes showing a low friction force (less than 0.4 N) and alginate showing a high friction
269 force (almost 1.6 N). The friction force of the alginate-coated liposomes was significantly lower
270 than the alginate solution and significantly higher than the positive liposomes. The chitosan-
271 coated liposomes had a very low friction force (less than 0.4 N). The chitosan-coated liposomes
272 are composed of negative liposomes showing a high friction force (more than 1.4 N) and
273 chitosan showing a low friction force (almost 0.6 N). The chitosan coated liposomes had a
274 statistically lower friction force than both the chitosan solution and the negative liposomes. The
275 HM-EHEC-coated liposomes showed a high friction force (around 1.3 N) however being
276 significantly lower than the friction force of the neutral liposomes (almost 1.8 N) but not
277 statistically different from the solution of HM-EHEC (1.5 N). At the low entrainment speed, the
278 commercial product had a relatively high μ (almost 1,4 N) being similar to several of the other

279 formulations such as the HM-EHEC solution, the negative liposomes and the HM-EHEC coated
280 liposomes.
281 At higher entrainment speed *i.e.* 50 mm/s (Fig. 3b) the friction force was slightly lower (in the
282 area 0-0.25 N) than at low speed for almost all the formulations except for the commercial
283 product. The commercial product had the highest decrease in friction force of all formulations
284 though still the value was considered high (1 N) (Fig 3c). Still the chitosan solution, the chitosan
285 coated liposomes and the positive liposomes had friction forces significantly lower than the other
286 samples.
287 The friction curves for the samples stored for more than 4 weeks were investigated (Fig. 4). The
288 HM-EHEC-coated liposomes and the alginate-coated liposomes had been stored for 4 months
289 while the chitosan coated liposomes had been stored for 4 weeks. The chitosan-coated liposomes
290 showed the same μ as the freshly prepared sample, while the μ value of the alginate-coated
291 liposomes were different. The μ was considerably lower for the alginate-coated liposomes stored
292 for the longest time (around 0.2-0.3 μ compared to 0.6-0.8 μ) and the coefficients started to
293 increase at higher speeds. The stored HM-EHEC-coated liposomes had a similar curve as the
294 fresh prepared sample but the decrease in the μ values at higher speeds were more pronounced
295 for the sample being stored for the longest time.
296
297 **4 Discussion**
298 Liposomes and polymer coated liposomes are interesting drug delivery systems and could have a
299 potential of adhering to the teeth of dry mouth patients acting like a saliva substitute by forming
300 an artificial enamel pellicle and by such protect the teeth [20]. One of the most important
301 properties of saliva is to lubricate both the oral cavity as well as the teeth. Several studies have

302 investigated saliva's lubricating properties [36]. The μ values reported in these studies varies
303 from 0.02 μ to as high as 0.45 μ.
304 The aim of the present study was to investigate the lubricating properties of polymer-coated
305 liposomes and compare it to the individual components namely naked liposomes and polymer
306 solutions. Fluid phase liposomes with different charge were chosen and also neutral liposomes
307 were investigated. Polymers of different charge, hydrophobicity and M_w were used to coat the
308 liposomes. The coating process of the liposomes is delicate, but we have standardized a
309 reproducible method where stable liposomes are prepared with a low polydispersity index (PDI)
310 obtained when the correct amount of polymer is used [22]. The amount of polymer in
311 combination with the amount of liposomes is crucial, however, the concentration range of
312 polymer to be used in order to obtain stable complexes is quite broad i.e between 0.04 wt % -
313 0.12 wt%. If the polymer complexes are not stable they will tend to aggregate by bridging
314 flocculation or depletion flocculation and the particles are visible with the naked eye. These
315 polymer-coated liposomes have been prepared and characterized in several of our previous
316 studies and on-going studies implying that the alginate coated liposomes are the smallest
317 (average hydrodynamic diameter ~ 200-300 nm), HM-EHEC coated liposomes and HM-HEC-
318 coated liposomes intermediate (~350-400 nm) and the chitosan coated liposomes being the
319 largest (~300-500 nm) [20, 25, 26]. Also, the chitosan coated liposomes tend to hold the highest
320 PDI-value (~0.25-0.4) compared to the alginate and the HM-EHEC and the HM-HEC coated
321 liposomes (PDI~0.15-0.25 and ~0.25). The size of the uncoated liposomes lies in the range
322 ~130-170 nm. The zeta potential of the chitosan coated liposomes was in the area 20-40 mV, the
323 alginate coated liposomes -45 mV - -55 mV and the HM-EHEC and the HM-HEC coated

liposomes close to 0 mV. The characteristics of the liposomal formulations in our study are summarised in Table 1.

Examining the friction coefficients (μ) of the polymer solutions

The friction curves indicated normal behavior for the polymer solutions i.e., at low speed the plateau of boundary lubrication could be identified while at higher speed the mixed regime was reached. This is usually seen for hydrophilic polymers [27, 29]. The friction coefficient, μ, at low speed for the neutral HEC, the hydrophobically modified polymer HM-EHEC and the negative polymer alginate was high and was not considered to be acting as boundary lubricants. Chitosan on the other hand had a μ around 0.25 at low speed (range 1 – 13 mm/s) decreasing to 0.14 μ at high speed (120 – 190 mm/s). This indicates that a solution of chitosan even at the low concentration investigated in this study tends to have excellent lubricating properties. Chitosan and alginate are polylelectrolyte polysaccharides, both being charged at the investigated pH value, chitosan having a positive charge (pK ~ 6.1) while alginate having a negative charge (pK ~ 2.8). Chitosan has been found to usually behave like a flexible rod type or stiff coil [37]. The chain stiffness and conformation is known to be dependent on degree of acetylation and Mw [38]. The flexibility reported for a chitosan of somewhat comparable characteristics (DDA 87% and Mw 112 kg/mol) to the sample used in our study, concurs with that of a semi-flexible linear chain [39]. Alginate is known as a stiff molecule and the stiffness is dependent on the content of guluronic (G) and mannuronic (M) acid. The stiffness is increasing in the order MG<MM<GG [40]. The alginate in this study is having an excess of guluronic residues, and will probably adopt a stiff conformation. In a previous study, the dependence of the stiffness of the chain on the lubricating properties in the boundary regime was investigated [41]. The study showed that μ

was highest for the rigid rod scleroglucan, while the extended coil carrageenan had a much lower μ. The high μ obtained for the alginate solution in our study could be due to a rigid rod conformation of the polymer while the lower μ of chitosan could perhaps be due to a more flexible chain. Also, when coating the liposomes with the polymer, the increase in size is quite small for the alginate-coated liposomes and the charge density is high, implying a rigid chain followed by a flat thin layer adsorption [20]. HM-EHEC takes a helical conformation in water and studies have shown that adsorption to solid surfaces (talc) is flat and driven by hydrogen bonding and the chains are evenly adsorbed to the surface. [42]. The high μ observed in the present study could be due to the similarities with alginate as a more rigid chain [43].

Also, the concentration dependence of different food hydrocolloids lubricating behaviour in the mixed regime has been investigated, from a low concentration of 0.1 wt % to a higher concentration of 1.0 wt % [41]. The hydrocolloids studied were gums such as locust bean gum and guar gum, but also λ-carrageenan and scleroglucan. This study showed that the highest concentration of the hydrocolloids decreased μ most. In our study, the concentration of the polymers was low, only 0.1 wt %. It might be speculated that a higher concentration would have decreased μ further, but this was beyond the scope of the present study and thus not investigated. The hydrodynamic volume of the chains are important in the boundary regime but also the ability of the chains to adsorb to the ball and plate is of high importance [29]. If the polymer strongly adsorbs to the surface either covalently or by electrostatic interactions, the degree of hydration and the amount of hydrated ions determine the friction. Previous studies have shown the ability of chitosan to adsorb and lower μ due to hydration and its polyelectrolyte character [44]. The findings in our study indicate poor adhesion properties to the steel ball and plate of both alginate as well as HM-EHEC. Quite interesting was the result from the commercial

product containing HEC indicating poor lubricating properties despite the product containing glycerol. Aqueous glycerol has previously been considered a good lubricant when mixed with the grafted polymer Poly(L-lysine)-graft-Poly(ethylene glycol) [45]. However, in this study the amount of glycerol was as high as 50%. The concentration of glycerol in the commercial product is unknown but is probably much lower than 50% and the effect may diminish. Also, the lubricating effect was more pronounced in the mixed regime which could also be seen for the commercial product, having the highest decrease in μ of all tested samples. The opposite was seen for an agar gel and the particulate gelled phase where μ increased with increasing amount of glycerol [46].

Examining the friction coefficients of the different liposomes

The neutral and negative liposomes had high μ values irrespective of entrainment speeds and only the boundary regime could be identified. For the positively charged liposomes, μ was lower and decreased during the whole investigated entrainment speed range. The main lipid of the liposomes was Soybean phosphatidylcholine (Soya PC) and the amount was as high as 90 mol %. The only difference between the investigated liposomes was the charged lipid, namely PG (negative charge) or DOTAP (positive charge) both 10 mol %, again implying that adsorption due to the positive charge may be a driving force for decreasing the friction coefficient. The size of the three types of liposomes was almost the same (140-185 nm), so the effect of size can possibly be ruled out. It has previously been shown that PC may substantially reduces the friction in aqueous systems but is dependant on the ions surrounding them [47]. There were no reduction in μ in the present study for the neutral and the negative liposomes. Interestingly, the viscosity of the dispersion of liposomes was very low at share rates from 10-

100 and was approximately 0.001 Pa s. This probably explains the non-appearance of the mixed regime for the negative and the neutral liposomes. When it comes to the positively charged liposomes, the low μ values might be associated with adsorption phenomena rather than viscosity contribution. It is important to note that it was not possible to increase the entrainment speed to higher values than 250 mm/s due to the formulations being so free flowing spattering out of the tribometer.

Examining the friction coefficients of the polymer coated liposomes

For the combination of polymers and liposomes *i.e.* the polymer-coated liposomes, only the boundary regime could be found. However, the chitosan-coated liposomes had a constant low μ showing promise as a lubricant. The results are interesting in many ways. The μ values of the polymer coated liposomes were dependent of the outermost layer of the formulation. The positively-charged liposomes with low μ changed to high μ when coated with alginate with a negative charge. The opposite was seen for the negatively charged liposomes originally having a high μ getting low when coated with chitosan with a positive charge. However, the μ values for the chitosan coated liposomes was even lower than the chitosan solution indicating a synergistic effect. This could be attributed to the possibility of building up a multi-layer consisting of some free chains of chitosan and the chitosan coated liposomes giving the possibility of acting by a "ball-bearing mechanism". This has previously been seen for whey protein microgel particles [27]. Also, the bigger size of the chitosan coated liposomes compared to the alginate coated liposomes could contribute to the ability of pushing the two surfaces apart and lowering μ. To investigate and explore any possible ballbearing effect, the formulations will in future studies be tested with different loads. The data from the viscosity measurements were interesting as the

alginate coated liposomes stand out as having a higher viscosity than the other polymer coated liposomes. The viscosity does not seem to play an important role in affecting the μ values of the polymer coated liposomes as the viscosity of the samples were so low and similar. Even plotting the entrainment speed multiplied with the viscosity indicated no effect of the viscosity on the friction curves (data not shown),

Examining the friction force in the boundary regime for all samples

When looking at the friction force values from the boundary regime (3 mm/s) and the mixed regime (50 mm/s), the values strongly reflect the friction curves. The values from the boundary regime (3 mm/s) indicate that a low friction force is dependent on the ability of the formulation to adhere to the ball and the disc. The formulations with positive charge tend to adhere more than the formulations with neutral or negative charge independent of the type of formulation (liposomes, polymer solution or polymer coated liposomes). The lowest friction force was found for the chitosan coated liposomes. Also, the positive liposomes had low friction force. In another study, the adhesive properties of polymer coated liposomes towards hydroxyapatite in phosphate buffer pH 6.8 were investigated [20]. Hydroxyapatite holds a negative charge at pH 6.8, so the adhesion is mainly electrostatically driven. The study revealed that the positive liposomes and chitosan coated liposomes were more adhesive than the negative liposomes and alginate coated liposomes. However, when the medium of the adhesion experiments was changed to artificial saliva, the alginate coated liposomes adhered to a high extent and even more than the negatively charged pectin coated liposomes investigated. This implies again that the alginate coated liposomes may have a very thin and flat coating, being altered when the medium is changed. This could perhaps also explain the higher viscosity of the alginate coated liposomes, as a flat

thin adsorption of alginate may leave some open spaces on the liposomes, or that the electrostatic forces between the liposomes and the alginate chains may not be that strong and alginate falls of. When stationary, these potential open spaces on the liposomes, free of alginate, can not prevent the steric repulsion of the particles exerted by alginate, while when shear is put on the particles there may be some bridging flocculation and building up of a network due to the particles being forced to be closer together and the attractive forces may start to dominate.

Examining the friction force in the mixed regime for all samples

The mixed regime is dependent on both the adhering properties of a sample and the viscosity of the sample. Almost all the formulations had a lower friction force at higher speed, but the reduction was rather modest. However, there was one formulation standing out behaving differently. The difference in the friction force at 3 mm/s and 50 mm/s was highest for the commercial product. The viscosity of this formulation has been investigated (*data not shown*), showing a shear independent viscosity with a constant value of 0.1 Pa s with shear rates from 0.1 – 100 s^{-1}. One possible explanation to the observed result could be that glycerol has higher lubricant activity at higher speed, as already discussed above. Also, a lowering of the friction force was seen for the positive liposomes although not as pronounced as the commercial product.

Long term stability

Selected samples were stored for 4 weeks and 4 months. The chitosan coated liposomes and the HM-EHEC coated liposomes showed similar results as the freshly prepared samples, while the stored sample of the alginate coated liposomes had lower friction coefficients than the fresh sample. This could again be due to the thin loosely bound coating layer of alginate around the liposomes. The alginate chains, being wrapped around the liposomes due to weak electrostatic

forces, will be even looser bound with time, and perhaps when the experiment starts the positive charge of the liposomes appear. The lubricating properties will then be dependent of both the liposomes having positive charge and alginate having negative charge.

The experiments conducted in this study is interesting in respect to how the formulations could potentially protect the teeth. The steel surface of the ball and disc are not completely comparable to the surface of the teeth composed of hydroxyapatite crystals having a negative charge, but it still gives some indications. Also, the surface of the oral cavity, the mucosa, with an outer layer of mucin being negatively charged, will act as a competitor to the adhesion to the teeth. Most probably a formulation of positively charged colloidal particles will adhere simultaneously both to the teeth and the oral mucosa and hence they might display a dual action. To understand the full potential of these formulations, the lubricating potential towards a dry mouth mimetic surface will, therefore, be investigated in the future [18, 48].

5 Conclusion

This study has shown that all the investigated formulations with positive charge had lubricating properties, with the chitosan coated liposomes lowering the friction force the most at low entrainment speed. Also, the chitosan solution and the positive liposomes had low friction force values. The low friction forces seem to be dependent on adsorption of the formulation to the ball and the disc and to a minor degree the viscosity of the samples. These formulations could have promising properties in a product intended to protect the teeth from erosion, attrition and abrasion and should be studied further with this in mind.

CRediT authorship contribution statement

Marianne Hiorth: Conceptualization; Investigation; Methodology; Project administration; Supervision; Visualization; Writing - original draft. Ljubica Mihaolovic: Data curation; Investigation; Methodology; Visualization; Writing - review. Malgorzata Adamczak: Investigation; Methodology; Visualization; Writing - review. Francisco Goycoolea: Conceptualization, Investigation, Writing- Reviewing & Editing. Prof. Anwesha Sarkar: Conceptualization, Methodology, Project administration; Writing- Reviewing & Editing.

Declaration of competing interest

None.

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605 Table 1. Characteristics of the uncoated and polymer-coated liposomes.

Formulation	Short name	Size (nm)	Zeta potential (mV)	PDI
Positive liposomes	PosLip	~135	33 – 38	0.12
Negative liposomes	NegLip	~135	-50 – -55	0.12
Neutral liposomes	NeuLip	~175	-2	~0.11
Alginate-coated liposomes[a]	AlgcLip	200 – 300	-45 – -55	0.15 – 0.25
Chitosan-coated liposomes[a]	ChitcLip	300 – 500	20 – 40	0.25 – 0.4
HM-EHEC*-coated liposomes[a]	HM-EHECcLip	350 – 400	0	0.25
HM-HEC**-coated liposomes	HM-HECcLip	~350	0	0.26

606 a The characteristics of the coated liposomes are taken from reference 20, 25 and 26

607 *HM-EHEC=hydrophobically modified ethyl hydroxyethyl cellulose

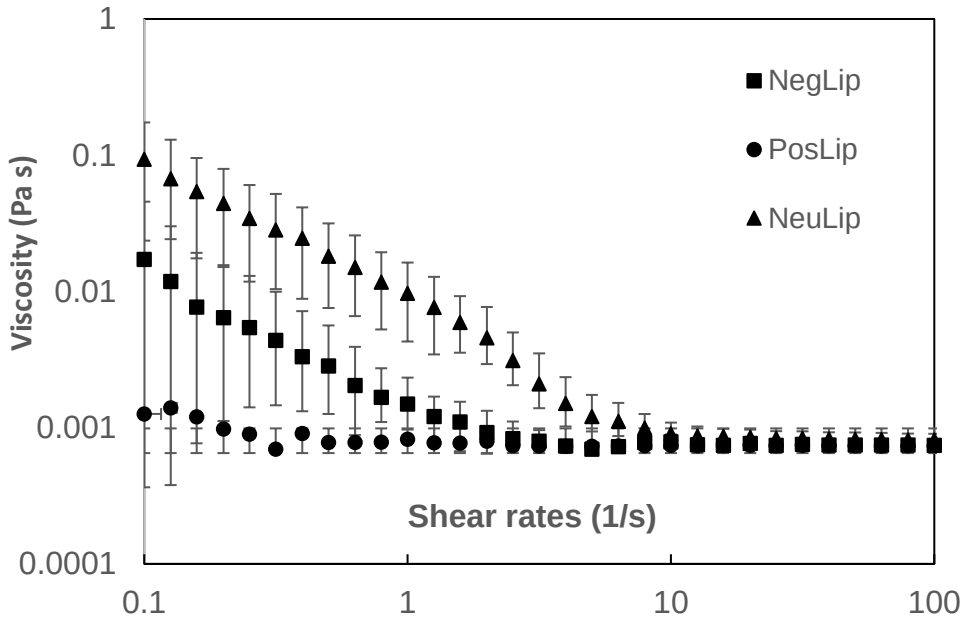
608 **HM-HEC= hydrophobically modified hydroxyethyl cellulose

609

610

Figure 1.

(a)


1
0.1
0.01
0.001
0.0001
Viscosity (Pa s)
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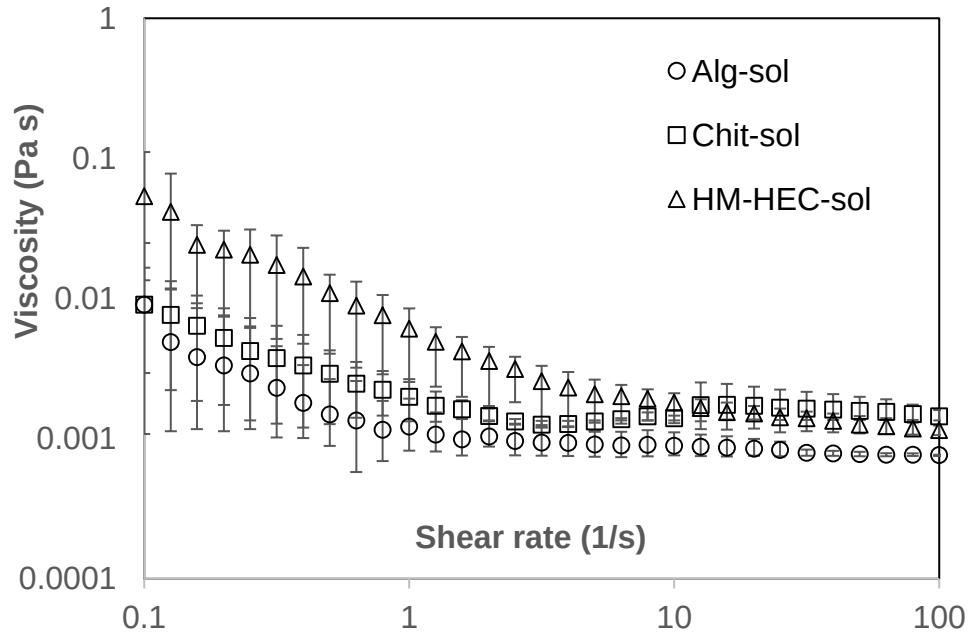
1
10
100
Shear rates (1/s)
NegLip
PosLip
NeuLip

(b)


1
0.1
0.01
0.001
0.0001
Viscosity (Pa s)
0.1
1
10
100
Shear rate (1/s)
Alg-sol
Chit-sol
HM-HEC-sol

(c)

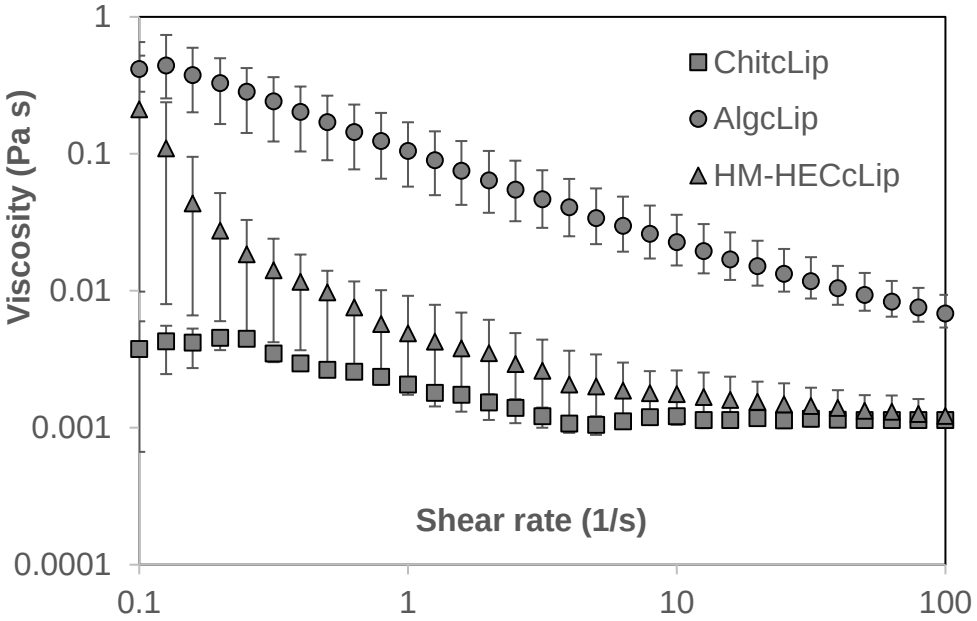
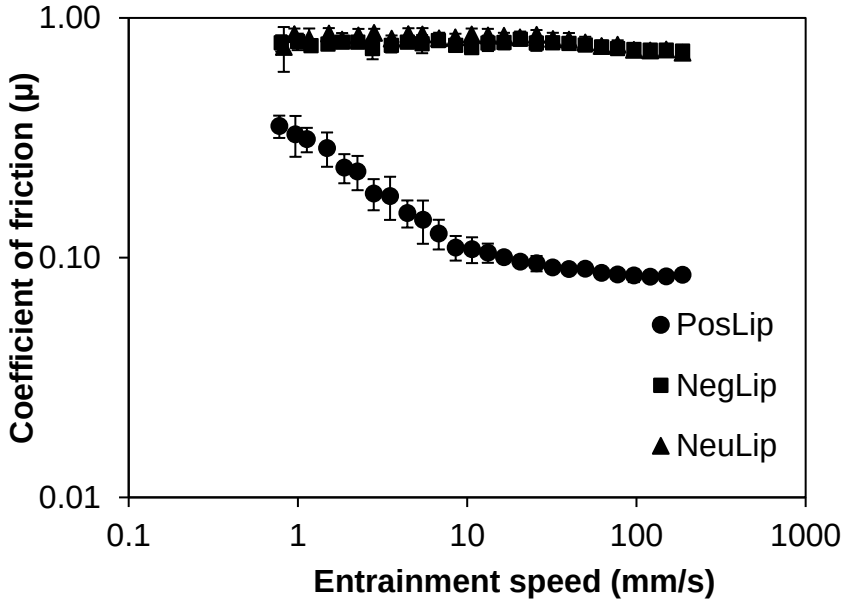


Figure 1. Viscosity as a function of the shear rate of liposomes (0.6 mM lipids) (a), polymer solutions (0.1 wt%), (b) and polymer-coated liposomes (0.1 wt% polymers and 0.6 mM lipids) (c), respectively. Error bars indicate standard deviation as obtained from three independent measurements.

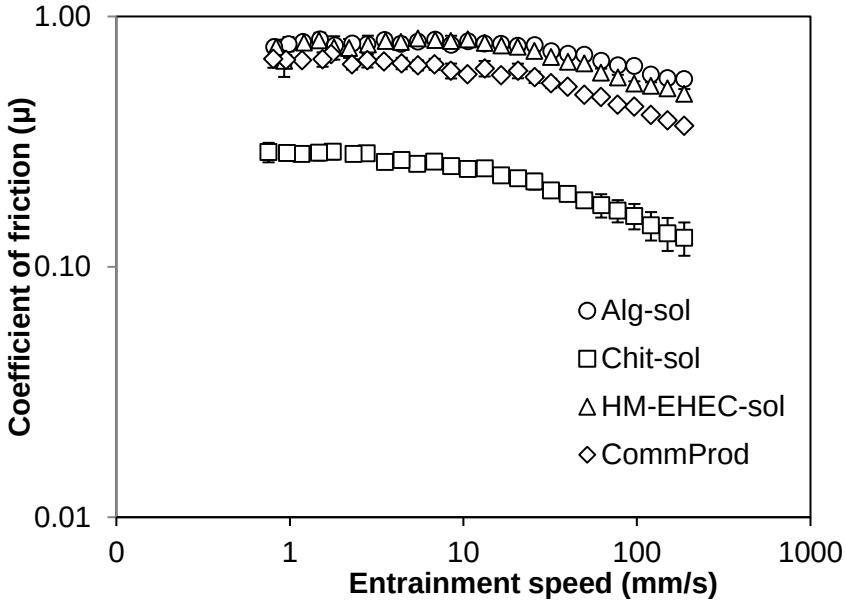
Size of the figures: 1 column

631 **Figure 2.**

(a)



(b)



(c)

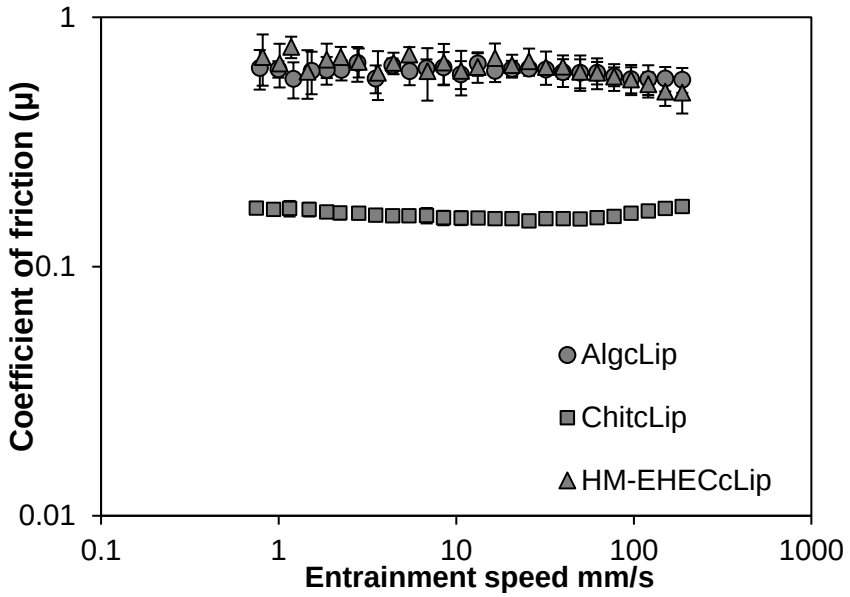
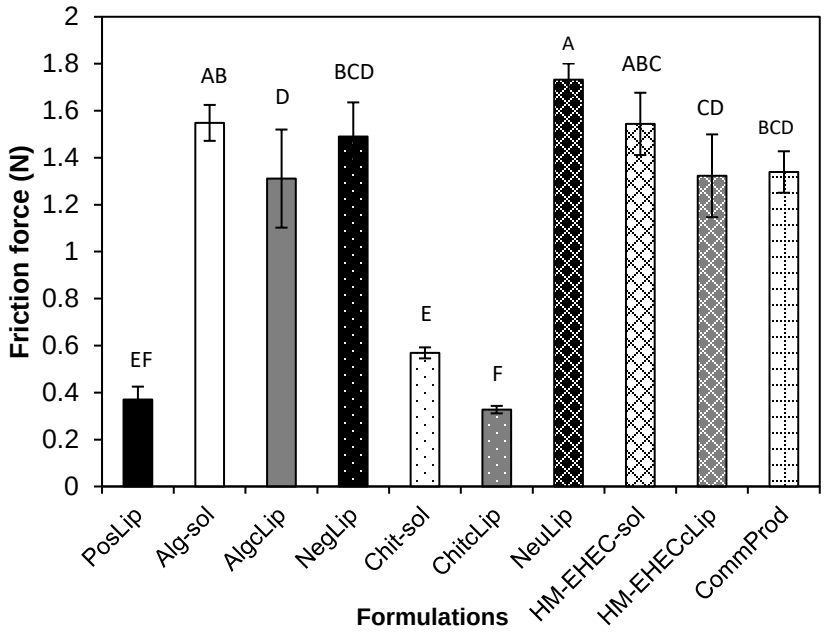


Figure 2. Friction curves showing coefficient of friction measured as a function of the entrainment speed of liposomes (0.6 mM lipids) (a), polymer solutions (0.1 wt%), (b) and polymer-coated liposomes (0.1 wt% polymers and 0.6 mM lipids) (c), respectively. Error bars indicate standard deviation as obtained from three independent measurements.

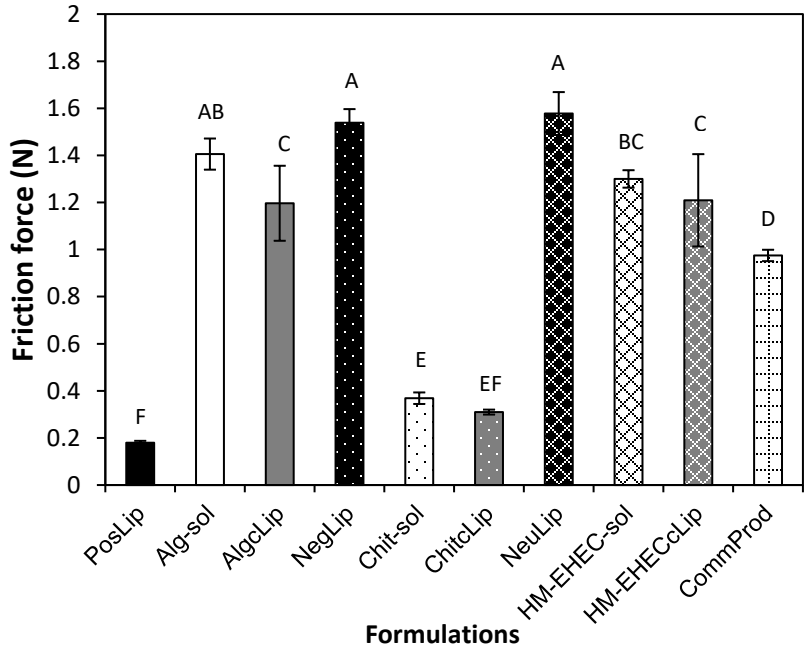
Size of the figures: 1 column

642 **Figure 3.**

(a)



(b)



643

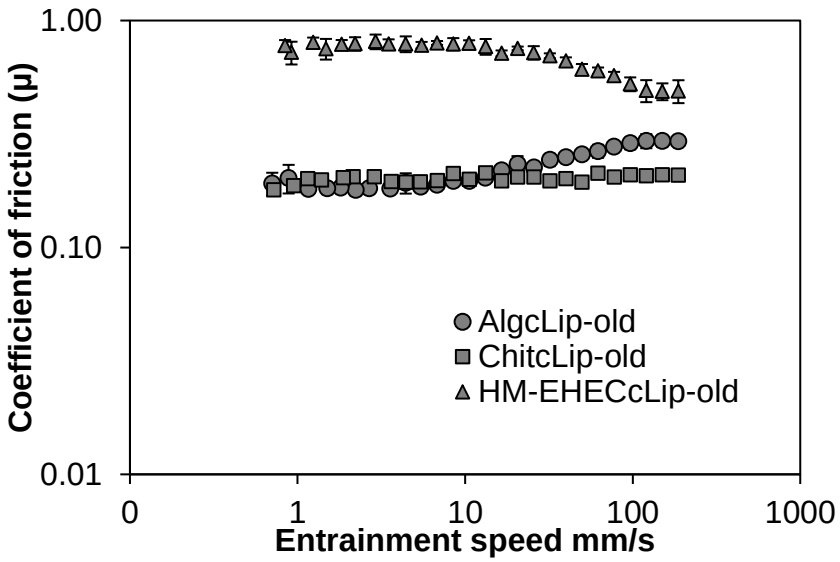
644 Figure 3. Friction force of different formulations at low (3 mm/s), (a) and high (50 mm/s),
645 (b) entrainment speeds, respectively. Error bars indicate standard deviation as obtained
646 from three independent measurements. Means that do not share a letter are
647 significantly different (Tukey's method 95% confidence).

648

649 Size of the figures: 1.5 column

650

651 **Figure 4.**



652

653

654

655 Figure 4. Friction curves showing coefficient of friction measured as a function of the
656 entrainment speed of polymer-coated liposomes 4 weeks after production for the chitosan
657 and the HM-EHEC coated liposomes and 4 months for the alginate coated liposomes.
658 Error bars indicate standard deviation as obtained from three independent measurements.

659

660 Size of the figure: 1 column

661

662