Statistical modelling of the rheological and mucoadhesive properties of aqueous poly(methylvinylether-co-maleic acid) networks: Redefining biomedical applications and the relationship between viscoelasticity and mucoadhesion

Statistical Modelling of the Rheological and Mucoadhesive Properties of Aqueous Poly(methylvinylether-co-maleic acid) Networks: Redefining biomedical applications and the relationship between viscoelasticity and mucoadhesion

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Abstract

Poly(methylvinylether-co-maleic acid) (PMVE/MA) is commonly used as a component of pharmaceutical platforms, principally to enhance interactions with biological substrates (mucoadhesion). However, the limited knowledge on the rheological properties of this polymer and their relationships with mucoadhesion has negated the biomedical use of this polymer as a mono-component platform. This study presents a comprehensive study of the rheological properties of aqueous PMVE/MA platforms and defines their relationships with mucoadhesion using multiple regression analysis. Using dilute solution viscometry the intrinsic viscosities of un-neutralised PMVE/MA and PMVE/MA neutralised using NaOH or TEA were 22.32 ± 0.89 dL g^{-1}, 274.80 ± 1.94 dL g^{-1} and 416.49 ± 2.21 dL g^{-1} illustrating greater polymer chain expansion following neutralisation using Triethylamine (TEA). PMVE/MA platforms exhibited shear-thinning properties. Increasing polymer concentration increased the consistencies, zero shear rate (ZSR) viscosities (determined from flow rheometry), storage and loss moduli, dynamic viscosities (defined using oscillatory analysis) and mucoadhesive properties, yet decreased the loss tangents of the neutralised polymer platforms. TEA neutralised systems possessed significantly and substantially greater consistencies, ZSR and dynamic viscosities, storage and loss moduli, mucoadhesion and lower loss tangents than their NaOH counterparts. Multiple regression analysis enabled identification of the dominant role of polymer viscoelasticity on mucoadhesion ($r>0.98$). The mucoadhesive properties of PMVE/MA platforms were considerable and were greater than those of other platforms that have successfully been shown to enhance *in vivo* retention when applied to the oral cavity, indicating a positive role for PMVE/MA mono-component platforms for pharmaceutical and biomedical applications.

1. Introduction

Poly (methylvinylether-co-maleic anhydride) is a 1:1 copolymer of methyl vinyl ether and maleic anhydride that is available in various grades, including the free acid form, poly (methyl vinyl ether-co-maleic acid) (PMVE/MA)[1]. The low toxicity and excellent biocompatibility of PMVE/MA have resulted in its widespread use throughout the pharmaceutical and cosmetic industry[2, 3]. In particular, this polymer has found use within toothpastes, mouthwashes, denture adhesives, hairsprays, transdermal patches, periodontal drug delivery systems and within stoma adhesive pastes[4-8].

PMVE/MA has been reported to form adhesive interactions with mucin-coated epithelial surfaces (termed mucoadhesion)[9-11] and, as a result, has been used as a component of a range of biomedical implants where retention at the site of application is important, e.g. as mucoadhesive nanospheres and microspheres, mucoadhesive buccal tablets, mucoadhesive implants for application to the periodontal pocket and microneedle transdermal systems. In such applications mucoadhesion has been shown to enhance the retention of the implant at the site of application, thereby facilitating controlled drug release and offering site specific mechanical properties[6, 9, 12, 13]. The mucoadhesive properties of PMVE/MA are accredited to its large molecular weight, its favourable chemical functional groups and anionic charge, all of which aid interaction with the mucus layer through polymer mucin interpenetration and the formation of various hydrogen bridges[9, 14, 15].

The successful pharmaceutical and biomedical applications of PMVE/MA have predominantly required the formation of networks with other polymers, most notably poly(vinylpyrrolidone, PVP). For example in a series of publications, Jones *et al.* described the formulation of networks of PMVE/MA and PVP that were designed for use as implants within the oral cavity[6, 12, 13]. In these studies it was shown that the rheological properties of the networks were engineered through modification of both the ratio of PMVE/MA to PVP and polymer concentration. Other studies have described the design of biomedical implants that involve the use of PMVE/MA in association with other polymers. For example, Moreno *et al.* described the formulation of thermosensitive hydrogels of PMVE/MA and Pluronic F127 that were designed for the controlled release of proteins[16]. Whereas the combined use of PMVE/MA and poloxamer 407 and hydroxypropylcellulose gels designed for the treatment of oropharyngeal cancer has been described[17]. Most recently however Jones *et al.* highlighted a significant concern regarding the use of interactive polymer networks involving PMVE/MA. The combination of PMVE/MA and poly(vinyl alcohol) produced rheologically structured, mucoadhesive networks. However, upon storage, the viscoelastic and mucoadhesive properties of the networks were observed to significantly and detrimentally change thereby obviating their use as biomedical implants. Conversely, there was no alteration of the rheological properties of mono-polymeric PMVE/MA systems on storage [18]. The use of binary polymeric networks involving PMVE/MA must therefore be treated with extreme caution.

Despite the (growing) number of publications that describe the use of PMVE/MA-based platforms for biomedical applications (particularly for drug delivery applications), there is a paucity of studies that have examined the physicochemical

properties of PMVE/MA regarding its suitability as a monopolymeric platform for biomedical applications. This oversight is of scientific relevance for two reasons. Firstly, without an understanding of the rheological and mucoadhesive properties of PMVE/MA, the formulation of existing biomedical implants may not be optimal, with detrimental consequences on their clinical usage. Secondly, full understanding and knowledge of the rheological and mucoadhesive properties will offer possibilities for the use of this polymer for an enhanced range of applications, e.g. as pharmaceutical implants, drug delivery applications and as mucoadhesive, viscoelastic implants designed to facilitate cataract removal[19].

Therefore, this study aims to provide a comprehensive description of the rheological properties of PMVE/MA and, for the first time, to specifically statistically examine the relationship of these properties to the mucoadhesive properties. In particular the generated data and the relationships between the various rheological and mucoadhesive properties will be statistically modelled, thereby providing a comprehensive characterisation of the relationship between these parameters. In so doing this study will offer a beneficial insight into the potential biomedical applications of PMVE/MA and of the contribution of physicochemical properties of PMVE/MA to mucoadhesion, an area as yet not fully clarified.

2. Materials and Methods

2.1 Materials

Poly(methylvinylether-co-maleic acid, PMVE/MA) (Gantrez® SBF97) with an average molecular weight of approximately 1,200,000 Da was kindly donated by ISP, Surrey, UK. Sodium hydroxide (NaOH) pellets and triethylamine (TEA) were purchased from Sigma Aldrich, Dorset, England. All other chemicals were purchased from BDH Laboratory supplies Dorset, England and were of AnalaR grade, or equivalent quality.

2.2. Methods

2.2.1 Manufacture of Dilute PMVE/MA Solutions

Stock solutions (0.2-0.6 g/dl) of PMVE/MA were prepared by adding the required mass of polymer to an appropriate volume of deionised water (pH 5.0-5.2). The polymeric solutions (five replicate batches) were subsequently agitated using a mechanical stirrer. Dilution of stock solutions was carried out to obtain the desired concentration, with the final volume being corrected after neutralisation of the relevant systems. Neutralisation of suitable solutions was carried out via the drop wise addition of sodium hydroxide solution (30% w/w NaOH) or Triethylamine (TEA) until a pH value of 7.4 was obtained (measured using a Hanna Instruments pH meter). The solutions examined reflected a range of un-neutralised, TEA and NaOH neutralised PMVE/MA systems.

2.2.2. Manufacture of Bulk Aqueous PMVE/MA Systems.

PMVE/MA systems were manufactured via the slow addition of the appropriate amount of polymer (5-30% w/w) to deionised water under mechanical mixing via a mechanical stirrer. A number of gels had their pH adjusted to pH 7.4 using either a 30% w/w NaOH solution or TEA; with gel pH determined using a flat faced gel pH probe. To remove air, all samples were stored for 24hrs prior to testing, with all testing being completed within a 72hr period. Bulk rheological measurements were performed using both continuous shear analysis and oscillatory analysis.

2.2.3. Viscometric Analysis of Dilute Solutions.

All viscometric analyses of PMVE/MA solutions (0.2-0.6 g/dl) were performed using Rheotek Ostwald U-tube viscometers sizes O-D. Solutions were added to the tube via a pipette until the required level was reached. The U-tube was then placed into a Rheotek water bath pre-set to 37°C±0.5°C and allowed to equilibrate for 15 minutes. The time required for the fluid to fall a predetermined distance was measured and used to calculate the kinematic viscosity (υ, mm^2s^{-1}) (Equation 1).

$$\upsilon = kt \qquad \text{Equation 1}$$

where: k refers to the U-tube multiplication factor and t refers the solution/solvent flow time (sec)

From this the relative viscosity (η_{rel}) was calculated

$$\eta_{rel} = \frac{\upsilon}{\upsilon_o} \qquad \text{Equation 2}$$

Where: υ and υ_o refer to the kinematic viscosities of the polymeric solution and the solvent in which the polymer is dispersed, respectively.

The specific viscosity (η_{sp}) is then calculated:

$$\eta_{sp} = \eta_{rel} - 1 \qquad \text{Equation 3}$$

The reduced viscosity (η_{red}) may be thus expressed as the ratio of the specific viscosity to the concentration:

$$\eta_{red} = \frac{\eta_{sp}}{C} \qquad \text{Equation 4}$$

Where *C* is the concentration of the polymer in g/dl[20].

U-tubes were chosen so that the efflux time for each solution was always above 200 sec (or 300 seconds for the O size tube) thus allowing greater accuracy within measured results. The viscometric properties of five replicate solutions were measured in all cases.

Calculation of the intrinsic viscosity of each system [η] was performed using the Huggin's equation (equation 5) or the equation described by Fuoss and Strauss (equation 6)[21] [22, 23].

$$\eta_{red} = \frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2 C \qquad \text{Equation 5}$$

Where (in addition to the previous descriptions) C is the polymer concentration and k' refers to Huggin's constant

$$\eta_{sp} = \frac{[\eta]}{(1+B\sqrt{C})} \qquad \text{Equation 6}$$

Where: C is the polymer concentration and B is a constant

185 *2.2.4. Continuous Shear Analysis.*

186 Continuous shear (flow) analyses were performed at 37°C on the PMVE/MA (5-30%
187 w/w) systems using a TA systems AR2000 rheometer. Flow rheograms were
188 determined using either a 6cm or 4cm parallel stainless steel plate (gap size 1000
189 μm), the choice of geometry being determined by sample consistency. Samples to
190 be analysed were applied to the lower plate and allowed 15 minutes to equilibrate to
191 negate any stresses induced during sample application. The shear stress was
192 applied over a predetermined range, with this range again being determined by
193 sample consistency. Mathematical modelling of the flow properties of the various
194 polymeric platforms was performed using the Rheology Advantage software (TA
195 Instruments) in conjunction with the Ostwald-de-Waele power law model (equation
196 7)[24] and the Cross model (equation 8)[25], as follows:

197 $$\sigma = k\dot{\gamma}^n$$ Equation 7

198 Where: σ refers to the shear stress, $\dot{\gamma}$ refers to the rate of shear, k refers to the
199 consistency and n represents a power law index

200 $$\eta = \eta_\infty + \frac{\eta_o - \eta_\infty}{1 + (K\dot{\gamma})^m}$$ Equation 8

201 Where: η is viscosity, η_∞ is the infinite shear viscosity, K is a structural relaxation
202 time, *m* is dimensionless and η_0 is the zero rate viscosity.

203 In each case, the flow properties of at least five replicates were determined.

204

205 *2.2.5. Oscillatory Analysis.*

206 Oscillatory analyses were performed at 37°C on PMVE/MA (5-30% w/w) systems
207 using a TA systems AR2000 rheometer, as previously reported by the authors[26].

Rheological analyses were conducted using either a 6cm or 4cm parallel stainless steel plate (gap size 1000 μm); the choice of geometry was determined by sample consistency. Samples to be analysed were applied to the lower plate and allowed 15 minutes to equilibrate to negate any stresses induced during sample application. For each sample, the linear viscoelastic region was determined via a stress sweep at a fixed frequency. Once determined, a frequency sweep from 0.1 to 10Hz was performed at a stress value selected from within the linear viscoelastic region. The linear viscoelastic region was identified as the region in which the stress and the strain were directly proportional and where the storage modulus (G`) remained constant. From the resulting relationships between modulus and oscillatory frequency, the storage modulus (G'), loss modulus (G"), dynamic viscosity (η') and the loss tangent (tan δ) were then determined using the Rheology Advantage software provided by T.A. Instruments. In each case the dynamic rheological properties of at least five replicates were determined.

2.2.6. Mucoadhesion Testing

Mucoadhesion testing was conducted using a TA XT2 Texture Analyser in adhesion mode as previously reported by Jones *et al.*[27-29]. In brief, 400mg mucin discs were manufactured using a 13mm IR press using a force of 10 tonnes for a period of one minute. The discs were then attached to the end of a 10mm diameter polycarbonate probe via double sided adhesive tape. Samples to be analysed were transferred into a three-sided mould with mucoadhesion testing being determined at 37°C. Prior to testing, samples were stored in sealed sample vials incubated at 37°C for 24hrs. Before testing, the disc was pre-wetted with 5% mucin solution with excess being removed via blotting. A downward force of 0.1N was applied to the

polymer platform, held for 30 seconds before being removed at a speed of 10mms^{-1}. Mucoadhesion, determined as the force required to detach the polymer platform from the mucin disc, of each formulation was obtained from five replicates measurements.

2.2.7. Statistical Analysis

Statistical modelling was performed using a General Linear Model. The effects of polymer concentration, type of pH neutralising agent and oscillatory frequency on the viscoelastic properties (storage modulus, loss modulus, tan δ and dynamic viscosity (η') were statistically examined using a two-way repeated measures Analysis of Variance. The effects of polymer concentration and type of pH neutralising agent on the gel strength, rheological exponent and on crossover frequency were statistically examined using a two-way Analysis of Variance. The effects of neutralising agent on the intrinsic viscosity and critical concentration (C*) of PMVE/MA was statistically analysed using a one way ANOVA. Post-hoc analysis of individual treatment differences was performed using Tukey's HSD test. In all cases $p<0.05$ was accepted as denoting significance and therefore the individual probability values have not been included in the text.

Statistical modelling of the relationship between polymer concentration and the type of neutralising agent on the viscoelastic parameters (at three defined frequencies, 2.37, 5.39 and 9.99Hz) and on mucoadhesion was performed using a multiple linear regression model (two-way ANOVA). As before, post-hoc analysis of individual treatment differences was performed using Tukey's HSD test ($p<0.05$ denoting

significance). The relationships between viscoelastic properties (G', G'', tan δ and η') and mucoadhesion were examined using multiple correlation analysis.

In all cases measurements and statistical analyses were performed on five replicate samples (n = 5).

3. Results and Discussion

Mucoadhesive polymers have been extensively used within the pharmaceutical industry, finding use within a wide range of formulations such as eye drops, vaginal gels, nasal inserts, buccal tablets and with some limited success in peroral drug delivery[9]. PMVE/MA has been reported to have strong mucoadhesive properties[8, 9, 30] however, this polymer has been limited to a component within implants that contain other polymeric systems. Whilst theoretically this strategy may have merit, the authors have recently reported that PMVE/MA networks may undergo rheological ageing, resulting in compromised rheological and mucoadhesive properties[18]. There are therefore concerns regarding this strategy. There has been a distinct paucity of reports that have examined the mucoadhesive properties and additionally the rheological properties of PMVE/MA monopolymeric systems. Furthermore, this manuscript has addressed a major deficiency in the scientific literature, namely the contribution of polymer viscoelasticity to mucoadhesion, a relationship that requires full understanding to enable the rational design of mucoadhesive systems as biomedical implants.

3.1. Dilute Solution Properties of PMVE/MA

In this study the intrinsic viscosities of PMVE/MA dilute solutions, (un-neutralised or neutralised using NaOH or TEA) were determined via extrapolation of the Huggins or the Fuoss and Strauss plots (Figures 1a and 1b, respectively). Modelling of the mathematical relationships revealed that the plot of reduced viscosity against concentration of PMVE/VA for neutralised systems was non-linear thereby invalidating the use of the Huggins model to determine intrinsic viscosity of these systems. Notably, the reduced viscosity was observed to decrease with increasing

289 concentration of polymer in a manner typical of polyelectrolyte solutions[31]. Fuoss
290 and Strauss[32, 33] reported an empirical expression from which the intrinsic
291 viscosity of polyelectrolytes may be determined from the linear plot of the reciprocal
292 of reduced viscosity against polymer concentration$^{0.5.}$ Using this relationship, the
293 intrinsic viscosities of un-neutralised PMVE/MA and PMVE/MA neutralised using
294 NaOH or TEA were 22.32 ± 0.89 dL g^{-1}, 274.80 ± 1.94 dL g^{-1} and 416.49 ± 2.21 dL g^{-}
295 ^{1}, respectively and were significantly different. Plotting the relationship between
296 log_{10} specific viscosity and log_{10} polymer concentration provides a further
297 understanding of the dilute solution properties of PMVE/MA. PMVE/MA solutions
298 that had been neutralised using NaOH or TEA exhibited statistically significant
299 inflection points (C*) at PMVE/MA concentrations of 0.26 ± 0.01 and 0.28 ± 0.01 g
300 dl_{-1}, respectively. No inflection point was observed for un-neutralised systems.
301 Below the critical overlap concentration (C*) within a polymer network, polymer
302 chains exist independently as single units without entanglement. At this dilute
303 concentration, association of polymer chains is negligible allowing analysis of the
304 polymer chain conformation[34]. It is this polymer chain conformation that eventually
305 influences the various topological constraints and supramolecular organisations that
306 will occur within a network eventually giving rise to gel formation[35]. The intrinsic
307 viscosity of a polymer represents the hydrodynamic volume which an individual
308 polymer chain occupies, thereby reflecting the dimensions of the polymer chain[36]
309 and indicates the role of factors, e.g. polymer/solvent interactions, pH and the
310 presence of other ions within solutions on gel networks formed from these
311 systems[37]. In this study the intrinsic viscosity increased following neutralisation
312 due to the expanded conformation of the polymer chains resultant from ionisation of
313 the pendant carboxyl acid groups of the polymer. Hence, when neutralised the
314 polymer chains are in an expanded conformation occupying a greater spatial
315 dimension than the compact un-neutralised PVME/MA polymer chains. The choice

316 of neutralising agent was also observed to have a significant effect on the intrinsic
317 viscosity values of PMVM/MA chains. TEA neutralised systems were observed to
318 have significantly higher intrinsic viscosities than those obtained via NaOH
319 neutralisation and hence TEA neutralised chains occupied the greater hydrodynamic
320 volume. Variation between the two neutralised polymeric solutions may be
321 attributed to shielding of these electrostatic charges by sodium ions within solutions
322 neutralised by NaOH, which restricted polymer chain extension. Ionic strength has
323 been shown to have a significant effect on polyelectrolyte gels containing
324 carboxylate groups. Previously Tam and Tiu[38] investigated the effect of various
325 cations on the rheology of solutions of the anionic polymer polyacrylamide. They
326 observed that the presence of cations such as Na^+ reduced polymer inter and
327 intramolecular interactions causing a significant reduction in solution viscosity.

328

329 *3.2. Flow Properties of PMVE/MA Gels*

330 The effects of polymer concentration, neutralisation and the type of neutralising
331 agent on the consistency and rate index of PMVE/MA gels, modelled using the
332 Ostwald-de-Waele power law model and on their zero-shear rate viscosities,
333 modelled using the Cross model, are shown in Table 1. Neutralised polymeric
334 platforms were pseudoplastic, evident from the rate indices being statistically less
335 than 1. Increasing polymer concentration and neutralisation of the PMVE/MA
336 systems significantly decreased the rate index and increased both the consistency
337 and zero-shear rate viscosities. Furthermore, the effect of TEA on these properties
338 was significantly greater than for NaOH. These observations accounted for a
339 statistically significant interaction term in the ANOVA, indicative of a non-additive
340 effect of the various variables. Hence, the effects of polymer concentration on the

aforementioned properties were dependent on the pH of the gels and the neutralising agent. The observed flow properties of the neutralised gels were expected, given that the polymer concentrations dramatically exceeded the C* concentration. The effect of polymer concentration on the observed flow properties is due to an increased number of molecular interactions between the polymer chains[39]. All platforms showed an increase in the zero rate viscosity (i.e. the viscosity a system would exhibit when at rest) upon increasing polymer concentration, denoting a stronger network due to increased interaction between polymer chains[40]. Increases in zero shear rate viscosity were observed to be non-linear in relation to increasing PVM/MA concentrations for all platforms; this being indicative of increasing interactions between constituent groups within the polymer chains which are disproportionate to increases in polymer concentration. The effects of neutralisation on the flow properties were in accordance with the observations from dilute solution viscometry, with TEA neutralised systems offering greater resistance to deformation (increased consistency and zero shear rate viscosity) than comparator systems neutralised using NaOH. For example a 25% w/w TEA neutralised system had a consistency value of 1191.00 ± 11.53 $Pa.s^n$, and a zero rate viscosity of 3397.67 ± 80.21 Pa.s, whilst a 25% w/w NaOH neutralised system had values of 510.10 ± 9.20 $Pa.s^n$ and 992.97 ± 11.22 Pa.s, respectively. Thus, electrostatic shielding of ionised carboxylate groups due to the presence of Na^+ ions restricts the extension of the PMVE/MA polymer chain within NaOH neutralised networks. As a result, the more highly expanded PMVE/MA polymer chains, present within TEA neutralised formulations, have a greater probability of entangling with one another thereby forming a more structured network than typically experienced within similarly concentrated NaOH neutralised formulations. The addition of small counterions can effectively reduce the mutual charge repulsion of carboxylate anions resulting in polymer chain coiling[41].

3.3. Oscillatory Rheometry of PMVE/MA Gels

The effects of frequency, PMVE/MA concentration and type of neutralisation agent on the storage and loss moduli of the systems under investigation are graphically displayed in Figures 2-4. Furthermore, a summary of the effects of the aforementioned parameters on the loss tangents and dynamic viscosities at specified frequencies is presented in Table 2. The frequency dependence of both the elastic and loss moduli indicate that the formation of viscoelastic networks are as a result of non-covalent intermolecular interactions and polymer chain entanglements[42-44]. Whilst increasing PMVE/MA concentration (in the un-neutralised state) increased the storage and loss moduli and the dynamic viscosity (at each frequency), the magnitudes of these parameters were small and, furthermore, the loss tangent exceeded 1 at all concentrations and frequencies examined. These observations are typical of an elastoviscous system[18]. The failure to determine an LVR for 5% and 10% w/w PVME/MA un-neutralised formulations can be directly attributed to their lack of suitable viscoelastic structure. These results are consistent with the presence of PMVE/MA in the coiled state, with minimal interactions between adjacent polymer chains[18, 41]. In the neutralised state, the moduli dramatically increased, complementing the findings from continuous shear analysis. The storage and loss moduli and dynamic viscosity exhibited within neutralised systems were significantly higher than those recorded for un-neutralised formulations, whilst tan δ values were significantly lower. For example, the storage and loss moduli of a 30% w/w un-neutralised formulation of PVME/MA were 523.03 ± 25.64 Pa and 633.20 ± 26.82 Pa, respectively, whereas the storage and loss moduli of the corresponding NaOH neutralised system were 5552.33 ± 134.75 Pa and 3310.00 ± 49.57 Pa (all measured at a frequency of 6.19

Hz). As observed previously within viscometric and continuous shear analyses, the choice of neutralising agent had a significant effect on measured viscoelastic parameters. TEA neutralised formulations possessed significantly higher elastic and loss moduli and dynamic viscosity values, whereas tan δ values were observed to be significantly lower than those of NaOH neutralised systems at comparable PMVE/MA concentrations and oscillatory frequencies. A 30% PMVE/MA TEA neutralised formulation had a tan δ value of 0.50 ± 0.01, whereas a 30% w/w PVM/MA NaOH neutralised formulation presented a significantly higher tan δ value of 0.57 ± 0.01 (frequency 8.50 Hz). Polyelectrolyte systems are known to be very sensitive to changes in pH, with neutralisation of acidic groups, as observed in this study, leading to the formation of a tighter gel network via non-covalent association of polymer chains[45]. The presence of various ions however can cause shielding of the negatively charged carboxylate groups resulting in a reduction in the repulsion between polymer chains, decreasing formulation viscosity and elasticity[46]. The net effect of this shielding effect that was apparent in this study was gels that had been neutralised by sodium hydroxide exhibited lower elasticity than their organic amine neutralised counterparts. This is apparent from the greater magnitude of the storage modulus, the lower loss tangent values, the lower gel strength and the lower crossover frequency of TEA-neutralised PMVE/MA gels, as depicted in Table 3. This table provides an insight into the frequency dependence of the elastic and loss moduli through the use of a power law model, which characterises the material strength (K) and the frequency dependency of the platform (n). Together, these parameters provide a further understanding of the nature of the platform under investigation[47, 48]. Power Law exponent values of moduli were observed to significantly decrease, whilst moduli gel strength values significantly increased upon increasing PMVE/MA concentration and/or following neutralisation. These observed trends indicate the movement towards more structured viscoelastic networks. An n

421 value close to or equal to zero is representative of a covalently crosslinked system,
422 whereas a network exhibiting an n value greater than zero indicates the presence of
423 a physical gel network[36]. All Power Law exponent values were markedly greater
424 than zero, consistent with the properties of polymer-entangled networks. According
425 to Winter and Chambon[49] a power Law exponent for both moduli equal or less
426 than 0.5 and where G'>G", indicates the presence of a gel. Using this principle only
427 a 30% w/w PVME/MA NaOH neutralised system along with 25% and 30% w/w
428 PVM/MA TEA neutralised systems can be termed gels.

429

430 *3.4. Mucoadhesion of PMVE/MA Gels*

431 The effects of polymer concentration and neutralisation/type of neutralising agent on
432 the mucoadhesive properties are presented in Table 3. The mucoadhesive method
433 employed in this study has been frequently reported and is accepted as a model
434 which reliably characterises the interaction between mucin and polymeric
435 platforms[9, 50, 51]. One benefit of this test is the ability to quantify the force (or
436 work) required to break the adhesive bond between the polymer platform and the
437 mucin substrate and therefore the mucoadhesive properties of polymeric platforms
438 may be effectively compared. Polymer concentration and neutralisation/neutraliser
439 type significantly affected mucoadhesion, with a statistical interaction between
440 concentration and neutraliser being identified. In this, the dependency of polymer
441 concentration on mucoadhesion was greater for TEA neutralised systems than for
442 NaOH neutralised systems. The mucoadhesive properties of un-neutralised
443 PMVE/MA systems could not be measured using this technique however, given the
444 ability of the method to reliably measure forces of detachment that are relevant to *in*
445 *vivo* retention of dosage forms[29, 52, 53], it may be inferred that the mucoadhesive

properties of these systems are negligible. The mucoadhesive properties of PMVE/MA systems significantly increased with increasing polymer concentration and were greater for TEA-neutralised gels than for their NaOH-neutralised counterparts. The mucoadhesive properties of neutralised PMVE/MA gels (15-30% w/w) were substantial and greatly exceeded those of mucoadhesive implants that have reported to be successfully retained *in vivo* within the periodontal pocket[29]. Furthermore, given the wide range of polymer concentrations associated with these enhanced mucoadhesive properties, it is expected that the retention of formulations *in vivo*, e.g. within the periodontal pocket, would be resistant to dilution from biological fluids. Given both the wide range of concentrations that exhibited mucoadhesive properties and the magnitudes of mucoadhesion exhibited, these mono-polymeric platforms would the expected to offer prolonged retention *in vivo*. This is the first report that suggests this role for mono-polymeric PMVE/MA platforms.

3.4. Statistical Modelling of the Relationship between Mucoadhesion and Rheological Properties of PMVE/MA Gels

Within this study a series of statistical methods has been employed to ensure that the effects of primary factors on the various rheological properties were fully ascertained within a factorial experimental design. However, importantly, this study additionally examined statistical interactions between the primary factors and explained these within the context of the physicochemical properties of the gel platforms. In so doing, a unique insight into the polymer state was derived. A key task of this manuscript was to understand the relationship between the various polymeric variables and mucoadhesion, a relationship that has not been fully

471 described in the scientific literature to date. This study therefore described the
472 application of multiple linear regression to model the relationships between the
473 various variables with mucoadhesion, a parameter that is indicative of retention *in*
474 *vivo*. Accordingly, using a general linear model three major variables were observed
475 to affect mucoadhesion, namely polymer concentration, pH and the type of
476 neutralising agent. There were strong statistical interactions between these
477 variables and their effects on mucoadhesion. In these, the effect of polymer
478 concentration on mucoadhesion was significantly greater in gels neutralised with
479 TEA than in gels neutralised using NaOH. Similarly there was an interaction
480 between the pH and polymer concentration on mucoadhesion in which polymer
481 concentration exhibited a significantly greater effect on the mucoadhesion of
482 PMVE/MA gels when neutralised. To understand these relationships further
483 stepwise multiple regression analysis between the viscoelastic parameters and
484 mucoadhesion was performed. Unsurprisingly, the correlation matrix identified
485 strong relationships between the various viscoelastic properties and therefore the
486 multiple regression analysis was simplified by considering the effect of storage
487 modulus on the mucoadhesive properties of neutralised PMVE/MA platforms over a
488 range of different oscillatory frequencies. At each frequency examined there was a
489 strong relationship between storage modulus and mucoadhesion ($r>0.98$).
490 Furthermore the relationship between polymer concentration and mucoadhesion
491 was linear ($r>0.99$ for TEA neutralised gels, $r>0.98$ for NaOH neutralised gels and
492 0.94 for the overall effect combining both types of neutralisation). These results
493 have enabled a re-imagination of the roles of polymer viscoelasticity, concentration
494 and neutralisation on mucoadhesion. The process of mucoadhesion is understood
495 to be complex, with a number of physicochemical properties being reported to
496 contribute to this phenomenon including polymer molecular weight, polymer
497 conformation, degree of cross-linking, the contribution of functional groups on the

polymer and the charge on these groups[9, 54]. In this study the contribution of polymer molecular weight and the type of functional group were constant across all polymer platforms; however, the contributions of charge on the groups and polymer concentration on mucoadhesion may be considered. Interestingly, there has been little attention paid to the relationships between the aforementioned properties, polymer viscoelasticity and mucoadhesion. This study has addressed this paucity of information. Mucoadhesion involves an adhesive interaction between a polymeric platform and mucin, facilitated through specific (hydrogen) bond formation[9]. In principal, this interaction should be enhanced whenever the functional groups in both polymers are unionised however this study has clearly shown that ionisation, facilitated through neutralisation of the carboxylate groups of PMVE/MA resulted in both marked and enhanced mucoadhesion. Notably, whilst the degree of polymer ionisation affected mucoadhesion, the contribution of polymer viscoelasticity was dominant, as defined within the statistical model. Thus, at identical levels of ionisation the role of neutralising agent on the mucoadhesive and viscoelastic properties was significant. Neutralisation with TEA resulted in a greater expansion of the polymer chains through charge repulsion than in platforms similarly neutralised using NaOH; the difference being accredited to charge shielding of the ionised carboxylate groups with the sodium counterion. At pH 7.4 the degree of ionisation of PMVE/MA was *circa* 99% and therefore, whilst there were differences in shielding of charge in the two neutralised, both systems exhibited predominantly anionic properties. With reference to the observed (strong) correlations between polymer viscoelasticity and mucoadhesion, this study has highlighted that the dominant factor that contributes to mucoadhesion is polymer viscoelasticity. Thus, as the elastic properties of PMVE/MA are increased, the mucoadhesion increased, notably in a linear fashion. Whilst not observed in this study, an upper limit in PMVE/MA concentration would be expected beyond which no further increase in

525 mucoadhesion is observed. In this scenario, the polymer interactions within
526 PMVE/MA would predominate thereby reducing the likelihood of adhesive
527 interactions with mucin. It should be clearly stated that, through the application of
528 statistical modelling, this study has clearly defined the dominant role of polymer
529 viscoelasticity on mucoadhesion, a conclusion that has not been directly defined in
530 previous studies. These observations explain, at least in part, the mucoadhesive
531 properties of non-charged polymers, e.g. cellulose ethers[9].

532

533 *3.5. Re-defining the clinical opportunities for PMVE/MA Gels*

534 Despite the use of PMVE/MA as a component within mucoadhesive platforms, there
535 has been limited use of this polymer as a single component system. Historically,
536 this may be related, at least in part, to the limited viscosity enhancement per unit
537 mass of polymer associated with this polymer (and the associated commercial cost).
538 Alteration of the concentration of PMVE/MA and/or neutralisation enhanced the
539 viscoelastic properties of the polymer platform and, in so doing, facilitated
540 enhanced mucoadhesion. Within the clinical domain, it is important that the
541 administration of implants is both not compromised by viscosity (associated with
542 increased polymer concentration) and is retained at the proposed site of
543 administration, the latter being facilitated by enhanced viscoelasticity (and polymer
544 concentration). Therefore a compromise is required between these two phenomena.
545 There is published evidence that clarifies the key rheological properties of gel
546 systems that have been successfully employed *in vivo* as implants for the treatment
547 of oral diseases, a potential application for the PMVE/MA systems under current
548 examination[29, 53, 55]. For example in these systems the storage modulus (at
549 *circa* 10Hz) and zero shear rate viscosity of a platform that was successfully applied

to and retained within the periodontal pocket using a periodontal syringe were *circa* 3.5kPa and 2.6kPa.s, respectively. Accordingly, PVME/MA 30% w/w, TEA neutralised gels, despite their excellent mucoadhesive properties would be inappropriate for delivery using a periodontal syringe, however in applications involving application of the gel to the site of action directly from a standard container (e.g. using an applicator or manually), this would not be as important. It should be noted that the rheological and mucoadhesive properties of the polymeric platforms described in this study would be suitable for other pharmaceutical and biomedical applications. For example, modification of the polymer concentration will enable the rheological and mucoadhesive properties of PMVE/MA gels to be engineered that are suitable for use as ophthalmic viscosurgical devices during phacoemulsification[19] or for vaginal drug delivery applications[51]. Finally, it is important to reiterate that by selection of polymer concentration and neutraliser type, PMVE/MA gels may be prepared that offer comparable rheological and mucoadhesive properties to multicomponent polymeric platforms that have been successful clinically [29, 53]. This is important as it will obviate potential issues with rheological ageing (structuring or destructuring) upon storage of multicomponent systems.

4. Conclusions

In this study the rheological and mucoadhesive properties of aqueous PMVE/MA platforms have been comprehensively characterised to establish their potential suitability for biomedical and pharmaceutical applications. To address this issue, the contributions of polymer concentration, pH and type of neutralising agent to the viscoelastic and mucoadhesive properties were statistically modelled. The

application of multiple linear regression analysis enabled the specific relationships between the viscoelastic and mucoadhesive properties to be explicitly defined, a feature that has yet to receive sufficient attention within the scientific literature. This study uniquely identified the dominant contribution of polymer viscoelasticity on mucoadhesion, an outcome that may be employed to rationally design mucoadhesive platforms. Manipulation of both the rheological (flow and oscillatory rheometry) and mucoadhesive properties was performed by changing both polymer concentration and pH and by choice of neutralising agent. In so doing mono-polymeric PMVE/MA platforms were prepared that, based on previous reports offered wide ranges of rheological and mucoadhesive properties that rendered them suitable for a range of pharmaceutical and biomedical properties where prolonged retention at the site of application is required.

588 **Legends for Figures**

589 **Figure 1a.** Huggins plot illustrating the relationship between reduced viscosity
590 and concentration of PMVE/MA. Circles refer to Unneutralised PMVE/MA whereas
591 squares and triangles refer to PMVE/MA that had been neutralised using either
592 NaOH or TEA, respectively. Standard deviations from five replicate measurements
593 are included.

594

595 **Figure 1b.** Fuoss and Strauss plot illustrating the relationship between log
596 reduced viscosity and the square root of concentration of PMVE/MA. Circles refer
597 to Unneutralised PMVE/MA whereas squares and triangles refer to PMVE/MA that
598 had been neutralised using either NaOH or TEA, respectively. Standard deviations
599 from five replicate measurements are included.

600

601 **Figure 2.** The relationship between modulus (storage and loss) and frequency
602 for unneutralised PMVE/MA platforms. Figure 2(a) relates to PMVE/MA 5% w/w
603 (circles), PMVE/MA 10% w/w (squares) and PMVE/MA 15% w/w (diamonds). Figure
604 2(b) relates to PMVE/MA 20% w/w (circles), PMVE/MA 25% w/w (squares) and
605 PMVE/MA 30% w/w (diamonds). Closed symbols relate to the storage modulus
606 whereas open symbols refer to the loss modulus. Standard deviations from five
607 replicate measurements are included.

608

609

Figure 3. The relationship between modulus (storage and loss) and frequency for PMVE/MA platforms that have been neutralised using NaOH. Figure 3(a) relates to PMVE/MA 5% w/w (circles), PMVE/MA 10% w/w (squares) and PMVE/MA 15% w/w (diamonds). Figure 3(b) relates to PMVE/MA 20% w/w (circles), PMVE/MA 25% w/w (squares) and PMVE/MA 30% w/w (diamonds). Closed symbols relate to the storage modulus whereas open symbols refer to the loss modulus. Standard deviations from five replicate measurements are included.

Figure 4. The relationship between modulus (storage and loss) and frequency for PMVE/MA platforms that have been neutralised using triethylamine. Figure 4(a) relates to PMVE/MA 5% w/w (circles), PMVE/MA 10% w/w (squares) and PMVE/MA 15% w/w (diamonds). Figure 4(b) relates to PMVE/MA 20% w/w (circles), PMVE/MA 25% w/w (squares) and PMVE/MA 30% w/w (diamonds). Closed symbols relate to the storage modulus whereas open symbols refer to the loss modulus. Standard deviations from five replicate measurements are included.

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802

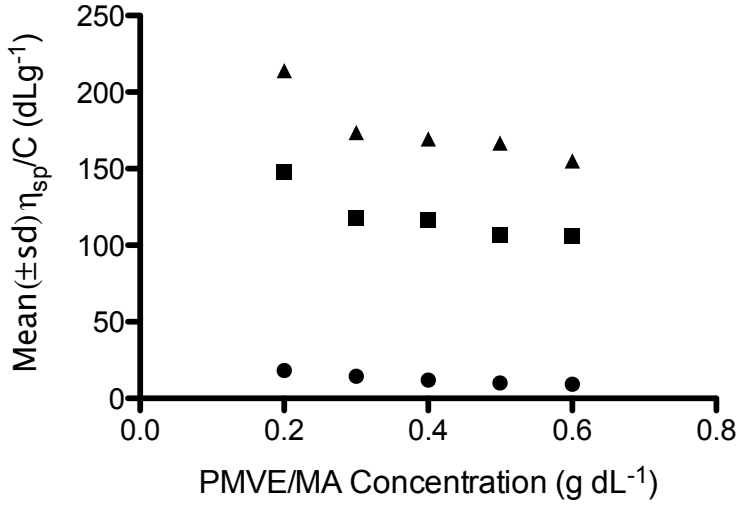


Figure 1a

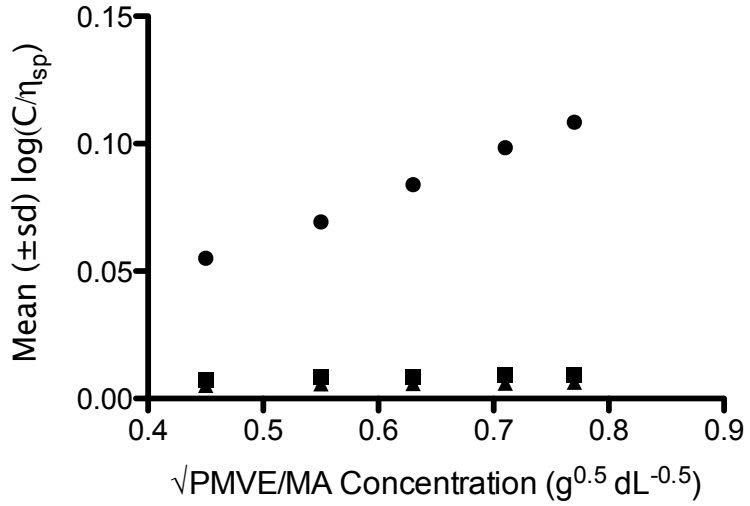


Figure 1b

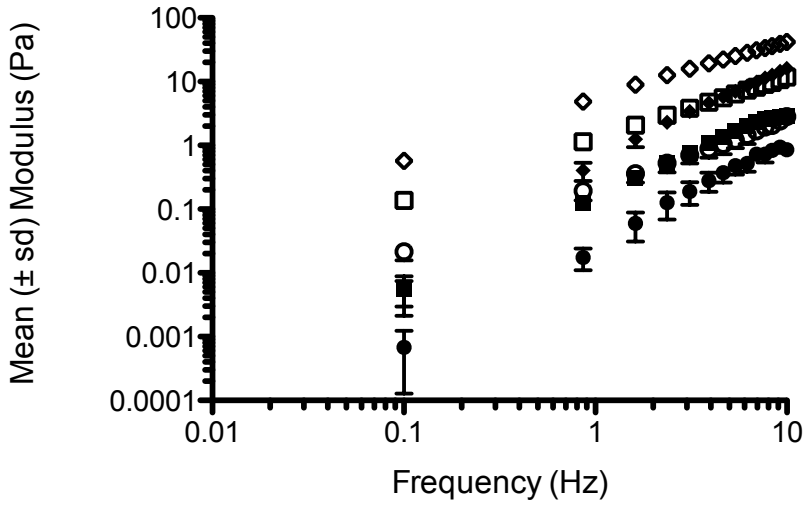
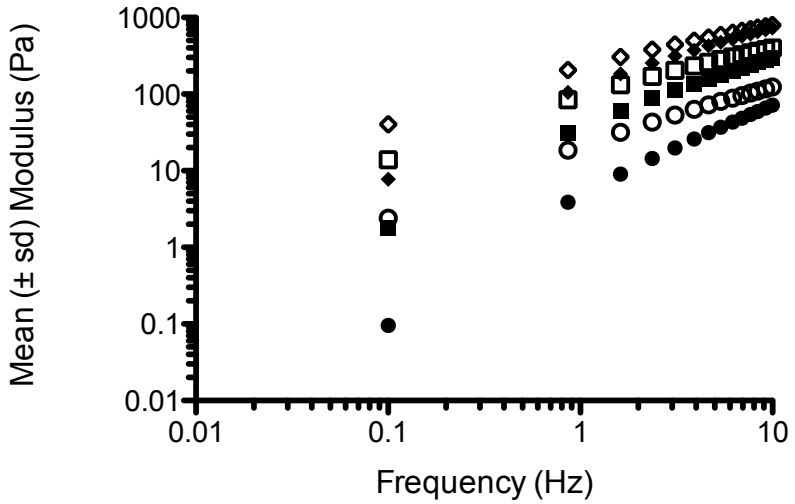


Figure 2(a)



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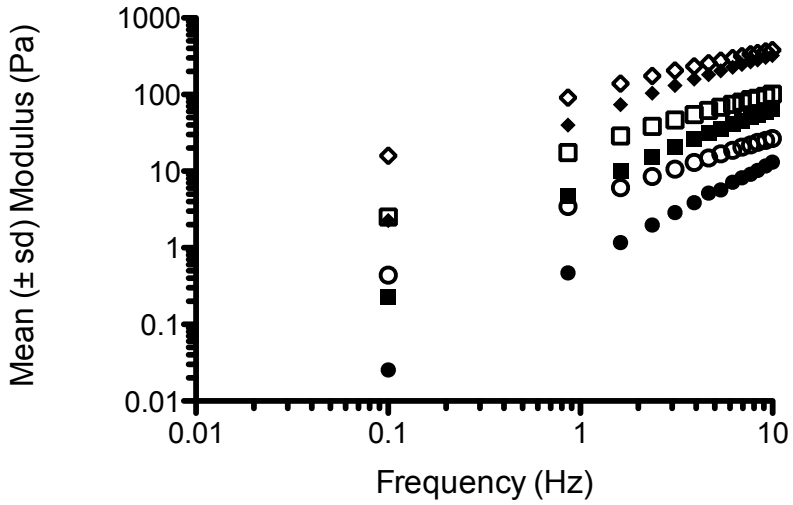
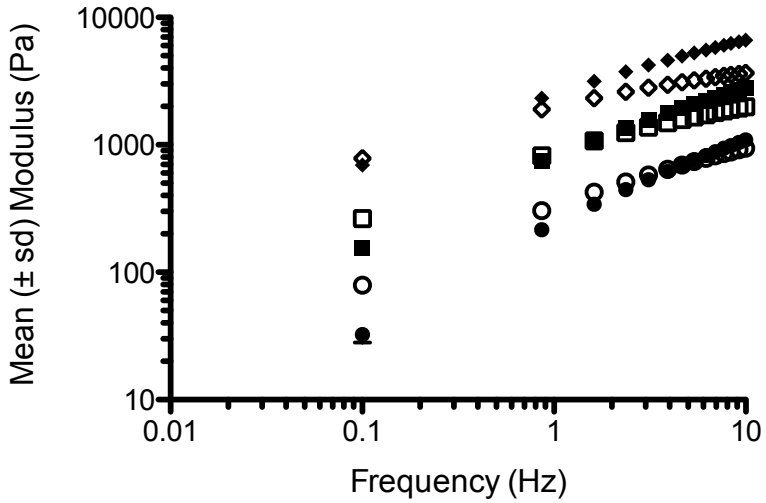


Figure 3a)



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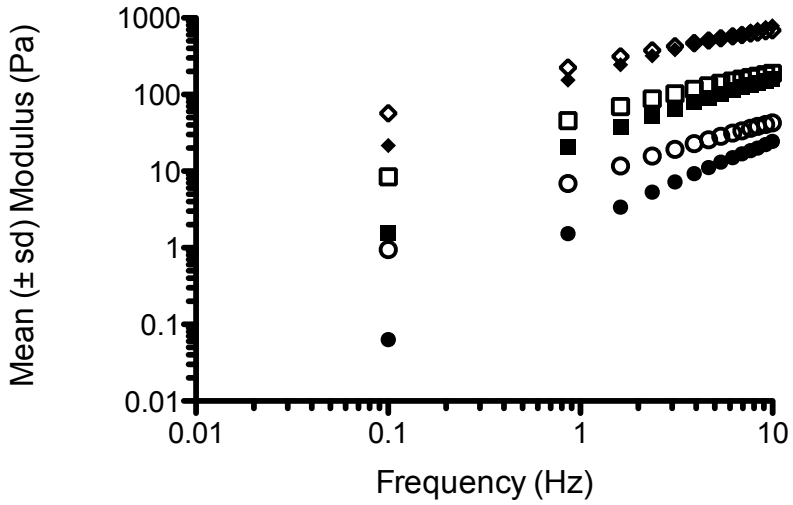

Figure 4(a)

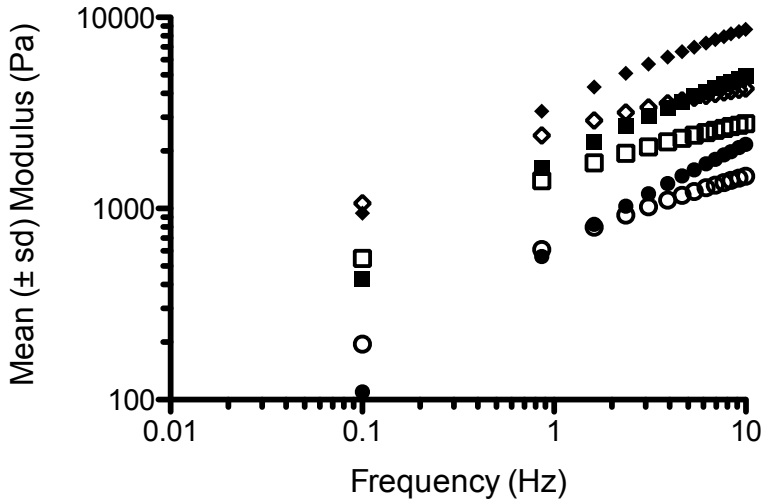

Figure 4(b)

Table 1. The effects of polymer concentration, neutralising agent and oscillatory frequency on the loss tangent (tan δ) and dynamic viscosity (η') of aqueous poly(methylvinyl ether-co-maleic acid) platforms

Concentration of PMVE/VA (% w/w)	Neutralisation	Mean (± sd) Consistency (Pa.s)^n	Mean (± sd) Rate Index	Mean (± sd) zero shear rate viscosity (Pa.s)
5	Un-neutralised	0.02 ± 0.00	0.98 ± 0.00	0.02 ± 0.00
10	Un-neutralised	0.25 ± 0.02	0.92 ± 0.02	0.20 ± 0.02
15	Un-neutralised	1.36 ± 0.03	0.87 ± 0.01	0.97 ± 0.05
20	Un-neutralised	6.21 ± 0.39	0.82 ± 0.01	4.37 ± 0.28
25	Un-neutralised	35.73 ± 0.70	0.67 ± 0.01	23.66 ± 0.12
30	Un-neutralised	105.80 ± 5.34	0.60 ± 0.01	89.41 ± 4.02
5	Neutralised with NaOH	0.96 ± 0.05	0.88 ± 0.01	0.76 ± 0.05
10	Neutralised with NaOH	5.23 ± 0.44	0.84 ± 0.00	4.52 ± 0.43
15	Neutralised with NaOH	35.48 ± 2.39	0.81 ± 0.00	43.76 ± 2.57
20	Neutralised with NaOH	133.93 ± 8.19	0.71 ± 0.03	207.70 ± 11.33
25	Neutralised with NaOH	510.10 ± 9.20	0.59 ± 0.00	992.97 ± 11.22
30	Neutralised with NaOH	1928.00 ± 73.70	0.57 ± 0.00	5548.33 ± 153.08
5	Neutralised with TEA	3.26 ± 0.03	0.85 ± 0.01	1.97 ± 0.09
10	Neutralised with TEA	19.86 ± 0.73	0.81 ± 0.01	21.39 ±1.18
15	Neutralised with TEA	109.53 ± 3.54	0.74 ± 0.01	159.90 ± 7.55
20	Neutralised with TEA	384.30 ± 5.15	0.59 ± 0.01	886.37 ± 54.38
25	Neutralised with TEA	1191.00 ± 11.53	0.53 ± 0.00	3397.67 ± 80.21
30	Neutralised with TEA	2471.00 ± 95.17	0.47 ± 0.01	13720.00 ± 469.36

Table 2. The effects of polymer concentration, neutralising agent and oscillatory frequency on the loss tangent (tan δ) and dynamic viscosity (η') of aqueous poly(methylvinyl ether-co-maleic acid) platforms

PMVE/MA Concetration	Oscilatory Frequency (Hz)	Mean (± standard deviation) viscoelastic parameters					
		Unneutralised		Neutralised (pH 7.4) with NaOH		Neutralised (pH 7.4) with Triethylamine	
		tan δ	η' (Pa.s)	tan δ	η' (Pa.s)	tan δ	η' (Pa.s)
5	2.37	NM*	NM*	4.19 ± 0.28	0.57 ± 0.02	2.94 ± 0.05	1.05 ± 0.01
	5.39	NM*	NM*	2.82 ± 0.20	0.50 ± 0.02	2.18 ± 0.03	0.84 ± 0.01
	9.99	NM*	NM*	2.01 ± 0.20	0.43 ± 0.01	1.75 ± 0.10	0.68 ± 0.02
10	2.37	NM*	NM*	2.54 ± 0.07	2.58 ± 0.03	1.68 ± 0.01	5.88 ± 0.34
	5.39	NM*	NM*	1.93 ± 0.03	2.03 ± 0.03	1.37 ± 0.01	4.15 ± 0.14
	9.99	NM*	NM*	1.60 ± 0.04	1.62 ± 0.03	1.18 ± 0.01	3.02 ± 0.11
15	2.37	5.60 ± 0.70	0.85 ± 0.02	1.68 ± 0.01	11.77 ± 0.13	1.17 ± 0.00	25.33 ± 0.12
	5.39	3.57 ± 0.12	0.74 ± 0.01	1.37 ± 0.01	8.23 ± 0.09	0.99 ± 0.00	16.02 ± 0.10
	9.99	2.62 ± 0.07	0.67 ± 0.01	1.18 ± 0.01	6.09 ± 0.07	0.88 ± 0.00	11.01 ± 0.05
20	2.37	3.00 ± 0.13	2.90 ± 0.16	1.15 ± 0.04	34.35 ± 0.51	0.90 ± 0.00	62.06 ± 0.37
	5.39	2.17 ± 0.08	2.37 ± 0.12	0.98 ± 0.03	21.81 ± 0.14	0.77 ± 0.00	36.24 ± 0.19
	9.99	1.73 ± 0.07	1.97 ± 0.09	0.86 ±0.02	15.02 ± 0.02	0.68 ± 0.00	23.50 ± 0.11
25	2.37	1.95 ± 0.01	11.38 ± 0.15	0.92 ± 0.00	83.15 ± 0.68	0.72 ± 0.00	129.90 ± 1.57
	5.39	1.57 ± 0.01	8.30 ± 0.11	0.79 ± 0.00	48.53 ± 0.29	0.63 ± 0.00	71.00 ± 0.80
	9.99	1.35 ± 0.01	6.35 ± 0.10	0.71 ± 0.00	31.60 ± 0.11	0.56 ± 0.00	44.22 ± 0.46
30	2.37	1.51 ± 0.02	25.53 ± 1.12	0.70 ± 0.01	173.90 ± 3.41	0.62 ± 0.01	212.03 ± 5.00
	5.39	1.25 ± 0.02	17.38 ± 0.74	0.61 ± 0.01	94.17 ± 1.50	0.54 ± 0.01	111.17 ± 2.34
	9.99	1.09 ± 0.01	12.66 ± 0.53	0.55 ± 0.01	58.21 ± 0.87	0.49 ± 0.01	67.53 ± 1.29

* Not Measureable

Table 3. The effects of polymer concentration, neutralising agent and oscillatory frequency on the loss tangent (tan δ), dynamic viscosity (η') and mucoadhesion of aqueous poly(methylvinyl ether-co-maleic acid) platforms

PVME/MA Concentration (% w/w)	Neutralisation	Mean (± sd) Gel Strength (Pa)	Mean (± sd) Rheological Exponent	Crossover Frequency (Hz)	Mean (± sd) Mucoadhesive Bond Strength (N)
5	Unneutralised	Not Measureable	Not Measureable	Not Observed*	Not Measureable
	NaOH	0.61 ± 0.13	1.35 ± 0.08	Not Observed*	Not Measureable
	Triethylamine	1.53 ± 0.08	1.27 ± 0.03	Not Observed*	Not Measureable
10	Unneutralised	Not Measureable	Not Measureable	Not Observed*	Not Measureable
	NaOH	4.62 ± 0.34	1.20 ± 0.03	Not Observed*	Not Measureable
	Triethylamine	19.42 ± 0.88	0.98 ± 0.00	Not Observed*	Not Measureable
15	Unneutralised	0.40 ± 0.09	1.72 ± 0.11	Not Observed*	Not Measureable
	NaOH	35.14 ± 0.90	1.04 ± 0.01	Not Observed*	0.54 ± 0.05
	Triethylamine	150.14 ± 0.72	0.76 ± 0.00	5.18 ± 0.03	0.70 ± 0.04
20	Unneutralised	3.50 ± 0.37	1.40 ± 0.02	Not Observed*	Not Measureable
	NaOH	213.10 ± 15.12	0.75 ± 0.02	5.12 ± 0.12	0.70 ± 0.03
	Triethylamine	547.52 ± 4.70	0.63 ± 0.00	1.34 ± 0.02	0.85 ± 0.04
25	Unneutralised	28.86 ± 0.70	1.08 ± 0.01	Not Observed*	0.12 ± 0.01
	NaOH	732.59 ± 10.26	0.62 ± 0.00	1.48 ± 0.03	1.02 ± 0.05
	Triethylamine	1596.97 ± 26.10	0.52 ± 0.00	0.37 ± 0.01	1.20 ± 0.04
30	Unneutralised	94.31 ± 5.70	0.96 ± 0.01	Not Observed*	0.21 ± 0.02
	NaOH	2322.43 ± 77.44	0.48 ± 0.00	0.24 ± 0.02	1.27 ± 0.04
	Triethylamine	3152.17 ± 145.75	0.47 ± 0.01	0.20 ± 0.01	1.58 ± 0.02

* Not observed over the frequency range studied