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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 dropwise 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 \( \nu \) (mm²s⁻¹) (Equation 1).

\[
\nu = kt
\]

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 \( \eta_{rel} \) was calculated

\[
\eta_{rel} = \frac{\nu}{\nu_o}
\]

Equation 2

Where: \( \nu \) and \( \nu_o \) refer to the kinematic viscosities of the polymeric solution and the solvent in which the polymer is dispersed, respectively.
The specific viscosity ($\eta_{sp}$) is then calculated:

\[ \eta_{sp} = \eta_{rel} - 1 \]  

Equation 3

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

\[ \eta_{red} = \frac{\eta_{sp}}{C} \]  

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 [$\eta$] was performed using the Huggin’s equation (equation 5) or the equation described by Fuoss and Strauss (equation 6)[21] [22, 23].

\[ \eta_{red} = \frac{\eta_{sp}}{C} = [\eta] + k'[\eta]^2C \]  

Equation 5

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

\[ \eta_{sp} = \frac{[\eta]}{(1+B\sqrt{C})} \]  

Equation 6

Where: C is the polymer concentration and B is a constant
2.2.4. Continuous Shear Analysis.

Continuous shear (flow) analyses were performed at 37°C on the PMVE/MA (5-30% w/w) systems using a TA systems AR2000 rheometer. Flow rheograms were determined using either a 6cm or 4cm parallel stainless steel plate (gap size 1000 µm), the choice of geometry being 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. The shear stress was applied over a predetermined range, with this range again being determined by sample consistency. Mathematical modelling of the flow properties of the various polymeric platforms was performed using the Rheology Advantage software (TA Instruments) in conjunction with the Ostwald-de-Waele power law model (equation 7)[24] and the Cross model (equation 8)[25], as follows:

\[ \sigma = k\dot{\gamma}^n \]  
Equation 7

Where: \( \sigma \) refers to the shear stress, \( \dot{\gamma} \) refers to the rate of shear, \( k \) refers to the consistency and \( n \) represents a power law index.

\[ \eta = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + (K\dot{\gamma})^m} \]  
Equation 8

Where: \( \eta \) is viscosity, \( \eta_{\infty} \) is the infinite shear viscosity, \( K \) is a structural relaxation time, \( m \) is dimensionless and \( \eta_0 \) is the zero rate viscosity.

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

2.2.5. Oscillatory Analysis.

Oscillatory analyses were performed at 37°C on PMVE/MA (5-30% w/w) systems 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 10 mms$^{-1}$.

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
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
concentration of polymer in a manner typical of polyelectrolyte solutions[31]. Fuoss and Strauss[32, 33] reported an empirical expression from which the intrinsic viscosity of polyelectrolytes may be determined from the linear plot of the reciprocal of reduced viscosity against polymer concentration\(^{0.5}\). Using this relationship, 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\(^{-1}\), respectively and were significantly different. Plotting the relationship between log\(_{10}\) specific viscosity and log\(_{10}\) polymer concentration provides a further understanding of the dilute solution properties of PMVE/MA. PMVE/MA solutions that had been neutralised using NaOH or TEA exhibited statistically significant inflection points (C\(^{*}\)) at PMVE/MA concentrations of 0.26 ± 0.01 and 0.28 ± 0.01 g dl\(^{-1}\), respectively. No inflection point was observed for un-neutralised systems.

Below the critical overlap concentration (C\(^{*}\)) within a polymer network, polymer chains exist independently as single units without entanglement. At this dilute concentration, association of polymer chains is negligible allowing analysis of the polymer chain conformation[34]. It is this polymer chain conformation that eventually influences the various topological constraints and supramolecular organisations that will occur within a network eventually giving rise to gel formation[35]. The intrinsic viscosity of a polymer represents the hydrodynamic volume which an individual polymer chain occupies, thereby reflecting the dimensions of the polymer chain[36] and indicates the role of factors, e.g. polymer/solvent interactions, pH and the presence of other ions within solutions on gel networks formed from these systems[37]. In this study the intrinsic viscosity increased following neutralisation due to the expanded conformation of the polymer chains resultant from ionisation of the pendant carboxyl acid groups of the polymer. Hence, when neutralised the polymer chains are in an expanded conformation occupying a greater spatial dimension than the compact un-neutralised PVME/MA polymer chains. The choice
of neutralising agent was also observed to have a significant effect on the intrinsic viscosity values of PMVM/MA chains. TEA neutralised systems were observed to have significantly higher intrinsic viscosities than those obtained via NaOH neutralisation and hence TEA neutralised chains occupied the greater hydrodynamic volume. Variation between the two neutralised polymeric solutions may be attributed to shielding of these electrostatic charges by sodium ions within solutions neutralised by NaOH, which restricted polymer chain extension. Ionic strength has been shown to have a significant effect on polyelectrolyte gels containing carboxylate groups. Previously Tam and Tiu[38] investigated the effect of various cations on the rheology of solutions of the anionic polymer polyacrylamide. They observed that the presence of cations such as Na+ reduced polymer inter and intramolecular interactions causing a significant reduction in solution viscosity.

3.2. Flow Properties of PMVE/MA Gels

The effects of polymer concentration, neutralisation and the type of neutralising agent on the consistency and rate index of PMVE/MA gels, modelled using the Ostwald-de-Waele power law model and on their zero-shear rate viscosities, modelled using the Cross model, are shown in Table 1. Neutralised polymeric platforms were pseudoplastic, evident from the rate indices being statistically less than 1. Increasing polymer concentration and neutralisation of the PMVE/MA systems significantly decreased the rate index and increased both the consistency and zero-shear rate viscosities. Furthermore, the effect of TEA on these properties was significantly greater than for NaOH. These observations accounted for a statistically significant interaction term in the ANOVA, indicative of a non-additive 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
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
value close to or equal to zero is representative of a covalently crosslinked system, whereas a network exhibiting an $n$ value greater than zero indicates the presence of a physical gel network\[36\]. All Power Law exponent values were markedly greater than zero, consistent with the properties of polymer-entangled networks. According to Winter and Chambon\[49\] a power Law exponent for both moduli equal or less than 0.5 and where $G' > G''$, indicates the presence of a gel. Using this principle only a 30\% w/w PVME/MA NaOH neutralised system along with 25\% and 30\% w/w PVM/MA TEA neutralised systems can be termed gels.

3.4. Mucoadhesion of PMVE/MA Gels

The effects of polymer concentration and neutralisation/type of neutralising agent on the mucoadhesive properties are presented in Table 3. The mucoadhesive method employed in this study has been frequently reported and is accepted as a model which reliably characterises the interaction between mucin and polymeric platforms\[9, 50, 51\]. One benefit of this test is the ability to quantify the force (or work) required to break the adhesive bond between the polymer platform and the mucin substrate and therefore the mucoadhesive properties of polymeric platforms may be effectively compared. Polymer concentration and neutralisation/neutraliser type significantly affected mucoadhesion, with a statistical interaction between concentration and neutraliser being identified. In this, the dependency of polymer concentration on mucoadhesion was greater for TEA neutralised systems than for NaOH neutralised systems. The mucoadhesive properties of un-neutralised PMVE/MA systems could not be measured using this technique however, given the ability of the method to reliably measure forces of detachment that are relevant to in 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 \textit{in vivo} within the periodontal pocket\cite{29}.

Furthermore, given the wide range of polymer concentrations associated with these enhanced mucoadhesive properties, it is expected that the retention of formulations \textit{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 \textit{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
described in the scientific literature to date. This study therefore described the application of multiple linear regression to model the relationships between the various variables with mucoadhesion, a parameter that is indicative of retention in vivo. Accordingly, using a general linear model three major variables were observed to affect mucoadhesion, namely polymer concentration, pH and the type of neutralising agent. There were strong statistical interactions between these variables and their effects on mucoadhesion. In these, the effect of polymer concentration on mucoadhesion was significantly greater in gels neutralised with TEA than in gels neutralised using NaOH. Similarly there was an interaction between the pH and polymer concentration on mucoadhesion in which polymer concentration exhibited a significantly greater effect on the mucoadhesion of PMVE/MA gels when neutralised. To understand these relationships further stepwise multiple regression analysis between the viscoelastic parameters and mucoadhesion was performed. Unsurprisingly, the correlation matrix identified strong relationships between the various viscoelastic properties and therefore the multiple regression analysis was simplified by considering the effect of storage modulus on the mucoadhesive properties of neutralised PMVE/MA platforms over a range of different oscillatory frequencies. At each frequency examined there was a strong relationship between storage modulus and mucoadhesion \((r>0.98)\). Furthermore the relationship between polymer concentration and mucoadhesion was linear \((r>0.99\) for TEA neutralised gels, \(r>0.98\) for NaOH neutralised gels and \(0.94\) for the overall effect combining both types of neutralisation). These results have enabled a re-imagination of the roles of polymer viscoelasticity, concentration and neutralisation on mucoadhesion. The process of mucoadhesion is understood to be complex, with a number of physicochemical properties being reported to contribute to this phenomenon including polymer molecular weight, polymer 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
mucoadhesion is observed. In this scenario, the polymer interactions within PMVE/MA would predominate thereby reducing the likelihood of adhesive interactions with mucin. It should be clearly stated that, through the application of statistical modelling, this study has clearly defined the dominant role of polymer viscoelasticity on mucoadhesion, a conclusion that has not been directly defined in previous studies. These observations explain, at least in part, the mucoadhesive properties of non-charged polymers, e.g. cellulose ethers[9].

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

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

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

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

Figure 2. The relationship between modulus (storage and loss) and frequency for unneutralised PMVE/MA platforms. Figure 2(a) relates to PMVE/MA 5% w/w (circles), PMVE/MA 10% w/w (squares) and PMVE/MA 15% w/w (diamonds). Figure 2(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 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.
References


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Figure 1a

Figure 1b
Figure 3a)

Figure 3(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

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

<table>
<thead>
<tr>
<th>PMVE/MA Concentration</th>
<th>Oscillatory Frequency (Hz)</th>
<th>Mean (± standard deviation) viscoelastic parameters</th>
<th>Unneutralised</th>
<th>Neutralised (pH 7.4) with NaOH</th>
<th>Neutralised (pH 7.4) with Triethylamine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>tan δ</td>
<td>η' (Pa.s)</td>
<td>tan δ</td>
<td>η' (Pa.s)</td>
</tr>
<tr>
<td>5</td>
<td>2.37</td>
<td>NM*</td>
<td>4.19 ± 0.28</td>
<td>0.57 ± 0.02</td>
<td>2.94 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>5.39</td>
<td>NM*</td>
<td>2.82 ± 0.20</td>
<td>0.50 ± 0.02</td>
<td>2.18 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>9.99</td>
<td>NM*</td>
<td>2.01 ± 0.20</td>
<td>0.43 ± 0.01</td>
<td>1.75 ± 0.10</td>
</tr>
<tr>
<td>10</td>
<td>2.37</td>
<td>NM*</td>
<td>2.54 ± 0.07</td>
<td>2.58 ± 0.03</td>
<td>1.68 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>5.39</td>
<td>NM*</td>
<td>1.93 ± 0.03</td>
<td>2.03 ± 0.03</td>
<td>1.37 ± 0.01</td>
</tr>
<tr>
<td></td>
<td>9.99</td>
<td>NM*</td>
<td>1.60 ± 0.04</td>
<td>1.62 ± 0.03</td>
<td>1.18 ± 0.01</td>
</tr>
<tr>
<td>15</td>
<td>2.37</td>
<td>5.60 ± 0.70</td>
<td>0.85 ± 0.02</td>
<td>1.68 ± 0.01</td>
<td>11.77 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>5.39</td>
<td>3.57 ± 0.12</td>
<td>0.74 ± 0.01</td>
<td>1.37 ± 0.01</td>
<td>8.23 ± 0.09</td>
</tr>
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<td></td>
<td>9.99</td>
<td>2.62 ± 0.07</td>
<td>0.67 ± 0.01</td>
<td>1.18 ± 0.01</td>
<td>6.09 ± 0.07</td>
</tr>
<tr>
<td>20</td>
<td>2.37</td>
<td>3.00 ± 0.13</td>
<td>2.90 ± 0.16</td>
<td>1.15 ± 0.04</td>
<td>34.35 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>5.39</td>
<td>2.17 ± 0.08</td>
<td>2.37 ± 0.12</td>
<td>0.98 ± 0.03</td>
<td>21.81 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>9.99</td>
<td>1.73 ± 0.07</td>
<td>1.97 ± 0.09</td>
<td>0.86 ± 0.02</td>
<td>15.02 ± 0.02</td>
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<tr>
<td>25</td>
<td>2.37</td>
<td>1.95 ± 0.01</td>
<td>11.38 ± 0.15</td>
<td>0.92 ± 0.00</td>
<td>83.15 ± 0.68</td>
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<td>5.39</td>
<td>1.57 ± 0.01</td>
<td>8.30 ± 0.11</td>
<td>0.79 ± 0.00</td>
<td>48.53 ± 0.29</td>
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<td>9.99</td>
<td>1.35 ± 0.01</td>
<td>6.35 ± 0.10</td>
<td>0.71 ± 0.00</td>
<td>31.60 ± 0.11</td>
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<tr>
<td>30</td>
<td>2.37</td>
<td>1.51 ± 0.02</td>
<td>25.53 ± 1.12</td>
<td>0.70 ± 0.01</td>
<td>173.90 ± 3.41</td>
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<tr>
<td></td>
<td>5.39</td>
<td>1.25 ± 0.02</td>
<td>17.38 ± 0.74</td>
<td>0.61 ± 0.01</td>
<td>94.17 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>9.99</td>
<td>1.09 ± 0.01</td>
<td>12.66 ± 0.53</td>
<td>0.55 ± 0.01</td>
<td>58.21 ± 0.87</td>
</tr>
</tbody>
</table>

* Not Measureable
Table 3. The effects of polymer concentration, neutralising agent and oscillatory frequency on the loss tangent (\(\tan \delta\)), dynamic viscosity (\(\eta'\)) and mucoadhesion of aqueous poly(methylvinyl ether-co-maleic acid) platforms

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

* Not observed over the frequency range studied