

Formulation and Characterisation of Tetracycline-Containing Bioadhesive Polymer Networks Designed for the Treatment of Periodontal Disease

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Abstract: This study described the drug release, rheological (dynamic and flow) and textural/mechanical properties of a series of formulations composed of 15% w/w polymethylvinylether-co-maleic anhydride (PMVE-MA), 0–9% w/w polyvinylpyrrolidone (PVP) and containing 1–5% w/w tetracycline hydrochloride, designed for the treatment of periodontal disease. All formulations exhibited pseudoplastic flow with minimal thixotropy. Increasing the concentration of PVP sequentially increased the zero-rate viscosity (derived from the Cross model) and the hardness and compressibility of the formulations (derived from texture profile analysis). These effects may be accredited to increased polymer entanglement and, in light of the observed synergy between the two polymers with respect to their textural and rheological properties, interaction between PVP and PMVE-MA. Increasing the concentration of PVP increased the storage and loss moduli yet decreased the loss tangent of all formulations, indicative of increased elastic behaviour. Synergy between the two polymers with respect to their viscoelastic properties was observed. Increased adhesiveness, associated with increased concentrations of PVP was ascribed to the increasing bioadhesion and tack of the formulations. The effect of increasing drug concentration on the rheological and textural properties was dependent on PVP concentration. At lower concentrations (0, 3% w/w) no effect was observed whereas, in the presence of 9% w/w PVP, increasing drug concentration increased formulation elasticity, zero rate viscosity, hardness and compressibility. These observations were ascribed to the greater mass of suspended drug in formulations containing the highest concentration of PVP. Drug release from formulations containing 6 and 9% PVP (and 5% w/w drug) was prolonged and swelling/diffusion controlled. Based on the drug release, rheological and textural properties, it is suggested that the formulation containing 15% w/w PMVE-MA, 6% w/w PVP and tetracycline hydrochloride (5% w/w) may be useful for the treatment of periodontal disease.

INTRODUCTION

Recently, it has been estimated that 36.8% (43 million) of adult Americans have periodontal disease, making it one of the world's most prevalent chronic diseases [1]. Periodontal disease, which encompasses gingivitis and periodontitis, results from plaque-associated bacterial infections, which cause inflammation in the surrounding tissue [2]. In periodontitis, inflammation causes the eventual destruction of the periodontal ligament, creation of a periodontal pocket and loss of the adjacent bone. Bacteria, mainly *Bacteroides* spp., *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, accumulate in the periodontal pocket that develops between the affected teeth and tissue [3]. If the treatment of periodontal disease is not initiated tooth loss will result [4].

Common treatments for periodontal disease aim to cure inflamed tissue, reduce bacteria and eliminate the periodontal pocket. Scaling (removal of calculus and plaque), root planing (removal of necrotic tooth tissue on root surface) and surgery (to remove tissue and reduce pocket depth) have been used in the mechanical treatment of

periodontal diseases; however, these procedures are time consuming and demanding on the patient [5]. Recent therapies for treating periodontitis have incorporated various antibiotic and antimicrobial agents. Due to the inadequacies of both peroral administration of antimicrobial agents and the use of antibacterial mouthwashes [2, 4], recent treatments have focused on the use of controlled release intra-pocket antimicrobial drug delivery systems. Examples of these include films [6-8], gels [1, 9, 10] and semi-solids [11-14]. In the development of implantable drug delivery systems for the treatment of periodontal diseases, several key physicochemical properties may be defined. These include, ease of administration into and prolonged retention within the periodontal pocket, controlled release of antimicrobial agent into the crevicular fluid and biodegradation/bioerosion, the latter property facilitating removal of the delivery system and reattachment of the gingiva [11, 14, 15]. However the design of currently available systems is sub-optimal. In a recent publication, the physicochemical properties of gels composed of a novel polymeric complex between poly(methylvinylether-co-maleic anhydride) and polyvinylpyrrolidone were described [16]. In particular, several of these systems exhibited rheological properties that were deemed suitable as platforms for topical drug delivery systems. Therefore, this study examined the physicochemical properties of gels composed of poly(methylvinylether-co-maleic anhydride) and polyvinylpyrrolidone and containing

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tetracycline, designed for the treatment of periodontal disease. Tetracyclines are commonly used for the treatment of periodontal disease and in this study tetracycline was chosen as a representative member of this class of antimicrobial agent [1, 2]. In particular the textural (mechanical) and flow and oscillatory rheological properties and release of tetracycline from these systems are described, due to the applicability of these properties to the clinical and non-clinical performance of periodontal drug delivery systems [2, 13, 14]. Therefore, it is anticipated that this study will define the suitability of these systems for use in periodontal disease.

MATERIALS AND METHODS

Materials

Poly (vinylmethylether-co-maleic anhydride) (PMVE/MA, Gantrez® S97) and poly (vinyl pyrrolidone) (PVP) K-90F were gifts from ISP (Surrey, England) and BASF (Ludwigshafen, Germany), respectively, whereas, tetracycline hydrochloride was purchased from Sigma Chemical Co (St. Louis, PA, USA).

All other chemicals used in this study were purchased from BDH Chemicals Ltd. (Gillingham, Dorset, U.K.) and were of AnalaR, or equivalent, quality.

Methods

Manufacture of Tetracycline-Containing Polymeric Gel Networks

Polymer gel networks were prepared by initially dissolving poly(vinylpyrrolidone), (PVP 0, 3, 6, 9% w/w) in distilled water containing the required amount of sodium hydroxide using a mechanical stirrer (2000 rev min⁻¹). PMVE/MA, (15% w/w) was then rapidly added to the vortex produced with stirring until fully dispersed. The polymeric gel networks were then placed on an ointment tile and tetracycline (1-5 % w/w) was manually incorporated using a spatula. All formulations were stored at 4°C for at least 24h (and less than 72 h) prior to all analyses.

Texture Profile Analysis of Tetracycline-Containing Polymeric Gel Networks

Texture profile analysis (TPA) of the formulations under investigation was performed using a TA-XT2 Texture Analyser (Stable Micro Systems, Surrey, UK) as previously described [2, 11, 13, 17]. In brief, each formulation (20g) was transferred into McCartney bottles to a fixed height (70 mm) and allowed to equilibrate at 20 ± 1°C in an oven. A cylindrical probe (10 mm diameter) was twice compressed into each sample to a depth of 15 mm at a rate of 10 mm s⁻¹, allowing a delay period of 15 s between the end of the first and the beginning of the second compression. For each formulation at least six replicate analyses were performed using a fresh sample in each case. From the resultant force-distance plot the following parameters were derived [11]:

- Hardness (the force required to attain a given deformation).
- Compressibility (the work required to deform the product during the first compression of the probe).

- Adhesiveness (the work required to overcome the attractive forces between the surface of the sample and the surface of the probe).

Flow Rheometry of Tetracycline-Containing Polymeric Gel Networks

Flow rheometry of each formulation was performed at a defined temperature (20 ± 0.1°C) using a Carri-Med CSL²-100 rheometer (T.A. Instruments, Surrey, UK) in flow mode in conjunction with a parallel plate geometry (1 mm plate gap), as previously described [11, 14]. The stress range and the plate diameter (2, 4 or 6 cm) were selected according to the consistency of the sample (Table 1).

In all cases, six replicate measurements were performed. Samples were applied to the lower stationary plate of the rheometer and allowed to equilibrate for 30 min prior to analysis. Rheograms were produced under controlled stress by gradually increasing the shearing stress from a minimum value to a maximum value in 60s, and then returning from the maximum to minimum stress in another 60s. Mathematical modelling of the flow curves was performed using the Cross model [11, 18], as follows:

$$\frac{\tau}{\dot{\gamma}} = (K \dot{\gamma})^m$$

where:

- = Viscosity
- τ_0 = Zero rate viscosity
- = Infinite shear viscosity
- K = Consistency index
- $\dot{\gamma}$ = Shear rate
- m = Slope of the curve at the inflection point

Evaluation of Drug Release

Tetracycline release from selected formulations was determined using a Caleva 7ST dissolution apparatus in conjunction with paddle stirrers as previously reported [2]. In brief, formulations were packed into plastic moulds and located at the bottom of 1L dissolution vessels. Prewarmed dissolution medium (phosphate buffered saline, pH 7.2 at 37°C) was added to the vessel, which was stirred at a constant rate (30 ± 1 rev min⁻¹) and maintained at 37°C. At pre-determined intervals, samples of dissolution fluid (5 mL) were removed, analysed using ultra-violet spectroscopy at 353 nm and an equal volume of fresh, pre-warmed PBS replaced into the dissolution vessels. The mass of tetracycline released at each sampling period was calculated following reference to a previously constructed calibration curve. At least five replicates of each release experiment were performed.

STATISTICAL ANALYSIS

The effects of increasing PVP and tetracycline concentration on the textural properties (hardness, compressibility, adhesiveness), drug release (times for the release of 30% and 50% of the original mass of drug, $t_{30\%}$,

Table 1. Shearing Stresses and Plate Diameters used in Continuous Shear Analysis of Tetracycline Hydrochloride-containing PVME/MA - PVP Systems

% PVP Incorporated	% Tetracycline hydrochloride Incorporated	Parallel Plate Diameter (cm)	Shearing Stress (Pa)
0	1	6	50 – 200
	3	4	100 – 400
	5	4	100 – 400
3	1	4	200 – 450
	3	4	200 – 450
	5	4	200 – 450
6	1	4	350 – 700
	3	4	350 – 700
	5	4	350 – 700
9	1	2	550 – 950
	3	2	550 – 950
	5	2	650 – 1000

$t_{50\%}$), flow rheological (zero-rate viscosity) and viscoelastic properties (storage modulus G' , loss modulus G'' , loss tangent $\tan \delta$, at four defined frequencies, 0.01, 0.38, 0.69, 1.0 Hz) were statistically compared using a three-way ANOVA (Statview, Abacus Concepts, CA, USA). In all cases, post-hoc comparisons of the means of individual groups were performed using Tukey's Honestly Significant Difference test. A significance level of $P < 0.05$ denoted significance in all cases [19].

RESULTS

The frequency dependency of the storage and loss moduli for formulations containing tetracycline hydrochloride (1, 3, 5% w/w), PVME/MA (15% w/w) and 0% w/w PVP, 3% w/w PVP, 6% w/w PVP and 9% w/w PVP are presented in (Fig. 1a-d), respectively. Table 2 presents the effects of increasing concentrations of PVP and tetracycline hydrochloride on the loss tangent of the PVME/MA – PVP systems at four representative frequencies. All formulations showed an increase in both the storage and loss moduli over the frequency range, whilst the loss tangent decreased with increasing frequency. Increasing PVP concentration increased both dynamic moduli, yet decreased the loss tangent. The effect of increasing the concentration of tetracycline hydrochloride on the viscoelastic properties of the various formulations was dependent on the concentration of the PVP (evident from the statistical interaction between these two factors within the ANOVA). In the absence of PVP or in the presence of 3% w/w of this component, increasing the concentration of tetracycline over the range of 1 – 5% w/w did not significantly alter G' , G'' and $\tan \delta$. Furthermore, over the frequency range examined, the above formulations exhibited $\tan \delta$ values that exceeded 1.0. Conversely in the presence of 6% w/w PVP increasing the concentration of tetracycline from 1-3% w/w significantly

increased G' and G'' , whereas a further increase in the concentration of this agent to 5% w/w decreased these parameters. Finally, increasing the concentration of tetracycline hydrochloride sequentially from 1 to 3 to 5% w/w in formulations containing 9% w/w PVP significantly increased G' and G'' and decreased $\tan \delta$. Therefore, at an oscillatory frequency of 1Hz, the maximum values of G' and G'' and the minimum value of $\tan \delta$ were associated with the formulation composed of 15% w/w PMVE-MA, 9% w/w PVP and 5% w/w tetracycline hydrochloride whereas minimum values of G' and G'' and maximum values of $\tan \delta$ were observed in formulations containing 15% w/w PMVE-MA and 1, 3 or 5% w/w drug. In Table 3 the crossover frequencies of the various formulations, i.e. the oscillatory frequency at which there was equality between G' and G'' are presented. In formulations either devoid of or containing 3% w/w PVP (independent of the concentration of tetracycline hydrochloride) the crossover frequencies exceeded 1 Hz. However, formulations containing 6 and 9% w/w PVP exhibited crossover frequencies that were less than 1Hz and decreased as the concentration of PVP increased.

In flow rheometry all formulations demonstrated pseudoplasticity without thixotropy. To enable comparison of the rheological properties of the formulations under investigation, the flow data was modelled using the Cross model, from which the zero-rate viscosity of the various formulations was determined (Table 4). As may be observed, the zero-rate viscosity significantly increased with increasing PVP concentration however, the effect of increasing concentration of tetracycline hydrochloride was dependent on the concentration of PVP (once more evident from the significant interaction between these two factors in the ANOVA). In the presence of 0, 3 or 6% w/w PVP, increasing the concentration of tetracycline hydrochloride from 1-5% w/w did not affect the zero-rate viscosity. Conversely, in

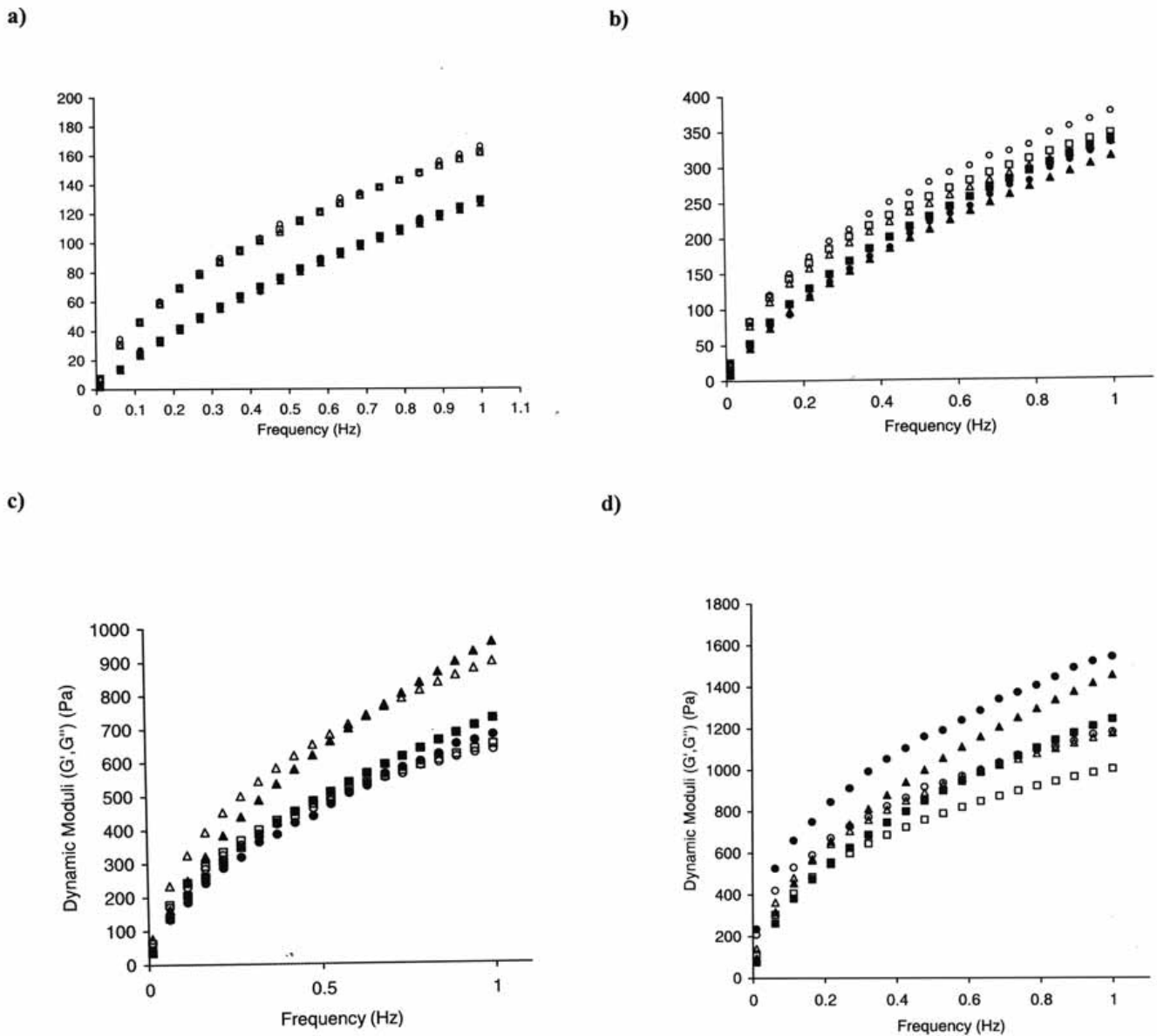


Fig. (1). The effect of oscillatory frequency on the storage (G' , closed symbols) and loss (G'' , open symbols) moduli of gels composed of PMVE-MA (15% w/w), tetracycline hydrochloride and PVP (0% w/w figure **1a**, 3% w/w figure **1b**, 6% w/w figure **1c**, 9% w/w figure **1d**). Key: squares refer to formulations that contained 1% w/w tetracycline hydrochloride; triangles refer to formulations that contained 3% w/w tetracycline hydrochloride; circles refer to formulations that contained 5% w/w tetracycline hydrochloride. On all occasions, six replicate measurements were performed, however, standard deviations have been omitted to retain clarity (coefficient of variation in all cases < 6%).

formulations containing 9% w/w PVP, increasing the concentration of drug from 1 to 3 to 5% w/w sequentially increased the zero-rate viscosity.

Table 5 presents the effects of increasing concentrations of PVP and tetracycline hydrochloride on the textural/mechanical properties of the various formulations. Significant increases in the hardness, compressibility and adhesiveness were observed as the concentration of PVP was sequentially increased from 0 – 9% w/w. As before, the effect of increasing concentration of tetracycline hydrochloride on these properties was dependent on the

concentration of PVP present in the formulation. Accordingly, increasing the concentration of this active agent in formulations containing 0 – 6% w/w PVP did not statistically affect the textural properties whereas in the presence of 9% w/w PVP, increasing the concentration of tetracycline hydrochloride significantly increased the hardness, compressibility and adhesiveness of the formulation.

The *in vitro* release of tetracycline hydrochloride, expressed as the cumulative mass of drug released as a function of time, from two candidate formulations,

Table 2. The Effects of Increasing Concentration of PVP and Tetracycline Hydrochloride (TH) on the Loss Tangent of Formulations Containing PVME/MA (15% w/w at Four Representative Frequencies)

Formulation		Mean (\pm s.d.) $\tan \delta$ at representative frequencies			
Conc of PVP	Conc of TH	0.01 Hz	0.3749 Hz	0.6873 Hz	1.001 Hz
0	0	3.05 \pm 0.27	1.97 \pm 0.05	1.70 \pm 0.01	1.59 \pm 0.01
	1	3.38 \pm 0.28	1.50 \pm 0.04	1.34 \pm 0.02	1.25 \pm 0.02
	3	4.31 \pm 0.15	1.53 \pm 0.04	1.36 \pm 0.02	1.28 \pm 0.02
	5	3.78 \pm 0.14	1.62 \pm 0.03	1.41 \pm 0.03	1.29 \pm 0.03
3	0	2.17 \pm 0.10	1.46 \pm 0.03	1.31 \pm 0.01	1.21 \pm 0.02
	1	2.44 \pm 0.10	1.18 \pm 0.02	1.08 \pm 0.02	1.02 \pm 0.02
	3	2.68 \pm 0.05	1.23 \pm 0.00	1.13 \pm 0.00	1.07 \pm 0.04
	5	3.50 \pm 0.31	1.34 \pm 0.04	1.21 \pm 0.02	1.13 \pm 0.06
6	0	1.68 \pm 0.15	1.18 \pm 0.10	1.09 \pm 0.10	1.03 \pm 0.10
	1	1.77 \pm 0.15	1.02 \pm 0.04	0.94 \pm 0.03	0.89 \pm 0.03
	3	2.08 \pm 0.03	1.09 \pm 0.01	0.99 \pm 0.05	0.94 \pm 0.04
	5	2.08 \pm 0.18	1.09 \pm 0.06	1.00 \pm 0.07	0.93 \pm 0.06
9	0	1.42 \pm 0.07	1.05 \pm 0.02	0.94 \pm 0.03	0.89 \pm 0.02
	1	1.51 \pm 0.05	0.92 \pm 0.03	0.85 \pm 0.01	0.81 \pm 0.01
	3	1.46 \pm 0.03	0.92 \pm 0.02	0.85 \pm 0.01	0.81 \pm 0.01
	5	0.98 \pm 0.04	0.79 \pm 0.07	0.78 \pm 0.06	0.77 \pm 0.04

Table 3. Effect of Increasing Concentrations of PVP and Tetracycline Hydrochloride on the Crossover Frequencies of the Various Formulations

% w/w PVP	% w/w Tetracycline hydrochloride	Cross-over Frequency* (Hz)
0	1	>1
	3	>1
	5	>1
3	1	>1
	3	>1
	5	>1
6	1	0.52 \pm 0.03
	3	0.64 \pm 0.04
	5	0.69 \pm 0.05
9	1	0.16 \pm 0.00
	3	0.15 \pm 0.00
	5	< 0.01

* the frequency at which $G' = G''$

Table 4. Cross Model Zero-Rate Viscosity (mean \pm s.d.) for gels/gel Networks Containing PVME/MA (15% w/w), PVP (0, 3, 6, 9% w/w) and Tetracycline Hydrochloride (1, 3, 5% w/w)

% w/w PVP	% w/w Tetracycline hydrochloride	Zero-rate Viscosity (Pa s)
0	1	96.25 \pm 5.68
	3	96.70 \pm 1.54
	5	97.21 \pm 2.31
3	1	354.68 \pm 21.97
	3	393.37 \pm 38.36
	5	350.23 \pm 30.00
6	1	1216.67 \pm 103.79
	3	1315.33 \pm 100.75
	5	1306.00 \pm 58.51
9	1	1709.17 \pm 63.38
	3	1913.00 \pm 32.70
	5	2212.33 \pm 85.41

Table 5. Textural/Mechanical Properties of Tetracycline Hydrochloride-containing PVME/MA gels

% w/w PVP	% w/w Tetracycline hydrochloride	Hardness (N)	Adhesiveness (N mm)	Compressibility (N mm)
0	1	0.38 \pm 0.02	2.74 \pm 0.16	2.95 \pm 0.15
	3	0.39 \pm 0.02	2.83 \pm 0.19	3.03 \pm 0.29
	5	0.41 \pm 0.02	3.11 \pm 0.16	3.33 \pm 0.27
3	1	1.12 \pm 0.04	8.21 \pm 0.28	8.75 \pm 0.35
	3	1.08 \pm 0.09	8.05 \pm 0.69	8.35 \pm 0.76
	5	1.19 \pm 0.07	9.13 \pm 0.66	10.17 \pm 0.93
6	1	2.05 \pm 0.26	16.76 \pm 2.38	17.18 \pm 1.83
	3	2.27 \pm 0.11	17.59 \pm 1.69	19.02 \pm 1.56
	5	2.67 \pm 0.11	18.83 \pm 1.45	19.13 \pm 1.78
9	1	2.89 \pm 0.51	19.64 \pm 2.16	20.82 \pm 1.87
	3	2.89 \pm 0.27	21.42 \pm 1.92	23.05 \pm 0.91
	5	3.76 \pm 0.18	27.86 \pm 0.28	28.81 \pm 0.94

composed of 15% PMVE-MA, 5% w/w tetracycline hydrochloride and either 6 or 9% w/w PVP, is shown in Fig. 2. As may be observed, increasing the concentration of PVP significantly decreased the mass of tetracycline hydrochloride released ($t_{30\%}$ and $t_{50\%}$). Furthermore, following application of the general release equation described by Peppas [20], the release exponent for each formulation ranged between 0.5 and 1.0.

DISCUSSION

Whilst several formulation strategies have been employed for the treatment of periodontal disease, more recently interest has been shown in the use of gel systems that may be directly administered into the pocket using a syringe [2]. Following implantation the formulations will be exposed to non-deformable stresses. In light of both this and

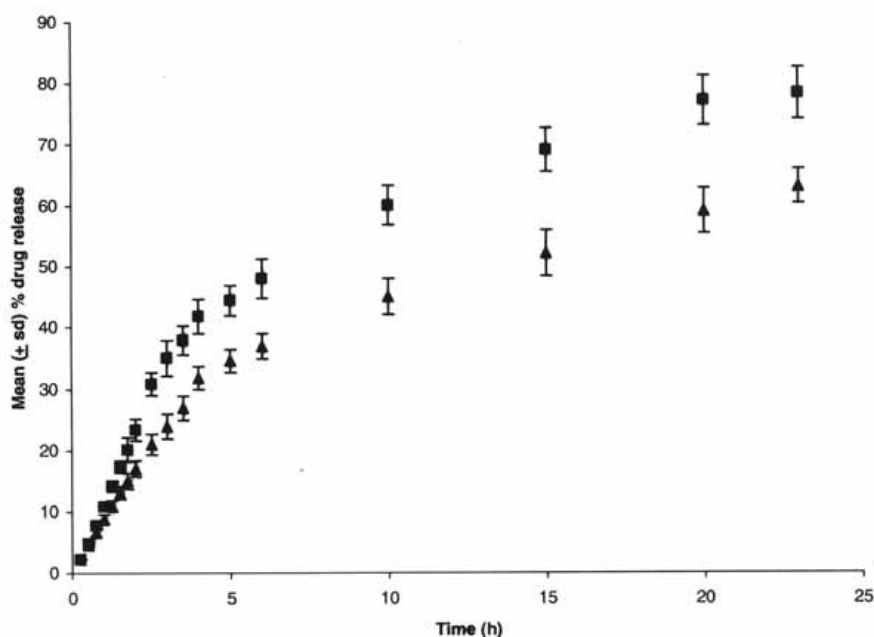


Fig. (2). The effect of PVP (6% w/w squares, 9% w/w triangles) on the release of tetracycline from formulations containing 15% w/w PMVE-MA and 5% w/w tetracycline hydrochloride. Each curve is the mean \pm one standard deviation of at least five analyses.

the chosen method of administration, the rheological properties of candidate formulations are important determinants of their clinical performance. Therefore, in this study a comprehensive examination of the rheological and textural/mechanical properties of candidate formulations was performed. Furthermore, following administration it is preferable that the formulations should be retained within the pocket and exhibit controlled release of antimicrobial agent into the crevicular fluid. The gel platforms that were examined in this study have previously been described as bioadhesive [16], however such systems have received little attention as topical drug delivery systems. Therefore, this study offers a comprehensive examination of the rheological properties of bioactive gel platforms composed of PMVE-MA and PVP, a novel polymeric complex that may be useful for the treatment of periodontal disease.

Two primary rheological analyses were employed in this study, namely oscillatory and flow rheology, as these techniques provide information on structural characteristics and the effects of applied stress on product deformation. Moreover, texture profile analysis characterises the textural/mechanical properties, which determine the ease of removal of a formulation from the container, ease of application and provides preliminary information concerning the possible retention at the site of application [11]. The viscoelastic properties (G' , G'' and $\tan \delta$) of the tetracycline-containing PVME/MA – PVP systems were dependent on the oscillatory frequency and were characteristic of physically cross-linked systems. In all cases, G' increased with increasing oscillatory frequency, indicating greater elastic behaviour. While G'' , a measure of viscous behaviour, also increased with increasing frequency, overall the loss tangent (ratio of G'' to G') decreased. This phenomenon is consistent with the Maxwellian model of viscoelastic

behaviour [14, 21], in which at higher frequencies there is sufficient time available for the Hookean spring to elongate and return to its original position; however, there is not enough time for the extended dashpots to contract. At lower frequencies, there is sufficient time available for both the spring and dashpot to deform and return to their respective original positions. Increasing PVP concentration increased G' and G'' , yet decreased $\tan \delta$, and may be accredited to the increased polymer entanglement. The decrease in the loss tangent further indicated that the elastic resistance was greater than the viscous resistance with increasing PVP concentration. At the concentrations employed in this study, the rheological properties of PVP have been reported to be minimal [16]. Accordingly, rheological synergy was observed that may be accredited to hydrogen bonding between PVME/MA and PVP. With respect to the clinical application of these gel platforms, it is preferable that the elastic properties predominate as these directly influence other primary determinants of product performance. For example, the relationship between retention at the site of application and product elasticity has been reported [2]. Within the periodontal pocket the drug delivery system will be exposed to non-destructive forces due to the flow of crevicular fluid and therefore, the elastic nature of formulations will resist deformation and product removal. In addition, it has been shown that the rate and mechanism of drug release are directly affected by the viscoelastic properties of topical formulations [2]. Therefore, formulations offering greater elastic properties would be more appropriate for use as drug delivery systems designed for the treatment of periodontal disease.

One of the favoured methods of administration of drug delivery systems into the periodontal pocket involves the use of a syringe and, as a result, knowledge of the flow

properties of candidate formulations is required to ensure optimal product administration. In this study two individual methods were employed to characterise the flow properties of the drug-containing gel platforms, namely flow rheometry and texture profile analysis. Typically flow rheometry may be employed to determine fundamental flow properties following application of a torsional stress, including flow phenotype, the presence/absence of thixotropy and viscosity (following the application of the appropriate rheological model) [11]. Conversely, texture profile analysis may be used to characterise product resistance to compressional shear stresses, which are more relevant to the proposed method of administration of the candidate formulations into the periodontal pocket. Using flow rheometry it was shown that the gels under investigation exhibited a pseudoplastic flow phenotype with minimal thixotropy. These properties are advantageous for the proposed application, as the viscosities of the candidate formulations will decrease as the shearing (application) stress is increased. This will therefore, facilitate administration of the gels into the periodontal pocket. The lack of thixotropy of the formulations is a further attribute as, following application into the pocket structural recovery will rapidly occur and, as a result, this will reduce the propensity for product removal from the site of application. Increasing the PVP concentration significantly increased the zero-rate viscosity which may be accredited to increased polymer entanglement. As before, synergy between the effects of PVP and PMVE-MA on the zero-rate viscosity was observed, indicative of complex formation between the two component polymers. In a similar fashion the hardness and compressibility of the formulations increased as the concentration of PVP increased, further illustrating polymer entanglement.

In textural analysis, adhesiveness is defined as the work required to remove the probe from the formulation following compression, a process which involves breakage of the bonds between the probe and the formulation, and under certain circumstances, cohesive forces within the polymeric gel [2, 11, 13]. Interestingly a relationship has been described between adhesiveness and bioadhesion for bioadhesive formulations [2, 16, 17]. Therefore, the increased adhesiveness that was observed as a function of increasing PVP concentration would suggest that the tetracycline-containing formulations would exhibit bioadhesive properties. This observation is in accordance with a previous report that described the mucoadhesive properties of these systems [16].

The incorporation of tetracycline hydrochloride significantly affected the elastic, flow and textural properties of the PVME/MA - PVP gel networks; however, these effects were dependent on the concentration of PVP. At lower concentrations of PVP (0 and 3% w/w), increasing drug concentration did not affect the rheological properties of the polymeric gel. As the concentration of PVP increased, the solubility of tetracycline hydrochloride within the gel systems decreased. Therefore, whilst the mass of insoluble drug will increase as the drug loading is increased, these differences did not affect the viscoelastic, flow and textural properties of these gels. Conversely, in the presence of 9% w/w PVP, the solubility of tetracycline hydrochloride will be decreased (in comparison to systems containing 0, 3 and 6%

w/w PVP) and therefore, as the drug loading is increased there will be a substantially greater mass of insoluble suspended drug within the various gels. The effect of increasing drug concentration on the rheological/textural properties of the gels may therefore, be accredited to the mass of insoluble suspended drug in this formulation. These observations are in accordance with previous reports that described increased viscoelastic, textural and flow properties in formulations in which the mass of suspended particles increased [11, 12, 14].

With respect to the proposed use of these formulations, full consideration of the rheological properties is required to ensure the selection of the optimal formulation for clinical assessment. The flow and textural properties of formulations containing 9% w/w PVP may be inappropriate for administration into the periodontal pocket using a periodontal syringe due to the high resistance to deformation. Conversely, formulations containing the highest concentration of PVP exhibited optimal viscoelastic properties (high G' and low $\tan \delta$) as these properties will ensure prolonged drug release and resistance to physiological stresses within the periodontal pocket. Therefore, based on the above the release of tetracycline from two candidate formulations containing 5% w/w tetracycline hydrochloride, 15% w/w PMVE-MA and either 6 or 9% w/w PVP was examined. As the concentration of PVP was increased the mass of tetracycline released decreased. The observed difference in release profiles may be accredited to several factors, principally, the greater mass of dissolved tetracycline hydrochloride in and the lower (visco)elastic properties of the gel containing 6% w/w PVP. Typically the time required for 50% release of the original mass of tetracycline was greater for the formulation containing the higher concentration of PVP. Interestingly, whilst the $t_{50\%}$ values for the two formulations were less than 24 h *in vitro*, it would be expected that the comparator values *in vivo* would be dramatically greater [22]. The limited flow of crevicular fluid in the pocket inevitably leads to a saturated solution of drug and therefore non-sink conditions predominate with the resultant prolonged release of drug. Furthermore, there is the potential for therapeutic levels of drug to be present even after dissolution/breakdown of the delivery vehicle. Therefore, the formulations investigated would be expected to have the potential to provide long-lasting controlled release facilitating effective therapeutic management of periodontal diseases.

In conclusion, using a selection of analytical methods the rheological properties of a series of bioadhesive formulations composed of 15% w/w PMVE-MA, 0 – 9% w/w PVP and containing tetracycline hydrochloride (1 – 5% w/w), designed for topical administration into the periodontal pocket have been characterised. By increasing the concentration of PVP, gels were produced that exhibited a wide range of rheological properties, due to interaction between the two polymeric components. Prolonged *in vitro* release of tetracycline hydrochloride (5% w/w loading) from two candidate formulations containing 6 and 9% w/w PVP was observed. Based on the drug release and rheological (dynamic and flow) and textural/mechanical properties the formulation containing 6% w/w PVP would seem to be particularly appropriate for the proposed application.

REFERENCES

- [1] Southard, G. L.; Godowski, K. C. *Int. J. Antimicrob. Agents*, **1998**, *9*, 239-253.
- [2] Jones, D.S.; Irwin, C.R.; Brown, A.F.; Woolfson, A.D.; Coulter, W.A.; McClelland, C. *J. Cont. Rel.*, **2000**, *67* (2-3), 357-368.
- [3] Slots, J.; Ting, M. *Periodontol.*, **1999**, *20*, 82-121.
- [4] Medlicott, N. J.; Rathbone, M. J.; Tucker, I. G.; Holborow, D. W. *Adv. Drug Deliv. Rev.*, **1994**, *13*, 181-203.
- [5] Philstrom, B. L.; Ammons, W. F. *J. Periontol.*, **1997**, *68*, 1246-1253.
- [6] Minabe, M.; Takeuchi, K.; Tamura, T.; Hori, T.; Umemoto, T. *J. Periodontol.*, **1989**, *60*, 552-556.
- [7] Medlicott, N.J.; Tucker, I.G.; Rathbone, M.J.; Holborow, D.W.; Jones, D.S. *Int. J. Pharm.*, **1996**, *143* (1), 25 - 35.
- [8] Agarwal, R. K.; Robinson, D. H.; Maze, G. I.; Reinhardt, R. A. *J. Controlled Release*, **1993**, *23*, 137-146.
- [9] Cho, C. S.; Ha, J. H.; Kim, S. H.; Han, S. Y.; Kwon, J. K. *J. Appl. Poly. Sci.*, **1996**, *60*, 161-167.
- [10] Radvar, M.; Pourtaghi, N.; Kinane, D. F. *J. Periodontol.*, **1996**, *67*, 860-865.
- [11] Jones, D.S.; Woolfson, A.D.; Brown, A.F. *Pharm. Res.*, **1997**, *14* (4), 450-457.
- [12] Jones, D. S.; Woolfson, A. D.; Brown, A. F. *Pharm. Res.*, **1998**, *15*, 1131-1136.
- [13] Jones, D.S.; Irwin, C.R.; Woolfson, A.D.; Djokic, J.; Adams, V. *J. Pharm. Sci.*, **1999**, *88* (6), 592 -598.
- [14] Jones, D.S.; Brown, A.F.; Woolfson, A.D. *J. Pharm. Sci.*, **2001**, *90*(12), 1978 - 1990.
- [15] Van der Ouderaa, F. J. G.; Cummins, D. *J. Dent. Res.*, **1989**, *68*, 1617-1624.
- [16] Jones, D.S.; Lawlor, M.S.; Woolfson, A.D. *J. Pharm. Sci.*, **2003**, *92* (5), 995 - 1007.
- [17] Jones, D.S.; Woolfson, A.D.; Brown, A.F. *Int. J. Pharm.*, **1997**, *151*, 223-233.
- [18] Cross, M. M. *J. Colloid Sci.*, **1965**, *20*, 417-437
- [19] Jones, D.S. *Pharmaceutical Statistics*, The Pharmaceutical Press, London, **2002**.
- [20] Peppas, N.A. *Pharmaceutica Acta Helv.*, **1985**, *60*, 110-111.
- [21] Ferry, J. D. *Viscoelastic Properties of Polymers* (3rd Edition) John Wiley & Sons, New York, **1980**.
- [22] Esposito, E.; Carotta, V.; Scabbia, A.; Trombelli, L.; D'Antona, P.; Menegatti, E.; Nastruzzi, C. *Int. J. Pharm.*, **1996**, *142*, 9-23.