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Research Paper

Formulation of Hydrophilic Non-Aqueous Gel: Drug Stability in Different Solvents and Rheological Behavior of Gel Matrices

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Purpose. This study was aimed at formulating a hydrophilic non-aqueous gel for topical delivery of the model moisture-sensitive drug, minocycline hydrochloride (MH).
Methods. Stability study of MH dissolved in water and various hydrophilic non-aqueous solvents was performed over a period of four months in order to select a suitable non-aqueous solvent for MH gel. To improve MH stability, the effect of different cation additives on MH stability in the selected solvent was investigated. Non-aqueous gel matrices were prepared from three different types of hydrophilic polymers in glycerin-propylene glycol mixture with Mg^{2+} cation additive. Oscillatory shear rheometry was performed on the gel matrices using a cone-and-plate rheometer.
Results. MH stability was affected by the type of solvent employed and the duration of storage. Different cation additives affected the extent of MH stabilization through MH-cation complex formation. Rheological properties of the non-aqueous gel matrices were significantly affected by the type and concentration of polymer, and the vehicle ratios in the formulations.
Conclusions. MH stabilization could be achieved using the selected glycerin-propylene glycol mixture containing $MgCl_2$. Gel matrix formulated using this solvent system and 3%w/w N-vinylacetamide/sodium acrylate copolymer had demonstrated the most favorable rheological properties as a gel for topical application.

KEY WORDS: minocycline hydrochloride; non-aqueous gel; rheology; stability; viscoelastic.

INTRODUCTION

The polymer-based semisolid gel has been one of the most popular means of topical drug delivery. The plethora of reported studies on aqueous gels pertaining to their use in topical applications could be attributed to their wide general usage and acceptability. Besides, water or any water-containing solvent system often exhibit good compatibility with hydrophilic gelling agents, thus facilitating gel formation. Despite its wide applications, aqueous gel may not be a vehicle of choice when formulating moisture-sensitive drugs as the presence of water in the delivery system often affects the chemical stability of such drugs (1,2). Thus, moisture-sensitive drugs are best formulated in non-aqueous gel vehicles to ensure drug stability.

Minocycline hydrochloride (MH) was selected as the model moisture-sensitive drug in this study (Fig. 1). MH, a member of the tetracycline antibiotics, is useful in the treatment of a host of topical bacterial infections. Oral administration of MH causes a number of systemic side-effects (3) that can be minimized by the use of topical preparations. The tetracyclines are known to be unstable under conditions of low or high pH, heat and high humidity (4,5). The most commonly reported transformation reactions are epimerization, a steric rearrangement in the configuration of the dimethylamino function at C_4 to form epi-tetracycline, and dehydration at $C_{5a,6}$ to form anhydrotetracycline (6). The activity of the epimer was found to be less than 5% of the normal analogue while the anhydro product has the potential to cause kidney damage (7). However, dehydration reaction does not occur in MH due to the lack of $-OH$ group at C_6 (6). Thus, a non-aqueous gel matrix was proposed to be a more appropriate gel vehicle for MH because the absence of moisture in the gel matrix would prevent MH degradation.

MH is ionic and freely water-soluble (8). The insolubility of MH in hydrophobic solvents required the use of non-aqueous but hydrophilic solvents to facilitate complete MH dissolution. A drug solution of reasonably high concentration in the gel matrix was preferable to provide a better drug release profile and bioavailability. Apart from the well-documented influences of pH, heat and humidity on the stability of tetracycline antibiotics, it was proposed that the nature of the formulation solvent played an important role in ensuring the stability of MH. The polymeric gelling agents employed in pharmaceutical gel preparations often possess relatively low chemical reactivity with drug compounds as compared to small molecule components such as solvents and salts. Thus, the stability of MH incorporated in the non-aqueous gel matrix was entirely influenced by the solvent.

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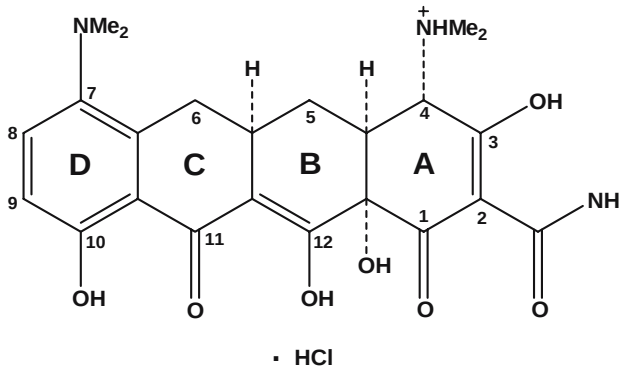

Fig. 1. Molecular structure of minocycline hydrochloride.

Preliminary studies carried out to identify physically compatible polymers that could dissolve in the non-aqueous solvents employed in this study led to the selection of three types of polymers, that is, PNVA, Gantrez S-97 and Plasdone S-630. PNVA is a copolymer of N-vinylacetamide and sodium acrylate. Gantrez S-97 is a synthetic copolymer of methylvinyl ether and maleic anhydride. It had been used as a drug delivery system for bromhexidine hydrochloride in the form of a polymeric film (9) and an ocular insert (10). Composite films containing diclofenac sodium had been formed using a Gantrez polymer and polyvinylpyrrolidone and evaluated in terms bioadhesion and drug release (11). Plasdone S-630 is a copolymer of N-vinyl-2-pyrrolidone and vinyl acetate with potential uses as a tablet binder, sustained release coating, film coating and film forming agent in topical sprays (Plasdone S-630 Technical Profile). Plasdone S-630 has recently been used as a novel film modifier for ethylcellulose films in coating application (12). The potential application of PNVA, Gantrez S-97 and Plasdone S-630 polymers as gelling agents has yet to be explored.

One of the fundamental properties for any gel system is rheological characteristics. Gel rheological property is a useful indicator of the type of gel present and provides structural information about the gel system. Apart from stability of the active ingredient, optimal rheological property has been well accepted as a desirable attribute required for topical formulations (13). These attributes are often associated with drug release kinetics, bioadhesion and the ability of the dosage form to withstand mechanical stresses due to body movements (14). Rheological properties of non-aqueous gel matrices are much less reported as compared to aqueous gels (14,15). Only few studies were found to report on the rheology of non-aqueous gels, on the lipophilic ethylcellulose gels (16,17) and a carbopol gel system (18). The latter is unusual as carbopol gel systems are mostly aqueous based.

This paper reports the study carried out to formulate a hydrophilic non-aqueous gel intended for topical delivery of the model moisture-sensitive drug, MH. Investigations carried out included (a) stability study of MH in water and hydrophilic non-aqueous solvents for the selection of a suitable vehicle for formulation of a gel matrix system for MH, (b) study of the effect of different cations on the stability of MH, with a view to improve drug stability in the vehicle, and (c) rheological characterization of the formulated gel matrices in order to obtain information about the gel physical properties. The stability studies were deemed to be a feasible approach to aid in the selection of a suitable solvent system for use in the formulation of the non-aqueous gel.

MATERIALS AND METHODS

Materials

Minocycline hydrochloride (MH) and propylene glycol (PG) were obtained from Sigma (St. Louis, MO, USA); glycerin from BDH (Poole, Dorset, England); and N-methylpyrrolidone (NMP) from ISP Technologies (Wayne, NJ, USA). Magnesium chloride hexahydrate, zinc chloride dihydrate, calcium chloride dihydrate, aluminium chloride hexahydrate, methanol and ethanol were obtained from Merck (Darmstadt, Hessen, Germany). These materials were used for stability study.

Methyl vinyl ether/maleic acid copolymer (Gantrez S-97) and vinyl pyrrolidone/vinyl acetate copolymer (Plasdone S-630) were obtained from ISP Technologies (Wayne, NJ, USA), and poly N-vinylacetamide/sodium acrylate copolymer (PNVA) from Showa Denko (Tokyo, Honshu, Japan). These polymers were used for formulation of hydrophilic non-aqueous gel matrices.

Stability Studies

A series of hydrophilic non-aqueous solvents suitable for pharmaceutical applications and could dissolve MH were identified from a preliminary screening study. A solution of 1%w/w MH was prepared in water and in the respective non-aqueous solvents such as ethanol, methanol, PG, glycerin and NMP. Samples were stored in sealed ampoules at room temperature (22±1°C) and ambient relative humidity of about 60%. In view of the light sensitivity of MH, care was taken to shield samples from light and experiments were carried out under subdued light. HPLC assay for MH remaining was performed at predetermined time intervals over approximately 4 months.

The influence of different cations on the stability of MH was studied using 1%w/w MH in a 50:50 mixture of PG and glycerin with an equivalent of 2 mol of cations for every mole of MH. The amount of cations employed (0.82%w/w) was completely soluble in the non-aqueous solvent. Control samples without any cation were also prepared for the purpose of comparison. Chloride salts of divalent cations, magnesium, calcium and zinc, and a trivalent cation, aluminium, were used. An accelerated stability study was carried out with samples in sealed ampoules, stored at 40°C. MH concentrations in the samples were assayed at predetermined time intervals for up to at least 4 weeks.

HPLC Analysis

HPLC assays of MH were carried out using the Hewlett-Packard LC 1100 Series (Agilent Technologies, Foster City, CA, USA) at 25°C using a 150×4.6 mm, 5 μm particle size reversed phase C_{18} column, Hypersil BDS (Thermo, Bellefonte, PA, USA) with a 20×4 mm, 5 μm particle size C_{18} guard column (Hypersil BDS) to remove impurities. The mobile phase employed was phosphate buffer (pH 3, 25 mM)-methanol-acetonitrile at a volume ratio of 85:10:5 at a flow rate of 1.5 ml/min. A 0.1 g sample was dissolved in

the HPLC mobile phase in a 10 ml volumetric flask and filtered through a 0.45 μm regenerated cellulose membrane filter. An injection volume of 40 μl and a detection wavelength of 255 nm were used. Calibration samples were freshly prepared each day for HPLC analysis. Linear calibration curves ($r^2 \geq 0.999$) were obtained over the drug concentration range of 5 to 100 μg/ml.

Gel Preparation

Three different types of polymers, PNVA, Gantrez S-97 and Plasdone S-630 were selected on the basis of their ability to dissolve in the chosen hydrophilic non-aqueous vehicles. Preliminary studies were carried out to establish the range of concentrations for each polymer to form a gel of reasonable consistency. The gels were then formulated using two levels of polymer concentration (low and high). The vehicle consisted of a binary mixture of PG and glycerin in three different ratios, namely low (10%w/w), medium (25%w/w) and high (50%w/w) levels with respect to PG. The compositions of the formulations studied are shown in Table I. Magnesium chloride was dissolved in glycerin with the aid of heat. PG was added upon cooling of the magnesium chloride-glycerin mixture to room temperature. Polymer was slowly added in small aliquots into the vehicle mixture with stirring to aid dissolution and gelation. The resultant mixture was kept for 24 h at 25±2°C to ensure complete swelling prior to any testing.

Oscillatory Rheometry

Oscillation measurements of gels were performed using a cone-and-plate rheometer, Haake RheoStress 1 (Thermo Electron, Baden-Württemberg, Karlsruhe, Germany). The experiments were performed using a cone angle of 1° and a cone diameter of 35 mm at a controlled temperature of 25±0.5°C (Haake Circulator DC30). Oscillatory stress sweeps were carried out at a constant angular frequency of 3 Hz (19 rad s^{-1}) in a stress range of 0.5 Pa to 1000 Pa. Oscillatory frequency sweeps were performed over a frequency range of 1 to 15 Hz (6 to 100 rad s^{-1}) at a constant stress amplitude of 10 Pa. The equilibration time before each run was 2 min. Viscoelastic parameters which included shear modulus (storage modulus, G', loss modulus, G'' and complex shear modulus, $G^*=[G'^2+G''^2]^{1/2}$), loss tangent (tan δ) and complex dynamic viscosity (η*) were obtained. The mean values of the various parameters of at least three samples were reported.

Table I. Compositions of Gel Formulations Investigated

Polymer Name	Formulation Code	Polymer (%w/w)	Propylene Glycol (%w/w)	Glycerin (%w/w)
PNVA	P1	3	10	q.s.
	P2	3	25	q.s.
	P3	3	50	q.s.
	P4	1	10	q.s.
	P5	1	25	q.s.
	P6	1	50	q.s.
Gantrez S-97	G1	10	10	q.s.
	G2	10	25	q.s.
	G3	10	50	q.s.
	G4	5	10	q.s.
	G5	5	25	q.s.
	G6	5	50	q.s.
Plasdone S-630	S1	20	10	q.s.
	S2	20	25	q.s.
	S3	20	50	q.s.
	S4	10	10	q.s.
	S5	10	25	q.s.
	S6	10	50	q.s.

Each formulation consisted of 0.82%w/w of magnesium chloride equivalent to 2 mol of Mg^{2+} cation for every mol of MH

Statistical Analysis

All results were evaluated statistically using one-way ANOVA. Post-hoc statistical analyses of the means of individual groups were performed using Tukey's test. For all analyses, $p<0.05$ denoted significance.

RESULTS AND DISCUSSION

Stability of MH in Pure Solvents

The % MH remaining in the pure hydrophilic non-aqueous solvents decreased with time and gradually leveled off indicating that the reaction was approaching an equilibrium state. On the contrary, there was a progressive decline in MH level in water with time without attaining any equilibrium state throughout the 4-month duration of the study. Water was included in the study to compare with the other non-aqueous solvents. Kinetics modeling was carried out using the equations describing the first-order kinetics, Eq. 1 (19) and the first-order reversible kinetics, Eq. 2 (7).

$$\ln(A/A_0) = -k_1 t \quad (1)$$

$$\ln[(A_0 - A_e)/(A - A_e)] = (k_1 + k_{-1})t \quad (2)$$

where A_0=% MH at time, t=0; A=% MH at time, t; A_e=% MH at steady state; k_1 and k_{-1}=forward and backward rate constant, respectively. The rate constants, k_1 and k_{-1} were determined using the following relationship (20):

$$(100 - A_e)/A_e = k_1/k_{-1} \quad (3)$$

The plot of ln $(A-A_e)$ *versus* *t* (Fig. 2) followed a straight line (correlation coefficient, r=0.980 to 0.999) with a slope of $-(k_1+k_{-1})$. Thus, the loss of MH at the initial phase before plateau in hydrophilic non-aqueous solvents was best described by first-order reversible reaction kinetics (pseudo first-order degradation), a reaction commonly observed for tetracycline (4,7). For MH in water, the rate of MH loss followed the first-order reaction kinetics as the plot of ln A *versus* t exhibited a straight line (r=0.997) with a slope of $-k_1$ (Fig. 2). A higher k_1 indicated a higher rate of MH transformation and faster attainment of equilibrium MH level over time. From the k_1 values of MH in various solvent systems (Table II), MH underwent the highest rate of transformation in NMP, followed by glycerin, PG, methanol, ethanol and water. Water demonstrated the lowest k_1 as this

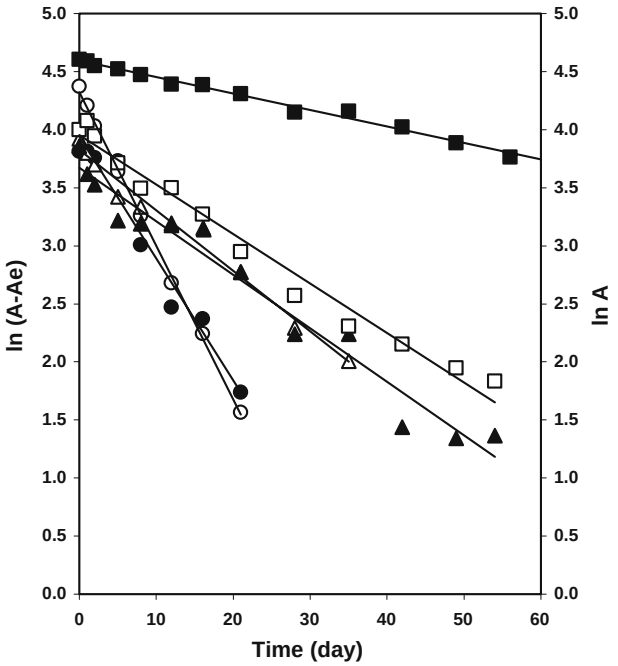

Fig. 2. First-order reversible kinetics and first-order kinetics for MH tranformation in non-aqueous hydrophilic solvents and water, respectively. NMP (*open circles*, $y=-0.132x+4.323$, $r=0.9993$), glycerin (*filled circles*, $y=-0.105x+3.938$, $r=0.9801$), propylene glycol (*open triangles*, $y=-0.052x+3.825$, $r=0.9905$), ethanol (*filled triangles*, $y=-0.046x+3.671$, $r=0.9819$), methanol (*open squares*, $y=-0.043x+3.952$, $r=0.9895$) and water (*filled squares*, $y=-0.013x+4.575$, $r=0.9974$).

value was derived from the first-order kinetic model where reaction end point was complete MH exhaustion instead of reaching an equilibrium level.

MH stability in the solvents studied could be interpreted in terms of extent and rate of MH transformation. Percent MH remaining in different solvents was compared at six different time points: day 2, 8, 28, 49, 70 and 105, selected to represent the entire study period (Table III). Percent MH remaining in NMP was significantly lower than the rest of the solvents throughout the study. Although water showed the lowest k_1 value, the first-order reaction kinetics eventually resulted in a significantly lower MH level than the rest of the solvents from day 49 onwards, with the exception of NMP. The steady state levels of MH obtained by day 70 and day 105 decreased in the order of glycerin>ethanol>PG>methanol> NMP (Table III). This reflected the extent of MH transformation in the non-aqueous solvents. However, the trend for extent of MH transformation did not correlate directly to its rate (k_1), which increased in the order of ethanol<methanol< PG<glycerin<NMP (Table II). Although equilibrium MH level was attained faster in glycerin, this solvent was able to maintain a higher amount of MH through a lower extent of MH loss over time.

Table II. Rate Constants for MH Transformation in Various Solvents

	k_1 (day^{-1})	k_{-1} (day^{-1})	k_1+k_{-1} (day^{-1})
NMP	0.106±0.002	0.028±0.001	0.133±0.003
Glycerin	0.047±0.001	0.057±0.002	0.105±0.004
Propylene glycol	0.026±0.001	0.026±0.000	0.052±0.001
Ethanol	0.021±0.000	0.024±0.000	0.045±0.001
Methanol	0.023±0.000	0.019±0.000	0.042±0.001
Water	0.013±0.000	N.A.	N.A.

The most likely mechanism to account for the decline of MH level in the non-aqueous solvents was C_4 epimerization, a first-order reversible reaction that had been associated with tetracyclines (6,7). In the HPLC run, collection and re-injection of the fraction corresponding to the epiminocycline (epiMH) peak gave rise to two peaks which corresponded to MH and epiMH. Thus, the epimer was able to revert back to its original form, further supporting the proposed mechanism of MH transformation by a reversible reaction. Such "back epimerization" reaction had been reported for MH (21) and tetracycline (4). For glycerin and PG, epimerization was the predominant mechanism responsible for the decline in MH levels as evident by the chromatograms showing only presence of MH and epiMH peaks (Fig. 3). The sum of the % MH and epiMH peaks was always approaching 100%, indicating the sole presence of these two compounds in these solvents. In contrast, for NMP or water, a progressive decline in total % MH and epiMH was evident over time (Table III). The epimer levels in NMP and water also started to decline after 35 and 56 days, respectively. Early extraneous chromatographic peaks which became more prominent with time indicated irreversible degradation of MH and epiMH into various low molecular weight degradants in water and NMP (Fig. 3). A number of compounds that eluted later than MH and epiMH were evident in NMP chromatograms indicating possible high molecular weight compounds formation through complexation. An insoluble dark colored precipitate was also formed over time as a result of MH oxidation, leading to MH polymerization through a free radical intermediate (22). These irreversible degradative processes accounted for the marked decline of MH concentration on prolonged storage in NMP or water. Although an equilibrium level was achieved for MH in NMP, on close examination of the stability data, the MH level was found to continue to decline very slowly. Thus, the rate constants of MH disappearance in NMP served only as an estimate for the rate of initial MH epimerization.

In summary, the stability of MH was affected by the type of solvent employed and the duration of storage. The results from the stability study were consistent with literature reports on the instability of tetracyclines in aqueous systems (6). The declining levels of MH in the non-aqueous solvents such as glycerin, PG, ethanol and methanol were primarily attributed to reversible C_4 epimerization. On prolonged storage in a solvent, equilibrium existed between MH and epiMH. NMP produced markedly lower levels of MH due to high rate of epimerization and irreversible degradation. Hence, the declining level of MH in a solvent was not directly related to the rate constant as these were essentially the rates of epimerization. Both the rate and extent of MH transformation must be taken into consideration when selecting a solvent system.

From the findings of the stability study, PG and glycerin were selected as the two exploratory solvents for the development of a gel formulation containing moisture-sensitive drugs such as MH. Although ethanol could offer advantages in terms of relatively good MH stability, it was

Table III. Percentage MH Remaining and epiMH Formed in Non-Aqueous Hydrophilic Solvents and Water Over Time

Day	2			8		
	MH	EpiMH	Total	MH	EpiMH	Total
NMP	77.1±0.6	15.4±0.1	92.5	46.7±0.6	37.2±0.3	83.9
Glycerin	97.4±4.3	13.6±1.1	111.1	74.9±0.5	30.7±1.7	105.6
Propylene glycol	88.9±0.2	10.5±0.2	99.4	77.6±0.7	24.2±2.0	101.8
Ethanol	85.6±0.4	7.0±0.4	92.6	77.1±0.4	14.5±0.2	91.5
Methanol	95.6±0.2	9.1±0.3	104.7	78.4±0.3	20.4±0.1	98.8
Water	94.7±0.4	6.5±0.1	101.2	87.6±1.2	12.9±0.2	100.5
	28			49		
NMP	20.8±0.1	50.7±0.4	71.5	15.6±0.2	46.4±3.6	62.0
Glycerin	55.3±0.1	49.7±0.4	105.0	51.5±0.1	49.4±0.1	100.9
Propylene glycol	59.4±0.2	42.7±0.2	102.1	50.9±0.1	54.7±0.4	105.7
Ethanol	62.1±0.4	28.5±0.1	90.6	56.6±0.3	38.5±0.3	95.0
Methanol	58.5±0.1	34.7±0.1	93.2	52.5±0.4	46.7±0.4	99.1
Water	63.4±0.2	21.6±0.2	85.0	48.7±0.1	24.3±0.4	73.0
	70			105		
NMP	13.0±0.8	38.9±5.5	51.9	11.0±0.2	26.5±0.0	37.5
Glycerin	54.7±0.5	51.9±0.7	106.6	54.0±0.1	47.0±0.1	101.0
Propylene glycol	44.2±0.1	55.3±0.3	99.5	48.4±0.8	56.2±0.6	104.6
Ethanol	48.7±0.4	41.3±0.1	89.9	51.9±0.1	47.9±0.2	99.8
Methanol	40.6±0.1	48.0±0.2	88.6	43.3±0.1	52.4±0.2	95.7
Water	37.2±1.1	23.1±1.4	60.3	25.3±0.1	15.8±0.1	41.1

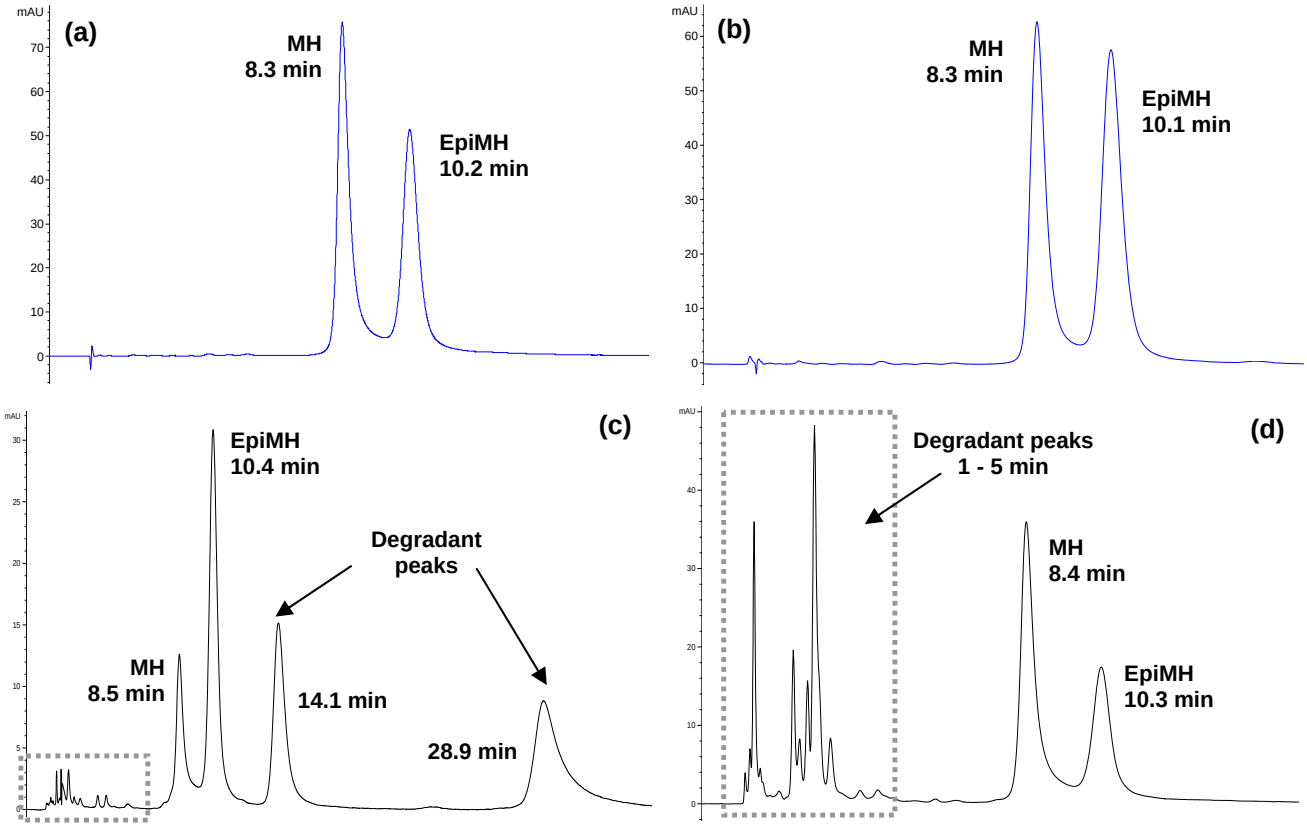

Fig. 3. Chromatograms of minocycline hydrochloride (MH) in glycerin (**a**), propylene glycol (**b**), N-methylpyrrolidone (**c**), and water (**d**) after 105 days of storage. The concentration of MH shown in the chromatogram is 49.2, 45.1, 10.6 and 25.3 μg/ml, respectively. The total analysis time of MH in N-methylpyrrolidone was increased to 35 min for all degradant peaks to elute. The total analysis times with other solvents were maintained at 12 to 17 min.

not selected as its high volatility poses potential problems, especially in maintaining a stable composition of the topical formulation. PG was selected for its relatively low rate of MH epimerization as shown by the rate constants. Besides, a study had demonstrated significant follicular deposition of pyridostigmine bromide formulated with PG due to the miscibility of PG with sebum (23). Glycerin was also chosen because it was able to maintain a relatively high level of MH at equilibrium. Thus, the combination of PG and glycerin would offer an advantage of low rate and extent of MH epimerization. As PG employed as a topical vehicle may potentially cause skin irritation (24–26), the use of glycerin as the major component in the vehicle could help to reduce such tendency, thus improving the acceptability of the gel formulation. Glycerin was shown to provide good skin tolerability (27,28) probably due to its good humectant property which absorbs moisture from the air, thus preventing skin dehydration (29). Its effectiveness in reducing skin dryness (27) implied the dominance of moisturizing property over its purported propensity of drawing moisture from the skin. Adverse skin reaction to glycerin has not been reported despite its wide application (27).

Effect of Different Cations on MH Stability in Hydrophilic Non-Aqueous Solvents

Further attempts were made to improve stability of MH in PG and glycerin by minimizing the epimerization reaction, the main mechanism responsible for MH decline in these solvents. Complexation reactions between tetracyclines and metal ions had been widely studied. Divalent cations such as Mg^{2+}, Ca^{2+} and Zn^{2+} and trivalent cation such as Al^{3+} were reported to form reversible complexes with the tetracycline antibiotics (30–32) and anhydrotetracycline (33). Successful stabilization of MH in polyhydric alcohols by Mg^{2+} had been reported (34). Therefore, the effects of different divalent and trivalent cations on the epimerization of MH in the selected solvent system were investigated. In view of the long equilibrium time needed for the transformation of MH in the pure solvents at room temperature, even longer times were expected with the introduction of complexing ions due to their stabilizing effect on the systems (34). Thus, an accelerated stability study at 40°C was carried out. Complexation reactions of MH with Mg^{2+} or Ca^{2+} were reported for cation-MH ratios of 1:1 comprising 1 mol of cation to 1 mol of MH or 2:1 comprising 2 mol of cation to 1 mol of MH (30,35). Complexes of 2:1 ratio were formed with increased cation concentrations. Thus, a cation-MH ratio of 2:1 was employed in this study. A combination of glycerin and PG in the weight ratio of 1:1 was employed as the solvent system.

From the plots of % MH remaining *versus* time (Fig. 4), the rates and extents of MH decline were different in the presence of different cations. The rate constants were not determined in this part of the study because the loss of MH in these systems did not follow any normally applied reaction kinetics. Hence, the % MH remaining in different samples were compared at four different time points over the duration of the study, namely 1, 7, 14 and 28 days. Over the four time points, samples added with either $MgCl_2$ or $ZnCl_2$ showed significantly higher % MH values than the samples

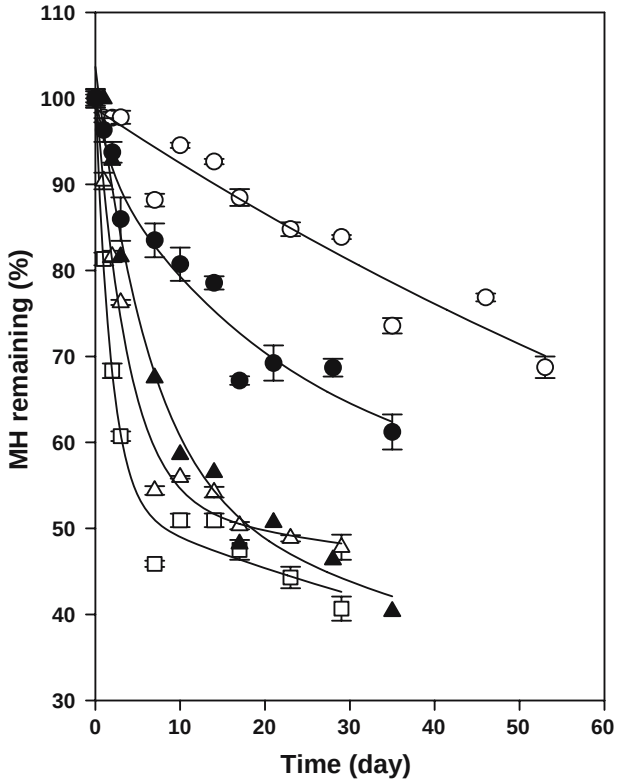

Fig. 4. Effect of various cations (2 mol) on stability of MH (1 mol) in propylene glycol–glycerin mixture of 1:1 ratio at 40°C. $MgCl_2$ (*open circles*), $ZnCl_2$ (*filled circles*), $CaCl_2$ (*open triangles*), $AlCl_3$ (*filled triangles*) and control (*open squares*). S.D. for $AlCl_3$ ranged from 3.7 to 26.8 (error bars were excluded for clarity).

containing either $CaCl_2$ or $AlCl_3$. For the first 7 days, the samples with $CaCl_2$, $AlCl_3$ and the control produced % MH remaining in the following order: $AlCl_3$ > $CaCl_2$ > control. After 14 days, % MH remaining in the samples containing $AlCl_3$ and $CaCl_2$ were not significantly different from the control. From day 14 onwards, the sample containing $MgCl_2$ had significantly higher % MH remaining than the rest of the samples. The extent of MH epimerization was significantly reduced in the presence of divalent and trivalent cations in the first 2 weeks of storage, with $MgCl_2$ being the most effective throughout the duration of the study.

The rate of MH epimerization observed in this study was influenced by the type of cation present. Differences in size (ionic radius) and ionic strength of the cations had caused differences in binding affinity between cations to MH molecule (34). The ionic radii followed the order of Ca^{2+} (0.99 Å)>Zn^{2+}(0.74 Å)>Mg^{2+} (0.72 Å)>Al^{3+} (0.535 Å; 36, 37). Calorimetric studies showed a stronger binding between tetracycline and Mg^{2+} than Ca^{2+} due to the higher charge density of Mg^{2+} (38), as evident from its smaller ionic radius. Thus, stronger Mg^{2+} binding with MH formed more stable complexes and this was contributory to the lower rate and extent of MH epimerization. However, ionic radius was not the sole factor responsible for MH stabilization through complexation. This was apparent by comparing the ionic radii with the cations' effectiveness in stabilizing MH which followed the order of Mg^{2+}>Zn^{2+}>Ca^{2+}≈Al^{3+}. This order was indicated by the % MH remaining towards the end of the

study. Ca^{2+} and Al^{3+} possessed the highest and lowest ionic radii, respectively, but both of these cations were essentially ineffective at stabilizing MH beyond 14 days. Thus, apart from forming stable complexes, another important factor, the binding action of the cation on MH molecule imparted stability to the configuration of MH at C_4 against epimerization. Depending on the pH and nature of the solvent as well as ionic strength and concentration, tetracyclines can adopt different modes of complexation with metal ions (30,39). Under different conditions, tetracyclines exist in different tautomeric forms which have binding sites of varying capabilities for complexation (39). While the lower effectiveness of Ca^{2+} for stabilizing MH could be attributed to the formation of a less stable complex due to its low charge density, the relative ineffectiveness of Al^{3+} and Zn^{2+} could likely be attributed to a less optimal binding site on MH in that the complexes formed was less able to resist epimerization as compared to MH-Mg^{2+} complexes.

From this study, Mg^{2+} was found to be the most effective cation in reducing the rate and extent of MH epimerization by forming more stable cation-MH complexes. On this basis, $MgCl_2$ was employed in the formulation of the non-aqueous gel matrix to minimize epimerization of MH in the eventual gel formulation.

Rheological Characterization

Preparation of Hydrophilic Non-Aqueous Gel Matrices

PNVA, Gantrez S-97 and Plasdone S-630 were able to form clear gels in the non-aqueous vehicles selected. Table I shows the amount of polymer employed to form a gel with suitable consistency based on visual observation. The minimum amount of PNVA needed for gel formation was the lowest followed by Gantrez S-97 and Plasdone S-630. Thus, PNVA demonstrated the best gel-forming property in the non-aqueous vehicles whereas Plasdone S-630 was the poorest.

Oscillatory Rheometry

Rheology is one of the most crucial physical characterizations for pharmaceutical gel systems as such characterization offers relevant information on the gel structure and gel behavior under different shear and stress conditions. Viscoelastic gel behavior had been reported to indicate the ability to withstand mechanical strain, influence on drug release, bioadhesion, gel spreading and retention on its substrate (17, 40–45).

The oscillatory stress sweep showed relatively constant values of the shear modulus and δ over 0.5 Pa to 100 Pa for most of the gel systems tested. Consequently, stress amplitude of 10 Pa was selected for the oscillatory frequency sweep experiments since this stress amplitude laid within the linear viscoelastic region for all the tested systems. The consistency of formulation S6 was very low and the shear modulus promptly decreased with the application of shear stress. Stable shear modulus was not attainable in the stress sweep. Thus, subsequent evaluation was not carried out for formulation S6.

The effects of different polymer types, polymer concentration and vehicle ratios on the viscoelastic properties of the gels were statistically evaluated at two representative frequencies, that is, 3 Hz and 10 Hz to represent the low and high frequencies, respectively (Table IV). The viscoelastic properties of the gels prepared from the three different polymers at different concentrations are illustrated in the oscillatory frequency sweep profiles (Figs. 5, 6 and 7). In general, there was an increase of G' and G'' as a function of increasing oscillatory frequency for all the formulations studied. A decrease in η* was observed for Gantrez S-97 and PNVA gels whereas η* remained relatively constant for Plasdone S-630 gels over the frequency range examined (Fig. 6).

The plots of oscillatory frequency profile for 3%w/w PNVA system at all the three PG levels tested demonstrated $G'>G''$ and tan $\delta<1$ for a frequency sweep of up to 10 Hz, indicating a predominant elastic behavior (Figs. 5 and 7). In oscillatory frequency sweep, $G'>G''$ and tan $\delta<1$ is typical of a structured, three-dimensional physical gel network (46). However, when the polymer concentration was reduced to 1%w/w, $G''>G'$ for all the different vehicle ratios used indicating a transition from structured gel behavior to that of a concentrated polymer solution. Low polymer concentration did not allow extensive entanglements of polymer molecules that would give the system a rigid three-dimensional gel structure. In the presence of higher polymer concentration, η* was significantly higher as higher extent of polymeric chain entanglements resulted in a higher resistance to flow (Fig. 6). For formulations with high polymer concentration (3%w/w), G' was significantly higher for gel systems with a low level of PG (10%w/w) than systems with a high level of PG (50%w/w). In systems with low polymer concentration (1%w/w), the level of PG did not significantly influence the values of G'. The same observation applied for G'', G^* and η*. PG level appeared to have a significant role in governing the viscoelastic characteristic of PNVA systems with a high polymer concentration.

For Gantrez S-97 formulations, $G''>G'$ (Fig. 5), indicating a more pronounced viscous behavior at all the polymer concentrations tested. They behave like concentrated polymer solutions, with both G' and G'' dependent upon frequency. The values of the viscoelastic parameters of Gantrez S-97 system were significantly different at various PG levels and polymer concentrations used. A higher polymer concentration increased solution viscosity and produced a more elastic character (higher G') to the gel system. Gantrez S-97 system was more sensitive to the level of PG in the formulations, in that, the viscoelastic parameters changed significantly with the change of PG level (Figs. 5, 6 and 7).

Plasdone S-630 formulations demonstrated strong dependency of G' and G'' on frequency with a much higher G'' compared to G', ranging from approximately 8 to 180 fold. It was known that for a strong, irreversible covalently cross-linked gel, G' and G'' are relatively insensitive to frequency. However, for a network of entangled polymer chains (pseudogel) or a polymer solution, $G''>G'$ where $G' \propto \omega^2$ and $G'' \propto \omega^1$ at low frequency (17). The slopes for G' and G'' in the double logarithmic plots of G', G'' *versus* frequency for formulations S1 and S2 were approaching 2 and 1,

Table IV. Rheological Parameters[b] of PNVA, Gantrez S-97 and Plasdone S-630 Gel Systems at Oscillatory Frequencies of 3 and 10 Hz

Formulation Code [a]	Frequency, 3 Hz				Frequency, 10 Hz			
	G' (Pa)	G'' (Pa)	tan δ	η* (mPa·s)	G' (Pa)	G'' (Pa)	tan δ	η* (mPa·s)
P1	155.2±13.0	102.7±11.6	0.660±0.023	9992±919	229.8±20.8	197.9±24.9	0.860±0.033	4827±508
P2	147.6±2.7	91.5±1.0	0.620±0.011	9328±136	215.2±2.3	173.5±1.7	0.807±0.012	4400±32
P3	102.7±3.1	64.2±1.0	0.624±0.011	6508±163	156.3±7.3	118.7±1.4	0.790±0.011	3049±55
P4	24.7±0.6	34.2±0.7	1.385±0.007	2257±50	44.6±0.9	77.3±1.6	1.735±0.006	1420±29
P5	17.6±0.3	24.6±0.3	1.398±0.003	1616±21	31.9±0.3	54.9±0.5	1.722±0.003	1011±9
P6	15.7±0.1	19.3±0.1	1.230±0.004	1332±7	28.2±0.2	40.3±0.3	1.427±0.007	783±5
G1	543.3±13.3	725.1±13.2	1.337±0.009	48453±970	1130.7±42.8	1356.7±50.3	1.200±0.001	28107±1053
G2	305.4±6.7	468.1±6.3	1.537±0.013	29860±474	679.4±15.7	902.1±19.6	1.328±0.006	17973±395
G3	154.2±2.9	282.9±2.8	1.841±0.017	17190±210	385.2±2.9	568.5±1.2	1.476±0.010	10930±40
G4	122.2±2.4	242.3±2.9	1.989±0.015	14473±194	303.2±3.1	514.6±2.3	1.697±0.011	9506±55
G5	86.2±2.2	190.0±3.3	2.212±0.019	11123±206	230.6±2.1	415.2±2.2	1.801±0.008	7559±46
G6	30.9±0.5	91.5±0.8	2.974±0.028	5140±46	99.1±1.0	216.0±0.8	2.179±0.018	3781±18
S1	25.7±1.9	433.2±12.1	17.126±0.823	23037±649	170.5±8.8	1359±36.4	7.984±0.377	21803±586
S2	5.2±0.3	200.5±2.4	39.029±1.907	10640±125	42.3±2.0	651.5±5.0	62.524±0.822	10390±76
S3	0.5±0.0	77.7±0.6	148.542±9.189	4121±34	4.1±0.3	258.6±1.0	62.524±4.958	4116±16
S4	1.3±0.1	91.7±2.9	73.027±4.744	4863±155	89.0±0.9	303.8±8.7	34.428±2.635	4837±139
S5	0.3±0.0	55.9±2.0	181.604±13.827	2967±107	1.6±0.5	186.3±5.5	120.791±29.922	2966±88

[a] The gel composition of each formulation is given in Table I.
[b] Coefficients of variation (%) ranged from 1.0–12.6 for P1–P3, 0.2–2.4 for P4–P6, 0.2–3.8 for G1–G3, 0.4–2.6 for G4–G6, 0.0–7.9 for S1–S3, and 0.0–31.3 for S4–S5.

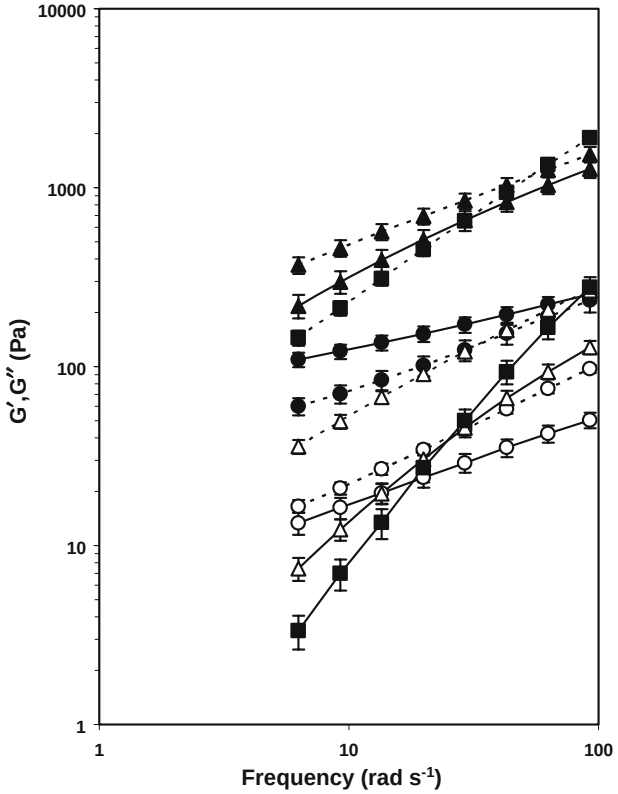

Fig. 5. Storage modulus, G' (*solid lines*) and loss modulus, G'' (*broken lines*) of PNVA (P1, *filled circles*; P4, *open circles*), Gantrez S-97 (G1, *filled triangles*; G6, *open triangles*) and Plasdone S-630 (S1, *filled squares*) gels as a function of radial frequency in the oscillatory frequency sweep. The gel composition of each formulation is given in Table I.

respectively (Fig. 5), indicating a typical rheological behavior of a polymer solution. The G'/G'' slope ratio of 1.64 for formulation S3 deviated slightly from 2, the theoretical slope ratio for a polymer solution, due to the presence of high level of PG, hence imparting dilution effect on the polymer solution. Formulations with 10%w/w polymer concentration showed extremely low G' (below 10 Pa) at all PG levels. The generally low G' values, large δ values approaching 90° and constant η* with respect to frequency (Fig. 6) for Plasdone S-630 systems suggested a purely viscous polymer solution behavior with insignificant elastic contribution to the system. There was a significant increase in η* with increased polymer concentration and/or decreased PG concentration. It was suggested that even if there were very little molecular entanglements as in a polymer solution, elasticity would still be demonstrated (47). Distortions of the coiled molecules by the shear regions gave rise to re-alignment of some of the molecular segments, resulting in flow. Upon cessation of flow, the deformed molecule will relax back to their normal coiled state. This phenomenon could justify the existence of G' for Plasdone S-630 systems even though they were considered as purely viscous solutions from their rheological profiles. Hence, a highly concentrated, viscous polymer solution could be obtained using high polymer concentration of Plasdone S-630 but gelation would not occur in the system regardless of polymer concentration.

In view of the different concentrations employed among the three different types of polymer, direct comparisons of the absolute values of G' and G'' were difficult. However, the different types of polymer resulted in different rheological profiles that allowed broad classification of the gels into different types in rheological terms. Gantrez S-97 systems lacked a physically crosslinked gel structure as indicated by $G''>G'$. However, the absence of characteristic slope values for G' and G'' curves, equivalent to 2 and 1 respectively, suggested that the state of true polymer solution was not

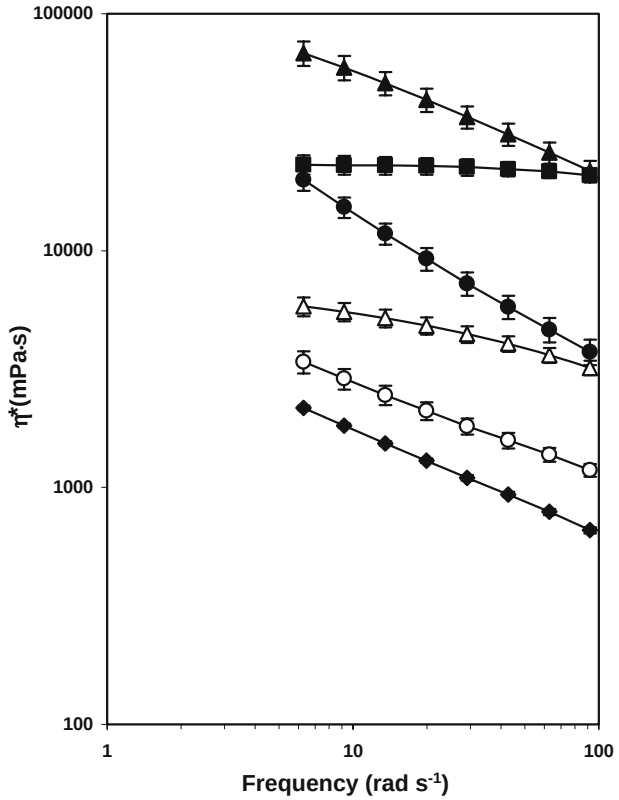

Fig. 6. Complex dynamic viscosity, **η*** of PNVA (P1, *filled circles*; P4, *open circles*; P6, *filled diamonds*), Gantrez S-97 (G1, *filled triangles*; G6, *open triangles*) and Plasdone S-630 (S1, *filled squares*) gels as a function of radial frequency in the oscillatory frequency sweep. The gel composition of each formulation is given in Table I.

attained. Thus, Gantrez S-97 systems exhibited physical characteristics in between the structured gel PNVA and the polymer solution Plasdone S-630. PNVA demonstrated the lowest frequency dependence with regard to G' and tan δ. Hence, PNVA systems were relatively more stable to oscillatory shear stresses as compared to Gantrez S-97 and Plasdone S-630 systems. The ability to withstand changes due to shear was a desirable property of the PNVA systems for the formulation of a topical gel. PNVA gels exhibited greater overall elastic contribution than Gantrez S-97. This was evident from the lower tan δ values of PNVA gels at the representative frequency range (3 to 10 Hz) which ranged from 0.8 to 1.7 as compared to that of Gantrez S-97 systems which ranged from 1.2 to 2.2.

The differences in rheological properties among the different types of gel systems could be attributed to differences in molecular structures of the polymers, resulting in different nature and strength of polymer interactions. The greater stability of PNVA gel systems under oscillatory shear stresses suggested the presence of stronger interactions between polymer molecules either by the formation of more contact points or stronger intermolecular attractive forces. PNVA is a neutralized system with the acidic hydrogen of the carboxyl group replaced by a sodium ion. The charge imparted to the polymer molecules by this neutralization process allowed the formation of stronger ionic bonds between the polymer molecules. Gantrez S-97 and Plasdone S-630 are unneutralized systems, hence intermolecular forces are composed mainly of hydrogen bonds that are more easily disrupted under shear stress (48). This observation is consistent with that of Barry and Meyer (18), and Tamburic and Craig (46) who found that unneutralized samples of the carbopol gels were significantly weaker than their neutralized counterparts. Jones *et al.* (14) investigated the rheological properties of the non-ionic hydroxyethylcellulose and the ionic sodium carboxymethylcellulose gels. Greater stability was observed in the ionic gel system.

The different rheological profiles exhibited by the three types of non-aqueous systems supported the notion that choice of polymer was the most important factor in determining the rheological properties of gel systems (46). Polymer concentration significantly affected the viscoelastic properties of PNVA gel such that typical three-dimensional gel structure was only formed at polymer concentration of 3%w/w. The polymer concentration and vehicle ratio exerted significant effect on the viscoelastic properties. Thus, a different PG-glycerin ratio and/or polymer concentration can be employed to change the rheological properties of PNVA and Gantrez S-97 gel formulations. Plasdone S-630 formed a purely viscous polymer solution and its viscosity was dependent mainly on the polymer concentration and PG-glycerin ratio. This property rendered pure Plasdone S-630 not ideal for formulation into a topical dosage form because oscillatory shear stresses encountered at the site of application will initiate flow, thus preventing product retention on the skin as an adhesive gel for drug delivery.

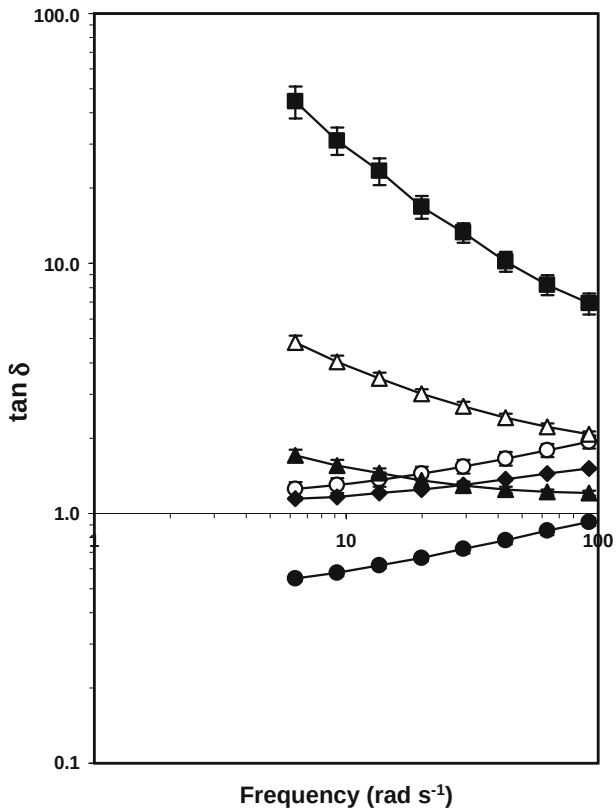

Fig. 7. Loss tangent, tan **δ** of PNVA (P1, *filled circles*; P4, *open circles*; P6, *filled diamonds*), Gantrez S-97 (G1, *filled triangles*; G6, *open triangles*) and Plasdone S-630 (S1, *filled squares*) gels as a function of radial frequency in the oscillatory frequency sweep. The gel composition of each formulation is given in Table I.

The reproducibility of the viscoelastic parameters (Table IV) as indicated by the coefficients of variation (CV) generally decreased as PNVA concentration increased. High PNVA concentration increased the tendency of cavitation or bubble retention within the gel matrices and complete bubble removal was always difficult. This could have created higher variability in measurement albeit still generally small. In contrast, the reproducibility of the viscoelastic parameters of Plasdone S-630 decreased as polymer concentration decreased due to lower measurement sensitivity of the cone-and-plate geometry when the sample became very diluted. The Gantrez S-97 systems did not exhibit any consistent trend of reproducibility with polymer concentration and its CV was generally low (≤3.8%).

CONCLUSIONS

This study has demonstrated that the stability of MH was affected by the types of non-aqueous solvents used. MH transformation (epimerization) in non-aqueous solvent was found to follow the first-order reversible kinetics. In the selection of a suitable non-aqueous solvent system for MH, both the rate and extent of MH transformation in these solvents, as given by the k_1 constant and steady-state MH level, respectively, need to be considered. Different types of divalent cations present in the non-aqueous solvent had imparted different extent of stabilization of MH through the formation of MH-cation complexes. The stability study of MH has led to the selection of PG and glycerin as the vehicle and magnesium chloride as the stabilizing agent to be employed in the gel formulations.

The three different types of polymers, PNVA, Gantrez S-97 and Plasdone S-630 had resulted in non-aqueous gel systems with different rheological properties reflected by the viscoelastic parameters. This was attributed to the different nature and strength of polymer interactions. Rheological properties were significantly influenced by the type and concentration of polymer as well as the vehicle ratios in the formulations. The extent of the vehicle ratio effect was dependent on the polymer used. PNVA was able to form a structured, three-dimensional gel network at high polymer concentration. Gantrez S-97 gel systems exhibited both the properties of a concentrated polymer solution and a viscoelastic gel. Plasdone S-630 only formed a purely viscous polymer solution. This polymer per se would not form a gel regardless of the polymer concentration used. It was found from the oscillatory rheological characterization that 3%w/w PNVA gels possessed the most favorable rheological properties as a gel for topical application.

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