Characterization of the Rheological, Mucoadhesive, and Drug Release Properties of Highly Structured Gel Platforms for Intravaginal Drug Delivery

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This investigation describes the formulation and characterization of rheologically structured vehicles (RSVs) designed for improved drug delivery to the vagina. Interactive, multicomponent, polymeric platforms were manufactured containing hydroxyethylcellulose (HEC, 5% w/w) polyvinylpyrrolidone (PVP, 4% w/w), Pluronic (PL, 0 or 10% w/w), and either polycarbophil (PC, 3% w/w) or poly(methylvinylether-*co*-maleic anhydride) (Gantrez S97, 3% w/w) as a mucoadhesive agent. The rheological (torsional and dynamic), mechanical (compressional), and mucoadhesive properties were characterized and shown to be dependent upon the mucoadhesive agent used and the inclusion/exclusion of PL. The dynamic rheological properties of the gel platforms were also assessed following dilution with simulated vaginal fluid (to mimic in vivo dilution). RSVs containing PC were more rheologically structured than comparator formulations containing GAN. This trend was also reflected in formulation hardness, compressibility, consistency, and syringeability. Moreover, formulations containing PL (10% w/w) were more rheologically structured than formulations devoid of PL. Dilution with simulated vaginal fluids significantly decreased rheological structure, although RSVs still retained a highly elastic structure ($G' > G''$ and $\tan \delta < 1$). Furthermore, RSVs exhibited sustained drug release properties that were shown to be dependent upon their rheological structure. It is considered that these semisolid drug delivery systems may be useful as site-retentive platforms for the sustained delivery of therapeutic agents to the vagina.

Introduction

The majority of new human immunodeficiency virus (HIV) infections occur via mucosal transmission during heterosexual intercourse.[1] Vaginally administered HIV microbicide and mucosal vaccine strategies are being investigated as a promising means of preventing HIV infection. One of the most logical and, hence, widely reported approaches to vaginal delivery of active agents is the use of topically applied bioactive semisolids that have been shown to be highly effective in treating/preventing local diseases.[2−4] Although conventional semisolids have found extensive use as vaginal drug delivery platforms due to their low cost and ease of use, they also suffer from relatively low patient acceptability and poor vaginal retention,[5,6] such that optimal delivery of therapeutic agents to the vagina remains a significant challenge. For example, it is understood that development of practical semisolid drug delivery platforms for vaginal application requires a fundamental understanding of their rheological, textural, and mucoadhesive properties within the vaginal vault.[7]

Of significant interest are the structural rheological properties of vaginal semisolids at equilibrium (low stresses/strains), particularly given the effect these may have on drug release properties and passive seepage between epithelial surfaces. Moreover, the flow rheological properties are of primary interest because they have been shown to largely govern the ease of application and dispersion of semisolids. Moreover, prolonged retention at the site of application leading to increased therapeutic efficacy may be achieved through knowledge of the mucoadhesive properties of the semisolid.[2] Mucoadhesion is particularly relevant because the self-cleansing mechanisms within the vagina, in addition to normal physiological functions, may limit the extent of contact between the vaginal mucosa and the applied semisolid. In an attempt to overcome the problems associated with poor retention and vaginal leakage, mucoadhesive polymers that offer the possibility of enhanced product retention and, thus, sustained delivery have received considerable attention.[8−10]

While simple, monopolymeric component systems may provide useful "first generation" platforms, enhanced functionality may be attained through the use of more complex multicomponent polymeric semisolids that offer high degrees of mucoadhesion and rheological structure to provide optimized retention under in vivo conditions. Ideally, a vaginal semisolid should possess suitable properties to facilitate application yet offer sufficient elastic structure in vivo to provide sustained drug delivery and enhanced retention.[11] Moreover, these drug delivery platforms should maintain rheological structure in vivo during usage (ambulation, seepage between epithelial surfaces, and during sexual intercourse) and following dilution with vaginal fluid.

Consequently, a logical yet previously unreported approach that may significantly improve the in vivo performance of vaginal semisolids would be to formulate rheologically structured platforms using multiple polymers so as to provide both an enhanced rheological structure and mucoadhesive character. This study investigates the use of rheologically structured vehicles (RSVs) in which structural synergy may be achieved due to polymer−polymer interactions.[12] Moreover, through judicious choice of the polymeric components, RSVs may be

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designed to control swelling of the mucoadhesive polymer, allowing interpenetration of the fluidized polymer chains with mucus and, hence, ensuring maximum physical and chemical adhesion.[13]

Given the complexity of RSV formulations and the range of shearing stresses experienced by these semisolids in vivo, characterization should involve a comprehensive rheological evaluation. The selection of semisolids intended for this purpose should be performed in the context of the rheological demands of these systems such that the presence of diluting vaginal fluids and the inherent effects on the rheological properties should be assessed. This would thereby provide an indication of the possible persistence of the rheological structure in vivo. The aim of this study is to characterize the rheological, mucoadhesive and compressional flow (textural) properties of RSV formulations to facilitate the development of novel vaginal semisolid delivery platforms. In so doing, we aim to achieve semisolids that enhance retention within the vagina to provide a platform suitable for the controlled delivery of therapeutic agents.

Materials and Methods

Materials. Poly(methylvinylether-*co*-maleic anhydride) (Gantrez S97) and polyvinylpyrollidone (Plasdone K-90, M_v 1.3 M) were kindly donated by International Specialty Products (Ohio, U.S.A.). Hydroxyethylcellulose (Natrosol 250-HHX-Pharm, M_v 1.3 M, DS 2.0) and polycarbophil (Noveon AA1, a divinyl glycol cross-linked poly(acrylic acid)) were also kindly donated by Aqualon (Warrington, U.K.) and Noveon Pharma GmbH & Co KG (Raubling, Germany), respectively. Pluronic (Lutrol F127, a copolymer of polyoxyethylene and polyoxypropylene, M_v 12600) was purchased from BTC Specialty Chemical Distribution Limited (Cheshire, U.K.). Erioglaucine disodium salt (792.85 MW) was purchased from Sigma (Poole, Dorset, U.K.). Replens was supplied by AAH Hospital Service (Belfast, U.K.). Replens is a commercially available vaginal moisturizer containing carbomer 934P, glycerin, hydrogenated palm oil glyceride, mineral oil, polycarbophil, purified water, and sorbic acid. All other chemicals were purchased from Sigma (Poole, Dorset, U.K.) and were of AnalaR grade or equivalent quality.

Preparation of Rheologically Structured Vehicles (RSV). Sorbic acid (0.1% w/w) and the required amount of sodium hydroxide to achieve a final pH = 6 were added to water in a HiVac mixing bowl (Summit Medical, Gloucestershire, U.K.). The mucoadhesive component, polycarbophil or poly(methylvinylether-*co*-maleic anhydride) (PC or Gantrez; 3% w/w), was subsequently introduced and mixed under vacuum. Following complete dissolution of the mucoadhesive component, Pluronic F127 (PL; 0 or 10% w/w), hydroxyethylcellulose (HEC; 5% w/w), and polyvinylpyrrolidone (PVP; 4% w/w) were added in a stepwise fashion and mixed under vacuum. To remove all entrapped air, formulations were transferred to McCartney bottles, gently centrifuged, and stored at 4 °C for 48 h prior to testing. Formulations containing erioglaucine (100 μg per 3 g dose) were prepared using a similar method with the exception that erioglaucine was added to the aqueous phase (1.36 mL of 11.18 mg/mL) prior to addition of F127.

Preparation of Simulated Vaginal Fluid. For the purposes of gel dilution studies, simulated vaginal fluid (SVF) was prepared as described by Owen and Katz.[14] NaCl (3.51 g), KOH (1.40 g), $Ca(OH)_2$ (0.222 g), bovine serum albumin (0.018 g), lactic acid (2 g), acetic acid (1 g), glycerol (0.16 g), urea (0.4 g), and glucose (5 g) were dissolved in 1 L of deionized water, followed by adjustment to pH 4.2 with HCl.

In Vitro Evaluation of Mucoadhesion. The mucoadhesive properties of RSVs were determined using a TA-XT2 Texture Analyzer (Stable Microsystems, Surrey, England) and a previously described mucin disk test.[15] In summary, porcine mucin discs (250 mg, 13 mm diameter), prepared by direct compression (10 tonne, 30s), were horizontally attached onto the lower face of an inert horizontal polycarbonate probe and immersed in a mucin solution (5% w/w) for 30s. The mucin disk was brought into contact with the formulation under examination and a force of 1 N was then applied for 30 s to ensure intimate contact between the disk and formulation. The probe was then elevated at a speed of 1.0 mm/s, and the mucoadhesive strength was determined from the force of detachment.

Measurement of Work of Syringeability. The work done to expel formulations from a model vaginal applicator was determined using a TA-XT2 Texture Analyzer in compression mode. RSVs (3 g) were packed into a model applicator and an inert polycarbonate probe was used to expel the syringe contents at a rate of 2.0 mm/s through a distance of 30 mm. The work done to expel the syringe contents was calculated from the area under the resultant force-time plot. In addition to RSVs, a commercially available formulation, Replens was also studied for comparison purposes.

Texture Profile Analysis (TPA). The compressional flow (hardness and compressibility) properties of RSV formulations were determined using a TA-XT2 Texture Analyzer in compression mode, as described previously.[15] In brief, samples (16 g) were packed into identical McCartney bottles and centrifuged to remove entrapped air. An analytical probe (10 mm diameter) was compressed twice into each formulation at a defined rate (2 mm/s) to a defined depth (15 mm), allowing a 15 s delay period between the end of the first and beginning of the second compression. From the resultant force-time plot the mechanical parameters including hardness (the force required to attain a given deformation) and compressibility (the work required to deform the formulation during the first compression of the probe) were derived.

Rheological (Dynamic) Analysis. Oscillatory (dynamic) rheometry was conducted using an AR2000 rheometer (T.A. Instruments, Surrey, England) at 25 ± 0.1 °C using a 2 cm diameter parallel plate geometry and a gap of 1000 μm, as previously reported.[16] Samples were carefully applied to the lower stationary plate and the upper plate was adjusted to the predefined gap size. Formulations were then retained for an equilibrium period to facilitate relaxation of internal stresses introduced during sample loading. During testing, samples were subjected to a predetermined oscillatory stress value (selected from within the linear viscoelastic region) over a frequency range from 0.1 to 10 Hz. Calculation of the storage modulus (G'), loss modulus (G''), loss tangent (tan δ), and dynamic viscosity (n') were performed using proprietary software (TA Instruments, Leatherhead, England).

In Vitro Gel Dilution. Given that vaginal semisolids will experience dilution in vivo it is important to characterize this effect by dilution of the prepared RSVs with simulated vaginal fluid (SVF). In summary, a defined mass (3 g) of RSV was thoroughly mixed in a HiVac mixing bowl (Summit Medical, Gloucestershire, U.K.) with 0.1, 0.3, 0.5, 0.7, and 0.9 mL of SVF. Gels were transferred to McCartney bottles, gently centrifuged to remove entrapped air and storage at +4 °C for 24 h prior to dynamic rheological analysis (37 °C). The dilution ratio was selected to represent that normally encountered in the vagina following insertion of the delivery vehicle.[17]

Flow Analysis under Continuous Shear. Flow rheometry was conducted at 25 ± 0.1 °C using an AR 2000 rotational rheometer operating in continuous flow mode using a parallel plate and a constant gap of 1000 μm. Sample geometry (2, 4, or 6 cm plate) was selected according to formulation consistency. Flow rheology was conducted using a loop test in which the shearing rate was increased gradually from a minimum (0.001 s^{-1}) up to a predetermined maximum (2 s^{-1}) within 60 s and then returned to the starting shear rate under the same conditions. For comparison purposes, the commercially available vaginal gel, Replens, was also studied. Modeling of the flow properties of the various formulations was performed using the Ostwald-de Waele equation, as follows:

$$\sigma = k\dot{\gamma}^n \tag{1}$$

where σ is the shearing stress, $\dot{\gamma}$ is the rate of shear, k is the consistency, and n is the pseudoplastic index.

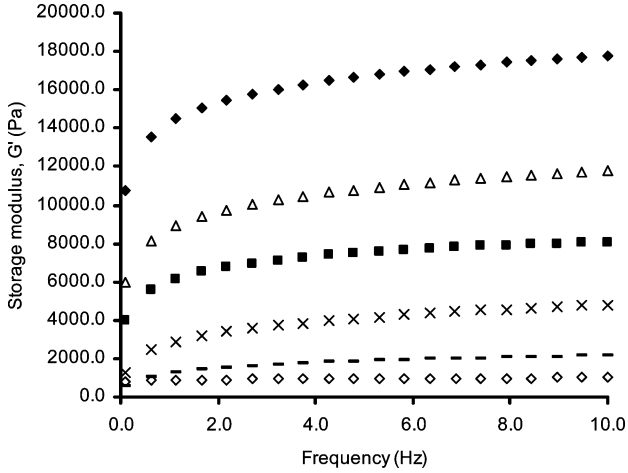

Figure 1. Effect of increasing oscillatory frequency on the storage modulus (G') of RSV formulations containing (◆) HEC 5%/PC 3%/PL 10%/PVP 4% (w/w); (Δ) HEC 5%/GANT 3%/PL 10%/PVP 4% (w/w); (■) HEC 5%/PC 3%/PVP 4% (w/w); (×) HEC 5%/GANT 3%/PVP 4% (w/w); (—) HEC 5%; (◊) PC 3% (w/w). Each value is the average of at least five replicates. Standard deviations have been omitted for clarity, however, and in all cases, the coefficient of variance was less than 4%.

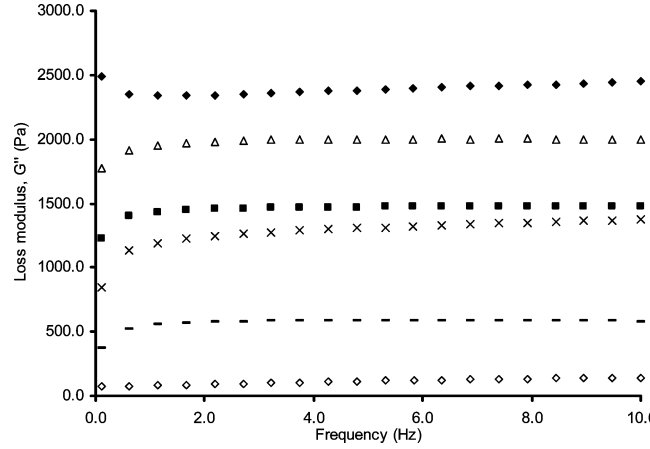

Figure 2. Effect of increasing oscillatory frequency on the loss modulus (G'') of RSV formulations containing (◆) HEC 5%/PC 3%/PL 10%/PVP 4% (w/w); (Δ) HEC 5%/GANT 3%/PL 10%/PVP 4% (w/w); (■) HEC 5%/PC 3%/PVP 4% (w/w); (×) HEC 5%/GANT 3%/PVP 4% (w/w); (—) HEC 5%; (◊) PC 3% (w/w). Each value is the average of at least five replicates. Standard deviations have been omitted for clarity, however, and in all cases, the coefficient of variance was less than 6%.

In Vitro Release of Erioglaucine from RSVs. A defined mass (5 g, $n = 6$) of each formulation was placed in a cylindrical vessel and anchored at the bottom of a stoppered 100 mL glass vial containing 50 mL of SVF, and the glass containers were placed in a shaking orbital incubator (Sanyo Gallenkamp, 100 rpm) maintained at 37 °C. The release medium was sampled (7 mL) at predetermined time intervals and analyzed by UV spectroscopy at 630 nm. At each sample point an equal volume of fresh prewarmed dissolution media was added to the dissolution vessels to replace the volume sampled. The mass of erioglaucine (EG) released was calculated with reference to a calibration curve (concentration range 0−8 mg/mL, $r > 0.999$).

Statistical Analysis. The effect of mucoadhesive type (PC or Gantrez S97) and PL concentration (0−10% w/w) on the mucoadhesive bond strength, percent release at 2, 6, and 24 h, syringeability, viscoelastic properties at defined representative frequencies (0.06, 0.27, 0.53, 0.74, 1.0 Hz) and mechanical (compressional flow) properties (hardness, compressibility) were statistically compared using a one-way ANOVA (Statview, Abacus Concepts, CA). In all cases, posthoc comparisons of the means of individual groups (following the ANOVA) were performed using Tukey's Honestly Significant Difference test. A significance level of $p < 0.05$ was accepted to denote significance in all cases.

Results

The variation in elastic and viscous modulus for each RSV formulation as a function of oscillatory frequency are illustrated in the representative rheograms shown in Figures 1 and 2, respectively, while the loss tangent and dynamic viscosities at five selected oscillatory frequencies are presented in Table 1. RSVs containing PC had a significantly greater G', G'', and η' and a significantly lower loss tangent than comparator formulations containing Gantrez S97. Also, formulations containing PL were more rheologically structured (statistically higher G', G'', and η' and lower tan δ) than formulations devoid of PL. Typically, physically entangled polymer gels exhibit storage and loss moduli that increase as a function of oscillatory frequency. However, RSVs exhibited relatively small increases in the storage and loss moduli as a function of oscillatory frequency. Interestingly, those formulations devoid of PL had a loss tangent that was significantly influenced by oscillatory frequency, whereas those formulations containing PL had a frequency independent loss tangent at oscillatory frequencies greater than 3 Hz. In addition, the storage modulus for all formulations was significantly higher than the loss modulus and, thus, the loss tangent (ratio of G''/G') was less than one.

The frequency dependence of the storage modulus was fitted to a general power law expression as follows (eq 2).

$$G_f = kf^n \tag{2}$$

In this model, G_f refers to the storage modulus (G) at a specified oscillatory frequency, k is the oscillatory consistency, f is the oscillatory frequency, and n is the oscillatory exponent.

The data obtained from this analysis is shown in Table 1 for all RSV systems and for the comparator single component systems. It was not possible to obtain oscillatory rheograms for the single polymeric component systems (PVP, Gantrez and PL) at the concentrations used, 4% w/w, 3% w/w and 10% w/w, respectively. Significant decreases in the oscillatory exponent (n) were observed as the mucoadhesive was changed from Gantrez S97 to PC (most notable in formulations devoid of PL), and upon the addition of PL. Moreover, the oscillatory exponent of 5% w/w HEC, the rheological structure building component, was statistically greater than that observed for any RSV. The oscillatory consistency values of the RSVs were affected by the addition of PL and the choice of mucoadhesive component. Formulations containing Gantrez S97 had a significantly lower oscillatory consistency than those RSVs containing PC, whereas the addition of PL significantly increased the oscillatory consistency. For the monopolymeric systems, HEC 5% w/w was shown to have a greater consistency than PC 3% w/w, whereas the oscillatory exponent (n) of PC 3% w/w was statistically lower than that obtained for HEC 5% w/w.

The temperature dependence of the storage modulus for the two RSVs containing PL is shown in Figure 3. The graph for the single component PL 10% w/w gel is not presented because it did not show a sol−gel transition temperature over the range 10−50 °C. There is a distinct increase in the storage modulus of the two formulations as a function of temperature with an obvious sol−gel transition temperature occurring at approxi-

Table 1. Rheological (Flow and Oscillatory), Mechanical (TPA) and Mucoadhesive Properties of RSVs[a]

conc. gel components (% w/w)					oscillatory rheology data					flow rheology	texture profile analysis data				in vitro release data	
HEC	PL	PC	GANT	PVP	freq. (Hz)	tan δ	η' (Pa·s)	consistency (Pa)	exponent	consistency (Pa·sn)	mucoadhesive strength (N)	syringeability (N·mm)	hardness (N)	compressibility (N·s)	exponent	% release (2, 6, and 24 h)
5	10	3		4	1.14	0.16 ± 0.01	326 ± 34	14070 ± 1851	0.11 ± 0.00	2933 ± 125	0.38 ± 0.04	58.8 ± 7.65	5.25 ± 0.89	5.45 ± 0.47	0.76 ± 0.01	
					3.23	0.15 ± 0.00	116 ± 13									4.53 ± 0.19
					5.31	0.14 ± 0.00	72 ± 8.4									9.94 ± 0.73
					7.40	0.14 ± 0.00	52 ± 6.5									30.16 ± 1.90
					10.00	0.14 ± 0.00	39 ± 5.2									
5		3		4	1.14	0.23 ± 0.00	200 ± 5.0	58880 ± 119	0.15 ± 0.00	3194 ± 177	0.37 ± 0.03	66.94 ± 9.19	6.28 ± 0.70	6.26 ± 0.52	0.74 ± 0.02	
					3.23	0.21 ± 0.00	72 ± 1.9									6.31 ± 0.36
					5.31	0.20 ± 0.00	44 ± 1.1									14.89 ± 0.34
					7.40	0.19 ± 0.00	32 ± 0.81									38.77 ± 1.28
					10.00	0.18 ± 0.00	23 ± 0.75									
5	10		3	4	1.14	0.22 ± 0.01	272 ± 13	8593 ± 409	0.14 ± 0.01	1622 ± 129	0.48 ± 0.03	37.4 ± 4.47	2.42 ± 0.54	2.51 ± 0.42	0.79 ± 0.05	
					3.23	0.19 ± 0.02	98 ± 4.7									7.02 ± 0.91
					5.31	0.18 ± 0.01	60 ± 2.8									19.71 ± 1.63
					7.40	0.18 ± 0.02	43 ± 2.1									49.97 ± 3.54
					10.00	0.17 ± 0.02	31 ± 1.5									
5			3	4	1.14	0.41 ± 0.00	166 ± 0.60	2646 ± 15	0.27 ± 0.00	1787 ± 89	0.50 ± 0.04	35.21 ± 2.96	3.28 ± 0.19	3.16 ± 0.74	0.80 ± 0.06	
					3.23	0.34 ± 0.00	63 ± 0.50									7.06 ± 0.92
					5.31	0.31 ± 0.00	39 ± 0.34									17.36 ± 0.66
					7.40	0.29 ± 0.00	29 ± 0.38									50.41 ± 1.40
					10.00	0.29 ± 0.00	22 ± 0.30									
5					1.14	0.43 ± 0.00	77 ± 2.1	1161 ± 34	0.29 ± 0.00							
					3.23	0.35 ± 0.00	29 ± 0.75									
					5.31	0.31 ± 0.00	18 ± 0.46									
					7.40	0.29 ± 0.00	13 ± 0.32									
					10.00	0.27 ± 0.00	9.2 ± 0.24									
		3			1.14	0.09 ± 0.00	11 ± 0.12	876 ± 4	0.05 ± 0.01							
					3.23	0.11 ± 0.00	5.0 ± 0.06									
					5.31	0.12 ± 0.00	3.5 ± 0.03									
					7.40	0.13 ± 0.00	2.8 ± 0.01									
					10.00	0.14 ± 0.00	2.3 ± 0.01									
		Replens								79 ± 4	0.19 ± 0.03	4.40 ± 4.60				

[a] In all cases, the values presented are the average ± standard deviation of at least three replicates.

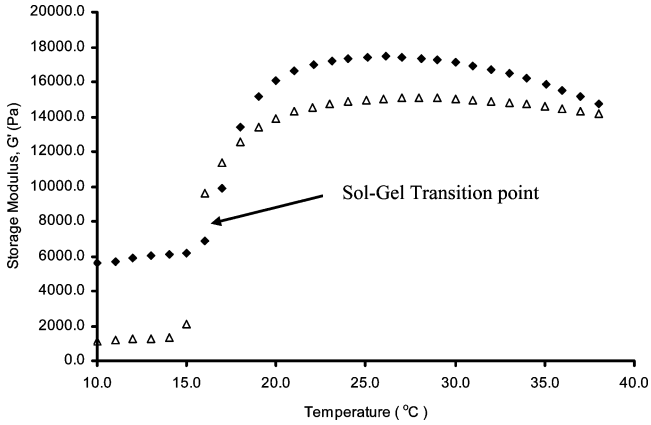

Figure 3. Temperature dependence of the storage modulus of the two RSV formulations containing PL, (■) HEC 5%/PC 3%/PL 10%/PVP 4% (w/w); (Δ) HEC 5%/GANT 3%/PL 10%/PVP 4% (w/w). Each value is the average of at least five replicates. Standard deviations have been omitted for clarity, however, and in all cases, the coefficient of variance was less than 5%.

mately 16 °C. The formulation containing Gantrez S97 had a storage modulus that was significantly lower than PC in the gel state (temperatures above 16 °C) and in the lower temperature sol state.

The effects of dilution with SVF on the rheological properties of the four RSV systems are demonstrated in Figure 4a−d. The ratios of the storage moduli of the diluted RSV systems (0.1−0.9 mL dilution) to that of their undiluted counterparts, across the frequency range investigated (0.1−10 Hz), are displayed. All formulations displayed a significant loss of storage modulus upon dilution. A significantly lower ratio of $G'_{gel+SVF}/G'_{gel}$ and, hence, decreased rheological structure was observed for all RSVs as the volume of SVF increased (from 0.1−0.9 mL). The ratio of the storage modulus of the diluted binary gels to the undiluted counterparts decreased when PC was substituted for Gantrez S97. Interestingly, the addition of PL to PC containing RSVs resulted in a formulation that was more robust to dilution at low SVF volume (0.1 mL), whereas at greater volumes (0.3−0.9 mL), the PC formulation devoid of PL was more capable of maintaining rheological structure. For RSVs containing Gantrez S97, at low SVF volume (0.1 mL) there was no significant difference between formulations containing and devoid of PL whereas at higher volumes (0.3−0.9 mL) formulations containing PL were more resistant to dilution. The RSVs containing PC (and devoid of PL) exhibited the highest ratio of diluted gel to gel and, hence, the greatest rheological structure following dilution at the maximum SVF volume (0.9 mL), whereas the lowest ratio was observed for the RSV containing 5% w/w HEC, 3% w/w Gantrez S97, and 4% w/w PVP (Figure 5).

All formulations displayed a nonlinear relationship between the shear stress and shear strain and, hence, a shear viscosity that decreased with increasing shear rate (pseudoplastic). In addition to being pseudoplastic, a shift in the upward and downward flow curves was observed, suggesting thixotropic behavior (data not shown). Decreases in viscosity as a function of increasing shear rate may be described in terms of shear stress and shear rate using an exponential function, referred to as the Ostwald-de Waele rheological expression (eq 1). This model has been shown to be appropriate for the physical characterization of many different materials,[18] providing information regarding the flow consistency (K) of the material, and that is an indication of the strength and structure of the material. Therefore, as the viscosity of the sample increases so will the consistency.[17] K may therefore be used to establish the effect of changes in formulation variables on material structure at a single shear rate value (1 s^{-1}). In this study we have investigated the effect of various mucoadhesive components (PC and Gantrez S97) and the inclusion of a thermogelling polymer (PL) on the rheological (destructive and transient), compressional flow (hardness and compressibility), and drug release properties of RSVs. In relation to flow rheology, changing the mucoadhesive from PC to Gantrez S97 significantly reduced RSV flow consistency, whereas there was no significant effect on the flow properties of RSVs upon the addition of PL (Table 1). The flow properties of a commercially available vaginal gel (Replens) was examined as a comparator to the RSV systems. Replens was shown to have a statistically lower consistency value (Table 1).

The formulations examined within this study displayed a wide range of mechanical and mucoadhesive properties that were significantly affected by changing the mucoadhesive polymer (Table 1). RSVs containing Gantrez S97 were more mucoadhesive than their PC counterparts. There was shown to be no significant difference in the mucoadhesive bond strength upon the addition of PL, however, all RSVs had a greater mucoadhesive ability than Replens. Formulations containing PC as the mucoadhesive component had significantly greater syringeability, hardness, and compressibility values than those formulations manufactured using Gantrez S97. As observed during flow analyses, the addition of PL had no significant effect on the syringeability and the mechanical properties (hardness and compressibility) of the RSV systems. The syringeability and mucoadhesive strength of Replens was much lower (statistically significant) than all RSV systems.

The release of a model compound, EG, as a function of time from the four RSV systems over a 24 h period is illustrated in Figure 6. The percentage EG released at 2, 6, and 24 h is also shown in Table 1. All gel systems displayed sustained release over the observed time period. The percentage released was significantly affected by the mucoadhesive component added to the formulation. For example, formulations containing PC released a lower percentage of total EG content compared to those containing Gantrez S97. Interestingly, the inclusion of PL had a significant effect on release for RSVs containing PC with the formulation containing 5% HEC, 10% PL, 4% PVP, and 3% PC displaying the slowest percent release. There was no statistical difference in the percentage of EG released between the two Gantrez S97 formulations. In an attempt to determine the mechanism by which the EG was released from the RSVs, a simple, semiempirical power law equation (eq 3)[19] to describe the release data was employed. Release data was fitted to the general release equation[20] using a double logarithmic transformation and subsequent least-squares regression. Modeling the release data using the Power law expression yielded release exponents ranging from 0.74−0.84, indicative of an anomalous transport mechanism.

$$M_t/M_\infty = Kt^n \quad (3)$$

M_t/M_∞ is the absolute cumulative amount of drug released at time t and infinite time, respectively, K is a constant incorporating structural and geometric characteristics, and n is the release exponent, which is indicative of the mechanism of release.

Discussion

Polymer gel systems are widely used to formulate pharmaceutical drug products and have been employed in dental,[21]

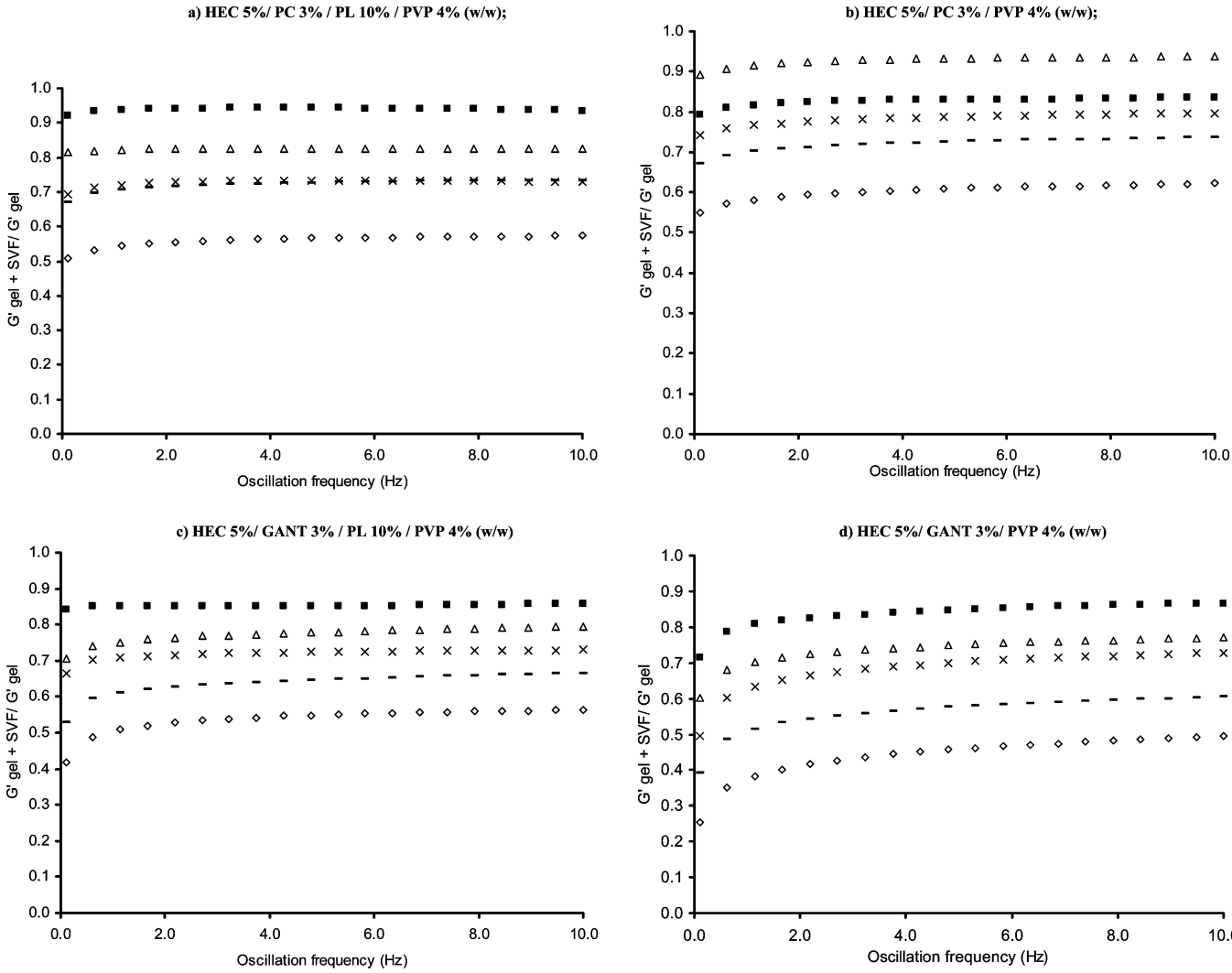

Figure 4. Frequency dependence of the ratio of the storage modulus of RSVs after and before dilution ($G'_{gel + SVF}/G'_{gel}$) with (■) 0.1 mL SVF per 3 g RSV; (△) 0.3 mL SVF per 3 g RSV; (×) 0.5 mL SVF per 3 g RSV; (−) 0.7 mL SVF per 3 g RSV; (Δ) 0.9 mL SVF per 3 g RSV. (a) HEC 5%/PC 3%/PL 10%/PVP 4% (w/w); (b) HEC 5%/PC 3%/PVP 4% (w/w); (c) HEC 5%/GANT 3%/PL 10%/PVP 4% (w/w); (d) HEC 5%/GANT 3%/PVP 4% (w/w). Each value is the average of at least five replicates. Standard deviations have been omitted for clarity, however, and in all cases, the coefficient of variance was less than 6%.

dermatological,[22] vaginal,[17] and ophthalmic applications.[23] Typically, these systems are easy to apply, offer enhanced patient compliance, and, in the case of aqueous-based systems, display reduced irritancy, which is essential for application to mucous membranes.[24] However, in the case of vaginal administration, gel mixing with cervicovaginal fluid and semen causes gel dilution and contributes to leakage of the formulation.[25] Retention of vaginal gels may be improved using mucoadhesive polymers, such as those based on poly(acrylic acid) or cellulose derivatives.[10,17] Although offering increased mucoadhesion, the rheological properties of these monopolymeric systems are usually insufficient to offer resistance to in vivo stresses, and hence, clinical performance is often suboptimal. The rheological and mechanical properties of vaginal gels should be tailored so that they are easy to apply (non-Newtonian, shear thinning), be well dispersed throughout the vaginal vault, and offer extended residence time to allow for controlled drug delivery.[17] It has been previously reported that optimal performance (controlled drug release and maximum retention) may be achieved for gel-based formulations where a high elastic modulus (exceeding the loss modulus) is maintained following dilution with vaginal fluids.[26] In this study, mucoadhesive, site-retentive RSV for-

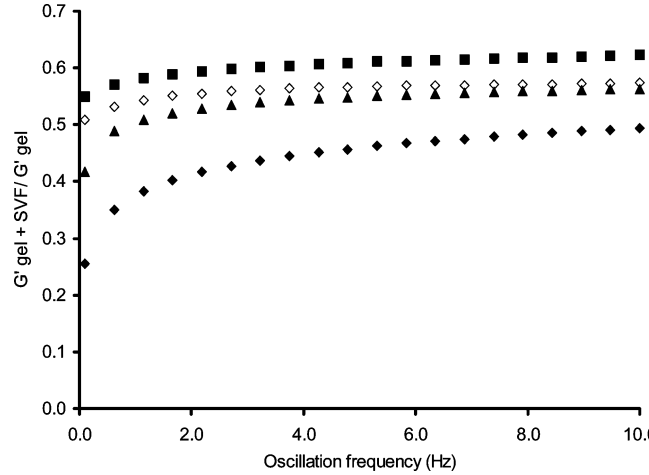

Figure 5. Comparison of RSVs after dilution with 0.9 mL SVF; (◊) HEC 5%/PC 3%/PL 10%/PVP 4% (w/w); (■) HEC 5%/PC 3%/PVP 4% (w/w); (▲) HEC 5%/GANT 3%/PL 10%/PVP 4% (w/w); (◆) HEC 5%/GANT 3%/PVP 4% (w/w). Each value is the average of at least five replicates. Standard deviations have been omitted for clarity, however, and in all cases, the coefficient of variance was less than 5%.

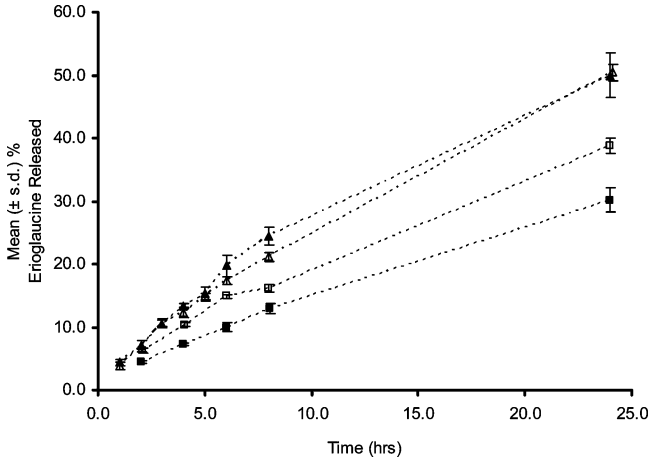

Figure 6. Cumulative release profile of erioglaucine from formulations containing; (■) HEC 5%/PC 3%/PL 10%/PVP 4% (w/w); (▲) HEC 5%/PC 3%/PVP 4%; (w/w); (Δ) HEC 5%/Gantrez 3%/PL 10/PVP 4% (w/w); (△) HEC 5%/GANT 3%/PVP 4% (w/w). Each value is the average ± standard deviation of six replicates.

mulations, comprising multipolymeric components, have been developed for sustained vaginal delivery of therapeutics agents.

The relationship between the nondestructive rheological parameters of gels and oscillatory frequency is often described using the Maxwell model.[27] Typically, the storage modulus will dominate the loss modulus at high oscillatory frequencies whereas at low frequency, viscous behavior will be the dominant relaxation process. Within this study, the storage (G') and loss moduli (G'') increased as a function of frequency. However, the extent of increase was small (n values in Table 1 tended toward zero). The response of the moduli to increasing oscillatory frequency was characteristic of the plateau region more often observed in chemically cross-linked systems.[16] All of the gels had storage moduli that exceeded the loss moduli (loss tangent < 1) and none displayed fluid (loss tangent > 1) or fluid/gel (loss tangent ~ 1) transition states.[28] The observed rheological response for RSVs may be attributed to significant physical interaction between the polymeric components, the formation of an enhanced physical network and hence an increased elastic response.[21] Moreover, the response of the RSVs to nondestructive stresses suggests the presence of a three-dimensional structure formed through the interpenetration of polymer chains and the formation of secondary interactions.

The physical response of a semisolid to external forces may be used to characterize the rheological behavior of polymer gels.[29] The frequency dependence of the storage and loss moduli is probably the most common technique used to identify the gel state. The presence of a plateau in the real part of the complex modulus (G') that extends over an appreciable window of frequencies is a typical characteristic of viscoelastic solids.[30] Although the formulations studied in this investigation did not display a plateau in the mechanical spectra, the dependence of the storage moduli to oscillatory frequency was low and tended toward zero ($n \leq 0.27$), a characteristic of polymer gels with a high level of interpenetration and entanglement between polymer chains.[26] Interestingly, the magnitude of the slope decreased after the addition of PL and when the mucoadhesive component was changed from Gantrez S97 to PC. In this study two different mucoadhesive components were used, Gantrez S97 and PC. Gantrez S97 is a linear polymer and possesses polymer cross-links in the gel state that are controlled by polymer chain overlap and secondary interactions such as hydrogen bonding.[29] Conversely, PC is a chemically cross-linked poly(acrylic acid) and hence the storage modulus is not highly dependent upon oscillatory frequency. Although the oscillatory consistency of PC 3% w/w was significantly lower than all other polymers ($K = 876.32 \pm 4.44$), the magnitude of the slope was extremely low ($n = 0.05 \pm 0.01$). Therefore, the slopes of the storage moduli of the RSVs containing PC were significantly lower than Gantrez S97. Furthermore, the inclusion of PL significantly increased the elasticity of the RSV systems and also resulted in a significant decrease in the slope of the storage moduli with respect to oscillatory frequency. PL (F127) is a long chain, nonionic triblock copolymer with a central hydrophobic region of polypropylene oxide (PPO) and two lateral hydrophilic segments of polyethylene oxide (PEO). These materials have an ability to undergo temperature-sensitive gelation, whereby the gel state "collapses" due to aggregation of the PPO segments upon reaching a critical temperature.[31] At a concentration of 10% w/w, PL did not show a sol−gel transition temperature over the temperature range examined (10−50 °C) and was extremely fluid. The addition of PL to the RSVs resulted in a significant increase in the storage modulus, loss modulus and dynamic viscosity, whereas the loss tangent decreased. Furthermore, the two RSVs containing PL at a loading of 10% w/w exhibited a distinct sol−gel transition temperature (Figure 3). In situ gelling of polymers has been used extensively for topical application and provides a convenient method for drug delivery. Here we have shown that the incorporation of PL within the RSVs reduces the critical gelation temperature, causing the formation of gel networks at approximately 16 °C. This effect may be ascribed to a reduction in the energy required to cause self-association of the PPO units. Moreover, the presence of highly entangled overlapping polymer chains, secondary interactions and permanent primary bonds within RSVs containing PL has yielded highly elastic rheological structures, higher than any of the individual polymer components used in their manufacture. The formation of these advanced gel systems decreased the dependence of the storage moduli on oscillatory frequency, enhanced the elastic moduli and generated in situ gelling properties that have been shown to be advantageous for vaginally administered gels.[32]

The most significant challenge for topically applied vaginal gel drug delivery systems is the detrimental effect of dilution by cervicovaginal fluids (and semen during intercourse) on formulation rheology and hence on product retention.[33] To assess this effect, dilution with SVF prior to oscillatory analysis at 37 °C was conducted. Current vaginal gels, such as those used in contraceptive applications, tend to be applied using volumes in the region of 1.5−3 mL. These gel volumes are typically subjected to dilution by approximately 0.75−1 mL of vaginal fluid.[17,34] Accordingly, the effects of dilution, representative of that encountered in vivo on the oscillatory properties of the candidate gel systems were examined by sequentially diluting 3 g of RSV with up to a maximum of 0.9 mL of SVF.[32] After dilution, all formulations exhibited a G' that exceeded G'' and, hence, a loss tangent that was less than 1 in all cases (data not shown). For each RSV investigated, consecutive dilutions significantly further reduced the storage modulus, although the effects of dilution on the RSVs in this study were less pronounced than in previously reported work.[32] This difference in performance may be directly attributed to a significant interaction between the components used to manufacture the RSVs, providing robust elastic networks capable of imbibing considerably larger volumes of vaginal fluid.[26]

Previous studies have focused on the effects of dilution by simulated vaginal fluid and semen simulant on the rheological

properties at a defined oscillatory frequency. The rheological performance of vaginal gels, following dilution, across a range of frequencies has not been comprehensively described. Following administration, a vaginal formulation will experience a range of stresses across a wide range of time scales.[17] Therefore, it is important to characterize and understand the time-dependent rheological behavior of vaginal gels following dilution to closely mimic in vivo conditions. Following dilution, an ideal response would be where the formulation preserves its rheological and mucoadhesive characteristics independent of oscillatory frequency and, hence, independent of the time scale over which the force is applied. In this study, the RSV platforms investigated had minimal dependence upon oscillatory frequency. At a maximum dilution of 0.9 mL, the magnitude of the slope of $G'_{gel+SVF}/G'_{gel}$ was ≤ 0.14. Therefore, these gels could be expected to maintain structure in vivo due to the presence of a highly elastic structure and an ability to imbibe fluid with a limited effect on the rheological structure.

Vaginal gel systems are typically administered via extrusion using an applicator. Understanding such a process requires a fundamental knowledge of the rheological behavior after exposure to complex compressional and torsional shearing stresses.[26] Moreover, shear-thinning behavior is highly desirable to aid expulsion from the applicator and ensure adequate spreading across the vaginal epithelia.[17] It has also been reported that gels designed for this purpose should possess a high resistance to deformation following application to minimize the deleterious effects of dilution and shearing experienced in vivo. In this investigation we have described the use of mechanical (texture profile analysis) and flow rheological tests to determine the compressional and torsional (shear) flow properties of the RSVs. We have also determined the ease of application of the RSVs from a syringe applicator. These techniques provide information on the relative change in material structure over a wide range of shearing stresses. This may aid in the fundamental understanding of the effect of physiological stresses on the formulation structure and the in vivo performance.[35] Additionally, flow rheology may simulate the stresses experienced during product usage (expulsion from an applicator) that are outside the linear viscoelastic region. All RSVs displayed a non-Newtonian pseudoplastic response and a shear viscosity that decreased with increasing shear rate. This nonlinear effect is typical of most pharmaceutical gels and arises primarily because of structural changes induced during the application of shearing stress. At low shear stresses, the polymer chains within the RSVs are present in a relaxed state, at concentrations greater than the critical concentration (c^*) for molecular overlap. As the sample is sheared, polymer chains disentangle and diffuse from the network, orientating in the direction of shear.[36] Realignment of the polymer chains enhances mobility and the shear viscosity decreases. The incorporation of PL had no significant effect on the consistency, hardness, compressibility, and syringeability of the RSVs, whereas changing the mucoadhesive from PC to Gantrez S97 significantly decreased these properties. The increased mechanical and flow rheological properties observed for RSVs containing PC may be attributed to the increased interaction between the polymeric components in these complex interactive blends.

The examination of mucoadhesive bond strength involved the use of a previously reported mucin disk test that determines the force required to separate a partially hydrated mucin disk from the surface of the formulation.[15] The ability of a vaginally applied gel system to adhere to the vaginal epithelium is essential to maximize residency and, hence, clinical performance.[37] The establishment of a mucoadhesive bond between polymeric components and a biological substrate may be influenced by the surface of the biological substrate, the surface of the bioadhesive layer and the interfacial layer between the two.[38] Assuming the surface of the mucin disk in each experiment is similar, then the differences occurring in the mucoadhesive ability of the polymer systems may be attributed to formulation surface effects. The RSVs investigated in this study were designed to provide enhanced retention in the vaginal vault to provide a means of sustained release of therapeutic agents to the vaginal mucosal surface. All RSV formulations exhibited high mucoadhesive bond strength ($>0.37 \pm 0.04$ N, Table 1), exceeding the value observed for the commercially available Replens formulation (0.19 ± 0.03 N). HEC has been reported previously to exhibit moderate mucoadhesive properties, whereas PC and Gantrez S97 are strongly mucoadhesive.[39,40] In this investigation, the RSV formulations containing Gantrez S97 were shown to be more mucoadhesive than PC (Table 1). Given that Gantrez S97 formulations were less elastic this effect may be attributed to increased diffusion, interpenetration and entanglement of these formulations with hydrated mucin on the surface of the disk.[41] Although there were small differences in mucoadhesive ability of PC and Gantrez S97 formulations, more importantly significant differences were observed between RSVs and a commercially available vaginal formulation, Replens. Moreover, RSVs displayed improved rheological properties that, when combined with the high mucoadhesiveness, may render these systems more acceptable.

The use of mucoadhesive polymers is a viable method of improving the residence time of vaginal gels. However, the design of mucoadhesive drug delivery systems with increased residence time and resistance to in vivo stresses will only be advantageous if the therapeutic agent is released over the retention period. In vitro drug dissolution demonstrated that all formulations provided sustained release of the model soluble drug EG. The variability in the dissolution profiles may be ascribed to the subtle rheological differences and, hence, the ability of the formulation to resist fluid ingress and thus dissolution of the active from the polymer matrix. The PC formulations that were more resistant to dilution effects by SVF also had significantly lower percent release at defined time intervals (Table 1). It was also noted during the dissolution study that PC formulations had a tendency to swell, whereas the Gantrez S97 did not. This may be attributed to the cross-linked nature of the PC component of the gels that allows the gel bolus to imbibe water without undergoing dissolution. Consequently, PC formulations would be less susceptible to dissolution and erosion and, hence, decreased release of EG.[42] The release exponent, n, indicative of the mechanism of drug release, was characteristic of anomalous transport; n was in the range $0.74 \pm 0.02 - 0.80 \pm 0.06$. This is typical for gels wherein a number of competing processes are occurring during drug dissolution.[43]

Conclusion

Due to their low cost, ease of manufacture, and precedence of use in the topical administration of drugs, conventional gel systems are commonly employed to administer drugs via the vaginal route, mainly for the treatment of vaginal infection, contraception, and hormone replacement therapy. More recently, gel-based formulations are being widely developed for sustained delivery of HIV microbicides and mucosal vaccines. The retention of vaginal gel formulations is fundamental to the improvement of clinical performance. Poor vaginal retention

of conventional gel formulations represents a significant challenge for those clinical indications where sustained delivery would enhance efficacy. This challenge is particularly pertinent for the HIV microbicide field, where coitially independent formulations are considered essential to overcome the potential issues surrounding user acceptability and compliance. Gels designed for this purpose should exhibit acceptable mechanical and rheological properties to ensure ease of application and retention. In this study, gels composed of interactive polymeric components have been formulated and the rheological, mechanical, mucoadhesive, and drug dissolution properties have been characterized. The dynamic (nondestructive) rheological properties following dilution, to simulate in vivo conditions, have also been investigated. RSVs displayed a wide range of mucoadhesive, mechanical (hardness, compressibility, and syringeability), and rheological (flow and dynamic) properties that were significantly affected by the mucoadhesive used (PC or Gantrez S97) and also the inclusion of a thermo-gelling polymer (PL). It is suggested that the rheologically structured gels described in this study possess suitable properties, even following dilution, to enhance clinical performance. In particular, the highly elastic structure of the PC formulations after dilution, the acceptable flow and mucoadhesive properties (thereby enabling ease of administration and retention) and the controlled release characteristics may provide significant clinical promise.

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