Dynamic Mechanical Behaviour of Nanoparticle Loaded Biodegradable PVA Films for Vaginal Drug Delivery

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ABSTRACT

In this study we investigated the viscoelastic and mechanical behaviour of polyvinyl alcohol (PVA) films formulated along with carrageenan, plasticizing agents (polyethylene glycol and glycerol), and when loaded with nanoparticles (NP) as a model for potential applications as microbicides. The storage modulus, loss modulus and glass transition temperature were determined using a dynamic mechanical analyzer (DMA). Films fabricated from 2% and 5% PVA containing 3mg or 5mg of fluorescently-labeled NPs were evaluated. The storage modulus and loss modulus values of blank films were shown to be higher than the NP-loaded films. Glass transition temperature determined using the storage modulus was between 40-50°C and 35-40°C using the loss modulus. The tensile properties evaluated showed that 2% PVA films were more elastic but less resistant to breaking compared to 5% PVA films (2% films break around 1N load and 5% films break around 7N load). To our knowledge, this is the first study to evaluate the influence of NP and film composition on the physico-mechanical properties of polymeric films for vaginal drug delivery.

KEYWORDS

Poly (vinyl alcohol), nanoparticles, dynamic mechanical analysis (DMA), modulus, glass transition temperature, thermal stability

1. INTRODUCTION

Polymeric films are increasingly gaining attention as drug delivery systems due to their ability to provide rapid drug release and bio-adhesive properties that may increase the retention time at the target tissue. Film-formulated products offer user compliance as well as ease of application. Recently, a number of researchers have focused on the development of polymeric vaginal films as contraceptives and microbicide formulations[1-5]. The potential of vaginal films for contraceptive or microbicide applications is mainly due to their ability to overcome several challenges related to acceptability, compliance, and efficacy in comparison to gel-based formulations[5-7]. For example, gels are messy and may leak resulting in reduced drug concentrations. Despite films demonstrating to be a promising drug delivery platform, the type of bioactive being delivered will affect the mechanical, chemical and physical properties of the film, which in turn will also alter drug release rates[7].

Polyvinyl alcohol (PVA) is a polymer that is widely used in a variety of applications due to its biodegradability and its ease in preparation[8]. PVA is a water-soluble and crystalline polymer. This material can form films that are biocompatible with good mechanical properties allowing its use in various industrial and medical applications. It has been used in pharmaceutics as a drug delivery system due to its high water solubility and rapid disintegration rate[3, 9]. Polymeric films can be designed to be highly resistant to repeated flexure or creasing[10]. Some of the film properties such as tensile strength, and permeability to gases or water vapour may be important features to consider when developing a drug delivery system[11]. Studies have shown that thin polymeric films tend to degrade in the presence of destabilizing forces such as heat and mechanical stress at the film's interface[10, 12]. Quantitatively, such instabilities affect the thermal and mechanical characteristics of the

film[10, 12]. Depending on the plasticizer, excipients, or drug loaded into the films, it can exhibit different physico-chemical behaviours.

Numerous studies are using polymeric film formulations to deliver nanoparticles (NPs)[13, 14]. The nanocarrier is used to protect the active compound from its external environment, can assist in active targeted delivery, and can provide controlled, sustained drug release[15]. They have been used to address the concerns of physicochemical instability of drugs, low cellular uptake and the need for multiple dosing[16]. For example, different PVA films with different thickness have been formulated to deliver varying concentrations of poly(D,L-lactic-co-glycolic acid)-poly(ethylene glycol) NPs containing siRNA as a therapy for preventing HIV infection within the female genital tract[4].

Hence, the objective of this study was to experimentally determine the influence of NP content and film composition on the physico-mechanical properties of NP-loaded PVA films subjected to varying temperature ranges.

2. MATERIALS AND METHODS

Degradex® fluorescent PLGA NPs were purchased from phosphorex (Hopkinton, Massachusetts, USA). PVA (98-99% hydrolyzed; MW 31,000-50,000) and λ-carrageenan was purchased from American Custom Chemicals Corp (San Diego, CA, USA). Phosphate-buffered saline (PBS; pH 7.4) was purchased from Lonza (Allendale, NJ, USA). Double distilled water (ddH_2O) was purified using Millipore Simplicity System, which was used for all the experiments.

2.1. Preparation of nanoparticle-loaded film

PVA films were prepared by a solvent casting method as previously described[4]. Briefly, PVA and carrageenan were dissolved overnight in distilled water at 80°C. The PVA solution was allowed to cool down to room temperature and diluted to either 5% or 2% solution (w/v). 240 μL of glycerol and 80 μL of PEG400 were mixed in 8 mL of the solution and allowed to stir for 1 h. Commercially available fluorescent NPs (200 nm) were added and mixed for an additional 1 h to prepare 5% film containing NPs with a w/w ratio of 1:88 [NP:(PVA+carrageenan)] for 5 mg NPs loading and 1:147 for 3 mg NP loading. For the 2% film, a w/w ratio of 1:40 and 1:67 were used for 5mg and 2 mg NP loading, respectively. The mixture was poured into a Teflon^{TM} dish (30 cm^2) and placed in the oven at 40°C overnight to evaporate the solvent and to form the film.

2.2. Dynamic mechanical analysis

Dynamic mechanical analysis (DMA) of the films was performed using a DMA Q800 (TA Instruments, New Castle, USA) in tensile mode. Film samples (2% and 5% PVA blank films or NP-loaded films) were cut into sizes between 5-6.5 mm in width, 20-30 mm in length, and 0.5-0.7 mm in thickness in order to conform to the dimensional limits required for the tensile film clamp test fixture. The average thickness for each film sample was based on three separate measurements, taken at the two ends and in the middle. The DMA test procedure was as follows: after mounting the film sample on the DMA tension film clamp, the furnace was sealed and the mechanical properties were measured from 25 ºC to 80 ºC at a ramp rate of 3 ºC per min under a preload of 0.5 N. Measurements were performed at a constant frequency of 1 Hz and strain amplitude of 10 μm. The glass transition temperature, the real (storage) modulus E', and imaginary (loss) modulus E'' was determined using the thermal analysis software (TA Instrument Universal Analysis 2000) included with the DMA apparatus. The software automatically detects signal change on the

analysis curve. The inflection point on the curve of the modulus can be considered the glass transition temperature and is determined using the tangent of the onset point and the end point[17, 18]. Three independent runs were performed for each film formulation throughout this paper.

2.3. In vitro drug release

PVA film samples (2% and 5% fluorescent NP-loaded films cut to around 20 mg) were suspended in 1 mL release medium (PBS pH 7.4). The fluorescent NP-loaded film suspension was transferred to an orbital shaker maintained at 37 ºC at a speed of 100 rpm (VWR, Edmonton, Canada). 200 µL aliquot of samples were periodically removed for analysis and were replenished with equal volume of fresh medium. The fluorescent NP concentration in the samples was analyzed on a microplate reader (BioTek Synergy) with an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Samples were taken and analyzed in triplicate for each time point. Films disintegration have been evaluated also by immersing 50 mg of each film in 3 ml of ddH_2O in 20 ml vial and place in the orbital shaker at 37 ºC at a speed of 100 rpm. Visual inspection is used to record the disintegration rate.

2.4. Determination of mechanical properties

Mechanical analysis was performed on an Instron tensile machine model 5943 with a crosshead speed of 100 mm/min and a force of 1 kN (load cell) at room temperature. All the film samples were cut into rectangular shapes with a length of 30±0.3 mm, a width of 6.80±0.7 mm, and a thickness of 0.07 mm for the 2% film and a thickness of 0.16 mm for the 5% film.

2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) studies were carried out using a Quanta 650 FEG equipped X-ray microanalysis with low-vacuum capabilities at a voltage of 10 keV. Film samples were cut into small pieces and attached to slab surfaces with double-sided adhesive tape.

Statistical analysis

Data are presented as mean ± standard deviation (SD). The n-value refers to number of replicates performed for each study. One-way ANOVA was performed on all results, with $P<0.05$ considered to be significant.

3. RESULTS

The storage and loss modulus of various 2% and 5% PVA film formulations were determined (Fig 1). Based on the results, the storage modulus for both 2% and 5% blank films were slightly higher than their counterpart films loaded with NP. The storage modulus of 2% blank film varied between 22.90±5.23 MPa to 13.87±2.91 for a temperature ramp from 30°C to 60°C and 143.24±14.18 to 82.63±5.43 MPa for 5% blank film at the same temperature ramp. Furthermore, the storage modulus for films containing 3 mg of NP was also higher than the ones containing 5 mg of NP. PVA films containing 3 mg and 5 mg vary between 15.67±2.08 to 10.07±1.35 MPa and 12.72±5.29 to 8.15±3.70 MPa, respectively. The storage modulus decreased gradually as the temperature increased. The mean storage and loss modulus are presented in Fig 2A and 2B, respectively. The sharpest slope of the storage modulus was used to determine the glass transition temperature where the film began its transition from a glassy state to a more rubbery state. The mean glass transition temperatures for each film as determined using the storage modulus is presented

157 in Fig 2A. As can be seen in Table 1, 2% PVA films loaded with either 3 mg (53.25±2.86
158 °C) or 5 mg (54.10±7.63°C) NP exhibited higher mean glass transition temperatures
159 compared to 5% PVA films with 3 mg (40.05±2.09°C) or 5 mg (42.07±2.21°C) NP,
160 respectively (Table 1), when using the storage modulus for determination. In contrast, no
161 significant differences were observed in any of the film groups when determining glass
162 transition temperature using the loss modulus (Table 1B).

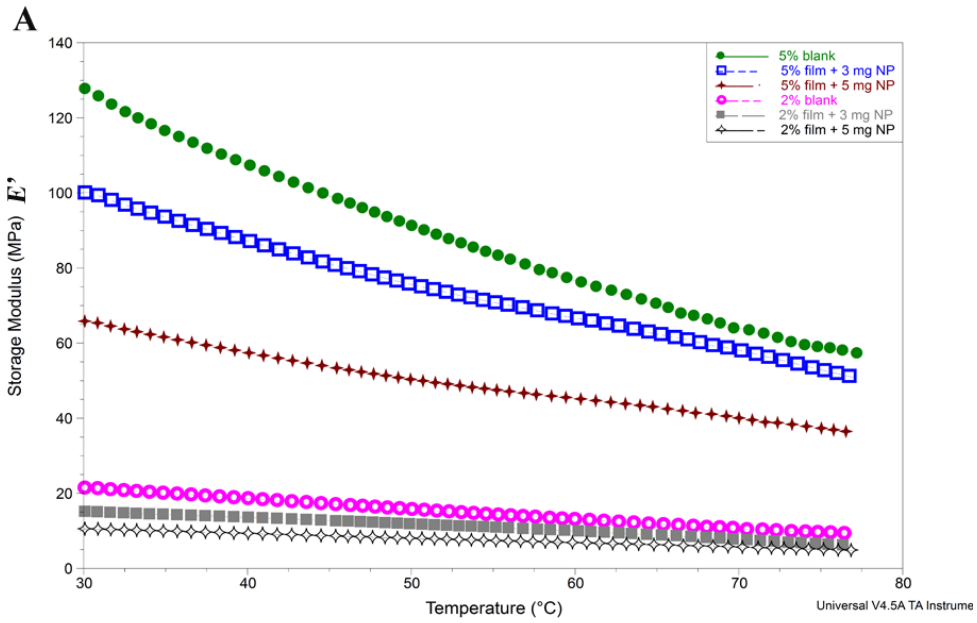
163 Disintegration studies were performed on blank PVA films and films loaded with 3
164 or 5 mg of NP. Around 50 mg of each film was immersed in 3 mL of PBS buffer and
165 placed in an incubator shaker at 37^{o}C and 100 rpm (Table 1). 2% PVA films with 3 or 5 mg
166 loading disintegrated within 3 hr making the medium cloudy. The 5% PVA films
167 containing 3 or 5 mg loading partially disintegrated with no changes to the transparency of
168 the medium.

169 In vitro release studies were performed on different film preparations. The amount of
170 fluorescent NPs released was evaluated using a micro plate reader (Fig 3). Fig 3 represents
171 the release obtained from 2% PVA films loaded with 3 mg or 5 mg of NPs and 5% films
172 loaded with 3 mg or 5 mg. 2% films demonstrated a release of 18.92±5.6 μg (8.7±0.7 %)
173 for 5 mg NP loading and 11.48±1.5 μg (9.8±0.5 %) for 3 mg NP loading after 4 hours
174 followed by a slow and negligible release up to 24 hours. For 5% film, there a slow release
175 of NPs up to 24 hours with 6.8±0.8 μg (3.25±0.39 %) for 5mg loading and 4.13±0.36 μg
176 (3.15±0.27 %) for 3mg loading.

177 Tensile properties such as Young's modulus, maximum load at break, tensile strain
178 at maximum load and tenacity (used to measure the overall strength) of the film at
179 maximum load has been calculated from stress-strain curves (Table 3). 2% blank films have
180 a tensile strain at maximum load of 0.162 mm/mm, and 0.151 mm/mm for films containing

3 mg NP loading and 0.134 mm/mm for films containing 5 mg NP loading. The 5% blank films demonstrated to have lower tensile strain and started to break at 0.037 and 0.043 mm/mm for the 3 mg and 5 mg NP loading, respectively. 2 % PVA films present more elasticity but start breaking at lower load, around 1N compared to 5% PVA film, which started to break around 7N. The 5% PVA film is less elastic, and broke at only 1 mm of the crosshead displacement distance compared to the 2% film which broke after 4 mm of extension.

The surface morphology of each film was visualized by SEM. The 2% blank film appeared to have a more porous structure, but became less obvious once loaded with 3mg or 5 mg NP. In contrast, the surface of 5% PVA films appeared more compact in structure with very few pores.



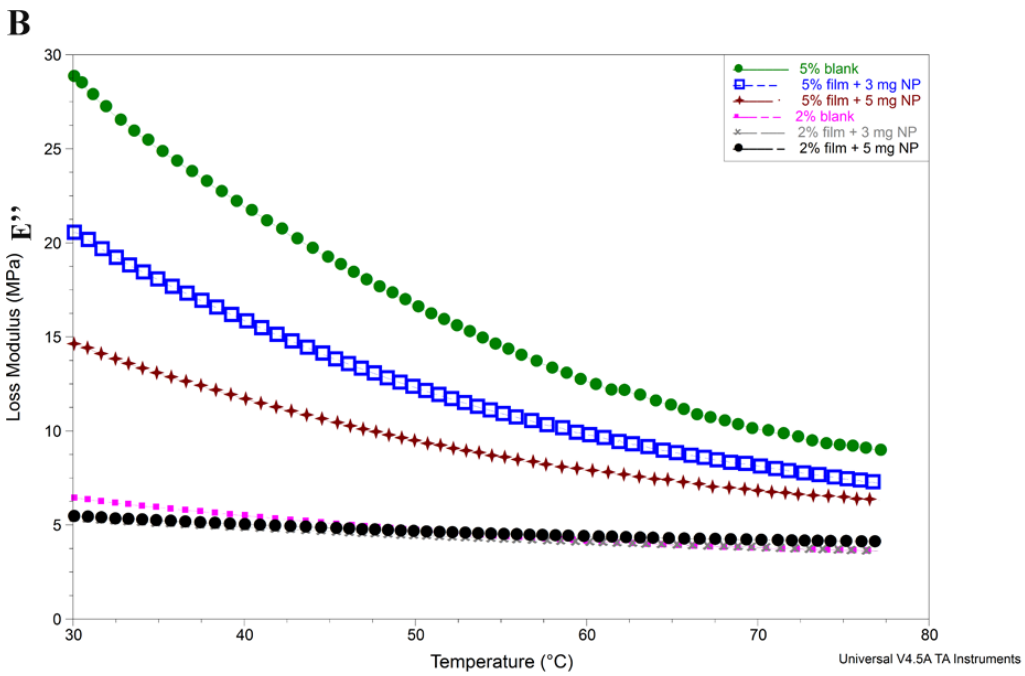
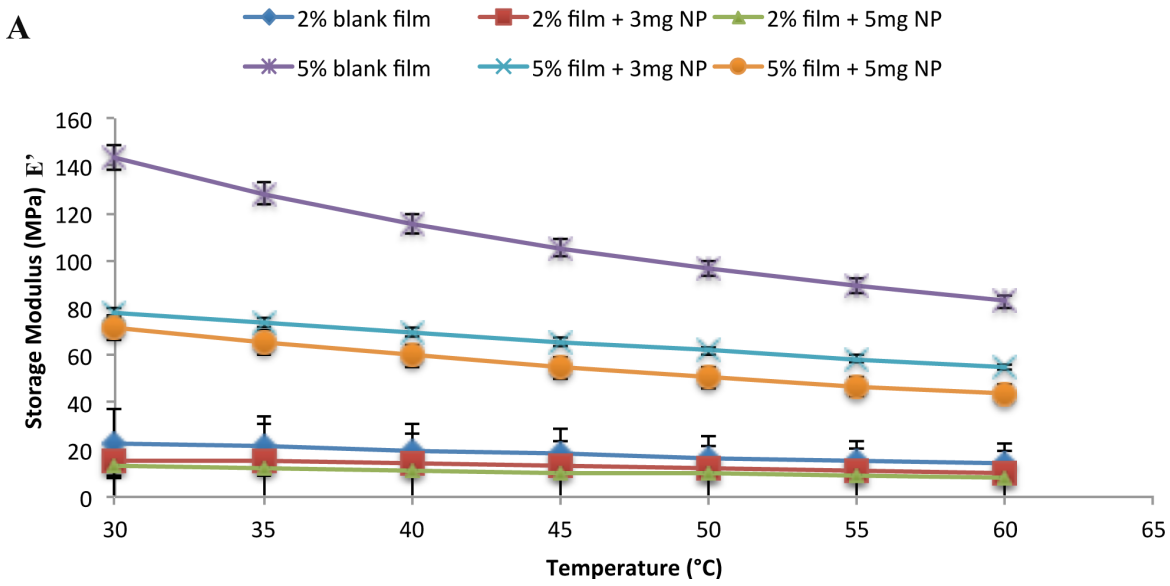


Figure 1. (a) Variation in storage modulus for 2 and 5% PVA blank films or films containing 3mg or 5 mg of NP. Data were plotted using TA Instrument Universal Analysis 2000 software. (b) Variation in loss modulus for 2 and 5% PVA blank films or films containing 3mg or 5 mg of NP. Data were plotted using TA Instrument Universal Analysis 2000 software.



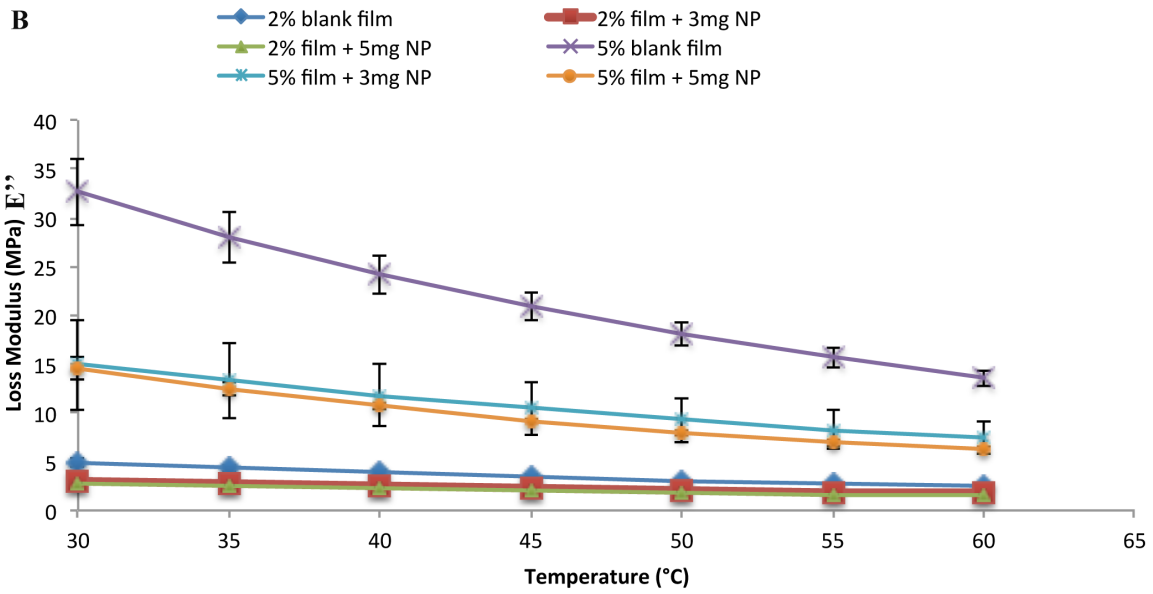


Figure 2. (a) Determination of storage modulus of 2% and 5% PVA films loaded with 3mg or 5 mg NPs. Data represent the mean +/-SD (N=3). (b) Determination of loss modulus of 2% and 5% PVA films loaded with 3mg or 5 mg NPs. Data represent the mean +/-SD (N=3).

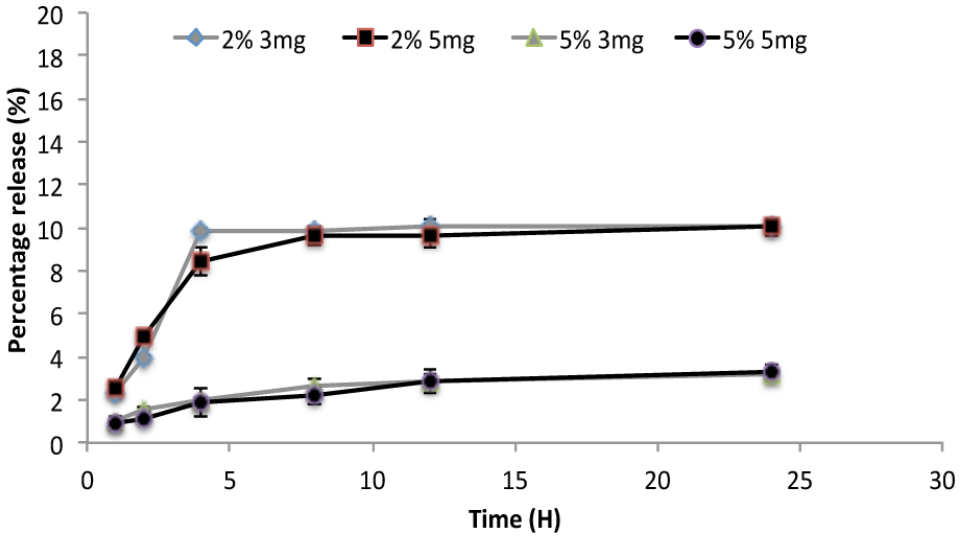


Figure 3. In vitro cumulative release of 2% and 5% PVA films loaded with 3mg or 5 mg fluorescent NPs. Percentage of NPs released. Data represent mean+/-SD (N=3).

215 **Table 1:** Glass transition temperature determined using two different parameters: (A)

216 storage modulus and (B) loss modulus. Data represents mean± SD (N=3).

A)

Glass Transition (°C)	Blank Film	3 mg NP loading	5 mg NP loading
2% PVA film	39.61 ±2.76	53.25±2.86	54.10±7.63
5% PVA film	37.57±0.28	40.05±2.09	42.07±2.21

217

B)

Glass Transition (°C)	Blank Film	3mg NP loading	5mg NP loading
2% PVA film	37.94 ±0.1	36.89±3.48	40.14±4.01
5% PVA film	36.88±1.4	37.81±0.12	37.7±0.32

218

219 **Table 2:** Disintegration time for PVA films.

PVA films	2% blank	2% film + 3mg NP	2% film + 5mg NP	5% blank	5% film + 3mg NP	5% film + 5mg NP
Disintegration time	3h	3h	3h	Partially after 24 h	Partially after 24 h	Partially after 24 h

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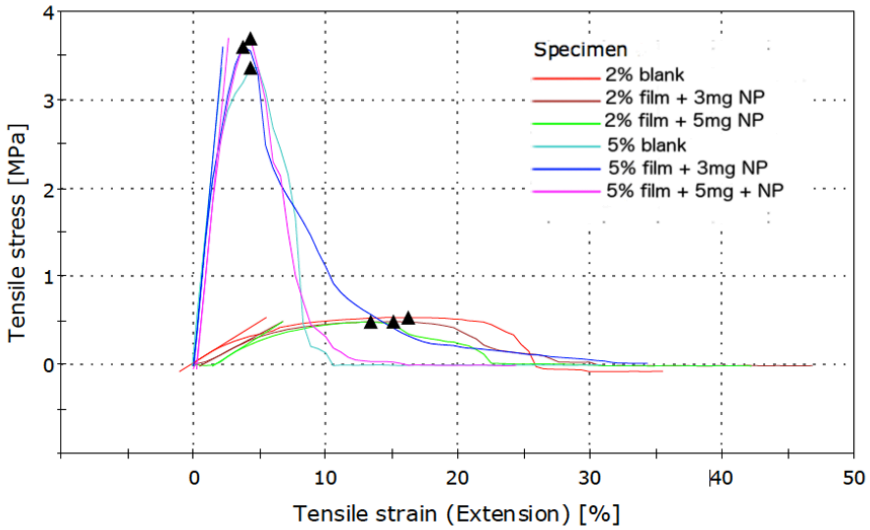

Figure 4. Tensile strain of 2% and 5% PVA films loaded with 3mg or 5 mg of NPs. The onset point shown by the dark triangle, represents the moment the film started to tear.

Table 3: Mechanical properties measured at room temperature for 2% and 5% PVA films loaded with 3mg or 5 mg NPs. Data represents mean± SD (N=3)

	Tensile strain (Extension) at Break (Standard) [%]	Maximum Load [N]	Modulus (Automatic Young's) [MPa]	Tensile strain at Maximum stress [%]	Tenacity at Maximum load [N/tex]
2% blank	36.83±5.03	1.2±0.1	10.5±2.4	13.1±2.78	1.14±0.14
2% film + 3mg NP	41.03±5.36	0.86±0.15	7.36±0.77	16.23±2.5	1.16±0.2
2% film + 5mg NP	41.36±4.92	1±0.2	8.33±1.15	13.8±2.42	1.06±0.05
5% film blank	28.76±1.5	7.23±0.49	128.2±18.37	5.24±0.87	7.68±0.72
5% film + 3mg NP	28.03±5.46	6.1±1.1	153.66±15.97	4.63±0.86	6.61±0.98
5% film + 5mg NP	25.76±2.03	7.16±0.3	133.03±18.94	5.76±2.1	6.52±1.14

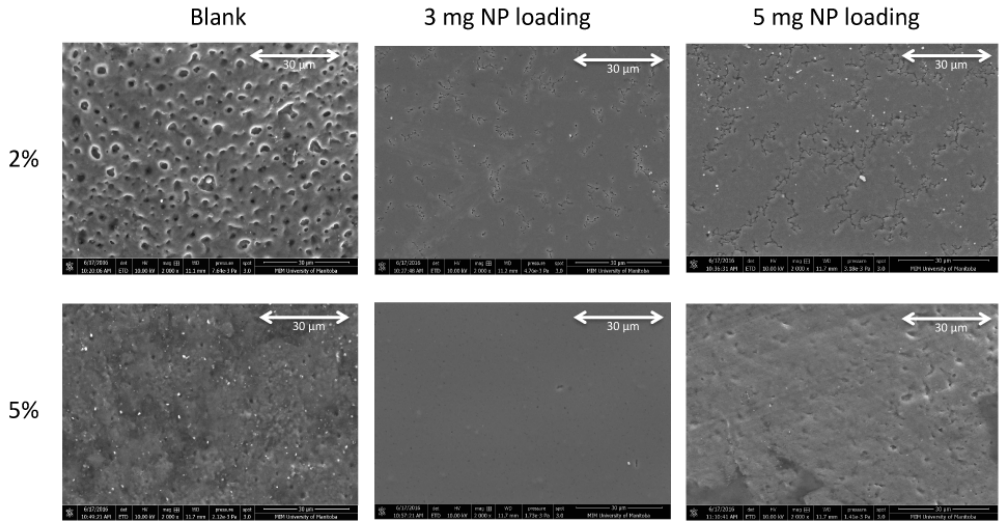

Figure 5. SEM images of 2% and 5% PVA films containing 3mg or 5 mg of NP loading (2000x).

4. Discussion

Vaginal films have been developed for multiple purposes and present many advantages, but the films must be designed in a specific way to be acceptable, usable, and stable[19]. For vaginal films, it must possess certain characteristics so that it will not cause irritation or toxicity, must be flexible enough to be easily handled by the patient and more importantly, must allow the active compound to be released at a given time frame for a therapeutic effect. In this study, we determined the mechanical properties of PVA films containing NPs as previously developed[4] . The film formulation using PVA and two different plasticisers (PEG 400 and glycerol) produce films that are smooth and slightly transparent. Different percentage of PVA produced different film thickness, which can play an important role in the thermodynamic properties of a film. In this study, we maintained the quantity of the plasticisers constant and changed the amount of PVA or NP loading and evaluated its effects on the mechanical properties of the films. The disintegration study presented in Table 2 showed that the 2% PVA film formulation broke down completely in 3 h. The amount of NPs loaded into the films appeared to have no impact on the rate of

disintegration but in contrast, the ratio of PVA/plasticiser used had an influence. Jun-Seo et al (2000), reported that the plasticising effect of PEG400 on PVA will make the film more flexible, and disintegrate more easily[8]. The film formulation containing 5% PVA was composed of a lower ratio of plasticiser/PVA. These films were thicker, can swell and partially break down after 24 h. A release study using fluorescent NP as presented in Fig 3 correlated with the disintegration study. The 2% film formulation, which disintegrated faster, showed the highest concentration of NPs released after 4 h. This release increased slowly until 8 h and remained constant until 24 h. The higher NP released represent a very low percentage which can probably be explained by a rapid decay of the fluorophore used to fabricate the commercial PLGA NP during the film fabrication process and the release study. Films loaded with 5mg of NP exhibited higher release of NP compared to 2% and 5% PVA films loaded with 3mg NP. Films formulated using 5% PVA exhibited a lower NP release profile which appears to be related to the slower disintegration rate in comparison to 2% films. To further analyze the mechanical properties of the films, we used DMA to evaluate the storage modulus, which is a measurement of the elastic response of a material, and the loss modulus, which is the measurement of the viscous response of a material. Storage modulus and loss modulus are two parameters that we used to determine the transition temperature of the films. TA Instrument Universal Analysis 2000 software is used to integrate all curves in order to determine the glass transition derived from the storage and loss modulus. From looking at Fig 1A, in addition to plasticizers that increasing the mobility of polymer chains[20], the presence of NPs in the film matrix affected the physical properties of the film possibly by reducing intermolecular forces[21]. This can decrease the storage modulus of the film and also affect the glass transition temperature as described by Ding et al.[20]. Less plasticizer gave a higher storage modulus as seen in with the 5% PVA film formulation compared to 2%. Based on the results, it appears that as we

incorporate more PLGA NPs into the matrix of PVA, it will tend to make the films less stiff. We also evaluated the loss modulus, which measures the energy dissipated as heat in order to determine the glass transition temperature of the polymeric film. Glass transition temperature determined using the storage modulus present a transition state of blank films around 40°C. This transition state remained constant for 3 mg and 5 mg NP film loading in 5% PVA film formulation. A small increase in glass transition temperature was observed for 2% PVA films loaded with 3 and 5 mg of NPs, which is around 50°C. This means that the 2 % PVA films loaded with NPs became more amorphous (semi-crystalline polymer) at a slightly higher temperature than 5% films containing the same loading of NPs. This might be due to the restriction of the NPs on the mobility of matrix segments of the film[22]. We also determined the glass transition temperature using the loss modulus. All the transition temperatures using the loss modulus occurred around 38°C (Fig 2B). There was no significant difference between 2% and 5% PVA film formulations, either loaded with or without NPs. The amount of NPs loaded in the films did not have a significant impact on the glass transition temperature range. This shows that a transition takes place within the 38-50°C temperature range. A wide temperature range was chosen (up to 80 ºC) to perform the thermal studies to mimic extreme conditions in which the film may potentially be subjected to e.g. countries with elevated ambient temperatures and inside a parked car where the temperature can rise up to 80 ºC[23]. Those conditions will affect the film stability and in some cases render them unusable. When looking at the mechanical properties of the films, 2% films, which are very elastic due the ratio of plasticizer present, were very fragile and broke at a load of 1 N. On the other hand, 5% films were less elastic because of the lower ratio of plasticizer but more rigid, so they break at a load of around 7 N. Compared to 2 % films, they supported a load higher than 6.5 N before breaking. The presence of NPs in the matrix of the films appeared to have no major effects on their elasticity. As revealed in

300 the SEM images (Fig 5), the blank 2% PVA films appeared to have a more porous structure
301 while 2 mg and 5 mg NP loaded films did not display this property. The porous structures,
302 as explained by Ping et al., are probably due to the shrinkage of the film due to rapid water
303 evaporation while exposed to heat during the film formation[24]. They further explained that
304 under heat, the molecular chains of PVA are inclined to rearrange to form crystalline
305 regions. This may also explain why NP loaded films do not reveal obvious pores since the
306 presence of the NPs in the polymer structure will interact with PVA molecules to prevent
307 shrinkage. The porous morphology is less obvious in 5% films since the PVA ratio is
308 higher but some small pores are visible. Vaginal Contraceptive Film® (VCF) (Apothecus
309 Pharmaceutical) is a commercially available PVA based filmed containing glycerine as a
310 plasticiser and nonoxynol-9 as a spermicide[25]. This film has been reported to have a
311 relatively hard texture and having sharp edges[26]. Those sharp edges and hard texture can
312 potentially cause trauma to the female genital tract before the film is dissolved. In an effort
313 to make our product softer and more pleasant for the user, we added PEG400 to supplement
314 glycerol. The VCF film on the other hand exhibits faster dissolution (data not shown)
315 compared to our formulation, which suggest that the viscoelasticity of PEG400 affects the
316 film disintegration time.

317 **5. Conclusion**

318 The present study focused on the viscoelastic and mechanical behaviour of PVA films
319 formulated along with NP for potential applications in vaginal drug delivery. The amount
320 of PVA in the film formulation plays an important role in film disintegration and the
321 release of the NPs. The presence of NPs in the film matrix did not significantly alter the
322 film's mechanical behaviour. On the other hand, presence of plasticisers such as PEG 400
323 and glycerol enhanced the film's flexibility, and played an important role in the rate of film

disintegration and the rate of NP release from the films. Hence, film formulations can be customized for the controlled release of NPs with the desired viscoelastic and mechanical properties suitable for vaginal drug delivery.

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