

2018

## Evaluating alternative crosslinking agents in poly(vinyl alcohol) hydrogels membranes

Naomi Deneke

Sarah Dohadwala

Quincy Moore

*Prairie View A&M University*

Felecia Nave

Audie Thompson

Follow this and additional works at: <https://digitalcommons.pvamu.edu/pursue>

---

### Recommended Citation

Deneke, N., Dohadwala, S., Moore, Q., Nave, F., & Thompson, A. (2018). Evaluating alternative crosslinking agents in poly(vinyl alcohol) hydrogels membranes. *Pursue: Undergraduate Research Journal*, 1(2). Retrieved from <https://digitalcommons.pvamu.edu/pursue/vol1/iss2/5>

This Article is brought to you for free and open access by Digital Commons @PVAMU. It has been accepted for inclusion in Pursue: Undergraduate Research Journal by an authorized editor of Digital Commons @PVAMU. For more information, please contact [hvkoshy@pvamu.edu](mailto:hvkoshy@pvamu.edu).

## Evaluating alternative crosslinking agents in poly(vinyl alcohol) hydrogels membranes

Naomi Deneke<sup>1</sup>, Sarah Dohadwala<sup>3</sup>, Quincy Moore<sup>2</sup>, Felecia Nave<sup>1</sup>, Audie Thompson<sup>1\*</sup>

<sup>1</sup>Department of Chemical Engineering, Prairie View A&M University, Prairie View, Texas; <sup>2</sup>Department of Biology, Prairie View A&M University, Prairie View, Texas  
<sup>3</sup>Seven Lakes High School, Katy, TX

Corresponding author: Audie Thompson, Tel: +1 936 261 9411; fax: +1 936 261 9417; [audiekthomposn@gmail.com](mailto:audiekthomposn@gmail.com)

### Abstract

Hydrogels are a network of polymer chains with properties that absorb, store and transport solutions. A hydrogel membrane has a permeability that allows influx and excretion. Therefore, it is the ideal material for medicated membranes. This study investigates the crosslinking of poly(vinyl alcohol) (PVA) hydrogel membranes using different agents and explores the usability of the candidate membranes as drug delivery systems. The model protein, bovine albumin serum (BSA), was used to test the stability and controlled drug release rate characteristics of the candidate hydrogel membranes. This investigation also evaluated the stability different crosslinkers for hydrogel membranes. Glutaraldehyde (GA) and an alternative crosslinking method of ultraviolet irradiation with the sensitizer, sodium benzoate (SB), were used to crosslink PVA containing BSA. In GA crosslinked membranes, BSA release diffusion experiments showed 48%, 45%, and 63% recovery of protein at pH 6.5, 7.4 and 8.0, respectively; this confirmed that this system is suited for physiological conditions and controlled release. Although SB has been used for membrane fabrication, our Fourier Transform Infrared Spectroscopy (FTIR) and Thermogravimetric Analysis (TGA) results indicate that UV(SB)-crosslinked films are not suited for drug delivery, despite the release of BSA.

**Keywords:** poly(vinyl alcohol), hydrogels, sodium benzoate, glutaraldehyde, cross-linking agents, ocular drug delivery

## Introduction

Over 30 years ago, polymeric hydrogels were introduced to the field of drug delivery and had been used ever since (Umesh K. Parida, 2011; Yang & Su, 2011). Hydrogel membrane micromatrices technologies have been developed for a number of applications. They have become very popular for use in treating diseases because their unique properties allow for the slow and sustained release of drugs through a porous surface (Umesh K. Parida, 2011; Yang & Su, 2011). Hydrogels are a hydrophilic polymeric network that can be cross-linked using a physical or chemical process to create a three-dimensional, porous matrix that can absorb aqueous solutions (Kamoun, Chen, Mohy Eldin, & Kenawy, 2015; Kiani & Asempour, 2012; Prabhu, Dubey, Parth, & Ghate, 2015).

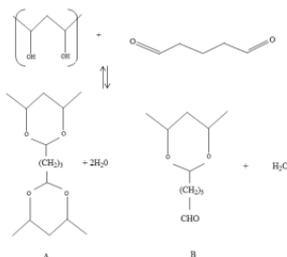
Hydrogels are hydrophilic biomaterials that can be composed of a variety of polymers, allowing for a range of chemical and physical properties (Mishra, Majeed, & Banthia, 2011; Prabhu et al., 2015; Yang & Su, 2011). Poly(vinyl alcohol) (PVA) hydrogels are an ideal material for drug delivery systems, because they are a linear hydrophilic polymer that is nontoxic and biocompatible (Kamoun et al., 2015; Mishra et al., 2011; Umesh K. Parida, 2011). Hydrogel membranes are fabricated by the chemical crosslinking of polymer chains using glutaraldehyde (GA) through intra/intermolecular interaction via hydrogen bonding. Therefore, medications can be incorporated into the porous structure of the membrane (Figueiredo, Alves, & Borges, 2009; Prabhu et al., 2015). The release rate is controlled through the permeability of the matrix (Lin & Anseth, 2009). A PVA hydrogel membrane drug delivery system that is applied topically has the advantage of bypassing the first-pass metabolism (Kamoun et al., 2015; Kiani & Asempour, 2012). Another advantage is the reduction in toxic symptoms or side effects, because the drug can be withdrawn by simply removing the membrane. As the hydrogel membrane allows for the slow and gradual release of the protein drug, this in turn allows the reduction of the overall dosage amount.

Crosslinking is the process that bonds polymer chains together to create a network mesh. The density of the crosslinking can

manipulated through different fabrication techniques (Hu et al., 2011; Kamoun et al., 2015; Sirousazar, Kokabi, & Hassan, 2012) to change the rate of drug release via the various sizes of pores. Thus, the network transiently houses small molecules that will easily diffuse out upon direct contact with a solution (Hsu, Fentzke, & Chauhan, 2013; Sittiwong, Niamlang, Paradee, & Sirivat, 2012). Utilizing different water-soluble polymers and cross linkers have paramount importance when researching the development of drug delivery patches and contact lenses.

Casting techniques are important to the fabrication of hydrogels, and depending on the method, can alter the structure of the pores. Throughout literature, different casting methods (such as freeze-dry method, autoclaving, freestanding solvent casting, UV photo polymerization and spin coater method) have been used (Mishra et al., 2011; Prabhu et al., 2015; Umesh K. Parida, 2011). In this research, PVA hydrogels were created via two different crosslinking methodologies and were utilized to fabricate hydrogel membranes loaded with model proteins to resemble a drug-loaded matrix.

This first methodology used GA to crosslink PVA by abstracting a proton from the hydroxyl group of PVA and forming an acetyl bridge between the two chains as shown in Figure 1 (Figueiredo et al., 2009).



**Figure 1.** Mechanism of the reaction between poly(vinyl alcohol) and glutaraldehyde.

Acidic catalysts increase the speed of the reaction, which makes abstracting the proton more favorable. Despite the wide usage of GA as a crosslinker (Figueiredo et al., 2009; Nave, Luo, & Coleman, 2008), it has been shown to cause respiratory and skin irritation from extended/repeated exposure, even at concentrations as low as 0.05 ppm (Endo, 2006). After membrane fabrication, a rinse is used to remove any remaining or unused GA. Therefore, GA is not a part of the final product.

The ocular environment is an immunologically privilege area which makes is highly sensitive to toxins. To use PVA-GA membranes in ocular environments, it is important to investigate whether the membrane will elicit an immune response. Human Corneal Epithelial cells (HCECs) cells were chosen due to their lack of defense mechanisms to danger. A PVA hydrogel membrane placed in HCEC was used to determine if the membrane alone would elicit an immune response. In other words, would the human cells recognize the membrane as harmful?

Photopolymerization uses a light source to create networks in drug delivery membrane matrices (Baroli, 2006; Mishra et al., 2011). UV polymerization is a viable alternative to GA crosslinking (J. Delville, 2002) and PVA has been shown to crosslink with UV irradiation in the presence of a sensitizer, sodium benzoate (Miranda, Gonçalves, & Amorim, 2001). Sodium benzoate's mechanism involves radical formation, but the specific pathway has not been elucidated. However, possible reaction pathways are proposed by Miranda et al. (Miranda et al., 2001), and the mechanism likely involves the splitting of the benzoate ring (Wittaya, 2012). This research seeks to evaluate whether UV-SB crosslinking is a feasible alternative to GA crosslinking by investigating the protein release behavior of BSA.

## Methods

### *Fabrication of GA-crosslinked membranes*

A solution of 12.5% by mass PVA was prepared by mixing 5 g of solid PVA in 40 mL of deionized water, while the water was heated and stirred continuously. 4.0 mg/mL BSA stock was prepared by

adding 1.0 g of crystallized protein to 250 mL sodium phosphate buffer at pH 7.4. To prepare the membranes, 0.960 mL PVA was added to 0.250 mL BSA, and the mixture was left to stir for approximately 5 minutes. Next, 0.218 mL of 10% sulfuric acid was added as a catalyst, 0.036 mL of 10% acetic acid was added as a buffer and 0.036 mL of 50% methanol as a quencher. Then, the cross-linker, 0.108 mL of 1% by mass GA, was added and stirred for 20 seconds. Finally, the solution was poured onto a petri dish, left to set for 15 minutes, and placed on a spin coater with increasing rpm to complete membrane fabrication. Lastly, the membrane was allowed to air dry overnight. The membrane was dislodged using deionized (DI) water.

#### *Fabrication of Ultraviolet (UV) Sodium Benzoate (SB)-crosslinked membranes*

Stock solutions of PVA and BSA were prepared as previously detailed above. The stock of SB was prepared by mixing 0.3 g solid SB in 50 mL of 30 mmol sodium phosphate buffer at pH 7. Three types of UV(SB)-crosslinked membranes were fabricated: (1) membranes lacking protein by mixing 0.750 mL of 12.5% PVA with 0.750 mL of 0.6% SB; (2) membranes with a 0.63 mg/mL concentration of protein by mixing 0.250 mL of BSA stock with 0.625 mL of PVA; and (3) 0.625 mL of SB stock. Membranes with a concentration of 1.26 mg/mL protein in the membrane were prepared by mixing 0.500 mL of protein, cross-linker, and polymer. These solutions were poured into 50 mL beakers or watch glasses (approximate diameter of membranes formed: 3.5 cm) and dried in an oven at 150 °C for approximately 2.5 hours. After drying, they were removed from the glass and placed under UV light at 254 nm for 1.5 hours.

#### *Attenuated Total Reflectance- Fourier Transform Infrared (ATR-FTIR)*

For FTIR analyses, the membranes were dried at 150 °C for approximately 2 hours. The UV(SB) membranes were dry upon formation and were used without additional heating. This technique was used to determine functional groups, such as hydroxyl groups present within the cross-linked membrane matrix.

### *Equilibrium Swelling*

Membranes were left in 20 mmol sodium phosphate buffer at pH 6.5, 7.4, and 8 for 24 hours. After equilibrating in solution, their weight was measured. Then, the samples were placed in an oven at 150 °C for approximately 2 hours to dry. The dry weight was measured, and the equilibrium swelling value was calculated as  $(\text{wet}_{\text{weight}} - \text{dry}_{\text{weight}})/\text{wet}_{\text{weight}}$ . From there, the mesh size and crosslinking density were calculated as described by Matsuyama (Hideto Matsuyama, 1997).

### *Thermogravimetric Analysis (TGA)*

The thermal properties and stability was analyzed through Thermogravimetric Analysis (TGA). Samples were weighed in an analytical balance, and the TGA was tared with the aluminum crucible. The pre-weighed sample was then placed in the crucible, and into the TGA. The TGA analyzes the samples from 30 to 575 °C at a rate of 10 °C/min.

### *Release Experiments*

BSA was used as the model protein to investigate the release profile. BSA is a 65 kD protein that has been used in research for drug delivery models (Censi, Di Martino, Vermonden, & Hennink, 2012; Kamoun et al., 2015). The release of protein was observed in 20 mL buffer solution either with (1) membrane sandwich between diffusion cell with only one side exposed to buffer or (2) membrane submerged in 20 mmol sodium phosphate buffer at pH 6.5, 7.4 and 8.0. The temperature was kept constant at 37 °C using a water bath, and samples were shaken at 83 rpm.

### *Quantitative Measurement of Release in GA membranes*

For the GA-crosslinked membranes, approximately 1 mL samples were taken out to be tested and then returned to the solution. Absorbance was measured at 279 nm to determine the concentration of BSA, and at 399 nm to establish a baseline measurement. The two readings were subtracted for the final absorbance used to calculate the concentration. The percentage of the protein mass recovered in solution was calculated with the Lambert-Beer Law.

*Qualitative Measurement of Release in UV(SB) Membranes*

In the UV(SB)-crosslinked membranes, absorbance measurements could not be used to calculate concentration due to the interference of sodium benzoate's absorption at 279 nm. Instead, membranes were removed from the buffer solution, and the time removed was noted. Then, the samples were concentrated using 20 mL (Corning) Spin X Concentrator centrifuge tubes, running the centrifuge for 15 minutes at 5000 rcf for three cycles until they were concentrated to approximately 500  $\mu$ L. The concentrating tubes were rinsed with DI water, for 15 minutes at 5000 (rcf) between samples. Then, the concentrated solutions were moved to Eppendorf tubes, and mixed with an equal volume of 10% trichloroacetic acid (TCA) to precipitate the protein; these samples were run in a micro-centrifuge for 25 minutes at 10 rpm. After that cycle, all liquid was removed from the tube, without disturbing the protein pellet, and 400  $\mu$ L of 0.3 M Tris buffer was added to the tube to rinse off the TCA; this mixture was run in the centrifuge for two minutes at 10 rpm. Finally, the Tris buffer was removed from the tube, and 50  $\mu$ L of loading buffer was added. These were spun for approximately 30 seconds at 10 rpm to ensure that the protein pellet was mixed with the buffer. The SDS PAGE gel was run for approximately one hour, at 120V, until the loading buffer reached the bottom of the gel. Afterwards, the gel was stained with comammassie stain for approximately 30 minutes. If necessary, the gel was destained by heating with 5% acetic acid until boiling, then rinsing with DI water thrice. The gel was then photographed using a lightbox.

*Human Corneal Epithelial Cells*

HCECs were purchased from Invitrogen and maintained in keratinocyte media supplemented with 0.2% v/v bovine pituitary extract (BPE), 1  $\mu$ g/mL recombinant human insulin-like growth factor-I, 0.18  $\mu$ g/mL hydrocortisone, 5  $\mu$ g/mL bovine transferrin and 0.2 ng/ml human epidermal growth factor. HCECs were grown to confluency and were introduced to the hydrogel without protein, for 24 hours in separate experiments. The supernatant was collected to use in an enzyme linked immunosorbent assay (ELISA).

### ELISA

HCEC exposed supernatants were analyzed with specific ELISA kits: Interleukin-6 (IL6), Interleukin-8 (IL8), Interferon gamma (IFN- $\gamma$ ), Tumor necrosis factor alpha (TNF- $\alpha$ ), Interleukin-12 (IL-12) P40, Interleukin-12 (IL-12) P70, Interleukin-16 (IL-16) and GRO- $\alpha$  (Ray Biotech). The homogenates were incubated in the ELISA plate at 4 °C overnight, which was followed by a wash according to the manufacturer's protocol. Diluted biotin-conjugated antibodies specific to the cytokine being evaluated were added to each well and allowed to incubate at 4 °C overnight. Wells were washed and incubated at 25 °C for 45 min with diluted horseradish peroxidase-conjugated streptavidin. The wells were washed, and 100  $\mu$ l of TMB One-Step Substrate Reagent was added to each well and allowed to incubate at room temperature for 30 min. The reaction was stopped by a solution and read at 450 nm immediately with the Biotech (Synergy HT) plate reader at 450 nm for ELISA analysis.

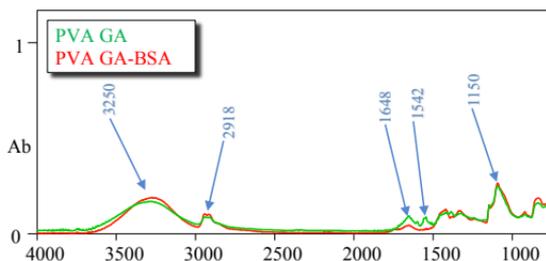
## Results and Discussion

### FTIR Studies

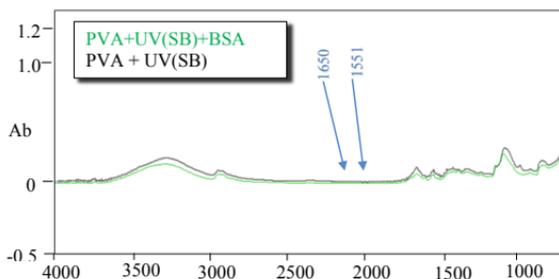
FTIR spectra of the GA-crosslinked membranes in comparison to the spectrum for pure PVA showed a reduced hydroxyl peak at 3330 – 3350 1/cm, shown in Figure 2. Furthermore, the C-H stretch peak at around 2800 1/cm, is reduced in both spectra. FTIR spectra of the UV(SB)-crosslinked membranes also showed a reduced hydroxyl peak. The UV(SB) membranes also showed new peaks at 1551 1/cm (aromatic stretch) and 1650 1/cm (alkenes) as shown in Figure 2a. Spectra of the GA-crosslinked membranes confirm the presence of BSA and crosslinking in the gel. Furthermore, visible peaks at 1648 1/cm and 1542 1/cm are indicative of amide I and amide II peaks in the gels containing protein confirmed protein integration (Figure 2a).

For UV(SB)-crosslinked membranes, crosslinking was not seen as dramatically; the characteristic duplet was reduced (Figure 2b). Wittaya (Wittaya, 2012) suggested various pathways of the SB reaction with UV light, and Miranda et al. suggested that ring-opening of the benzoate is probable (Miranda et al., 2001). However, the FTIR spectra obtained from UV(SB) membranes were unable to confirm either pathway – peaks at 1650 suggest that the aromatic

ring did not open up, and peaks at 1551 suggest that the ring was opened during radical formation. Furthermore, absorbance at 220 nm indicated the presence of SB in the release solution, confirming that some of the sensitizer was not consumed in the reaction. Thus, the crosslinking was incomplete, and SB remained intact. More irradiation time has shown the decomposition of the sodium benzoate (Miranda et al., 2001) and more crosslinking may have occurred.



**Figure 2a.** FTIR spectra comparison of poly (vinyl alcohol) membrane (PVA) crosslinked with glutaraldehyde (GA) without protein and poly (vinyl alcohol) (PVA) crosslinked with glutaraldehyde (GA) with protein,BSA membrane.

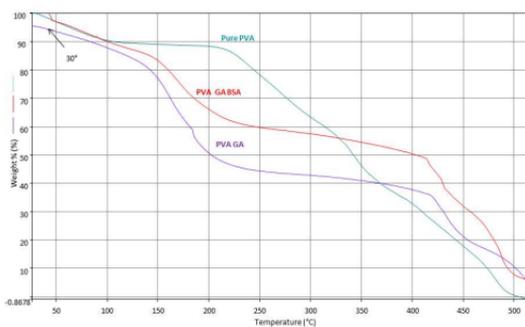


**Figure 2b.** FTIR spectra comparison of poly (vinyl alcohol) PVA UV crosslinked with sodium benzoate (SB) as sensitizer without protein and poly (vinyl alcohol) PVA UV crosslinked with sodium benzoate (SB) as sensitizer with protein, BSA.

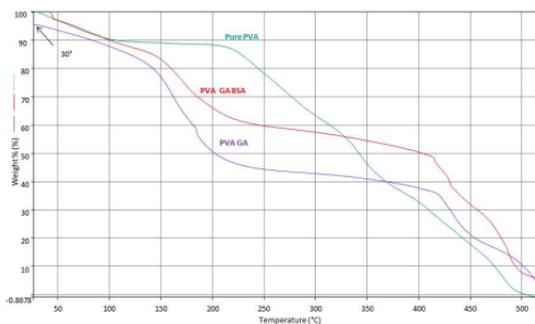
*Thermogravimetric Analysis (TGA)*

TGA results, seen in Figure 3a, of glutaraldehyde (GA)-crosslinked membranes show a gradual change in mass over temperature change, as compared to pure PVA. Results of UV-crosslinked with sensitizer, sodium benzoate (SB), membranes, seen in Figure 3b, show that UV(SB) films have similar rates of mass change as pure PVA. However, TGA curves for UV (SB)-crosslinked membranes indicated rapid thermal degradation under heat. This decreased stability is likely due to the inconsistent and random crosslinking of the polymer found in UV (SB)-crosslinked films.

a)



b)



**Figure 3.** Thermogravimetric analysis results of polyvinyl alcohol chemically crosslinked with glutaraldehyde (GA) (a) and UV crosslinked with sensitizer, sodium benzoate (SB) (b) incorporating model protein, bovine serum albumin.

*Equilibrium Swelling (ES)*

The values in Tables 1 and 2 are for both sets of membranes and reveal the low standard deviations calculated, assuring the consistency in the results. For all pH values, the GA-crosslinked membranes showed an average swelling percentage of 86.5%, while the UV(SB) membranes showed an average of 73.8%. The GA membranes had larger mesh sizes and lower crosslink densities in comparison to the UV(SB) films, indicating that the UV(SB) can load less protein in each pore than the GA gels. Similar values were found among protein concentrations of 0.63 mg/ml and 1.26 mg/ml in the UV(SB) membranes, suggesting that doubling the protein loading does not affect mesh size.

**Table 1.** Equilibrium swelling of poly (vinyl alcohol) (PVA) crosslinked with glutaraldehyde (GA) membranes with or without protein, bovine serum albumin (BSA).

pH	Swelling	Mesh Size (Å)
12.5 % PVA, 1 % GA, 0.63 mg/mL BSA		
6.5	86.0±0.001%	155
7.4	90.5±0.007%	155
8.0	85.7±0.036%	290
12.5 % PVA, 1 % GA, No BSA		
6.5	87.4±1.60%	212
7.4	89.0±0.21%	148
8.0	82.2±0.03%	108

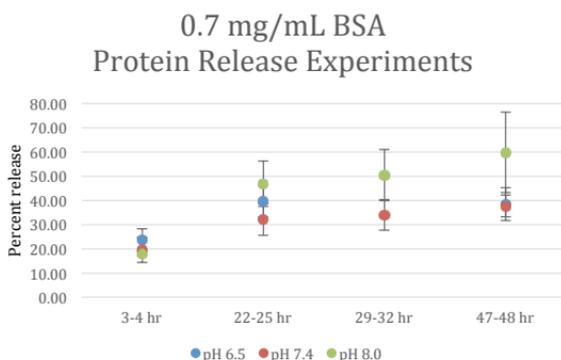
**Table 2.** Equilibrium swelling of poly (vinyl alcohol) (PVA) UV crosslinked with sensitizer, sodium benzoate (SB), membranes with and without protein, bovine serum albumin (BSA).

pH	Swelling	Mesh Size (Å)
12.5 % PVA, 0.6 % SB, 0.63 mg/mL BSA		
6.5	70.9±0.005%	41
7.4	74.0±0.009%	53
8.0	73.1±0.022%	48
12.5 % PVA, 0.6 % SB, 1.26 mg/mL BSA		
6.5	76.8±0.030%	66
7.4	75.3±0.022%	57
8.0	71.7±0.052%	46
12.5 % PVA, 0.6 % SB, No BSA		
6.5	75.8±0.017%	59
7.4	72.2±0.004%	45
8.0	75.0±0.018%	56

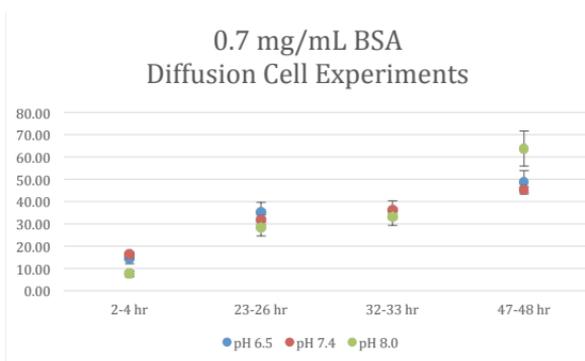
### Release Studies

Release kinetics were evaluated using Higuchi's equation and protein percent release over 48 hours (Siepmann & Peppas, 2011). Protein concentration taken at various time points are used to calculate the amount of protein diffused with only one side of the membrane exposed to buffer or protein release with the membrane submerged in buffer. The BSA release resulted in a curve graph indicating a gradually for both types of experiments as shown in Figures 4 and 5. The diffusion experiments showed averages of 48%, 45%, and 63% percent recovery of protein for GA-crosslinked membranes at pH 6.5, 7.4, and 8.0 respectively, as seen in Figure 4. The release experiments showed slightly lower averages of 36%,

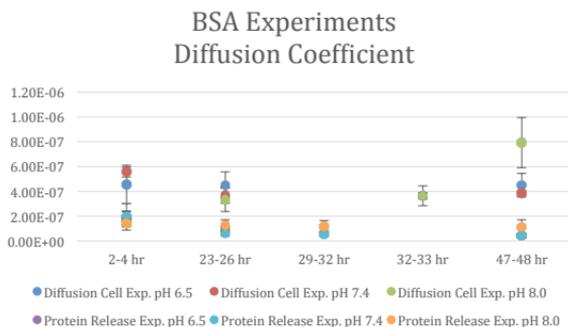
38%, and 60% percent recovery of protein for GA-crosslinked membranes at pH 6.5, 7.4, and 8.0 respectively, as seen in Figure 5. The diffusion coefficients for all pH values demonstrate a decreased rate during the 32 hour period and showed a burst afterwards. The concentration gradient is the driving force of the initial release. As the protein leave unoccupied pores, other protein will occupy these spaces before releasing from the protein. Resulting in a rate increase as the BSA protein moved out seen in Figure 6. The protein release experiments did not show an increase after 32 hours due to both faces being exposed to buffer. Therefore, the rate has decay due to the protein hovering around the surface of the membrane and interrupting the concentration gradient. SDS-PAGE was used to confirm BSA release from the UV(SB) membranes. The release SDS-PAGE, depicted in Figure 7, shows the presence of protein between 40 – 75 kD markers confirming that BSA (65 kD) was detected. Furthermore, the protein samples were taken at various time points over 24 hours, and the gel demonstrates that protein was released (detected) over time. Though UV(SB) films gradually release protein, as confirmed by the gel, they were unstable in the buffer, and the crosslinking appeared to be incomplete as indicated by FTIR. Some membranes became semi-solid during the 24-27 hour experiment.



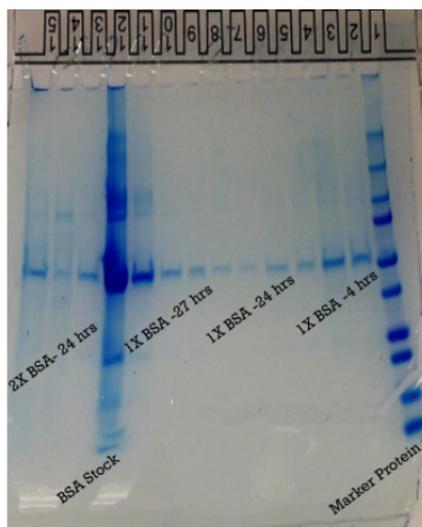
**Figure 4.** Bovine Serum Albumin (BSA) release experiments at various pHs from poly (vinyl alcohol) crosslinked with glutaraldehyde (GA) membranes.



**Figure 5:** Bovine Serum Albumin (BSA) diffusion experiments at various pHs from poly (vinyl alcohol) crosslinked with glutaraldehyde (GA) membranes.



**Figure 6.** Bovine Serum Albumin (BSA) diffusion coefficient for both release and diffusion experiments at various pHs from poly (vinyl alcohol) crosslinked with glutaraldehyde (GA) membranes.



**Figure 7.** Poly (vinyl alcohol) PVA UV crosslinked with sodium benzoate (SB) Release SDS-PAGE at various times.

BSA release experiments confirmed the gradual release of the protein over a 48-hour period with GA-crosslinked membranes. The protein release rate demonstrates a plateau due to the migration of protein to the exposed surface of the membrane after the amount at the surface was exhausted. Though UV(SB) films gradually released protein as confirmed by the SDS-PAGE, they were unstable in water, and the crosslinking appeared to be incomplete. Some membranes began to disintegrate while in the buffer, thus, indicating the instability of the UV(SB) membranes for drug delivery at 37°C.

### *Cytokines ELISA*

Cytokine ELISA was utilized to investigate host immune factors that are involved in the ocular environment when the hydrogel is present. The immune response was investigated utilizing specific ELISA assays of cytokines present during a bacterial infection. The corneal homogenates collected at post-exposure to bacteria were analyzed using human inflammatory cytokine arrays. The cytokine difference relative to the media and hydrogel membrane was determined and

listed in Table 3. The results demonstrated no contrasts in the cytokine expression profiles in the corneal homogenates collected from exposure to media alone and hydrogel.

**Table 3.** Cytokine profiles of supernatants from human corneal epithelial cells exposed hydrogel.

Sample	INF-gamma	IL-12 P40	IL-12 P70	IL-16	IL-6	Gro-alpha
Control	0.055±0.007	0.311±0.066	0.254±0.068	0.436±0.091	0.167±0.072	0.048±0.0033
Hydrogel membrane	0.062±0.006	0.291±0.056	0.231±0.043	0.410±0.216	0.151±0.059	0.046±0.0004

Numbers are expressed as Mean ± Standard Error of the Mean (SEM)

## Conclusion

The GA-crosslinked membranes were far more suitable for drug delivery than the UV(SB)-crosslinked membranes, which were unstable. The GA-crosslinked membranes retained their structural integrity in release buffer, while the UV (SB)-crosslinked membranes did not. They degraded in physiological temperatures, showed rapid mass changes in TGA, and the FTIR was unable to confirm crosslinking in the UV(SB) membranes. Overall, though UV(SB) was able to partially crosslink the membranes; it did not do so to the same degree as GA. However, UV(SB) crosslinking may still retain potential in other areas of drug delivery, such as in skin patches with longer irradiation and additional freeze/drying cycles. The results from this study give insight into the minimum presence of cytokines, which are indicators of an innate immune response. Based on these findings, the presence of the hydrogel will not trigger a human immune response that could potentially hinder the action of the drug therapy. This study provides the groundwork for applications of this hydrogel membrane delivery system in various infection models, specifically ocular models.

## Acknowledgements

This research was supported by the Research Experience for High School (REH) Program at Prairie View A&M University. Mr. Tony Grady and the Chemistry Department for the use and assistance with the Thermogravimetric Analysis.

## References

- Baroli, B. (2006). Photopolymerization of biomaterials: issues and potentialities in drug delivery, tissue engineering, and cell encapsulation applications. *Journal of Chemical Technology & Biotechnology*, 81(4), 491-499. doi:10.1002/jctb.1468
- Censi, R., Di Martino, P., Vermonden, T., & Hennink, W. E. (2012). Hydrogels for protein delivery in tissue engineering. *J Control Release*, 161(2), 680-692. doi:10.1016/j.jconrel.2012.03.002
- Endo, T. T. a. Y. (2006). Effects of Glutaraldehyde Exposure on Human Health. *J Occup Health*, 48, 75-87.
- Figueiredo, K. C. S., Alves, T. L. M., & Borges, C. P. (2009). Poly(vinyl alcohol) films crosslinked by glutaraldehyde under mild conditions. *Journal of Applied Polymer Science*, 111(6), 3074-3080. doi:10.1002/app.29263
- Hideto Matsuyama, M. T., Hiroshi Urano. (1997). Analysis of solute diffusion in poly(vinyl alcohol) hydrogel membrane. *Journal of Membrane Science*, 126, 151-160.
- Hsu, K. H., Fentzke, R. C., & Chauhan, A. (2013). Feasibility of corneal drug delivery of cysteamine using vitamin E modified silicone hydrogel contact lenses. *Eur J Pharm Biopharm*, 85(3 Pt A), 531-540. doi:10.1016/j.ejpb.2013.04.017
- Hu, X., Hao, L., Wang, H., Yang, X., Zhang, G., Wang, G., & Zhang, X. (2011). Hydrogel Contact Lens for Extended Delivery of Ophthalmic Drugs. *International Journal of Polymer Science*, 2011, 1-9. doi:10.1155/2011/814163
- J. Delville, C. J., P. Cole, C. Bliard. (2002). Solid state photocrosslinked starch based films: a new family of

- homogeneous modified starches. *Carbohydrate Polymers*, 49, 71-81.
- Kamoun, E. A., Chen, X., Mohy Eldin, M. S., & Kenawy, E.-R. S. (2015). Crosslinked poly(vinyl alcohol) hydrogels for wound dressing applications: A review of remarkably blended polymers. *Arabian Journal of Chemistry*, 8(1), 1-14. doi:10.1016/j.arabjc.2014.07.005
- Kiani, A., & Asempour, H. (2012). Hydrogel membranes based on gum tragacanth with tunable structures and properties. II. Comprehensive characterization of the swelling behavior. *Journal of Applied Polymer Science*, 126(S1), E478-E485. doi:10.1002/app.36782
- Lin, C. C., & Anseth, K. S. (2009). PEG hydrogels for the controlled release of biomolecules in regenerative medicine. *Pharm Res*, 26(3), 631-643. doi:10.1007/s11095-008-9801-2
- Miranda, T. M. R., Gonçalves, A. R., & Amorim, M. T. P. (2001). Ultraviolet-induced crosslinking of poly(vinyl alcohol) evaluated by principal component analysis of FTIR spectra. *Polymer International*, 50(10), 1068-1072. doi:10.1002/pi.745
- Mishra, R. K., Majeed, A. B. A., & Banthia, A. K. (2011). Fabrication and characterization of Chitosan/Poly (vinyl alcohol)-co-(vinyl acetate)-co-(itaconic acid) hydrogel membranes. *International Journal of Plastics Technology*, 15(1), 21-32. doi:10.1007/s12588-011-9012-2
- Nave, F. M., Luo, Y. Z., & Coleman, R. M. (2008). Impact of Mobile Phase Parameters on Transport Properties of Metal Affinity Hydrogel Membranes. *Separations Science and Technology*, 43, 4075-4098.
- Prabhu, P., Dubey, A., Parth, V., & Ghate, V. (2015). Investigation of hydrogel membranes containing combination of gentamicin and dexamethasone for ocular delivery. *Int J Pharm Investig*, 5(4), 214-225. doi:10.4103/2230-973X.167684
- Siepmann, J., & Peppas, N. A. (2011). Higuchi equation: derivation, applications, use and misuse. *Int J Pharm*, 418(1), 6-12. doi:10.1016/j.ijpharm.2011.03.051

- Sirousazar, M., Kokabi, M., & Hassan, Z. M. (2012). Swelling behavior and structural characteristics of polyvinyl alcohol/montmorillonite nanocomposite hydrogels. *Journal of Applied Polymer Science*, 123(1), 50-58. doi:10.1002/app.34437
- Sittiwong, J., Niamlang, S., Paradee, N., & Sirivat, A. (2012). Electric field-controlled benzoic acid and sulphanimide delivery from poly(vinyl alcohol) hydrogel. *AAPS PharmSciTech*, 13(4), 1407-1415. doi:10.1208/s12249-012-9869-1
- Umesh K. Parida, A. K. N., Birendra K. Binhani, P.L. Nayak. (2011). Synthesis and Characterization of Chitosan-Polyvinyl Alcohol Blended with Cloisite 30B for Controlled Release of the Anticancer Drug Curcumin. *Journal of Biomaterials and Nanobiotechnology*, 2, 414-425. doi:10.4236/jbnb.2011
- Wittaya, T. (2012). Rice Starch-Based Biodegradable Films: Properties Enhancement. In *Structure and Function of Food Engineering*.
- Yang, J. M., & Su, W. Y. (2011). Preparation and characterization of chitosan hydrogel membrane for the permeation of 5-Fluorouracil. *Materials Science and Engineering: C*, 31(5), 1002-1009. doi:10.1016/j.msec.2011.02.025