Content Uniformity, Drug Quantification and Cross-reactivity Analysis of Protein Loaded PGSU Implants Using Raman Spectroscopy and Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)

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PURPOSE

Content uniformity and accurate quantification of drug loading (DL) are important parameters in evaluating the performance of medical devices. **Secant Group** has developed a flexible elastomer made of poly(glycerol sebacate) urethane (PGSU) for next-generation delivery of active pharmaceutical ingredients **(APIs)** that allows a steady release for several months to years. The purpose of this study is to develop methods that can provide information regarding uniformity of DL and accurately quantify the amount of protein-loading within these implants.

OBJECTIVE(S)

The main objectives of this study were to 1) determine the accurate amount of drug loading of proteins in PGSU implants using acid hydrolysis and amino-acid content analysis and 2) utilize Raman spectroscopy to analyze content uniformity of protein loaded PGSU implants.

METHOD(S)

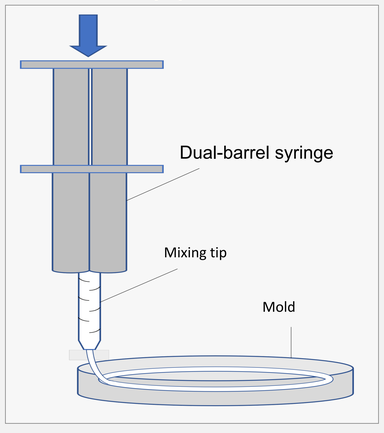


Figure 1. API-loaded PGSU rods were manufactured by injection molding. API, prepolymer resin, cross-linker and catalyst were mixed and extruded into molds. [1,2]

Figure 2. API-loaded PGSU rods were digested In 6 N HCl for 48 hrs at 110 °C. Samples were analyzed using LC-MS for cross-reactivity and to quantify the drug loading

RESULT(S)

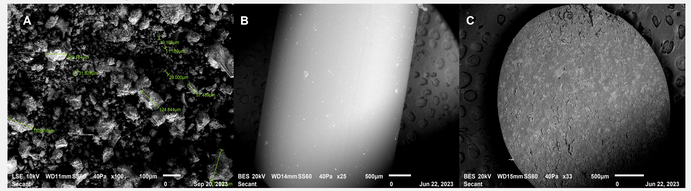
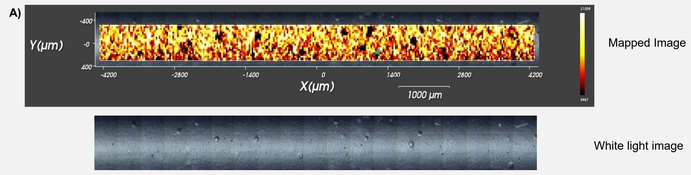


Figure 3. SEM Images A) BSA used as model API. B) Lateral image of 20% loaded BSA-PGSU implant with no visual surface defects C) Cross-section of 20% loaded BSA-PGSU implant showing API pockets and cured PGSU around the BSA particles.



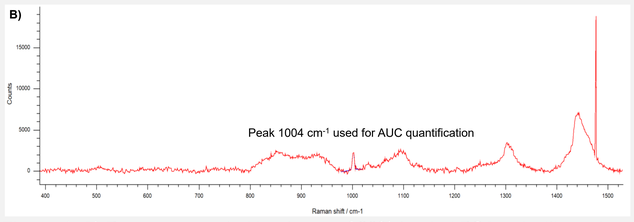


Figure 4. A) Raman generated heat map of 20%-loaded BSA rod with peak area quantified for phenylalanine along with the white light image. B) 1004 cm^{-1} peak, specific for proteins, was used for peak quantification.

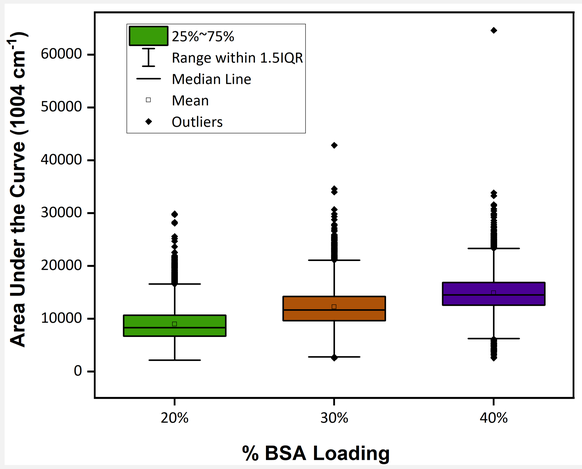


Figure 5. Box plot of three formulations with three different drug loadings (20,30 and 40%), quantified for phenylalanine peak area. Each box plot represents all the data points collected and plotted for the surface analysis for the respective formulations. While no statistical difference was observed between the groups, however, a slight linear increase in the amount of protein incorporated on the surface can be observed.

% Drug Loading	Mean	STD	RSD
20	8989.85	3154.80	35.09
30	12421.97	3771.55	30.36
40	16603.35	3817.99	23.00

Table 1. Mean, standard deviation and relative standard deviation (RSD) values for the three formulations with different drug loadings are presented. An increase in the mean values of peak area of phenylalanine can be observed with increase in drug loading.

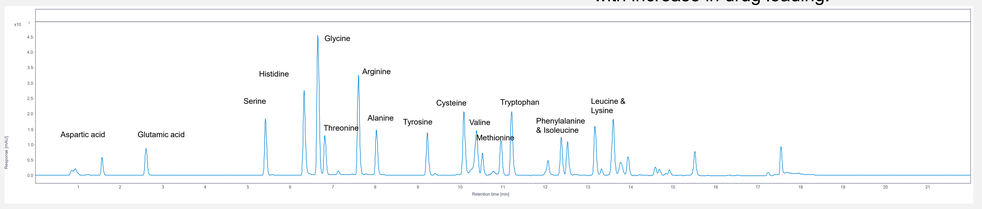


Figure 6. Amino acid standards on an Agilent HPLC using Zorbax® Eclipse Plus C18 column at 338 nm. Digested protein samples will be run to quantify the amount of protein loading in the PGSU samples.

CONCLUSION(S)

Content Uniformity

- Raman spectroscopy reveals a linear increase in the amount of phenylalanine present in the PGSU rods with increased drug loading
- Currently, phenylalanine is being used as a model API to manufacture PGSU rods with different drug loadings in order to generate a calibration curve
- Protein quantification will then be accurately determined for various protein loadings by normalizing it to the number of phenylalanine residues present in the protein

Drug quantification and cross-reactivity

- Acid hydrolysis coupled with LC-UV and LC-MS methods are being developed to accurately quantify the protein loading and to study the effects of the cross-linker (an isocyanate) and its reactivity with the proteins of interest

REFERENCES

1. Reed, S., Smoot, C., Shull, D., Crumbling, T., D'Ottavio, J., Gabriele, P. D., Ely, J. "Tunable, controlled-release, urethane-containing elastomers and processes of forming the same" (2020). *U.S. Patent Application No. 16/547,175.*
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3. AdvanceBio Amino Acid Analysis. (Agilent). https://www.agilent.com/en/product/biopharma-hplc-analysis/amino-acid-cell-culture-analysis/advancebio-amino-acid-analysis-aaa

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