Evaluating Poly(anhydride-ester) Encapsulation Characteristics for Delivery of Hydrophobic Small Molecules

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ABSTRACT

Biodegradable salicylic acid-based poly(anhydride-ester)s (SAPAE) have proven to be effective in many biomedical applications including controlling inflammation, promoting bone growth, and preventing biofilm formation due to the release of salicylic acid upon hydrolysis of the polymer anhydride and ester bonds. Microspheres of SAPAE polymer are one fabrication option available for the encapsulation and controlled release of hydrophobic small molecules. This project aims to evaluate and characterize the ability for SAPAE microspheres to encapsulate, protect, and deliver retinol, a small hydrophobic molecule which is highly used in dermatological and cosmetic products for anti-aging purposes. The SAPAE of interest is a copolymer of salicylic acid (SA), adipic acid, and a diphenylene acetic acid (PAA). Due to supply chain limitations, the polymers used to form microspheres were of two variations, low molecular weight and high molecular weight. Nonetheless, this allowed for comparison of microspheres characteristics including size, morphology, and retinol loading efficiency. Through scanning electron microscopy (SEM), it was confirmed that the unloaded and retinol-loaded microspheres had a spherical shape, and the sizes were similar between the low molecular weight and high molecular weight polymer versions. Residual methylene chloride solvent was successfully reduced in all samples which increases the viability for biological applications. Finally, ultraviolet-visible spectroscopy detected a maximum of 4% w/v loading of retinol in the microspheres.

KEYWORDS: microspheres, salicylic acid, retinol

FACULTY MENTOR - Dr. Kathryn Uhrich, Department of Chemistry

Dr. Uhrich, a distinguished polymer chemist, is a Professor of Chemistry and the Dean of the College of Natural and Agricultural Sciences at UCR. She received her PhD in organic chemistry at Cornell University. Her work focuses on creating new materials and delivery systems with biodegradable polymers for various applications including drug delivery, food safety, and personal care. Currently in her lab, she has collaborations within the university, across the nation, and spanning the globe including partnerships with BASF through the California Research Alliance program. She has been issued over 70 U.S. and international patents and has been elected fellow of AAAS, ACS, AIMBE, CRS and NAI. She is also editor-in-chief of Journal of Bioactive and Compatible Polymers.

Kaitlyn Ngo

Kaitlyn Ngo is a fourth-year Biology major. She studied biodegradable polymers for various applications including medicine as a sustainable bioactive. Her research was conducted under the direction of Dr. Kathryn Uhrich and in collaboration with BASF and the California Research Alliance (CARA). Currently co-president of the Mustard Seed Project at UCR, a medical scribe in Loma Linda, and mentor for Big Brothers and Big Sisters of the IE. She plans to pursue a career as a pediatric physician.

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INTRODUCTION

Retinol, a naturally occurring derivative of Vitamin A, is widely used in cosmetic and dermatologic treatments for skin conditions including acne and psoriasis **(Figure 1)**. 1 This small, hydrophobic molecule is particularly effective as an anti-aging agent by minimizing the appearance of wrinkles and reducing hyperpigmentation on skin. [2–6] However, retinol's therapeutic potential is limited by its tendency to degrade upon exposure to ultraviolet light, heat, and oxygen. [7] This work highlights a biodegradable and environmentally friendly delivery system for retinol that would avoid this complication.

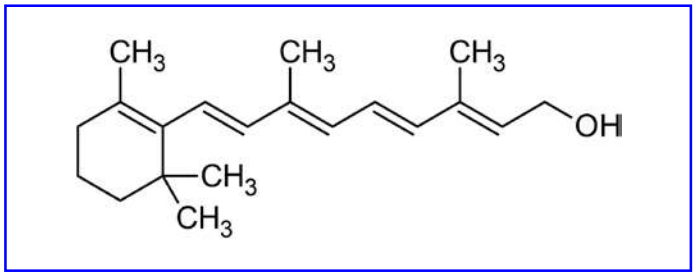

Figure 1. Chemical Structure of Retinol

Previously, our laboratory has reported the synthesis of salicylate-based poly(anhydride-ester)s (SAPAEs) homopolymer that release two naturally occurring compounds upon degradation, adipic acid and salicylic acid **(Figure 2)**.[8] Adipic acid is a biocompatible and biodegradable moiety widely utilized in biopolymers.[9–11] Salicylic acid imparts this material with important antipyretic, anti-inflammatory, and analgesic properties for use in a variety of clinical applications. [12]

Salicylate-based poly(anhydride-esters) (SAPAEs) can be copolymerized and formed into microspheres, which are of particular interest for drug delivery and controlled release of sensitive therapeutic agents such as retinol. The copolymer of SAPAE was synthesized in a 4:1 ratio of salicylic diacid monomer and phenylene diacetic acid linker **(Figure 3)**. In theory, the reduced steric hindrance surrounding the anhydride bonds in the copolymer should make the material more susceptible to degradation versus the homopolymer. The ability to alter the rate of degradation for SAPAEs provides a method for creating SAPAE microspheres that can be customized to release bioactive agents within various time frames based on the desired application.

Overall, the aim of this study is to encapsulate retinol and expand on what is known about SAPAE copolymers as a drug delivery system. It is hypothesized that microspheres made from the SAPAE copolymer will successfully encapsulate retinol while maintaining the characteristic smooth surface morphology of microspheres.

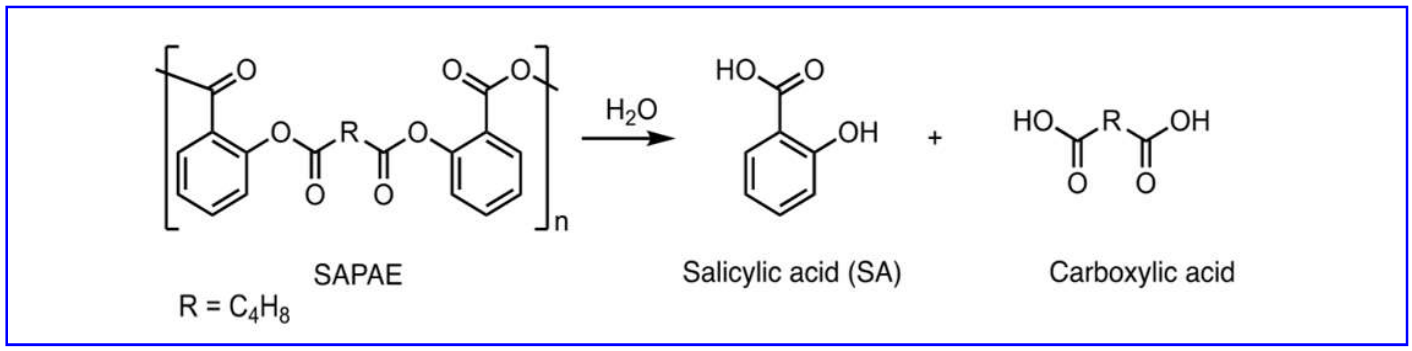

Figure 2. Degradation of SAPAE by Hydrolysis

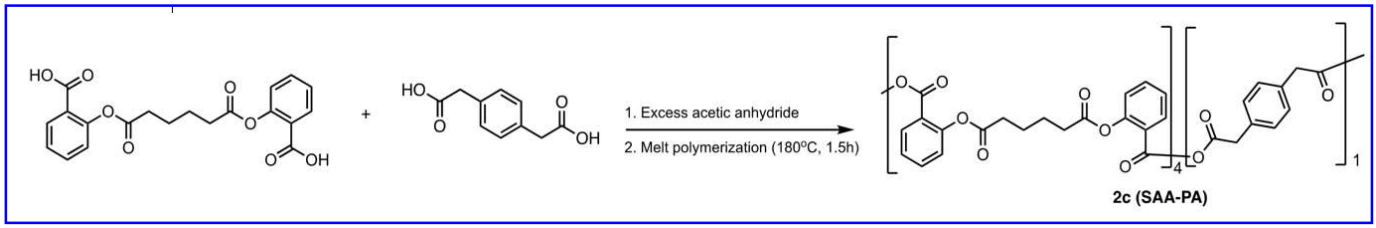

Figure 3. SAPAE copolymer with phenylene diacetic acid (SAA-PA) linker

MATERIALS:

Salicylic acid (Reagentplus®, ≥99%), acetic anhydride with high purity (Reagentplus®, ≥99%), anion traces (GR ACS, 97%), and higher purity (99.5%), poly(vinyl alcohol) (87-90% purity hydrolyzed, powder), anhydrous dichloromethane (>99.8% purity), and retinol (CAS 68-26-8, synthetic, >95% purity by HPLC) were purchased from Sigma-Aldrich. Acetic Anhydride (CAS 108-24-7) with 99+% purity was purchased from Acros Organics. Whatman™ filters Grade 40, 12.7mm) were purchased from GE Healthcare Life Sciences. Dimethyl Sulfoxide (>99.9% purity) was purchased from Fisher Scientific.

METHOD

Polymer Synthesis:

The SAPAE-phenylene acetic acid copolymer (SAPAE-PAA) were prepared based on a previously published method. [8] In brief, a salicylate-based diacid (MW 386.36, 2.0g) and with the addition of p-phenylene diacetic acid (MW 194.18, 0.25g) were added to an excess of acetic anhydride (40mL). The reaction mixture was stirred in a round-bottom flask in an oil bath, cooled, and then reheated while being mixed by an overhead stirrer under vacuum. Polymerization was complete once the viscosity of the melt increased and remained constant. A dark caramel color was also characteristic of a completed reaction. The polymer was cooled to room temperature, stirred with in a minimal amount of DCM to dissolve (~8mL), and precipitated in an excess of diethyl ether (~50mL) isolated, and dried overnight under vacuum.

Molecular Weight Determination:

Gel permeation chromatography (GPC) was used to determine the weight-averaged molecular weight (Mw) of the polymer prior to formulation. The TOSOH EcoSEC HLC-8320 GPC system contains a dual-flow refractive index detector and a porous stationary phase to allow for size exclusion of the polymer samples. The EcoSEC System Control and Analysis software was used to collect and analyze the size distribution of the polymer. The samples of polymer were dissolved in DCM (5-20mg/mL) and the solution was passed through syringe filters with 0.45um pore size. The elution rate was 0.6 mL/min for a total run time of 50 min.

Microsphere Preparation:

Microspheres preparation were based on previously published methods. [13] An oil-in-water emulsion solvent evaporation technique was used to prepare retinol-loaded microspheres with SAPAE-PAA. A retinol/SAPAE/DCM/PVA mixture was homogenized and the resulting emulsion stirred for 1 hour and 30 minutes at room temperature to evaporate DCM. The microspheres were collected by centrifugation at 1500 rpm for 2 minutes and washed three times with deionized water to remove residual PVA before filtering the solution to collect the solids. The samples were stored at -18ºC between further studies to minimize exposure to the environment including moisture. Non-loaded microspheres were prepared as a negative control via

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the same protocol without the initial addition of retinol.

Microsphere Size and Surface Morphology:

A minuscule sample of microspheres were distributed on aluminum studs, sputter-coated with gold-palladium for 20 seconds using a Cressington 108 Manual Sputter Coater and imaged with a TESCAN Vega3 SBH scanning electron microscope (SEM). The acquired images were useful for visualizing the surface morphology and size of the spheres. SEM images were analyzed with NIH ImageJ Software to measure the area of the microspheres. Subsequently, the mean particle diameter in the SEM image was calculated to compare the unloaded and retinol-loaded microspheres.

Retinol Concentration Determination:

When in a solution with DMSO, retinol had a unique absorbance peak in the presence of salicylic acid **(Figure 4)**. Using the Cary 60 Ultraviolet-visible spectroscopy (UV-Vis) and quartz cuvette, a calibration curve was created by measuring the absorbance of retinol dissolved in DMSO with a starting concentration of 200ug/mL. Nine more subsequent concentrations via serial dilution were also measured. The concentrations of retinol in the loaded microspheres were calculated by applying referencing the calibration curve based on an absorbance of 360nm.

RESULTS AND DISCUSSION

In this work, we demonstrate that SAPAE microspheres are capable of encapsulating retinol. Non-loaded microspheres were used as a negative control to compare the microspheres size and morphology.

Melt condensation polymerization yielded SAPAE's with varying molecular weights. In a batch of acetic anhydride with 99% purity, the molecular weight as measured by GPC was 8.0 kDa. However, a batch made with acetic anhydride with reports anion traces at 97% purity resulted in polymers with lower molecular weights of about 4.6 kDa. The predominant impurity in acetic anhydride is likely acetic acid which can hinder the formation of SAPAE's with the molecular weights that are 8.0 kDa or greater which has

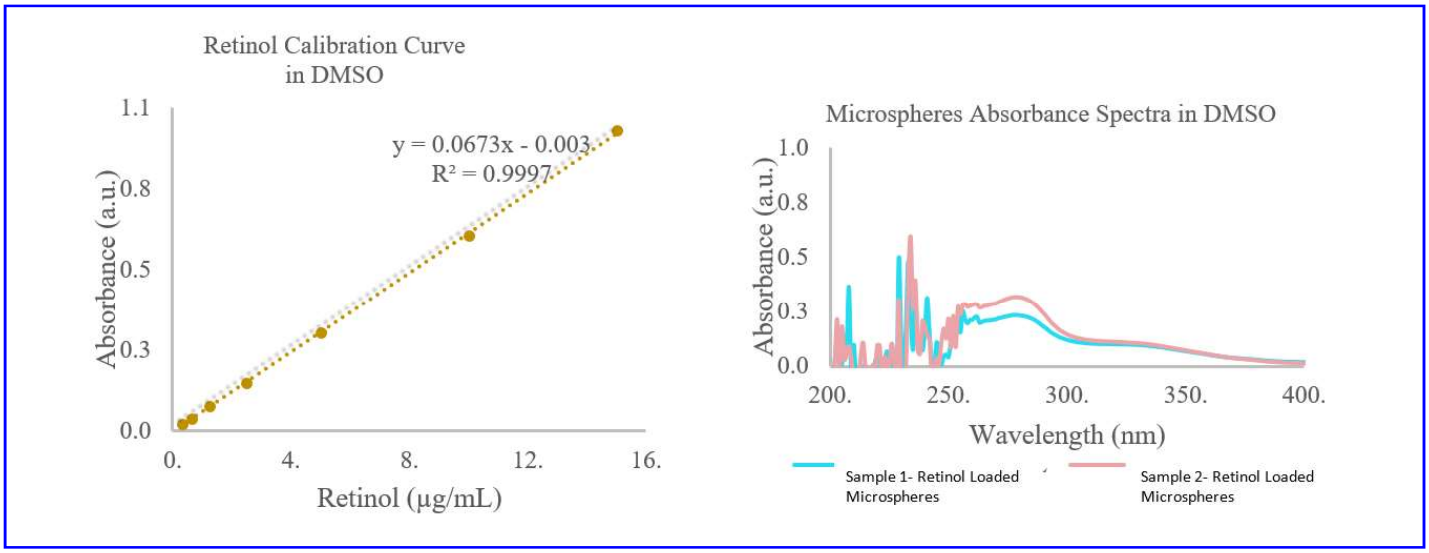

Figure 4. Ultraviolet-visible spectroscopy (UV-Vis) calibration curve for retinol in DMSO at absorbance of 360nm (**left**). Absorbance spectrum of retinol-loaded microspheres (20 mm/mL) in DMSO (**right**).

previously been consistent when using acetic anhydride with higher purity. Due to supply chain issues, only acetic anhydride with 97% purity was available. Nonetheless, the study proceeded with the understanding that the lower purity of acetic anhydride likely has had significant impacts on the quality of SAPAE's and ultimately for the subsequent microsphere studies.

An oil-in-water emulsion solvent evaporation method yielded microspheres with smooth, surfaces **(Figure 5)**. Two separate batches of microspheres, both with loaded and unloaded negative controls, were compared as they were made from SAPAE's with different acetic anhydride purities. Despite differences in the SAPAE molecular weight, the microspheres were similar in morphology and the unloaded microspheres had a greater average diameter than the retinol-loaded microspheres. In addition, both batches of loaded particles were smooth and spherical. On average the low-molecular-weight, unloaded microspheres had a smaller diameter, 9 μm, compared to 15 μm in the sample of high-molecular-weight, unloaded microspheres. The retinol-loaded microspheres were more similar in size between the low molecular-weight and high-molecular-weight microspheres, 7 μm and 6 μm respectively.

The precision of retinol loading studies in DMSO were verified with standards of known retinol and salicylic acid concentrations. Solutions of microspheres dissolved in DMSO were analyzed for retinol loading and the low molecular weight polymer and high molecular weight polymer had similar loading, 4.0% and 4.2% respectively **(Table 1)**. The attempted loading percentage of retinol in the microspheres was 10% by weight thus the experimental encapsulation efficiency is lower than expected. However, since retinol can cause skin irritation at high concentrations, the concentration of this strong bioactive in cosmetic products is between 0.0015% and 0.3%.[1] Thus, the retinol-encapsulating microspheres show potential for formulations at the current encapsulation efficiency. Future analysis could benefit from verifying that all the retinol added into the formulation is accounted for. This would require measuring the amount of retinol that remains in the solution of PVA and residual DCM before the washing step of the microspheres. Changes in the parameters of the microspheres preparation method can be made to achieve higher efficiencies. Even though such modifications have not been explored yet they can be evaluated in the future.

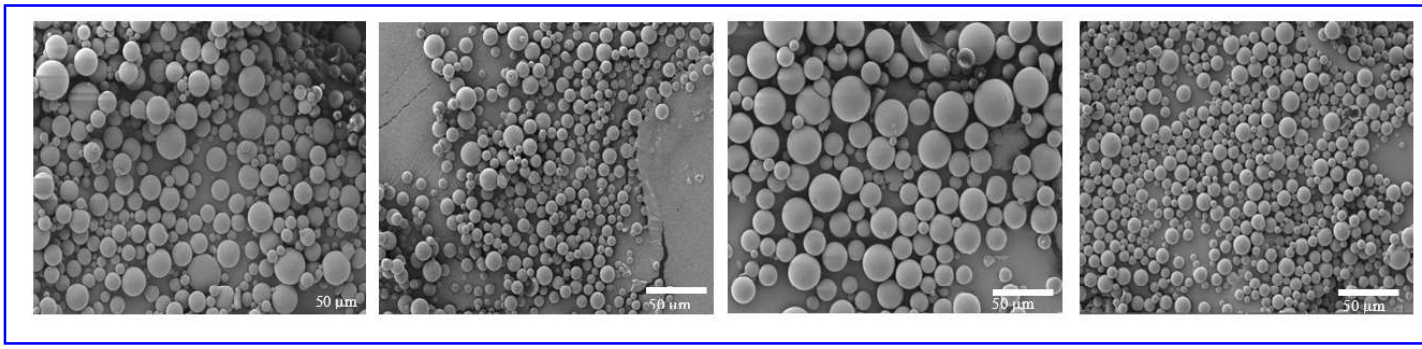

Figure 5. SEM images of microspheres, from left to right, made from SAPAE Mw 4.7kDa, unloaded and loaded with retinol, and SAPAE Mw 8.0kDa, unloaded and loaded with retinol.

Loading Calculation		
Microspheres Preparation	**Sample 1**	**Sample 2**
Abs at 360 nm (a.u.)	0.051	0.054
Calculated [Retinol] (µg/mL)	0.80	0.85
[Microspheres] (µg/mL)	20.0	20.0
Calculate %Loading	4.0	4.2

Table 1. Table of Retinol Loading Calculations including absorbance values by UV-Vis. 10% of retinol by weight was incorporated into the formulation mixture and 3.98% ad 4.24% were the calculated values for loaded retinol from Sample 1 and Sample 2, respectively.

CONCLUSION

In this study, we confirm that 2c SAPAE polymer can successfully form microspheres and encapsulate retinol at 4% encapsulation efficiency. Future experiments are aimed to improve the SAPAEs microspheres encapsulation efficiency. Retinol mass balance can also be done including measuring the amount of retinol left behind in the DCM/PVA solution in the microspheres' formulation mixture before the washing step. Other expansions of this current work include optimizing a standard addition method for quantifying salicylic acid in the retinol-loaded microspheres as well as evaluating the retinol release rate under varying media conditions.

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