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Formulation of salicylate-based poly(anhydride-ester) microspheres for short- and long-term salicylic acid delivery

Roselin Rosario-Meléndez[#], Michelle A. Ouimet[#], and Kathryn E. Uhrich^{*}

Rutgers, The State University of New Jersey, Department of Chemistry and Chemical Biology, 610 Taylor Road, Piscataway, New Jersey 08854, United States

Abstract

The formulation of salicylate-based poly(anhydride-ester) (PAE) microspheres was optimized by altering polymer concentration and homogenization speed to improve the overall morphology. The microspheres were prepared using three salicylate-based PAEs with different chemical compositions comprised of either a heteroatomic, linear aliphatic, or branched aliphatic moiety. These PAEs broadened the range of complete salicylic acid release to now include days, weeks and months. The molecular weight (M_w), polydispersity index (PDI) and glass transition temperature (T_g) of the formulated polymers were compared to the unformulated polymers. In general, the M_w and PDI exhibited decreased and increased values, respectively, after formulation, whereas the T_g changes did not follow a specific trend. Microsphere size and morphology were determined using scanning electron microscopy. These microspheres exhibited smooth surfaces, no aggregation, and size distributions ranging from 2-34 μ m in diameter. *In vitro* release studies of the chemically incorporated salicylic acid displayed widely tunable release profiles.

Keywords

drug delivery; microspheres; salicylic acid; biodegradable

Introduction

Polymer microspheres are widely used as delivery systems for encapsulated drugs, [1-4] proteins, [5] and genes/DNA [1-3,6] for a variety of biomedical applications. Microspheres are preferred over other formulations as they can be injected into the body rather than surgically implanted [7]. Previously, our laboratory reported the formulation of salicylate-based poly(anhydride esters) (PAEs) (Figure 1, 2) into microspheres for controlled delivery of salicylic acid (SA) [8], a non-steroidal anti-inflammatory drug.

These polymers are unique in that SA is chemically incorporated into the polymer backbone via a “linker” molecule [9-11]. SA is released in a near zero-order fashion as the labile anhydride and ester bonds within the polymer are hydrolytically cleaved. These polymers have been extensively studied for the past decade and are biocompatible [12-15], stable under storage conditions [16], and can be exposed to ionizing radiation for sterilization without changing their physicochemical properties [17]. Furthermore, these PAEs allow for a higher percentage of drug loading (60-80 %) in microsphere formulations, compared to

^{*}Corresponding author: Dr. Kathryn E. Uhrich, Department of Chemistry and Chemical Biology, Rutgers, The State University of New Jersey, 610 Taylor Road, Piscataway, NJ 08854, United States, Tel.: +1 732 445 0361, Fax: +1 732 445 7036, keuhrich@rutgers.edu.

[#]Both authors have equally contributed to this manuscript

other biodegradable polymers such as poly(lactic-co-glycolic) microspheres encapsulating SA (20 %) [18].

Previous work on salicylate-based PAEs using discs has shown that drug release can be tuned to last over varying time periods by using different linkers within the polymers (Figure 1) [9], but the formulation and characterization of polymer microspheres on the extreme ends of the drug release timing spectrum (days and months) are reported herein for the first time. The use of a heteroatom, specifically an oxygen-containing linker (Figure 1, **1**), within the polymer allows for a faster drug release rate (days) [19] whereas a linear aliphatic linker (Figure 1, **2**) and a branched aliphatic linker (Figure 1, **3**) can prolong the release to weeks and months [9], respectively. It is important to note that as PAEs are surface-eroding polymers, the geometry of the device (discs vs. microspheres) will likely have an effect on the release profile [20].

The goal of this work was to optimize a previously published microsphere preparation method that would release SA over a wide-range time frame (e.g., days vs. months), while not significantly altering physicochemical properties. The initial PAE-based microsphere formulation was prepared by a well-established oil-in-water single emulsion solvent evaporation method [8]. However, the initial microsphere formulation exhibited significant aggregation and non-spherical morphology, which are undesirable for uniform and predictable drug release. Therefore, to improve the quality of the microspheres, homogenization speed was decreased and polymer concentration was increased during formulation.

Microsphere molecular weight (M_w), polydispersity index (PDI) and glass transition temperature (T_g) were measured and compared to the pre-formulated polymer to determine the formulation effect on the polymer. Scanning electron microscopy (SEM) was used to determine the microsphere size and morphology. *In vitro* studies were performed on the polymer microspheres to investigate SA release.

Methods

Materials

All chemicals and reagents were purchased from Sigma-Aldrich (Milwaukee, WI) and used as received unless otherwise noted.

Polymers synthesis

Salicylate-based PAEs were synthesized and characterized using previously reported methods [9,10,19,13]. In brief, SA (2 equivalents (eq)) was dissolved in THF and pyridine (4 eq). Diacyl chloride (R = “linkers” **1-3**, Figure 1) (1 eq) was diluted in THF and added drop-wise to the stirring reaction mixture to give a white suspension. The reaction mixture was allowed to stir overnight at room temperature and subsequently quenched by pouring over excess water and adding concentrated HCl until pH = 2. The obtained solid was filtered, washed with water, and dried *in vacuo* at room temperature. Polymer precursor (linker **1**) was polymerized using previously described solution polymerization methods [13,19]. Polymer precursor (linkers **2** and **3**) was activated in an excess of acetic anhydride (Fisher, Fair Lawn, NJ) at room temperature and polymerized by previously described melt-condensation polymerization methods [13,9,10].

Microsphere preparation

Polymers were formulated into microspheres using a modified procedure of a published oil-in-water single emulsion solvent evaporation technique [8]. In general, salicylate-based

PAEs (0.50 g) were dissolved in dichloromethane (3 mL) and added drop-wise to 1% aqueous poly(vinyl alcohol) (PVA) solution (80 mL) at room temperature. The emulsion was homogenized for 2 min using an IKA Ultra-Turrax T8 homogenizer at approximately 10,000 rpm. The homogenized solution was left stirring for 2 h to allow microsphere formation by solvent evaporation. Microspheres were transferred to sterile 50 mL polypropylene conical tubes (30 × 115 mm style, BD Falcon, Franklin Lakes, NJ), washed with acidic water (pH 1) to remove residual PVA, and isolated by centrifugation at 3,000 rpm for 10 min. Microspheres were frozen by placing the conical tubes in a dry ice/acetone bath and lyophilized for 24 h at -40°C and 133×10^{-3} mBar (LABCONO Freeze Dry System/Freezon 4.5).

Molecular weight

Gel permeation chromatography (GPC) was used to determine the M_w and PDI of polymers and microspheres. A Perkin-Elmer LC system consisting of a Series 200 refractive index detector, a Series 200 LC pump, and an ISS 200 advanced sample processor were used. A Dell OptiPlex GX110 computer running Perkin-Elmer TurboChrom 4 software was utilized for data collection and control. The connection between the LC system and the computer was made using a Perkin-Elmer Nelson 900 Series Interface and 600 Series Link. Samples were dissolved in dichloromethane (10 mg/mL) and filtered through $0.45\ \mu\text{m}$ polytetrafluoroethylene (PTFE) syringe filters (Whatman, Clifton, NJ) prior to elution through a Jordi divinylbenzene mixed-bed GPC column ($7.8 \times 300\ \text{mm}$) (Alltech Associates, Deerfield, IL) at a rate of 1 mL/min for a total run time of 30 min. M_w was calculated relative to narrow molecular weight polystyrene standards (Polysciences, Dorval, Canada).

Thermal analysis

Thermal analysis was performed using differential scanning calorimetry (DSC) to obtain T_g values. DSC was performed using a Thermal Advantage (TA) DSC Q200 running on an IBM ThinkCentre computer equipped with TA Universal Analysis software for data collection and processing. Samples (5-8 mg) were heated under nitrogen from -10°C to 200°C at a rate of $10^{\circ}\text{C}/\text{min}$. A minimum of two heating/cooling cycles were used. TA Instruments Universal Analysis 2000 software, version 4.5A was used to analyze the data.

Size and morphology

Size and morphology of the microspheres were determined using SEM. Images for each set of microspheres were obtained using an AMRAY-1830I microscope (AMRAY Inc.) after coating the samples with Au/Pd using a sputter coater (SCD 004, Blazers Union Limited). SEM images of each polymer microsphere sample were then analyzed using NIH ImageJ software. Distributions of particle diameter were obtained by evaluating > 50 particles per sample.

In vitro drug release

SA release from polymer microspheres was studied at 37°C in phosphate buffered saline (PBS) at pH 7.4 with agitation (60 rpm) to mimic physiological conditions. Triplicate samples of each set of microspheres (20.0 mg) were suspended in 20 mL of PBS. At predetermined time points, samples were centrifuged at 3,000 rpm for 5 min (Hettich Zentrifugen EBA12) to allow microspheres to settle to the bottom. Aliquots of the supernatant (15 mL) were collected and replaced with fresh PBS (15 mL). Spent media was analyzed via ultraviolet/visible spectroscopy at $\lambda = 303\ \text{nm}$ (wavelength at which SA is the only degradation product to absorb) using a Perkin Elmer Lambda XLS spectrophotometer

to monitor SA release. Data was analyzed against known concentrations of SA and normalized to the amount of SA in the sample.

Results and Discussion

Previous work on salicylate-based PAEs using the free powder or pressed discs demonstrated that the physical properties and the SA release rate can be significantly altered by changing the chemical composition of the polymer via the linker [9]. Salicylate-based PAEs with linear aliphatic linkers (including **2**) were previously formulated into microspheres, but were relatively small in size (i.e., 1-10 μm in diameter), exhibited aggregation, and achieved drug release for 12 days [8]. In this study, the formulation was improved to overcome the aforementioned issues in addition to formulating polymers **1** and **3** into microspheres to achieve short- and long-term (days and months, respectively) SA release.

To enhance polymers **1-3** microsphere formulation, a modified oil-in-water single emulsion solvent evaporation technique was used [8]. The formulation was first attempted using polymer **2** at a concentration of 0.10g/mL and homogenization speed of $\sim 25,000$ rpm. To remove residual PVA, acidic water (pH 1) was used instead of neutral pH water to decrease potential polymer degradation after water exposure as the salicylate-based PAEs degrade at a slower rate under acidic conditions [11]. Two methods were used to isolate the microspheres: 1) vacuum filtration and drying *in vacuo* overnight at room temperature and 2) centrifugation and freeze-drying overnight. Microspheres of 1-10 μm in diameter with minimal aggregation were obtained (data not shown), but the yield was higher using centrifugation (80 %) compared to vacuum filtration (60 %). In an attempt to negate aggregation and increase microsphere size (surface area-to-volume ratio increases with decreasing particle size thus increasing degradation rates) [21,3], the formulation method was further optimized by increasing polymer **2** concentration to 0.16g/mL and decreasing homogenization speed to 10,000 rpm. With this, larger microspheres (4-34 μm in diameter) were obtained in 80% yield without aggregation. This optimized method (0.16g/mL polymer concentration, homogenization speed of 10,000 rpm, wash with acidic water, and isolation by centrifugation) was used to formulate microspheres of polymers **1** and **3**.

To investigate the different time frames of complete SA release, polymer **1** was used to create faster-degrading microspheres that achieve release within days, whereas polymer **3** was used to extend SA release into the month time frame. Polymer **2** was used as a reference point to compare microsphere properties against previously published data [8]. When evaluating morphology, narrow size distribution and smooth surfaces are essential properties to ensure uniform microsphere degradation and therefore uniform drug release [22]. Also, microspheres exhibiting no aggregation are important because aggregated microspheres could be detrimental to the injection process by not passing through the needle [22]. Microspheres comprised of polymer **1**, **2**, and **3** demonstrated size distributions of 2-30 μm , 4-34 μm , and 2-31 μm , respectively, as depicted in the representative SEM images (Figure 2 a-c). The size distribution of microsphere diameters are demonstrated in Figure 3 that exhibit mean values of 11 ± 7 μm , 19 ± 7 μm , and 11 ± 6 μm for polymer **1**, **2**, and **3**, respectively. Overall, these microspheres are larger in size as compared to previously published data (1-10 μm in diameter) [8]. No signs of aggregation were noted and all microspheres displayed smooth surfaces, and are therefore viable candidates as drug delivery systems.

The M_w and T_g of the microspheres were determined and compared to the values obtained for the unprocessed polymers (Table 1). A consistent decrease in M_w and increase in PDI compared to the unprocessed polymer was observed for all microspheres. These changes were expected for these hydrolytically degradable polymers, as the polymers were exposed

to water during formulation. However, polymers **2** and **3** microspheres preserved a relatively high M_w (only decreasing 7 % and 19 %, respectively). The larger decrease observed for polymer **1** microspheres (40 %) can be attributed to the more hydrophilic nature of the linker compared to linkers **2** and **3**. The changes in T_g values did not follow a specific trend after formulation for all sets of microspheres. All samples had T_g values above 37 °C, which ensure their shape will remain when placed *in vivo*.

In vitro drug release from the microspheres was studied to determine the SA release profiles shown in Figure 4. Microspheres prepared from polymers **1** and **2** released 100 % SA without the presence of a lag period (i.e., an initial time period where SA is not released), whereas polymer **3** microspheres released SA after a 10 day lag period. Release rates for all microspheres correlate to the linkers used: the microspheres containing the relatively more hydrophilic polymer **1** (contact angle 50°) [19] released 100 % of the drug in a shorter period of time (3 days), followed by polymer **2** (21 days). The microspheres containing the polymer with branched aliphatic linker, **3**, being relatively more hydrophobic (contact angle 93°) [9] and sterically hindered, released the drug at a slower rate; polymer **3** microspheres released 21 % SA over 21 days, which is projected to reach 100% SA release within 3.5 months at this rate.

Conclusion

Polymer microspheres were prepared using three different salicylate-based PAEs. The modified oil-in-water single emulsion solvent evaporation method was successfully used to obtain microspheres with size distributions ranging from 2-34 μm and smooth surfaces without aggregation. The M_w , PDI and T_g of the microspheres were studied and compared to the polymer prior to formulation where decreases in M_w , increases in PDI, and only minor changes in the T_g were observed. The release profiles showed SA release can be altered to last from days to months, a feature not yet attained from previous salicylate-based PAE microspheres, by changing the chemical composition of the polymer used via the linker molecule. The ability to control SA release over a wide time range using these microspheres provides great promise for the treatment of inflammatory diseases. Future studies will focus on the *in vivo* testing of the microspheres, overcoming the lag period observed for the slower degrading polymer microspheres, and encapsulating bioactives.

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Fig. 1.
Structure of polymers **1-3** used to formulate salicylate-based PAE microspheres and their hydrolytic degradation to release SA

Fig. 2.
Representative scanning electron microscopy images of microspheres prepared from polymer **1** (a), **2** (b), **3** (c)

Fig. 3.

Box plots demonstrating the percentage of microsphere size for polymers **1-3** from representative SEM images using NIH ImageJ Software where top and bottom bars represent maximum and minimum values, respectively, black and grey portions represent the 25-50 % and 50-75 % portion of the sample set, respectively, and the white line represents the median value

Fig. 4. Normalized *in vitro* SA release from polymer microspheres (SA $\pm$ standard error): polymer **1** (filled diamonds/dashed line), polymer **2** (filled squares/dotted line), and polymer **3** (filled triangles/solid line)

Table 1

Molecular weight (polymer and microspheres), polydispersity index (polymer and microspheres), glass transition temperature (polymer and microspheres), and % yield (microspheres)

	M_w (Da) polymer	M_w (Da) microspheres	PDI polymer	PDI microspheres	T_g (°C) polymer	T_g (°C) microspheres	% Yield microspheres
1	12,000	7,200	1.2	1.9	68	70	90
2	18,000	16,800	1.3	1.9	50	51	80
3	11,000	9,000	1.4	1.7	57	54	70