Senior Thesis Proposal:

Evaluating the estrogenic activity and redox potential of gallate esters with varying lipophilicity

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Abstract:

In practice, parabens have been used as preservatives in a variety of cosmetics and foods. While their antimicrobial properties are useful, they are also known xenoestrogens and may increase risk of developing hormone receptor positive breast cancer. Previous synthesis and evaluation of 3,5-substituted parabens have found even weaker estrogenic activity and comparable antimicrobial properties; however, other health concerns, such as redox potential must be considered. Gallate esters with varying carbon chains have been previously synthesized and will be evaluated as a potential alternative to traditional parabens. Cyclic voltammetry and colorimetric assays will be performed to determine the redox potential of gallate esters, while an ELISA assay will be performed in order to evaluate their estrogenic activity. Antioxidant activity is expected due to the presence of polyphenols on the gallate esters, and binding of the estrogen receptor without activation is expected due to their 3,5-substitution.

Introduction:

Parabens, a class of preservatives, are alkyl esters of *p*-hydroxybenzoic acid (Figure 1). Because of their antimicrobial properties, these preservatives can be found in a wide variety of cosmetic products, with an FDA allowance of up to 0.1% in food and food packaging, and up to 1% in cosmetics [1]. Their antimicrobial properties are not completely clear as there are multiple ways in which parabens cause cell death; however, their main mode of action is agreed to be through the disruption of the cell membrane of microbes [2]. This is achieved by embedding themselves in the cell membranes of microbes, causing a disruption of homeostasis through the efflux of ions such as potassium and ultimately lead to cell death (Figure 2).

While parabens are useful for their antimicrobial properties, they are also known xenoestrogens and can affect estrogen-regulated gene expression and physiological responses

[3]. The estrogen receptor (ER) is typically activated *via* hydrogen bonds at two different locations, which can then trigger gene-expression of estrogen or cell proliferation [4]. On the other hand, parabens only bind to one location of the ER, making their interactions with the ER much weaker than that of estrogen (Figure 3). Despite this, parabens are still able to bind to and activate the ER and cause cell proliferation at concentrations as low as 10^{-6} M [5]. Since approximately 80% of breast cancers are hormone receptor positive, with malignant growth resulting from increased levels of estrogen, the estrogenic activity of parabens may increase the risk of developing breast cancer caused by the overproduction of estrogen or abnormal cell growth [6]. Thus, finding an alternative to traditional parabens is crucial for ensuring the safety of consumers who use products that require preservatives.

Our lab has previously synthesized a variety of substituted parabens in hopes to discover a safer alternative for traditional parabens [7]. In this study, the 3,5 positions on the phenol ring were substituted with a multitude of blocking groups (Figure 4). This substitution resulted in even weaker binding to the ER, to the point where the substituted parabens no longer activated the ER and showed antagonistic activity (Figure 3) [7,8]. Also, the antimicrobial activity of the substituted parabens was found to be comparable to that of traditional parabens. While all of the substituted parabens synthesized could be safer in terms of their abilities to act as xenoestrogens, there are other possible health concerns that must be considered.

One of the biggest health concerns that should be considered in determining the safest alternative is the ability to participate in redox chemistry. All parabens contain a phenol, which consists of a hydroxyl group bound to an aromatic benzene ring. Phenol has been found to increase oxidative stress in organisms through its involvement in free radical generation through redox reactions [9,10]. On the other hand, the presence of multiple hydroxyl groups in

polyphenols act as antioxidants through free radical scavenging [11]. Since gallate esters are 3,5-substituted with multiple hydroxyl groups, our lab believes they could act not only as a preservative, but also an antioxidant. Previous studies have found gallic acid and a few of its ester derivatives, such as dodecyl gallate, to have antioxidant tendencies [9,10, 11]. For this reason, our lab is interested in further elucidating the potential of gallate esters as alternatives to traditional parabens.

While gallate esters have been synthesized and studied in previous research, there are many gaps in the literature in terms of their various properties. First, previous studies have attempted to evaluate the antimicrobial properties of some gallate ester derivatives but fail to perform biological assays on the full range of gallate ester derivatives. This is likely due to the lack of commercial availability of many gallate ester derivatives, especially those consisting of odd-numbered carbon chains. In response to this, our lab has synthesized many derivatives of gallate esters that are not commercially available, including pentyl, hexyl, heptyl, and decyl gallate esters. Our lab has recently tested the antimicrobial properties of the synthesized derivatives, as well as the commercially available ones, in order to understand the antimicrobial properties of the full range of gallate esters. Despite this, there is still little known about the estrogenic activity and redox potential of gallate esters.

Proposal:

First, to evaluate the redox potential of the gallate esters, we propose performing cyclic voltammetry. Cyclic voltammetry will be used to measure the oxidation potentials of gallate esters, which will provide valuable insight into their antioxidant properties. This method can be used for the determination of antioxidant capability, in the same manner as the more widely used DPPH radical scavenging assay, because of the correlation between oxidation potentials and

anti-radical power [15]. Also, cyclic voltammetry has previously been used to evaluate the antioxidant properties of parabens and their analogues, including dodecyl gallate [16]. This makes cyclic voltammetry a suitable method for evaluating the redox potential for the whole range of gallate esters being studied.

Second, to support the results found through cyclic voltammetry, we propose performing colorimetric assays to determine the antioxidant properties of gallate esters. One of the proposed colorimetric assays is DPPH radical scavenging assay, which is an accepted mechanism for screening the antioxidant activity of many phenolic compounds [17]. In this assay, absorbance spectroscopy is used to measure color change to calculate the radical scavenging activity of a compound. Another proposed colorimetric assay is ferric reducing antioxidant power (FRAP) assay, which can measure antioxidant potential through the reduction of ferrous ions [17]. These assays are more frequently used in determining antioxidant potential and will be used to confirm the results found through cyclic voltammetry.

Last, to evaluate the estrogenic activity of the gallate esters, we propose performing an enzyme-linked immunosorbent assay (ELISA). This method utilizes antibodies to detect the activation of the estrogen receptor, resulting in fluorescence. Our lab has previously used ELISA assays to determine the estrogenic activity of 3,5-substituted parabens, including a few gallate ester derivatives [7]. This makes an ELISA assay a suitable method for determining the estrogenic activity of the entire family of gallate esters being studied.

Specific Goals:

- **Goal #1 –** Perform cyclic voltammetry for determination of re-dox potential of gallate esters.

- **Goal #2 –** Perform supporting colorimetric assays for determination of antioxidant properties of gallate esters.
- **Goal #3 –** Perform ELISA assay for determination of estrogenic activity of gallate esters.

Materials & Methods:

Cyclic voltammetry for determination of redox potential

Cyclic voltammetry will be performed under the advisory of Dr. Andrew Yeagley with guidance from Dr. Melissa Rhoten. The procedures for cyclic voltammetry will be carried out according to the methods from of the Gil et al publication in which cyclic voltammetry was used in order to determine the redox potential of traditional parabens. In this procedure, a phosphate buffer solution was used as a solvent and a glassy carbon electrode was used as the working electrode. This methodology would allow us to better mimic the aqueous environment of the human body if the gallate esters easily dissolve in the buffer solution. If the gallate esters are insoluble in the phosphate buffer, acetonitrile will be used as the solution and tetrabutylammonuim perchlorate as the supporting electrolyte, as used in the Masek et al publication for the cyclic voltammetry of dodecyl gallate.

Colorimetric assays for determination of antioxidant properties

To support the redox potential data found during the cylic voltammetry of the gallate esters, a DPPH assay or a ferric reducing antioxidant power (FRAP) assay will be performed under the advisory of Dr. Andrew Yeagley. Both assays will be carried out according to the methods of Zielinska and Turemko [18].

ELISA assay for determination of estrogenic activity

An ELISA assay will be performed under the advisory of Dr. Amorette Barber. Two 17β-Estradiol ELISA kits (ADI-900-008) were purchased from Enzo Life Sciences and will be used. The methods will precisely follow the manufacturer instructions provided with the purchase of these kits.

Timeline:

The timeline for the proposed research can be seen in Figure 5. Weeks 1-16 in the fall semester of 2021 will focus on the evaluation of the redox potential and estrogenic activity of gallate esters. During this time, the proposed cyclic voltammetry, colorimetric assays, and ELISA assays will be performed. With the results from the proposed assays, weeks 17-32 of the spring semester of 2022 will be focused on writing the complete thesis document.

Figures

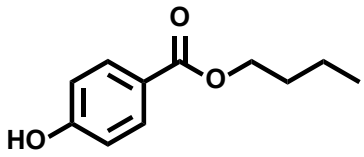

Figure 1. Structure of a traditional paraben.

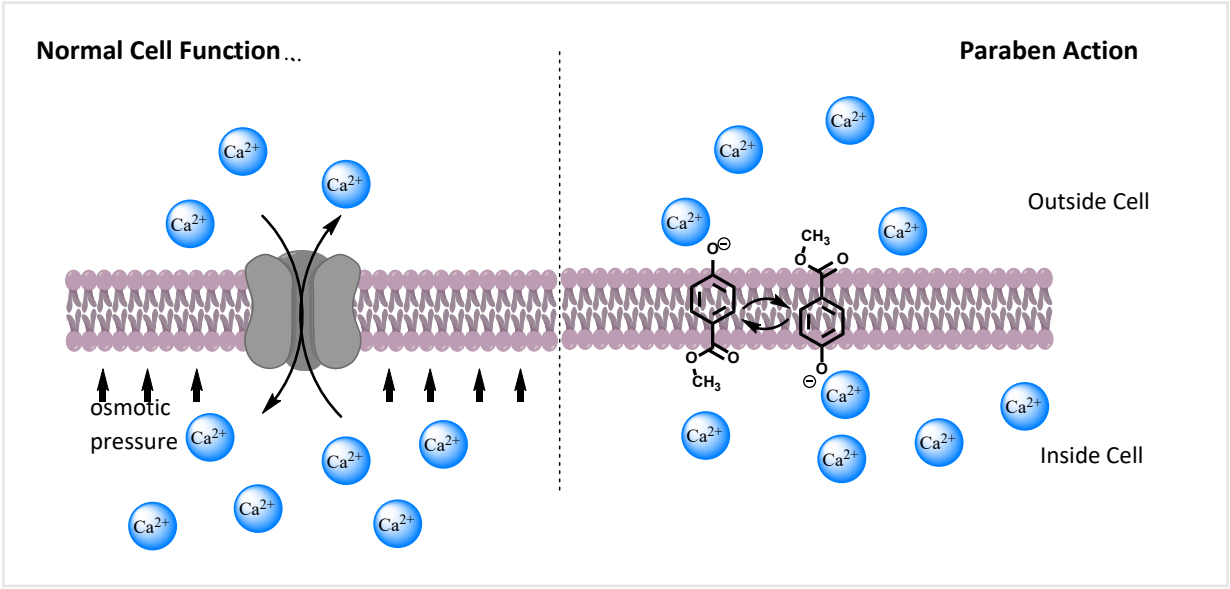

Figure 2. Paraben mode of action.

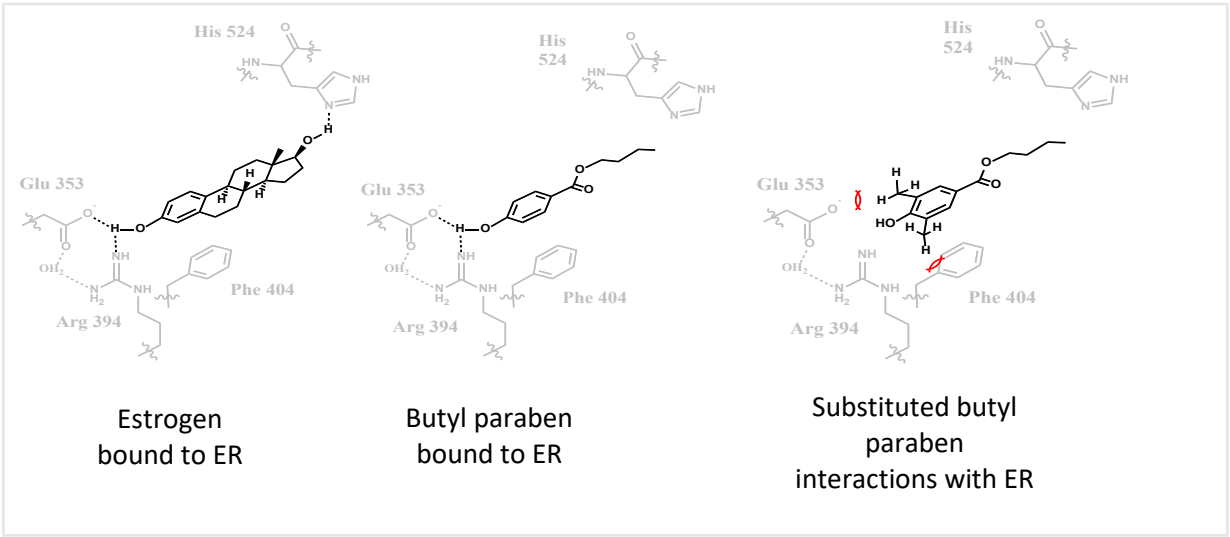

Figure 3. Interactions and binding with the estrogen receptor. This figure shows interactions of different molecules with the ER.

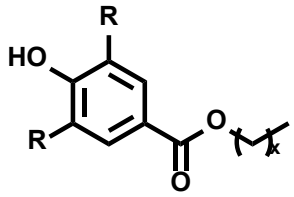

Figure 4. Structure of 3,5-substituted parabens *R= Br, Cl, I, OMe, and OH (gallate esters), *X=0-7, 9, 11

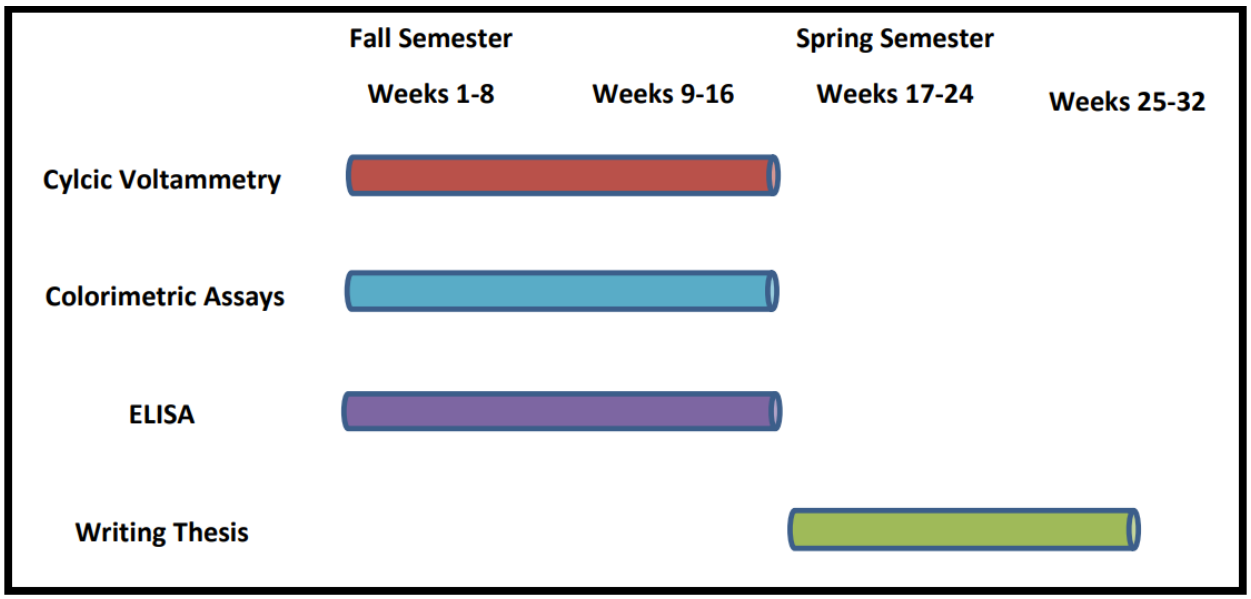

Figure 5. Timeline for proposed research project

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