Non-Covalent Derivatives

by Emily Stoler

B.S in Chemistry, Tufts University
M.S. in Chemistry, Brandeis University

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Dissertation directed by

Graham Jones
Professor of Chemistry

&

John Warner
President and CTO of WBI
Dedication

This dissertation is dedicated to my daughter in the hopes that she will inherit a world that is brighter, cleaner and safer.
Acknowledgements

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Abstract of Dissertation

Non-covalent derivatization is a process by which one or more molecules are incorporated into the solid-state matrix of the target molecule by way of non-covalent forces such as ionic Van der Waal forces, hydrogen bonding or pi-pi interactions. This incorporation produces a new material, a non-covalent derivative (NCD), which has properties that are distinctly different from those of the parent molecules. When faced with the need to modify the physical properties of a molecule, one would traditionally employ a series of familiar synthetic modifications. Examples of these modifications include halogenations for improved stability, oxidation for increased water-solubility, alkylations for lipophilic compatibility, or addition of double bonds for improved conjugation. In some cases, these (potentially) toxic, waste-producing and labor-intensive modifications can be replaced with non-covalent derivatization. The NCD, when compared to the parent molecules, can show dramatic changes in thermal properties, solubility, optical properties and stability while maintaining the desirable properties of the target molecule. This dissertation explores several specific, practical applications of NCDs. A study is presented that demonstrates the capacity of NCDs to impart oxidative stability to solid-state materials. This capacity has far-reaching applications in a diversity of specialty chemical areas where shelf-stability and product performance are vital. Two case studies are presented that explore the application of NCDs for product development. In the first case, the color formation of a biomimetic hair dye is altered by the use of an NCD. In the second, a peroxycacid is stabilized in water by way of an NCD. These three studies demonstrate the utility and versatility of non-covalent derivatization as a tool in material development and optimization.
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<tr>
<td>NCD</td>
<td>Non-covalent derivatives</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>LAG</td>
<td>Liquid-assisted grinding</td>
</tr>
<tr>
<td>CBZ</td>
<td>Carbamazepine</td>
</tr>
<tr>
<td>PXRD</td>
<td>Powder X-ray diffraction</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>SS-NMR</td>
<td>Solid-state NMR</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
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<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>AFM</td>
<td>Atomic force microscopy</td>
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<tr>
<td>API</td>
<td>Active pharmaceutical ingredient</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet-visible</td>
</tr>
<tr>
<td>HQ</td>
<td>Hydroquinone</td>
</tr>
<tr>
<td>DETA</td>
<td>Bis-((N,N\text{-diethyl})) terephthalamide</td>
</tr>
<tr>
<td>DPTA</td>
<td>Bis-((N,N\text{-propyl})) terephthalamide</td>
</tr>
<tr>
<td>DBTA</td>
<td>Bis-((N,N\text{-butyl})) terephthalamide</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>L-DOPA</td>
<td>L-3,4-dihydroxyphenylalanine</td>
</tr>
<tr>
<td>DHICA</td>
<td>5,6-Dihydroxyindole-2-carboxylic acid</td>
</tr>
<tr>
<td>DHI</td>
<td>5,6-Dihydroxyindole</td>
</tr>
<tr>
<td>PHF</td>
<td>Potassium hexacyanoferrate (III)</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>DART</td>
<td>Direct analysis in real time</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel permeation chromatography</td>
</tr>
<tr>
<td>Gln</td>
<td>Glutamine</td>
</tr>
<tr>
<td>L-Arg</td>
<td>L-Arginine</td>
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DSC  Differential Scanning Calorimetry
SCXRD  Single crystal X-Ray diffraction
PA  Peroxyacid
CA  Carboxylic acid
GRAS  Generally regarded as safe
NFPA  National fire protection association
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CHAPTER 1: Non-Covalent Derivatives (NCDs)

1.1 Introduction to NCDs

Non-covalent derivatization is a process by which a material is formed when one co-former molecule or more is incorporated into the solid-state matrix of the target molecule by way of non-covalent forces. These forces can include ionic, Van der Waals forces, hydrogen bonding or pi-pi interactions. The resulting material, a non-covalent derivative (NCD), has properties that are distinctly different from those of the parent molecules.

There are two material forms that fall under the umbrella of NCDs: cocrystals and eutectics. After some initial debate, the definition of a cocrystal has developed as a homogenous crystalline material that is made up of two or more molecules in definite stoichiometric amounts held together by non-covalent forces. The cocrystal is distinguished from a salt by the degree of proton sharing. In the FDA’s guideline toward the classification of cocrystals, a cocrystal is required to have a pKa difference between coformer pairs that is less than 1, thus indicating a non-ionic species and minimal proton sharing. However, this is a limiting definition, as it has been seen that cocrystals rather than salts can occur at pKa differences as large as 4. A cocrystal is also differentiated from a solvate by the state of matter of the starting materials. If both the starting materials are solid then the NCD product is a cocrystal. If one of the materials is a liquid then, despite the similarity in binding forces, the materials combine to form a solvate. A eutectic can also be formed from two solids, but the resulting NCD is a homogenous material that is recognizable on a phase diagram as a single minimum point.

A non-covalent derivatization will result in a cocrystal or a eutectic depending on the predominant driving force. If the enthalpic advantage outweighs any entropy loss, then the system is enthalpically driven and the result is a cocrystal. If, on the other hand, the entropy gain outweighs the enthalpy changes, then the eutectic predominates. The conditions for entropic versus enthalpic control can be attributed in part to the topography of the parent molecules. In cases where the functional groups are compatible for effective non-covalent bonding and the size and shape of the parent molecules are similar enough for good crystal packing, cocrystals will result. In cases where only the non-covalent bonding is favored but there is poor packing shape compatibility of the parent molecules then only the binding forces predominate and a eutectic is formed.
1.2 Preparation of NCDs

Non-covalent derivatization can produce dramatic changes in the target molecule’s solubility, melting point, optical properties, bioavailability, or stability. When compared with traditional synthetic modifications used to effect these same types of property changes, the preparation of NCDs is an alternative that can be less toxic, produces less waste and is, in many cases, less labor-intensive.11

NCDs can be prepared through a variety of solvent techniques, though the majority of these methods result in cocrystals. Traditional methods of precipitation12-15 cooling crystallization16, 17 and slurry formation18 have been reported for the preparation of cocrystals. However, evaporation19-26 has emerged as the most common solvent-based method for cocrystal preparation.27 Solvent evaporation has also been reported in the preparation of eutectics.28, 29 However, solvent preparation methods can be challenging because they not only require the target molecules to be compatible with each other for NCD formation, but also to have solubility relationships with the solvent that allow for concurrent precipitation as the solution cools or evaporates.

Partly because of this limitation with solvent-based methods, solid-state grinding has become a well-established method for the preparation of NCDs. Numerous examples can be found in the literature of cocrystals13, 30-33 and eutectics34,35 formed when the neat solids were ground by mortar and pestle or automated grinders. Three mechanisms are recognized to be in effect with grinding in the solid state: grind molecular diffusion, eutectic formation, and cocrystallization mediated by amorphous phase.36 The grind molecular diffusion is described as a process by which the surface of the solid is made mobile either by vaporization or energy transfer to the surface of the solids. In the case of the eutectic, the eutectic liquid is first formed which then crystallizes to the final solid. Lastly, the cocrystals is described as coalescing out of the amorphous phase of the parent solids. In each of these cases, the intermediate bulk phase (gas liquid or amorphous solid) has a higher mobility and/or energy with respect to starting material forms.37

It was observed that the addition of catalytic amounts of solvent to the grinding matrix, improved the efficiency of preparation37 and increased the crystallinity of the final cocrystal product.38 This observation and subsequent studies confirming the positive effect of solvent 36 has led to the common practice of liquid-assisted grinding (LAG), originally referred to as solvent drop grinding, for the preparation of NCDs.39-41 In a study comparing method preparations, it was demonstrated that it was possible to prepare the same carbamazepine cocrystals achieved with evaporation by using solvent-assisted grinding.42 More importantly, grinding can produce cocrystals that are not available by solvent means.43,13
Spray dry evaporation technique was also observed to produce different cocrystals forms from the same parent compounds. This was seen in the preparation of cocrystals formed from urea and succinic acid. With spray drying, a previously unidentified cocrystal structure was formed. It was found to have a 1 to 1 molar ratio and a laminar sheet form. With other preparation methods, the same materials produced a 2 to 1 urea to succinic acid structure with a complex three-dimensional structure that contains to ring-structure repeat units. The two forms had different stabilities and dissolution rates. This study is indicative of the versatility of NCD formation, not only in choice of coformer but also in method in order to produce NCDs with the desired properties.

There are issues associated with the practical industrial manufacture of cocrystals. In most cases, a cocrystal is prepared in small quantity and studied. The most common methods for the preparation of cocrystals, solid-state grinding or slow evaporation, don’t lend themselves easily to large scale processes. However, some limited promise was seen in the case of carbamazepine/saccharin in which the cocrystal was prepared in a 30 g scale by solution crystallization. Several references discuss the importance of the preparation of ternary phase diagrams for the interaction of coformers and the solvent in order to facilitate large batch cooling crystallization for the preparation of cocrystals. A solvent free process for the formation of carbamazepine (CBZ)-trans-cinnamic acid cocrystals, in stoichiometric ratios, was reported using continuous hot melt extrusion. In a similar set of studies, CBZ-nicotinamide cocrystals were prepared in the presence of a polymer matrix using hot melt extrusion. Another interesting option for large scale production is demonstrated through the preparation of cocrystals through vapor deposition of triiodotrifluorobenzene onto a layer of 1,4-bis (E)-2-(pyridin-4-yl) vinyl benzene.

1.3 Characterization of NCDs

The most direct and conclusive method for NCD characterization is through the analysis of cocrystals via crystal structure analysis. A wide variety of single crystal structures of cocrystals can be found in the literature. However in many cases, particularly those where grinding preparation methods are employed, a cocrystal of the appropriate size for single crystal analysis is not achievable. In these cases, powder X-ray diffraction (PXRD) has been turned to as an alternative characterization technique. Indexing software was used to identify the crystal structures from the PXRD data of theobromine and trifluoroacetic acid or malonic acid. Because PXRD alone cannot differentiate between hydrates, solvates, and cocrystals, the data is often considered in conjunction with another analysis method. For example, by combining PXRD, Nuclear Magnetic Resonance (NMR) and calculation theory, theophylline-nicotinamide cocrystal structures were solved from the powder cocrystal data.
In addition to solid-state NMR (SS-NMR), other spectroscopic methods are frequently employed for the identification of non-covalent derivatives. These methods are not generally predictive of the structure of the cocrystals or eutectic, but can indicate the presence of novel interactions (usually novel peaks) in the NCD that are not seen in the pure parent compounds. In one example, terahertz spectroscopy was used to follow the mechanochemical construction of a two-component cocrystal by grinding together phenazine and mesaconic acid. In some cases, especially those where the coformer has a carboxylic acid, infrared (IR) spectroscopy can be used because the frequency of some stretches will change when in a cocrystal environment. Raman spectroscopy has also proven to be an effective tool for NCD analysis as exemplified by the analysis of cocrystals prepared from salicylic acid and a series of coformers.

Differential Scanning Calorimetry (DSC) can be used to identify the melting point NCDs. Phase diagrams can be developed by plotting the mole percent of the coformer against the melting point changes. These phase diagrams can be powerful tools for differentiating between the NCD’s cocrystal and eutectic forms. Phase diagrams prepared by plotting the percent composition against DSC thermal data results in a classic “V” shape for eutectic mixtures (Figure 1) and a classic “W” shape for cocrystals (Figure 2).

![Figure 1: Ideal binary phase diagram for a eutectic.](Figure Used with Permission)

The binary-phase diagram for two components exhibits a more complex behavior which contains two eutectic points (where the solid and liquid phase are in equilibrium) and a region of “cocrystal” as seen below:
Additionally, the creation of a phase diagram for cocrystals identifies the stoichiometric relationship between the coformers. This is pointed out by the maxima of the curve in the cocrystal region. 

Microscopy has also been used to indicate the formation of a cocrystal, though this method can only indicate a significant change in morphology. In one example, the interface of cocrystal preparation for lamotrigine and phtalimide was observed by hot-stage microscopy. In another study, atomic force microscopy (AFM) was used to differentiate between two forms of caffeine-glutaric acid cocrystals.

1.4 Applications of NCDs

Pharmaceuticals

Much attention has been paid to the potential utility of cocrystals in the pharmaceutical industry. A variety of active pharmaceutical ingredients (APIs) have been incorporated into cocrystals and
eutectics with the result of remarkable changes in physical properties without the loss of pharmaceutical activity. Improvements to the solubility and hydro-stability have been reported for cocrystals with APIs. Dovetailing with the changes in solubility, pharmaceutical cocrystals have been hailed as one of the most effective methods available for the challenging task of improving bioavailability. A number of review articles are in print on pharmaceutical NCD cocrystals. Likewise, pharmaceutical eutectics have been recognized as a powerful means for altering API properties.

In an illustrative and often referenced study, several cocrystals were prepared from fluoxetine hydrochloride and the solubility was measured. The fluoxetine hydrochloride solubility was determined to be 11.6 mg/mL. The benzoic acid cocrystal solubility was measured at 5.6 mg/mL. Both the fumaric acid and succinic acid cocrystal were found to increase the solubility to 14.8 mg/mL and 20.2 mg/mL, respectively. In a similar study, the cocrystal formed from the anti-fungal drug, Tegafure, was shown to have solubility comparable to that of the amorphous drug and much better than the pure crystal. The advantage of the cocrystal in this case was the improvement of the solubility without the loss of stability associated with the amorphous phase. A number of similar examples showing changes in solubility and dissolution for cocrystals can be found in the literature and several reviews have been published on the subject.

This change in solubility has implications on the bioavailability of APIs. In one study, glutaric acid cocrystals were prepared with the API, 2-[4-(4-chloro-2-fluorophenoxy) phenyl]pyrimidine-4-carboxamide. The cocrystals showed and increased aqueous dissolution and subsequently improved bioavailability when compared to the pure API. In another example, 1:1 danazol:vanillin cocrystals were prepared in formulation and tested to show improved bioavailability by comparison the poorly available pure danazol.

Enhancement in stability has also been reported with cocrystals. Superior humidity stability was also reported for cocrystals for theophylline: oxalic acid cocrystals. Cocrystals of carbamazepine also showed a higher resistance to hydrate formation.

**Cosmetics**

Typical cosmetic formulation concerns involve preparing the active ingredient in a format that is both lends itself to easy application and is stable. Eutectic mixtures and cocrystals have been widely reported to facilitate this preparation. In one example, a eutectic mixture is utilized to
incorporate solid fragrances into perfumes and colognes. Typically, these solid fragrances are a challenge to include into formulations because the high temperatures necessary to melt the solids results in degradation of the other formulation components. The invention involves preparing the standard solid fragrances as eutectic mixtures with benzophenone to result in liquids that can be readily incorporated into the formulations. This patent displays the versatility and tailorability of these types of mixtures by including a table of the multi-component eutectic mixtures and their corresponding liquid points. In a similar example, eutectic mixtures were prepared from 12-hydroxysteric acid. While 12-hydroxysteric acid itself has well-known benefits to the skin, it is a solid of high-melting point with limited bioavailability. The eutectic mixtures provided lower melting point solids that could provide more availability. In a companion patent, Butyl methoxydibenzoylmethane (BMDM), a UVB absorber was incorporated into a eutectic with or without the 12-hydroxysteric acid to result in liquids that overcome the challenge of the high melting point of both. In another anti-sun formulation, eutectic mixtures were used, but this time to provide stability. Eutectic mixtures of n-butylthalamide and isopropylthalamide were prepared with the 1,3,5-triazine derivatives resulting in unusual stability. A eutectic mixture was used in scalp itch treatment formulations that showed improved scalp deposition of the eutectic mixtures of monoethanolamides versus the pure amides.

Cocrystals of 3-iodopropynyl butylcarbamate, an antifungal agent used in personal care products, were reported. The cocrystals compositions were reported to have better physical and chemical properties, in particular greater solubility in water and greater heat stability. Interestingly, the cocrystal compositions were also reported to have better “workability” properties such as better powder flowability and better compressibility for tablet formation. In another patent, cocrystal of p-coumaric acid and nicotinamide were reported as preparations for acne treatment, though no additional performance parameters were reported in the patent. In an example coming from our labs, it was shown that NCDs prepared from the colorant for a hair dye were more stable on the hair then the pure colorant.

Agrochemicals

Numerous patents for cocrystals used for agricultural products, such as fungicides, fertilizers and insecticides have been filed. For example, cocrystals of metalaxyl, a fungicide, were prepared with prothioconazole, another broad spectrum fungicide, to produce a material with dramatically decreased water solubility versus that of pure metalaxyl. The advantage in the decreased water solubility was described as serving to reduce the run off of the fungicides and thus limit the amount of fungicides necessary for efficacy as well as prevent excessive run-off into ground water streams. This is a particularly interesting example because both partners in the cocrystal are active ingredients and are working to form a mutually beneficial product.

Cocrystals of the herbicide, 3,6-dichloro-2-methoxybenzoic acid (decamba) were prepared with a variety of nitrogen containing heterocycles. These cocrystals were reported to not only address
the issue of excessive water solubility, but also that of stability. In particular, some herbicides were reported to suffer from the “Ostwald effect” over time, i.e. the precipitation of large crystals during the aging process. These large crystals are considered to have a deleterious effect during both the workability of the material at production level and on the efficacy of the herbicide during use. The cocrystals were reported to have improved stability against this aging process. In a similar patent, 4-hydroxybenzoic acid was reported to be effective with numerous pesticides and herbicides when prepared as a cocrystal.\textsuperscript{92}

Cocrystals were also used to raise the melting point of an insecticide, imidacloprid, using oxalic acid.\textsuperscript{93} The raised melting point allowed for better shelf stability and prevented the melting and clumping of the pure insecticide with aging. The same group published a similar patent reporting increased stability with raised melting point with 4-\{[(6-chloropyrid-3-yl) methyl](2,2-difluoroethyl)amino\}furan-2(5H)-one with salicylic acid.\textsuperscript{94}

**Chromophores**

Pigments are a particularly interesting chromophore application of NCDs. Bucar, et al. makes a compelling argument for the use of mechanochemical grinding to create novel pigments in quantitative yields that would otherwise be unachievable by solvent methods. In his paper on the subject, he demonstrates the effective preparation of three colour-tuned fluorescein cocrystals as model studies to support this argument.\textsuperscript{30} In a series of patents, cocrystals of titanyl fluorothalocyanine with titanyl fluorocyanine are described\textsuperscript{95} and prepared by heating\textsuperscript{96} and dry milling.\textsuperscript{97} The resulting cocrystals not only had a novel spectrum but more importantly had improved electrophotographic sensitivity and low dark decay. Red textile pigments were prepared as two component dizao-based eutectics. These pigments were shown to have performance equal to those of more toxic dyes in terms of color fastness, heat resistance, acid resistance, alkali resistance and solubility.\textsuperscript{98} In a 2011 paper, Yan, et al., demonstrate that cocrystals can be used to not only tune the color, stability and solubility of chromophores. They prepared a series of stilbene-type molecules with different co-formers and showed remarkable differences in the luminescence emissions, UV-Vis absorbance and quantum yield.

**Food Additives**

NCDs have also found a place in food additives. Cocrystals prepared from the antioxidant yerba mate and sucrose resulted in powders with lowered hygroscopicity when compared to yerba mate and good flowability for processing.\textsuperscript{99} It was confirmed that the yerba mate did not lose its antioxidant activity in the cocrystal form. Cocrystals were also used to solve another processing problem. While the mixture of ethylvanilla and vanilla is desirable from a taste and fragrance perspective, the simple mixture gives clumping that makes it unusable in a manufacturing
process. Cocrystals prepared from the same however were shown to produce powders that had good flow and good potential in the food and fragrance industry.\textsuperscript{100} Cocrystal of menthol and xylitol were prepared as another flavor additive with better processing properties. The cocrystals were reported to show lower hygroscopicity compared with xylitol and a higher solubility in water compared with menthol.\textsuperscript{101}

**Other Potential Applications**

NCDs have enormous commercial potential in other less-developed areas as well. They are worth mentioning, despite limited patent activity, because the effects are notable. Cocrystals have been used to alter electrical properties and shown to have potential as organic semiconductor.\textsuperscript{102} In the referenced publication, it was shown that cocrystallization resulted in a material that more electrically conductive than the parent molecule. This is an area of great interest for power production and should be further explored. In another interesting application, hydroquinones were prepared with diamine coformers and used in instant photograph development. The hydroquinone cocrystals were less soluble in water and were therefore able to be coated from an aqueous media.\textsuperscript{103}

Cocrystal reagents are also an interesting opportunity. By preparing cocrystals from olefins, solid-state reactions were run in high yield. The indications are that it is possible to use cocrystals to direct reactivity.\textsuperscript{104} In one example, unique photochemical reactions were possible with diarylethene due to the conformation induced by the cocrystal.\textsuperscript{105} Cocrystals were also used to effect chiral resolution. Cocrystal were prepared from the racemic mixture DL-arginine. The difference in solubility between the D and L based cocrystals results in enantiomeric separation.\textsuperscript{106}

1.5 Summary

NCDs, encompassing both cocrystal and eutectic forms, possess properties that are unique to their supramolecular matrix. These properties include critical product performance factors such as solubility, stability and bioavailability. NCDs have been used to tailor materials for a variety of applications and have the potential to be valuable in an even broader range of materials and processes. NCDs can be prepared using little or no solvent and none of the reagents typical to synthetic modifications. Thus, non-covalent derivatization is both a powerfully versatile and environmentally-friendly and cost effective tool.
1.6 References


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CHAPTER 2: NCDS FOR SOLID STATE OXIDATIVE STABILITY

2.1 Introduction

Hydration and oxidation are among the two major pathways for degradation of organic materials in general and pharmaceuticals in particular.\(^1\) Oxidation of pharmaceuticals leads to limitations in shelf-stability and can result in the loss of drug efficacy and the formation of harmful degradation products.\(^2,3\) Stabilizing solid-state pharmaceuticals against oxidation is currently addressed in several ways. When possible active pharmaceutical ingredients (APIs) can be designed that avoid susceptible functional groups, such as alkenes, thioethers and amines.\(^4\) The addition of antioxidant excipients can also be employed to sacrificially protect the pharmaceutical or prevent propagation.\(^4,5\) Typical antioxidants include ascorbic acid,\(^4\) cysteine, sodium metabisulfite, propyl gallate, butylated hydroxytoluene, butylated hydroxyanisole.\(^5\) Appropriately engineered packaging can also serve as a barrier between the pharmaceutical and sources of oxidative catalysis such as oxygen or water.\(^4\) Where the oxidative formation of peroxides via ultraviolet light is the catalyst, light blocking packaging such as amber bottles can be employed.\(^4\)

Both the development of inherently more stable pharmaceutical molecules and the use of more resistant packaging have obvious negative implications for the environment. Increasing evidence has indicated that bioactive pharmaceuticals are accumulating in the environment, including drinking water. These bioactive pollutants are responsible for a variety of troubling effects\(^6-9\) ranging from reproductive effects on zebrafish\(^10\) to the near extinction of the Asian vulture.\(^11\) Building more stable drug molecules will increase their accumulation in the environment and thus their availability for unintended side effects. More oxygen stable packaging has a similar downside. If the packaging is more stable against oxygen, then it will be less likely to degrade and more likely to accumulate in our landfills. The inclusion of antioxidant excipients in pharmaceuticals comes with its own particular challenges.\(^5\) Since antioxidants are an additional chemically active ingredient, the possibility of side reactions exists. Also, antioxidant themselves have been associated with a number of undesirable side effects, including gastric irritation, tumors, colic and diarrhea.\(^12-14\)

It is at this point that the argument for the use of NCDs for imparting oxidative stability becomes apparent. As opposed to the addition of antioxidants or use of more degradation resistant materials, a benign unreactive coformer can be incorporated in the API to provide the necessary stability. The NCD can be designed so that it dissociates upon administration or during the metabolism process. Any un-metabolized API would be excreted into the environment without the stabilization provided by the coformer. The excreted API would thus be more likely to degrade then accumulate in the environment. The same argument for the
environmental advantage of NCD stabilization can be made for any number of solid state organic material products. The potential environmental advantages are far-reaching, making the concept worthy of exploration and implementation.

There are reasons to anticipate the efficacy of NCD oxidative stability from a mechanistic standpoint. A variety of examples exist in the literature exploring the stabilizing effect of cocrystals against hydrolysis. However, there is little research that explores the effect of cocrystals on oxidative stability. Oxidation can occur through a variety of mechanisms including, auto-oxidation (mediated by free radicals), electron transfer, photochemically induced oxidation (singlet oxidation), trace impurities (peroxides or transition metals), or direct oxidation of functional groups. In the case of a crystal’s susceptibility to autoxidation, it has been suggested that the ability of oxygen to infiltrate and degrade the structure is directly related to the size of the spaces available within the crystal structure that allow or prevent the diffusion of a di-oxygen molecule. When considering these mechanisms, it becomes apparent that altering the rate of oxidation could be achieved by changing the electronic environment of the target molecule (i.e. the bonding arrangement) and/or the size and shape of the crystal structure. Non-covalent derivatives offer the opportunity to impart these types of changes to parent compounds in the solid state and subsequently and thus affect oxidative stability.

2.2 Development of a Model System

Hydroquinone was chosen as a model material for oxidative stabilization of NCDs for two reasons. To begin, hydroquinone is known to convert to benzoquinone by way of oxidation. This process has been extensively studied and consists of an electron abstraction to form a radical structure. This radical structure can exist as either a semiquinone radical or, with the loss of an additional proton, as a semiquinone radical anion. The loss of a second electron results in the p-benzoquinone. It is interesting to note that benzoquinone and hydroquinone create a stable charge transfer system referred to as a quinhydrone which is one of the earliest identified non-covalent derivative systems.

The second reason hydroquinone made for an ideal model molecule is its ability to form non-covalent derivatives with bis-(N,N-dialkyl) terephthalamides. These non-covalent derivative have been well-established and are known to exist in the form of a 1:1 cocrystal. They can be readily formed by way of both solvent recrystallization and mechanochemical means.

2.2.1 Preparation of Hydroquinone and Bis-(N,N-dialkyl)terephthalamides NCDs
Bis-(N,N-dialkyl) terephthalamides were prepared by condensing terephthaloyl chloride and the appropriate dialkyl amine. Three alkyl derivatives were prepared: ethyl, propyl and butyl (Scheme 1).

\[
\begin{array}{c}
\text{O} \\
\text{Cl} \\
\text{Cl} \\
\text{O} \\
\end{array} + \begin{array}{c}
2 \text{H} \\
\text{R-N} \text{R} \\
\text{R} \\
\end{array} \rightarrow \begin{array}{c}
\text{O} \\
\text{R-N} \text{R} \\
\text{R} \\
\end{array}
\]

\[\text{R} = \text{CH}_2\text{CH}_3, \text{CH}_2\text{CH}_2\text{CH}_3 \text{ and CH}_2\text{CH}_2\text{CH}_2\text{CH}_3\]

**Scheme 1**: Preparation of Bis-(N,N-dialkyl) terephthalamides

Products were purified by solvent recrystallization and confirmed by NMR. The preparation of the three alkyl derivatives allowed for the preparation of three types of NCD cocrystals with hydroquinone, each with varying crystal packing shapes and melting points.

NCD cocrystals between hydroquinone and bis-(N,N-diethyl) terephthalamide were prepared using both solvent and mechanochemical processes. Cocrystals prepared from solvent were recrystallized from ethanol. Equimolar amounts of hydroquinone and bis-(N,N-diethyl) terephthalamide were dissolved in ethanol with heat and left to precipitate overnight. The resulting white needle crystals were collected and dried in a vacuum oven at room temperature for a subsequent night. Analysis of the white crystals by NMR confirmed a 1:1 relationship between the hydroquinone and amide in the solid. Differential scanning calorimetry (DSC) was also used to compare the hydroquinone and bis-(N,N-diethyl) terephthalamides with the cocrystal (*Figure 3*).
Figure 3: Differential Scanning Calorimetry (DSC) showing the cocrystal melting point of 150°C which falls between those of the parent compounds, hydroquinone (174°C) and bis-(N,N-diethyl) terephthalamide (131°C).

The phase transition of the cocrystal was seen as a single peak in the DSC indicating a pure homogenous material. The melting point of the cocrystal was seen to fall between those of hydroquinone and bis-(N,N-diethyl) terephthalamide. This melting point behavior is consistent with the majority of cocrystals where the melting point of the cocrystal falls between those of the parent compounds.³⁵

NCDs were also prepared by way of mechanochemical means using an automatic amalgamator. The amalgamator was employed with the hope of developing more reproducible grinding conditions. To begin, a series of grinds were prepared with a 1:1 molar ratio of hydroquinone to bis-(N,N-diethyl) terephthalamide at different grind times. DSC analysis was used to follow the formation of the cocrystal. Comparison of DSC showed that samples ground for four hours produced materials with DSCs consistent with the 1:1 cocrystal prepared from solvent re-crystallization (Figure 4).
Figure 4: DSC comparison of 1:1 molar ratios of hydroquinone and bis-(N,N-diethyl) terephthalamide showing the effective preparation of the NCD cocrystals as grind time increases from 0 minutes to 4 hours.

Next, a series of 15 samples was prepared using varying ratios of hydroquinone (HQ) and bis-(N,N-diethyl) terephthalamide (DETA). Three vials of each samples type were prepared and each vial was sampled for three DSCs. Two things were confirmed with these experiments. First, the reproducibility of sample preparation method was confirmed. It was seen that a high degree of reproducibility existed not only between samples from a single vial, but also for each iteration of the ratio sample. Interestingly, this was the case not only for the 1:1 ratio, as would be expected for the preparation of the homogeneous cocrystal, but for each of the ratios. This high degree of reproducibility is shown below for the 9 runs of 2:1 HQ:DETA and the 9 runs of 1:1 HQ:DETA (Figure 5).
**Figure 5:** The high degree of reproducibility of the cocrystals prepared by amalgamator demonstrated in the 2:1 HQ to DETA and 1:1 HQ to DETA DSCs.

A phase diagram was prepared by plotting the mole percent of hydroquinone for each ratio against the dominant endothermic peaks in the DSC run on each ratio (**Figure 6**).

**Phase Diagram for HQ and DETA**

**Figure 6:** Phase diagram plotting the mole percent of hydroquinone versus DETA for each ratio against the dominant endothermic peaks in the DSC run on each ratio and showing the cocrystal as the maxima between two eutectic points. Each symbol represents dominant peaks on the individual DSC scan and are represented as shapes to highlight the shape of the curve.
This plot revealed the expected behavior for a cocrystal, that of two eutectic points surrounding the cocrystal maxima. The position of the cocrystal maxima is indicative of the ratio at which the cocrystal occurs. The cocrystal of hydroquinone and bis-(N,N-diethyl) terephthalamide was seen to occur reproducibly at a 1:1 molar ratio.

Two more 15 sample sets were prepared using the same method above with hydroquinone and bis-(N,N-propyl) terephthalamide (DPTA) and bis-(N,N-butyl) terephthalamide (DBTA). Phase diagrams were also prepared for these two systems and showed a cocrystals with DPTA of 104.68°C (Figure 7) and with DBTA of 88.89 °C (Figure 8).

**Figure 7**: Phase diagram plotting the mole percent of hydroquinone versus DPTA for each ratio against the dominant endothermic peaks in the DSC run on each ratio and showing the cocrystal as the maxima between two eutectic points. Each symbol represents dominant peaks on the individual DSC scan and are represented as shapes to highlight the shape of the curve.
**Figure 8**: Phase diagram plotting the mole percent of hydroquinone versus DBTA for each ratio against the dominant endothermic peaks in the DSC run on each ratio and showing the cocrystal as the maxima between two eutectic points. Each symbol represents dominant peaks on the individual DSC scan and are represented as shapes to highlight the shape of the curve.

The phase diagrams for the propyl and butyl amides showed curve shapes very similar to that of the hydroquinone and bis-(N,N-diethyl) terephthalamide series. This makes sense when considering that the systems rely on the same carboxylic acid to amide synthons and differ only in carbon chain length. As also might have been predicted when considering the increasing steric hindrance from ethyl to propyl to butyl, the cocrystal’s melting points decrease with increasing chain length. This is in keeping with the idea that a closer packed crystals results in a more stable system, as indicated by the higher melting point.

The three phase diagrams identified the hydroquinone cocrystals with bis-(N,N-diethyl) terephthalamide as the most stable cocrystal based on its higher melting point. Thus the ethyl based system was chosen for subsequent oxidation studies.

### 2.3 Oxidation of Hydroquinone and Bis-(N,N-Diethyl)terephthalamides NCDs

#### 2.3.1 Oxidation under Ambient Conditions
A series of samples was prepared for a time study of oxidation at ambient conditions. A comparison of the oxidation of differing 1:1 molar mixtures of hydroquinone to bis-(N,N-diethyl) terephthalamide was performed. Equal gram aliquots of materials were prepared with differing preparation methods in order to explore the efficacy of the cocrystal in preventing the oxidation of hydroquinone. It was previously identified that the cocrystal was prepared by grinding for four hours. Samples of varying ratios were thus prepared accordingly. Samples were also prepared with grind times of less than four hours in order to show that oxidative stability was imparted by the cocrystal and not simply the presence of the amide as a mixture. A sample of cocrystal prepared by precipitation from ethanol was also included in the oxidation sample matrix along with samples of pure hydroquinone and bis-(N,N-diethyl) terephthalamide, both as received and with four hours grinding, as controls. Samples were then subjected to long-term ambient condition degradation studies. Over the course of more than two years, samples were monitored by DSC, NMR and High performance liquid chromatography (HPLC). During the course of that time a color change was observed.

At 2.4 years, the color change was measure by densitometer. Densitometers provide a measure of the density of color. This is a measure of the absorbance of light over a range of visible wavelengths compared to the control of the total reflection of white control. Thus in this data, an increased density of blackness corresponds to a more colored solid. This color change is most likely due to the formation of benzoquinone and the concurrent formation of the highly colored quinhydrone complex. However, attempts to quantify the benzoquinone using NMR, IR and HPLC indicated that the benzoquinone did not reach measurable quantities. The color changes at 2.4 years as recorded by densitometer are reported below in Figure 9.
Figure 9: Densitometer readings of the color changes on the samples after 2.4 years of ambient oxidation. The results show that color change decreased with respect to increasing grind time.

Densitometer data indicated that the formation of the cocrystal between hydroquinone and bis-(N,N-diethyl)terephthalamide did inhibit the oxidation of hydroquinone as evidenced by the formation of the colored complex. The data was particularly remarkable with respect to the stepwise decrease of color-related oxidation with increased grind time. This supports the idea that the presence of the cocrystal provides the hydroquinone with oxidative stability. However, because the benzoquinone was not identifiable and quantifiable by typical analysis methods, it was desirable to develop accelerated oxidation conditions to provide further confirmation.

2.3.2 Oxidation under Accelerated Conditions

Development of an accelerated oxidation method began with a survey of current literature on solid state oxidative degradation methods. Often times, especially in the case of pharmaceuticals, liquid state oxidation studies are run and the data is used to predict solid state behavior. In one example, 2,2-azobis(- amidinopropane) dihydrochloride was simply added to a mixture of a drug and excipients. Additional examples of the limited solid state oxidation methods included several in which hydrogen peroxide was spiked into a mixture of a drug and excipients before tablet formation. More applicable were several studies in which a drug was exposed to hydrogen peroxide vapor. The source of vapor was either from hydrogen peroxide dissolved in
water in a sealed container filled with oxygen\textsuperscript{38} or from the introduction of a urea-hydrogen peroxide adduct in a closed system with the drug solid.\textsuperscript{39}

Procedures that required inclusion of an additional material into the solid matrix were rejected as these would obviously disrupt or alter the nature of the cocrystal. Efforts to initiate oxidation of hydroquinone by exposure of the solid to hydrogen peroxide vapor, using either the hydrogen peroxide solution or the urea-hydrogen peroxide adduct failed to induce oxidation.

Ozone has been reported for treatment of wastewater treatment, specifically phenols.\textsuperscript{40, 41} Additionally, kinetic and mechanistic work has been done on the effective oxidation of phenol in the solid state.\textsuperscript{42} Thus, it was hoped that ozone might be effective for solid state oxidation of hydroquinone. It was observed that hydroquinone was converted upon exposure to ozone to the highly colored complex usually associated with benzoquinone. IR and HPLC were used to confirm that the oxidation resulted in (and was limited to) benzoquinone. A reaction set up was developed wherein the solid on the bottom of a glass vial was treated with a constant stream of ozone for varying times. The reaction was monitored by both IR (infrared) spectroscopy and HPLC (high performance liquid chromatography). Over the course of 2 hours of ozone exposure of 1 gram of hydroquinone, the concentration of benzoquinone was seen to increase dramatically, as observed by HPLC. However, at this concentration the benzoquinone only reached 5\% conversion with respect to the total hydroquinone weight. When a second set of experiments was performed using a smaller starting aliquot of hydroquinone, the conversion increased to nearly 20\% benzoquinone. This difference is likely attributable to the greater surface area of the lesser bulk of solid, then any limiting molar relationship of the reactants (\textbf{Figure 10}).
Figure 10: Percent benzoquinone over the course of 2 hours of ozone exposure for 1 gram of hydroquinone. When a smaller initial hydroquinone aliquot of 25mg was used, the conversion increased to nearly 20% benzoquinone.

Ozonation was then run on a series of cocrystals. The sample of 1:1 hydroquinone to DETA ground for 4 hours, corresponding to the cocrystal as discussed above, showed no conversion to benzoquinone. The cocrystal prepared from ethanol precipitation showed very limited oxidation by comparison to the pure hydroquinone and the unground mixture (Figure 11).
Figure 11: Comparison of benzoquinone in 1:1 HQ to DETA samples exposed to ozone. Material ground for 4 hours showed no conversion to benzoquinone. The cocystal prepared from ethanol precipitation also showed very limited oxidation by comparison to the pure hydroquinone and the unground mixture.

A series of 15 ratios of hydroquinone to bis-(N,N-diethyl)terephthalamide were also oxidized with ozone. The resulting in a curve indicating the cocystal regime prevented the oxidation of hydroquinone (Figure 12).
Figure 12: Percent benzoquinone produced from the ozonation of a series of 15 ratios of hydroquinone to bis-(N,N-diethyl) terephthalamide. The resulting data indicated the cocrystal regime prevented the oxidation of hydroquinone.

These procedures rely on the ozonation of the surface of the solid. Despite efforts to retain consistency by maintaining the same solids content as well as sample area, these experiments can be expected to contain a range of results. However, within these limitations, these experiments effective demonstrate that the 1:1 cocrystal of hydroquinone and bis-(N,N-diethyl) terephthalamide does prevent the oxidation of hydroquinone.

2.4. Summary

It was anticipated that NCDs would be able to effect oxidative stability in a more ecologically sound manner. A model study was developed to explore this possibility. The study demonstrated the ability of the non-covalent derivative cocrystals between hydroquinone and bis-(N,N-diethyl) terephthalamide to effectively prevent the oxidation of hydroquinone. The introduction of a coformer into the target compound as the source of oxidative stability indicates the ability to stabilize APIs or other commercially valuable targets via a benign, solvent-free mechanochemical method.
2.5 References


CHAPTER 3: DEVELOPMENT OF A NOVEL BIOMIMETIC HAIR DYE

3.1 Introduction

Commercial hair dye formulations have recently come under scrutiny due to their association with serious health issues, such as bladder cancer\(^1\) and leukemia.\(^2\) Though these association are a subject of debate,\(^3\) the concern remains that amines, a class of compounds common to oxidative hair dyes, have known carcinogenicity.\(^4\) Phenylene diamine (PPD) in particular serves as a primary intermediate in many dyes and has been associated with a variety adverse health effects, including induced hypoglycemia and disturbed renal, hepatic and cardiac functioning.\(^5\) Thus, there was the need to develop a novel hair color system that did not rely on the typical dye molecules. The development and optimization of this dye demonstrates the way in which non-covalent derivatives (NCDs) can be employed to tailor specific properties without the use of hazardous or wasteful derivatizations.

Biomimicry is a school of thought that suggests turning to nature for inspiration when developing novel materials and systems.\(^6\) The advantage of a biomimetic approach is that nature has evolved functionalities using efficient, largely benign systems through millions of years of iterative research. Thus, in designing a novel biomimetic hair dye, natural mechanisms for coloration were considered. Insect sclerotization is a process by which the shell or exoskeleton of an insect becomes progressively harder. Sclerotization begins with tyrosine and proceeds through a series of enzyme-catalyzed oxidation reactions to yield dopamine derivatives.\(^7\) In sclerotization, these dopamine derivatives proceed to quinones that are incorporated into the cuticle matrix. However, in the case of colored insects shells, some of these dopamine derivatives are converted to indoles that ultimately form highly colored melanins.\(^8\) During his work toward developing a unified mechanism of sclerotization, Sugumaran reported a series of experiments in which catechol derivatives were oxidized with mushroom tyrosinase enzyme to produce highly colored species.\(^9\) These experiments indicated a possible route to synthetic color that would be compatible with a hair system. Natural hair color is comprised of two forms of melanin oligomeric structures: eumelanin and pheomelanin. Pheomelanin is responsible for red hair color and consists of a structure incorporating amino acid derived sulfurs. Eumelanin is responsible for the majority of hair color shades ranging from brown to black. Like sclerotization, hair melanogenesis begins with tyrosine and proceeds through a series of enzymatically-catalyzed oxidation reactions.

Raper initially published the biosynthetic pathway for eumelanin in 1926 and it was subsequently refined by Mason in 1948.\(^10\) The resulting Raper-Mason pathway remains the basis for the understanding of eumelanin production, both in mammalian hair and insect shells.\(^8,11\) This pathway begins with the oxidation of tyrosinase (I) to yield L-3,4-dihydroxyphenylalanine (L-DOPA) (II). Enzymatic oxidation is then responsible for the quinone derivative (III) which then rearranges to the related indole, leucodopachrome (IV).\(^12\) Further oxidation yields the 5,6-dihydroindol-2-carboxylic acid (DHICA) (V) which then decarboxylates to 5-6-dihydroxyindole (DHI) (VI) (Figure 13).
As it stands, a complete picture of the final structure of natural hair eumelanin remains elusive. This is the case for several reasons. First, the structure varies between animal species and even from individual to individual.\(^\text{13}\) Also, there is evidence that the eumelanin is capable of incorporating additional molecules or proteins into its structure.\(^\text{14}\) Further complicating the issue, the process of extraction of the eumelanin from hair can lead to changes in the original morphology of the material.\(^\text{15}\) In addition, the insoluble and resistant nature of eumelanin makes it difficult to apply traditional methods of structure identification. Lacking a true crystal of melanin, the X-Ray crystallography results provide indications about the material, but not a complete picture of the structure.\(^\text{16, 17}\) Solid-state NMR gives only minimal information about the oligomeric.\(^\text{18}\) Digestion experiments provides fragments, but can only point to possible structures, rather than concrete information, about the morphology of the eumelanin.\(^\text{19}\)

However, some information has been gleaned about the elements of a general eumelanin structure. One interesting paper suggests that a porphyrin-type structure of four indole units best matches the data for a DHI-rich oligomeric structure.\(^\text{20}\) The initial Raper-Mason pathway suggested that eumelanin was a result of the polymerization of DHI, though it was later found that DHICA degraded in the extraction process and so had not been initially identified as part of the eumelanin structure.\(^\text{21}\) Current evidence supports the idea that both DHI and DHICA are incorporated into the mammalian’s oligomeric eumelanin structure. The inclusion of both DHI and DHICA has a profound effect on the structure of eumelanin. Branching and larger molecules tend to occur with DHI, whereas DHICA tends to produce linear polymers. This is due to available bonding sites. DHI can form covalent bonds between carbons 7 and 4 as well as 2 and 3. The carboxylic acid on DHICA blocks position 3 and deactivates position 3, leaving only positions 4 and 7 for bonding.\(^\text{13}\) With this information on natural pigmentation in mind, a hair color system based on pseudo-melanin was developed.

**3.2 Design of a Biomimetic Hair Dye**

Both natural eumelanin color formation and insect sclerotization begin with tyrosine and proceed to L-DOPA. The additional hydroxyl on L-DOPA results in better water solubility when
compared to tyrosine. With a mind towards Sugumaran’s catechol experiments and the desire for a water-based system, L-DOPA was chosen as the starting material for a biomimetic hair dye. Initial experiments followed a literature procedure using potassium hexacyanoferrate (III) as a catalyst at room temperature. The literature procedure describes air-free conditions employed to prepare and isolate the indole. These experiments were run in the air with the hopes of encouraging further oxidation to the colored oligomer complex. The resulting crude black solid was characterized by NMR to reveal a mixture of indole (VI) and L-DOPA.

Concurrent dyeing experiments were also run using hair samples. Potassium hexacyanoferrate (PHF), PHF with potassium carbonate, pure L-DOPA and L-DOPA with potassium carbonate controls were run (3A, 4A, 7A and 8A). In keeping with the above procedure, L-DOPA, PHF and potassium hydrogen carbonate were combined and immediately added to the hair (5A). The previously prepared indole was also explored, along with additional PHF and potassium hydrogen carbonate (6A). Finally, hair was soaked in L-DOPA solution (7B) or L-DOPA in potassium carbonate (8B) followed by PHF and additional base. These last two methods were to create a situation of in situ oxidation on the hair sample. Evidence of effective hair dying was rated on a visual scale 1-10 scale for initial assessment (Figure 14) and analyzed by solid probe UV-Vis spectroscopy (Figure 15). As seen by both visual inspection and UV-Vis, the in situ oxidation beginning with an L-DOPA solution (7B) created the greatest color change in the hair, as seen by the UV-Vis results and visual inspection.
<table>
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<th>Result</th>
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<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>3A</td>
<td>PHF</td>
<td>1</td>
</tr>
<tr>
<td>4A</td>
<td>PHF and KHCO₃</td>
<td>2</td>
</tr>
<tr>
<td>5A</td>
<td>Oxidized L-DOPA Applied to Hair: PHF, KHCO₃ and L-DOPA</td>
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</tr>
<tr>
<td>6A</td>
<td>PHF, KHCO₃ and DHI</td>
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<tr>
<td>7A</td>
<td>L-DOPA in water</td>
<td>2</td>
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<td>7B</td>
<td>In situ oxidation, L-DOPA soak followed by PHF and KHCO₃</td>
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</tr>
<tr>
<td>8A</td>
<td>L-DOPA and KHCO₃</td>
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<tr>
<td>8B</td>
<td>L-DOPA and KHCO₃ soak followed by PHF and KHCO₃</td>
<td>8</td>
</tr>
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**Figure 14:** Visual inspection and grading of the initial hair dye conditions indicating that L-DOPA soak on the hair followed by introduction of the initiator (**in situ** oxidation) resulted in the most effective hair color.
Figure 15: Solid State UV-Vis quantifying the color on dyed hair samples. Increased absorbance indicated darker hair dying. These results showed that L-DOPA soak on the hair followed by introduction of the initiator (in situ oxidation) resulted in the most effective hair color.

Scanning electron microscope (SEM) images was used to characterize the morphology of the hair dye on the hair shaft. As might be expected, a much smoother and uniform coating was produced when the oxidation was run in the presence of the hair shaft as opposed to applying the oxidized material to the hair post-reaction (Figure 16).

Figure 16: SEM showing the difference between applying a polymerized L-DOPA sample to the hair versus the smooth coating on the hair shaft achieved by running the polymerization in the presence of the hair sample.
Conditions of increasing L-DOPA concentration and time were explored for their effect on hair dye efficacy. As might be expected, increasing time led to darker hair samples. Increased L-DOPA concentration also resulted in darker samples, despite the fact that the increased concentrations exceeded the initial solubility of L-DOPA and resulted in slurries. Additional catalysts, in particular horse radish peroxidase/peroxide and mushroom tyrosinase, were also explored. Both produced color, though tyrosinase was more effective at producing color on the hair. In fact, in experiments with successive dye treatments, a direct comparison between potassium hexacyanoferrate catalyst and tyrosinase showed tyrosinase to be capable of producing better hair color (Figure 17). This encouraging result indicated the possibility for optimization using the preferred tyrosine initiator. Tyrosinase was thus employed as the initiator for subsequent hair dyeing experiments.

**Figure 17:** Repeat dye experiments showing that repeat tyrosinase dyeing (blue) resulted in darker color than those with the repeat PHF catalyst dyes (green).

### 3.3 Optimization of Dye Stability

In keeping with standard hair dye testing, the stability of color was explored by repeat washing experiments.\(^23\) It was determined that the L-DOPA/tyrosine system did not have the necessary stability when run at the initial reaction conditions for pH 7 at room temperature for 24 hours.

In traditional oxidative hair dyes a portion if not all of the dye chemistry takes place within the hair fiber. The process of oxidative hair dyeing consists of swelling the hair cuticle followed by diffusion of the primary intermediate, typically phenylene diamine or a derivative, into the hair fiber. The primary intermediate is oxidized by hydrogen peroxide in an alkaline environment. It has been suggested that this initial oxidation takes place within the hair shaft and therefore occurs more rapidly than it would occur in solution.\(^24\) The explanation given for this is that, while hydrogen peroxide is not a particularly good oxidant for phenylene diamine, in the closed high concentration environment of the hair shaft, the hydrogen peroxide decomposes to oxygen.
and thus becomes a markedly better oxidant for the diamine. The oxidized phenylene diamine then reacts with a coupler, typically aromatic compounds with electron donating groups arranged meta to one another. After initial coupling, the dyes can continue to develop sometimes with two or even three more couplers.\textsuperscript{25} This final step serves both to mature the color and lock in the dye molecules. This locking process is in part a function of size increase and serves to give the color the needed stability. It was hoped that taking advantage of this locking process would facilitate the development of an L-DOPA based color that displayed the necessary stability. For this to be possible, it was necessary to determine conditions under which either the L-DOPA or a partially oligomerized derivative could diffuse into the hair shaft before becoming large enough to become locked in the cuticle hair space.

Studies exploring the movement of dye into the hair fiber\textsuperscript{26-28} identify several intertwined conditions that effect the diffusion mechanism. The size and shape of the molecule obviously play a role. However, there is some discussion as to how predictable the relationship is between the molecule and the hair morphology. The pore size of hair is typically quite small. In a model considering the hair shaft as a sieve, size exclusion via the pore holes could restrict the molecules to as small as 9.5 Å.\textsuperscript{28} However, the hair pore system is likely more dynamic then a solid sieve model would suggest. Under appropriate conditions, the hair pores can be expected to temporarily expand to allow the penetration of larger molecules.\textsuperscript{27} Increased temperature and pH can be employed to increase swelling of the hair fiber. The increased temperature will also increase the movement of the dye to the hair surface via increased kinetic energy of the molecules in solution.\textsuperscript{27} In addition, hair at an alkaline pH shows significant swelling, which has been attributed as likely due to the ionization of diacidic amino acid residues.\textsuperscript{28}

To begin the optimization for stability, a series of experiments was performed exploring the effect of increasing temperature and pH on the L-DOPA/mushroom tyrosinase system. Experiments were initially run in glass vials and the products were characterized. Conditions were chosen that remained within the pH and temperature activity limits of mushroom tyrosinase\textsuperscript{29} and could be used on the human head. The reactions were run for 1 hour. Results were initially analyzed by Beckman Coulter particle size (Figure 18).
The particle size results showed an increased size in the filtered material and distribution as the pH was increased from 7 to 9.

The particle size results showed an increased size in molecules as the pH was increased from 7 to 9, as well as an increasing distribution and size with increase of temperature from 35 to 45 to 55 °C. However, the Beckman Coulter analyzes particle size by determining the solid particles that pass by the eye of the analyzer and indicates the count as relative percent of the whole. This means that while larger water insoluble molecules are observed, water insoluble molecules less than 40 nm and water-soluble components are not observed. A simple weight experiment was run to determine the effect of the conditions on the percent of water-soluble material. The above conditions were run and the filterable fraction was filtered, dried and weighed (Figure 19).
Figure 19: Measure of filterable material with changing conditions for the L-DOPA/tyrosine system. These results indicate a maximum fraction of water-soluble materials at pH 9 and 55 °C.

These results showed that while some of the product of oligomerization at high pH and high temperature were indeed larger, this only accounted for a small fraction of the overall material. It can be inferred that at pH 9, the product is either made up unreacted L-DOPA (due to a significantly slowed reaction time) or a higher fraction of water-soluble oligomerized species. This water solubility could be due to smaller sized molecules or an increased presence of polar groups, such as carboxylic acids, on the molecule. Either way, these experiments indicated that a pH of 9 at higher temperature would produce the largest fraction of water soluble materials which could lead to more effective diffusion into the hair shaft.

Experiments were then run on hair samples. Increased temperature of 55 °C did not improve the stability for an hour. Even a single wash resulted in dramatic fading of the color (Figure 20).

Figure 20: Stability tests of hair samples at pH 7 and 55 °C. Results showed significant fading over the course of 30 washes at 45 °C.

However, experiments were run dyeing hair at pH 9 and 55 °C for an hour. In keeping with expectations developed from the particle size analysis, stability tests showed the hair to have both good color and good stability (Figure 21).
Figure 21: Stability tests of hair samples at pH 9 and 55 °C. Results showed stability over the course of 30 washes at 45 °C.

A colorimeter was used to measure the degree of lightness (L) between washes and compare those to the lightness results of hair samples dyed at pH 7 at 55 °C (Figure 22).

![Colorimeter data testing the color stability by measure of lightness comparing samples dyed at pH 7 versus pH 9.](image)

Figure 22: Colorimeter data testing the color stability by measure of lightness comparing samples dyed at pH 7 versus pH 9.

SEM images of the stability samples of hair dyed at pH 9 (Figure 23) showed no visible coating on the hair shaft. This, coupled with the high color density and good stability, is indicative of the desired absorbed into the hair fiber, as opposed to coated on the hair shaft.

![SEM images of the stability samples of hair dyed at pH 9 and 55 °C with successive washes showing no visual evidence of coating on the hair fiber and no significant change over the course of washes.](image)

Figure 23: SEM images of hair dyed at pH 9 and 55 °C with successive washes showing no visual evidence of coating on the hair fiber and no significant change over the course of washes.
NMR of the final reaction product after heating for an hours showed only L-DOPA in the soluble fractions in both chloroform and dimethylsulfoxide. The dark black insoluble solid was found to be insoluble in chloroform, dimethoxysulfoxide, acetonitrile, acetone, pyridine, isopropanol, N-methyl-2-pyrrolidone or dimethylformamide. The tyrosinase-induced oxidation of L-DOPA at pH 9 with sodium bicarbonate was run in D2O in NMR tubes and NMR experiments were run periodically in the hopes of seeing evidence of the transitory indole formation. However, only L-DOPA was apparent in solution throughout these studies.

Thermogravimetric analysis (TGA) was run on materials prepared from L-DOPA comparing the solid products of reactions run at pH 7, 8 and 9 at 35 °C, 45 °C, 55 °C. Materials were combined according to Table 1. The vials were heated with stirring for one hour. The solids were filtered and dried under vacuum at room temperature overnight. The dried solids were analyzed by TGA with scans run under nitrogen at a ramp rate of 20 °C/minute up to 1000 °C.

<table>
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<tr>
<th>Sample #</th>
<th>T (°C)</th>
<th>pH</th>
<th>NaHCO3 solution</th>
<th>mL water</th>
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<td>5</td>
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<td>5A</td>
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<td>8A</td>
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<td>8</td>
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<td>2.5</td>
</tr>
<tr>
<td>17A</td>
<td>55</td>
<td>9</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1: Description of Sample Preparations for TGA Studies on the Effect of pH and Temperature. An initiator solution of 0.0004 mg/mL tyrosinase in water and 0.25 g L-DOPA were used.

TGAs of the starting material, milled L-DOPA, versus products showed minimal difference, supporting the idea that the product is a small oligomeric structure rather than a large polymer. The only unusual point was a 2.5% greater decrease in weight % loss at 400 °C for solid collected from pH 9 and 55 °C. This does indicate that pH 9 and 55 °C does mark a set of conditions that produces a unique material, though minimal information on this difference. Mass Spectrometry using Direct Analysis in Real Time (DART) analysis of the product did show evidence of the 5,6-dihydroxyindole as well as L-DOPA, amid a variety of additional unidentified peaks. Gel permeation chromatography (GPC) showed little difference between the starting material and product, indicating that the product could be of very low molecular weight, possibly of only 1, 2, 3 or 4 repeat units of the indole. However, it should be remembered that these characterizations were run on samples run in glass vials. Because of the nature of oxidative hair dyes, it should be noted that the physical restrictions brought on by the small interstitial space of the hair follicle are thought to affect the final morphology and thus color of
the dye.\textsuperscript{28} Thus, any characterization of the material run outside the hair fiber may not be representative of the structure of the hair dye that ultimately colors the hair fiber.

3.4 Use of NCDs for Hair Dye Optimization

In a series of experiments parallel to the stability work, NCDs were prepared from L-DOPA and a variety of coformers. It was thought that the rate at which L-DOPA diffuses into the water would directly affect the rate at which it oligomerizes. The ability of NCDs to effect dramatic changes on solubility are well known.\textsuperscript{30} Thus, it was expected that NCDs from L-DOPA would serve as a useful way of tailoring the L-DOPA coloring system. NCDs were prepared using a variety of potential coformers. Each coformer was prepared in varying molar ratios with L-DOPA and ground via the amalgamator. These amalgamations were then used to dye hair samples according to a standardized dyeing method.

In an initial set of experiments, equal gram amounts of the amalgamations were used for each hair sample. This was done with the hope of identifying ways to reduce the necessary L-DOPA to effect the desired color change. As this was early in the project, these initial experiments were run at pH7 at room temperature for 24 hours in keeping with initial reaction conditions. The full range of ratios of L-DOPA:bisdiethylterephthalamide (DETA) and alanine produced the same hue as the control, but reduced intensity. However, L-DOPA:arginine grinds were seen to be effective at altering the color of the dye. The solid was observed to dissolve significantly faster than pure L-DOPA and the resulting color was a much lighter brown. It was apparent that arginine effected a change on the color formation from L-DOPA. The SEM images for the amalgamations showed coating on the outside of the hair sample for both L-DOPA: DETA and L-DOPA:arginine, but no coating was visible for L-DOPA:arginine (Figure 24). This indicated that the presence of L-arginine might have allowed for the penetration of color into the hair fiber.

![Figure 24: SEM images showing the coatings on the outside of the hair fiber created by both the DETA and alanine based NCDs. Coatings were not evident with the arginine NCDs indicating possible incorporation of the dye into the hair.](image)

While it was ultimately decided that increasing the pH and temperature was a preferable method for optimizing the dye uptake and stability, the effect of arginine remained of interest. A kinetics
experiment was run by way of a series of UV-Vis spectroscopy scans taken over the period of an hour (Figure 25). These scans were run in triplicate on a series of materials comparing pure L-DOPA and the potential NCDs. To maintain comparability, all samples, including the controls, were ground for thirty minutes using the amalgamator. A comparison was made between the molar ratios of the materials ground together (co-ground) and the simple mixtures of those same combinations made with simple stirring instead of grinding. This permitted a comparison of effects due to an NCD interaction (co-ground) and effects simply due to the presence of the coformer (mixture). In all experiments, the concentration of starting L-DOPA was maintained. The results indicated a significant change in the kinetic rate of the color formation of 1:2 L-DOPA:arginine. The differences between the kinetic rate of color formation with pure L-DOPA and any of the mixed samples, or those samples containing glutamine, were not as significant. The concentration of L-DOPA was maintained for each sample.

![Kinetic Comparison of NCD vs Grind by UV-Vis](image)

**Figure 25:** A comparison of the rate of color formation between L-DOPA and co-grinds versus mixtures of L-DOPA and both glutamine (Gln) and L-arginine (L-Arg) as cofactors.

Characterization by standard methods of the NCD interaction between L-DOPA and arginine proved difficult. Comparison by infrared (IR) spectroscopy of the parent compound showed the no absence nor gain of a peak in 1:1 L-DOPA:arginine sample or 1:2 L-DOPA:arginine samples, as might be indicative of an NCD. By solid state NMR, there were no novel peaks evident in the 1:1 L-DOPA:arginine sample when compared to the pure parent compounds. Differential Scanning Calorimetry (DSC) analysis proved more interesting (Figure 26). A comparison of the parent compounds to the 1:1 L-DOPA to arginine showed only a broad melting peak slightly lower than the L-DOPA. This could simply be indicative of the broadening and depression
associated with the inclusion of arginine as an impurity in the L-DOPA. The 1:2 L-DOPA:arginine scan proved a bit more interesting. There is evidence of a novel peak at 228°C, which is notably different from the melting points of pure L-DOPA (283°C) and pure arginine (241°C). However, the base line for this peak shows significant noise and melt before the peak and so this can be regarded as indicative of, but not conclusive proof of the preparation of an NCD at 1:2 L-DOPA: arginine.

![DSC scans](image)

**Figure 26:** A comparison of the DSC scans for L-DOPA and arginine compared to the 1:1 and 1:2 molar ratio amalgamations. The 1:1 L-DOPA to arginine showed only a broad melting peak slightly lower than the L-DOPA, however the 1:2 L-DOPA:arginine showed evidence of a novel peak.

A recent publication on the development of cocrystals of L-DOPA for its medicinal application adds additional information to this discussion. During a wide range screening for cocrystals, they reported no evidence of a cocrystal between L-DOPA and a variety of coformers. However, they do point to a novel dihydrate of L-DOPA that was detected and analyzed by SCXRD (single crystal X-Ray diffraction). The authors report that this novel dihydrate was only formed when attempting to make the ascorbic acid or glutamine cocrystals. They report that this dihydrate to be unstable and that reversion back to the anyhydrous L-DOPA form occurs in less than two days. Arginine was not one of the coformers tested during their screening, so no report exists in this effort for the formation of either the dihydrate or cocrystal. However, this study does point to additional interesting questions in the understanding the supramolecular behavior of L-DOPA and any potential cocrystals.
3.5 Summary and Conclusions

The development of a novel biomimetic hair color based on L-DOPA proved successful. Optimization of stability was accomplished by increased pH and temperature. The characterization of the final dye structure was hampered by the difference between hair dye prepared in reaction vessel and that which results from a hair dye development within the hair fiber. A novel non-covalent derivative between L-DOPA and Arginine was identified at a 1:2 molar ratio. This NCD was identified by DSC and kinetics experiments. The NCD was used to produce a hair color of reasonable stability that was different from that of the pure L-DOPA. This provides an example of the way in which an NCD can be used to alter the properties of a solid state system using a benign and effective technique.

3.6 References


CHAPTER 4: NCDs FOR STABILIZATION OF REACTIVE FUNCTIONAL GROUP

4.1 Introduction

Organic peroxyacids (or peracids) are a class of compounds that have found value in industry, often as cleaning agents and cosmetic ingredients owing to their bleaching and disinfecting abilities. Peroxyacids are also employed as initiators for radical polymerizations, cross-linking agents for polyolefins and hardeners for elastomers. Because this valuable high reactivity is coupled with the predictable low shelf stability, peroxyacids are often generated in situ. However, in some situations, this is not an ideal option and the problem of how to stabilize these compounds in formulations presents itself. This chapter focuses on the stabilization of a commercially important peroxyacid as a dispersion in water using NCDs. For reasons of maintaining client confidentiality, the complete structure of this peroxyacid is withheld. However, the discussion of NCD stabilization can still be effectively made by considering a general peroxyacid structure.

Peroxyacids decompose exothermically to their corresponding carboxylic acid. This exothermic behavior leads to further instability in peroxyacid compounds due to a self-catalyzing accelerated decompositions as the temperature increases. The temperature at which this occurs, the self-accelerating decomposition temperatures (SADTs), varies with the structure of the organic peracid. However, in their solid form, peroxyacids can self-stabilize due to their ability to form a herring-bone type crystal packing structure that stabilizes the oxygens within the peroxy groups. Peroxyacids are somewhat stable at low pH by creating an equilibrium between peroxide and carboxylic acid. Conversely, alkaline pH rapidly destroys peroxyacids.

A survey of stabilization techniques in the patent literature includes methods such as encapsulation of granules of peroxyacid via materials such as polyvinyl alcohol, polyethylene glycol, cross-linked sodium alginate, oligosaccharides and paraffin. Other methods address the exothermic self-catalysis via the addition of exotherm control agents, citing citric acid and urea as examples.

4.2 Development of a Screening Method

NCDs were prepared with peroxyacid to impart stability. It was desired that the final formulation of the peroxyacid was as a dispersion in a proprietary formulation of additives in water. The aim of these experiments was to develop and perform a screening method with different coformers to identify candidate coformers that were capable of stabilizing the peroxyacid in water.

Evaluation by HPLC was limited by the peracids poor stability in organic solvents. During the time it took to prepare and run a sample on the HPLC, the peracid was already showing significant degradation to its corresponding carboxylic acid. Thus, it was desirable to develop an additional characterization method. In NMRs run in chloroform, the methylene peaks for both peracid and the corresponding carboxylic acid are readily integrated and a relative amount of peracid to acid could easily be determined (Figure 27).
**Figure 27:** NMR showing clear distinction between methylene peaks for both peracid and the corresponding carboxylic acid which are readily integrated to determine relative amount of peracid to carboxylic acid.

It was found that samples could be run in chloroform on the NMR quickly enough to show only a very minimal degradation of the peroxy acid. Pure samples of peracid were suspended in water and subjected to heating with stirring. Samples were removed at timed intervals and immediately evaluated by NMR. The NMRs indicated no other degradation products aside from the carboxylic acid thus permitting the calculation of percent peracid relative to carboxylic acid to be calculated from the integration of the methylene peaks. The peracid showed surprising stability in the studies run in pure water. This was likely due to self-stabilization of the peracid in solid form and its relative insolubility in water. However, once the degradation began, it was rapid. By following the degradation of peracid by NMR, 80°C was identified as the temperature at which peracid degraded to 60-80% in the screening period of 24 hours (**Figure 28**).

**Figure 28:** Degradation studies of peracid in proprietary formulation with heat as determined by integration of NMR.
4.3 Coformer Screening

NCD preparation is done using the two pure materials (in this case the peracid and the co-
molecule). These pure materials are either combined in the solid state or in the presence of a co-
solvent. To this end, a scaled-up synthesis of the target peracid starting from the inexpensive
readily available starting materials was developed. The synthesis proceeded by preparing the
carboxylic acid analogue followed by oxidation to the peracid via a typical acid-catalyzed
hydrogen peroxide reaction. The synthesis was completed over the course of several days and
structure and purity were confirmed by NMR.

With a large quantity of the pure peroxyacid (PA) in hand, NCDs were prepared by grinding. To
begin, a series of peracid samples were ground on the amalgamator over the course of several
hours and evaluated for degradation. This was to insure that preparation of the NCD did not
result in premature degradation (Figure 29).

Figure 29: Study of effect of grinding on amalgamator to insure peracid (PA) sample stability
and constant carboxylic acid concentration (CA) during NCD preparation.

A series of coformers was chosen that comprised a variety of functional groups and carbon chain
lengths with the potential capacity to form a noncovalent derivative with the target peroxyacid.
The initial list of complexing materials was screened from the FDA GRAS (generally regarded
as safe) or related compounds whose National Fire Protection Agency (NFPA) health rating is 2
or below. NCDs prepared from this list were evaluated for stabilization of peroxyacid under
various conditions by evaluating the change in peroxyacid concentration.

These grinds were then prepared in 20% solids in aqueous dispersions. This solids percent is
consistent with the proprietary formulation and served as a model for the formulation. The aim
was to prepare a material with similar solids percent but a reduced need for PA. For the standard formulations, a decrease in PA concentration for the 20% solids resulted in a more rapidly degrading material. This was attributed to the reduced self-stabilization of the solid peroxyacid at lower concentrations. Samples were heated while monitoring the peracid (PA) degradation. This information was used to identify two co-molecules which showed PA stabilization over time: adipic acid and glutaric acid (Figure 30).

**Figure 30:** Peracid concentration monitored in heated aqueous dispersions identifying two co-molecules that showed some PA stabilization over time: adipic acid and glutaric acid.

Stabilization of the adipic acid was confirmed by HPLC as well. This served to both validate the NMR method and reproduce the stabilization results (Figure 31).
Figure 31: Peracid concentration monitored in heated aqueous dispersions showing stabilization of the peracid by adipic acid as shown by both NMR and HPLC.

A series of degradation experiments were run comparing varying ratios of peracid showing that 1:1 peroxyacid to adipic acid to have the least degradation (Figure 32).

Figure 32: Degradation experiments comparing varying ratios of peracid showing reduced degradation of 1:1 peracid to adipic acid.

As might be anticipated, similar stabilization was seen with glutaric acid whose similar structure to adipic acid makes it a reasonable candidate (Figure 33).
Figure 33: Degradation experiments comparing varying ratios of peracid showing reduced degradation in samples that included glutaric acid.

The inherent insolubility of the peroxy acids provides them with some stability in pure water. However, the addition of any organic solvent leads to increased dissolution and subsequent degradation. In a subsequent set of studies, the pure peroxyacid was tested in the product’s target formulation. This formulation contained ingredients that increased the dissolution of the peroxyacids.

Figure 34: Degradation studies with peroxyacid and adipic acid grinds in formulation showing some improvement in stability at the 1:1 and 1:2 ratios.
As was seen in the pure water samples (Figure 34), both 1:1 and 2:1 peroxyacid: adipic acid showed promise as a stabilizer. It was important to establish that this stabilization effect was not simply a function of pH. This question was explored using the adipic acid system. First, a comparison was made between adipic acid as an NCD and adipic acid simply added to the peracid solution. The use of the NCD of adipic acid showed a two-fold improvement in stability as compared to the simple addition of adipic acid. An additional argument is made for the value in the formation of the NCD when comparing the results of adipic acid versus suberic acid at varying ratios (Figure 35). Suberic acid is a dicarboxylic acid of eight carbon chain length and so makes an interesting comparison to adipic acid as a six carbon chain length dicarboxylic acid. Suberic acid and adipic acid have nearly identical pKa’s. In these studies, a series of grinds were prepared at varying ratios of peracid to dicarboxylic acid. The grinds were then subjected to our standard accelerated degradation studies and evaluated for peracid degradation.

![Image](image-url)

**Figure 35:** Degradation studies with peroxyacid and suberic acid in formulation showing a more rapid degradation of PA with decreasing PA concentration.

It can be seen suberic acid, despite its similar pKa provided no stabilization for the peracid. The differences seen here between the ratios can most readily be associated with the self-stabilization of the peracid. As the concentration of peracid is lowered, without the presence of a stabilizing factor, the peracid degrades more rapidly.

### 4.4 NCD Characterization

Having determined that adipic acid and glutaric acid showed promise as stabilizing co-formers, characterization of their interaction with the peracid became of interest. Both differential scanning calorimetry (DSC) and powder X-Ray diffraction (PXRD) were employed with the hopes of elucidating the nature of the interaction. A series of grinds were run comparing 15 ratios of the peracid to coformer. DSC were run and the results were used to create phase diagrams. Both adipic acid (Figure 36) and glutaric acid (Figure 37) showed indications of non-
covalent derivative interaction between the peracid and the coformer. By contrast, Glutamic acid provides an example of a coformer that has no interaction with the peracid (Figure 38). PXRD was also employed. A comparison of the 1:1 PA to adipic acid amalgamation to the parent compounds showed no novel peaks (Figure 39). The PXRD for glutaric acid proved more interesting. The PXRD scans for 1:2 peracid to glutaric acid showed significant shifts in the peaks when compared to the parent compounds (Figure 40). This is supportive of an NCD interaction between the peracid and glutaric acid.

**Figure 36:** Phase diagram of adipic acid in peracid showing NCD interaction between the two solids.

**Figure 37:** Phase diagram of glutaric acid in peracid showing NCD interaction between the two solids.
**Figure 38:** Phase diagram of glutamic acid in peracid showing no NCD interaction between the two solids.

**Figure 39:** PXRD showing no novel peaks in the 1:1 PA to adipic acid (purple) when compared to the parent compounds PA (blue) and adipic acid (red).
Figure 40: PXRD showing novel peaks in the 1:2 PA to glutaric acid (green) when compared to the parent compounds PA (blue) and glutaric acid (red) or the 1:1 PA to glutaric acid (purple).

4.5 Summary

This work demonstrates a screening for NCD coformers for stabilization of a target peroxy acid. Both adipic acid and glutaric acid were identified as potential stabilizers for the peracid. The DSC for both adipic and glutaric indicated an NCD interaction between the coformers. The powder XRD further supported the presence of an NCD interaction for the 1:2 peroxyacid to glutaric acid.

4.6 References


CHAPTER 5: EXPERIMENTAL METHODS

5.1 Experimental Methods for Chapter 2

Chemicals: All materials were purchased from Sigma Aldrich and used as received.

Instruments: Amalgamations were ground using a SPEX amalgamator, 5100 Mixer Mill. Solid state IR measurements were made using a ThermoScientific Nicolette 6700 FT-IR with a Smart Golden Gate Diamond Attenuated Total Reflectance (ATR) accessory. NMR scans were made using a JEOL NMR AS400. DSC was run on a TA Instruments Q200 in hermetically sealed T-Zero aluminum pans. An A2Z ozone generator, Aqua 6, was used to produce ozone.

Bis-(N, N-diethyl) terephthalamide In a round-bottomed flask was combined 200 mL deionized water and 20 mL methylene chloride. The solvents were cooled using an ice bath. 51mL of diethylamine was then added to the cooled solvents. 20.06g terephthaloyl chloride was then added slowly over the course of fifteen minutes to avoid excess heat formation. The reaction was left to stir overnight and come to room temperature. The next day, the reaction mixture was transferred to a separatory funnel and additional methylene chloride was added. The mixture was washed with a 10% hydrochloric acid solution. The organic layer was collected and combined with 3X methylene chloride extraction of the aqueous layer. The organic layer was dried over sodium sulfate, dried to a solid on the rotovap and then dried overnight at RT under vacuum. The solid was then recrystallized from ethanol. Structure was confirmed by NMR.

Bis-(N, N-dipropyl) terephthalamide In a round-bottomed flask was combined 200 mL deionized water and 20 mL methylene chloride. The solvents were cooled using an ice bath. 135.5 mL of dipropylamine was then added to the cooled solvents. 20.06g terephthaloyl chloride was then added slowly over the course of fifteen minutes to avoid excess heat formation. The reaction was left to stir overnight and come to room temperature. The next day, the reaction mixture was transferred to a separatory funnel and additional methylene chloride was added. The mixture was washed with a 10% hydrochloric acid solution. The organic layer was collected and combined with 3X methylene chloride extraction of the aqueous layer. The organic layer was dried over sodium sulfate, dried to a solid on the rotovap and then dried overnight at RT under vacuum. The solid was then recrystallized from water and methanol. Structure was confirmed by NMR.
Bis-\((N,N\text{-dibutyl})\) terephthalamide: In a round-bottomed flask was combined 200 mL deionized water and 20 mL methylene chloride. The solvents were cooled using an ice bath. 173.2 mL of dibutylamine was then added to the cooled solvents. 20.06g terephthaloyl chloride was then added slowly over the course of fifteen minutes to avoid excess heat formation. The reaction was left to stir overnight and come to room temperature. The next day, the reaction mixture was transferred to a separatory funnel and additional methylene chloride was added. The mixture was washed with a 10% hydrochloric acid solution. The organic layer was collected and combined with 3X methylene chloride extraction of the aqueous layer. The organic layer was dried over sodium sulfate, dried to a solid on the rotovap and then dried overnight at RT under vacuum. The solid was then recrystallized from ethanol. Structure was confirmed by NMR.

Solvent Preparation of Hydroquinone and Bis-\((N,N\text{-diethyl})\) terephthalamide NCD Cocrystals: Equal molar amounts of hydroquinone (2.84 g, mmols) and diethylterephthalamide (7.15 g, mmols) were dissolved in ethanol with heat and then left to cool and recrystallize overnight. The resulting white needle crystals were collected via filtration and dried in a vacuum oven at room temperature overnight. Analysis of the white crystals by NMR confirmed the 1:1 relationship between the coformers (Figure 1). DSC was also used to confirm the novel single peak of the cocrystal. The melting point of the cocrystal was seen to at 150°C, between those of the hydroquinone (174°C) and bis-\((N,N\text{-diethyl})\)terephthalamide (131°C).
Figure 1: Analysis of the white crystals by NMR confirmed the 1:1 relationship between hydroquinone and bis-\((N,N\text{-diethyl})\) terephthalamide.

Figure 2: Differential Scanning Calorimetry (DSC) showing the cocrystal melting point of 150°C which falls between those of the parent compounds, hydroquinone (174°C) and bis-\((N,N\text{-diethyl})\) terephthalamide (131°C).
Optimization of Mechanochemical Preparation of Hydroquinone and Bis-(N,N-diethyl) terephthalamide NCD Cocrystals: Samples of 1:1 molar ration terephthalamide and hydroquinone were weighed out at 500 mg increments into plastic amalgamator vials. Vials were equipped with a methylmethacrylate ball and ground for increasing intervals of: 10 minutes, 20 minutes, 40 minutes, 1 hour, 2 hour, 3 hour and 4 hour. These samples were evaluated by DSC to identify the condition best able to prepare the NCD comparable to that prepared via ethanol cocrystallization.

Figure 3: Comparison of 1:1 molar ratios of hydroquinone and bis-(N,N-diethyl) terephthalamide shows the effective preparation of the NCD cocrystals as grind time increases from 0 minutes to 4 hours.
Mechanochemical Preparation of Ratio Series Hydroquinone and Bis-(N,N-diethyl) terephthalamide NCD Cocrystals: A series of 15 samples was weighed using varying ratios of hydroquinone and bis-(N,N-diethyl) terephthalamide according to Table 1.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Ratio HQ:DETA</th>
<th>g HQ</th>
<th>g DETA</th>
</tr>
</thead>
<tbody>
<tr>
<td>093-20-1</td>
<td>1:0</td>
<td>0.4500</td>
<td>0.0000</td>
</tr>
<tr>
<td>093-20-2</td>
<td>9:1</td>
<td>0.3519</td>
<td>0.0981</td>
</tr>
<tr>
<td>093-20-3</td>
<td>4:1</td>
<td>0.2765</td>
<td>0.1735</td>
</tr>
<tr>
<td>093-20-4</td>
<td>3:1</td>
<td>0.2450</td>
<td>0.2050</td>
</tr>
<tr>
<td>093-20-5</td>
<td>7:3</td>
<td>0.2168</td>
<td>0.2332</td>
</tr>
<tr>
<td>093-20-6</td>
<td>2:1</td>
<td>0.1996</td>
<td>0.2504</td>
</tr>
<tr>
<td>093-20-7</td>
<td>3:2</td>
<td>0.1683</td>
<td>0.2817</td>
</tr>
<tr>
<td>093-20-8</td>
<td>1:1</td>
<td>0.1282</td>
<td>0.3218</td>
</tr>
<tr>
<td>093-20-9</td>
<td>2:3</td>
<td>0.0944</td>
<td>0.3556</td>
</tr>
<tr>
<td>093-20-10</td>
<td>1:2</td>
<td>0.0748</td>
<td>0.3752</td>
</tr>
<tr>
<td>093-20-11</td>
<td>3:7</td>
<td>0.0656</td>
<td>0.3844</td>
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<td>093-20-12</td>
<td>1:3</td>
<td>0.0528</td>
<td>0.3972</td>
</tr>
<tr>
<td>093-20-13</td>
<td>1:4</td>
<td>0.0408</td>
<td>0.4092</td>
</tr>
<tr>
<td>093-20-14</td>
<td>1:9</td>
<td>0.0191</td>
<td>0.4309</td>
</tr>
<tr>
<td>093-20-15</td>
<td>0:1</td>
<td>0.0000</td>
<td>0.4500</td>
</tr>
</tbody>
</table>

Table 1: Preparation conditions for amalgamations prepared as a series of rations between hydroquinone and bis-(N,N-diethyl) terephthalamide

The appropriate amount of material, as listed in Table 1, was added to a plastic amalgamator vial along with a methylmethacrylate grinding ball. The sample was then ground using the amalgamator for 4 hours. Three vials of each samples type were prepared and each vial was sampled for three DSCs. The results were compared to determine reproducibility between samples (Figure 5).
Figure 5: The high degree of reproducibility of the cocrystals prepared by amalgamator is demonstrated in the 2:1 HQ to DETA and 1:1 HQ to DETA DSCs. A phase diagram was prepared by plotting the mole percent of hydroquinone versus DETA for each ratio against the dominant endothermic peaks in the DSC run on each ratio and showing the cocrystal as the maxima between two eutectic points.

![Phase Diagram for HQ and DETA](image)

Figure 6: Phase diagram plotting the mole percent of hydroquinone versus DETA for each ratio against the dominant endothermic peaks in the DSC run on each ratio and showing the cocrystal as the maxima between two eutectic points.

Preparation of Ratio Series Hydroquinone and Bis-(N,N-dipropyl) terephthalamide and Hydroquinone and Bis-(N,N-dibutyl) terephthalamide Amalgamations and Phase Diagrams: Two sets of 15 samples was weighed using varying ratios of hydroquinone and bis-(N,N-diethyl) terephthalamide according to Table 2 and 3. The appropriate amount of material
was added to a plastic amalgamator vial along with a methylmethacrylate grinding ball. The sample was then ground using the amalgamator for 4 hours. Phase diagrams plotting the mole percent of hydroquinone versus each of the amides for each ratio against the dominant endothermic peaks in the DSC were prepared and showed the cocrystal as the maxima between two eutectic points for DPTA (Figure 7) and DBTA (Figure 8).

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Ratio</th>
<th>g HQ</th>
<th>g DPTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>093-36-1</td>
<td>1:0</td>
<td>0.450</td>
<td>0.000</td>
</tr>
<tr>
<td>093-36-2</td>
<td>9:1</td>
<td>0.337</td>
<td>0.113</td>
</tr>
<tr>
<td>093-36-3</td>
<td>4:1</td>
<td>0.256</td>
<td>0.194</td>
</tr>
<tr>
<td>093-36-4</td>
<td>3:1</td>
<td>0.224</td>
<td>0.226</td>
</tr>
<tr>
<td>093-36-5</td>
<td>7:3</td>
<td>0.196</td>
<td>0.254</td>
</tr>
<tr>
<td>093-36-6</td>
<td>2:1</td>
<td>0.179</td>
<td>0.271</td>
</tr>
<tr>
<td>093-36-7</td>
<td>3:2</td>
<td>0.149</td>
<td>0.301</td>
</tr>
<tr>
<td>093-36-8</td>
<td>1:1</td>
<td>0.112</td>
<td>0.338</td>
</tr>
<tr>
<td>093-36-9</td>
<td>2:3</td>
<td>0.081</td>
<td>0.369</td>
</tr>
<tr>
<td>093-36-10</td>
<td>1:2</td>
<td>0.064</td>
<td>0.386</td>
</tr>
<tr>
<td>093-36-11</td>
<td>3:7</td>
<td>0.056</td>
<td>0.394</td>
</tr>
<tr>
<td>093-36-12</td>
<td>1:3</td>
<td>0.045</td>
<td>0.405</td>
</tr>
<tr>
<td>093-36-13</td>
<td>1:4</td>
<td>0.034</td>
<td>0.416</td>
</tr>
<tr>
<td>093-36-14</td>
<td>1:9</td>
<td>0.016</td>
<td>0.434</td>
</tr>
<tr>
<td>093-36-15</td>
<td>0:1</td>
<td>0.000</td>
<td>0.450</td>
</tr>
</tbody>
</table>

*Table 2:* Preparation conditions for amalgamations prepared as a series of rations between hydroquinone and bis-(N,N-dipropyl) terephthalamide.
Figure 7: Phase diagram plotting the mole percent of hydroquinone versus DPTA for each ratio against the dominant endothermic peaks in the DSC run on each ratio and showing the cocrystal as the maxima between two eutectic points.

<table>
<thead>
<tr>
<th>Sample Number</th>
<th>Ratio</th>
<th>g HQ</th>
<th>g DBTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>093-38-1</td>
<td>1:0</td>
<td>0.4500</td>
<td>0.0000</td>
</tr>
<tr>
<td>093-38-2</td>
<td>9:1</td>
<td>0.3232</td>
<td>0.1268</td>
</tr>
<tr>
<td>093-38-3</td>
<td>4:1</td>
<td>0.2391</td>
<td>0.2109</td>
</tr>
<tr>
<td>093-38-4</td>
<td>3:1</td>
<td>0.2068</td>
<td>0.2432</td>
</tr>
<tr>
<td>093-38-5</td>
<td>7:3</td>
<td>0.1791</td>
<td>0.2709</td>
</tr>
<tr>
<td>093-38-6</td>
<td>2:1</td>
<td>0.1628</td>
<td>0.2872</td>
</tr>
<tr>
<td>093-38-7</td>
<td>3:2</td>
<td>0.1342</td>
<td>0.3158</td>
</tr>
<tr>
<td>093-38-8</td>
<td>1:1</td>
<td>0.0994</td>
<td>0.3506</td>
</tr>
<tr>
<td>093-38-9</td>
<td>2:3</td>
<td>0.0715</td>
<td>0.3785</td>
</tr>
<tr>
<td>093-38-10</td>
<td>1:2</td>
<td>0.0558</td>
<td>0.3942</td>
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<td>093-38-11</td>
<td>3:7</td>
<td>0.0487</td>
<td>0.4013</td>
</tr>
<tr>
<td>093-38-12</td>
<td>1:3</td>
<td>0.0388</td>
<td>0.4112</td>
</tr>
<tr>
<td>093-38-13</td>
<td>1:4</td>
<td>0.0298</td>
<td>0.4202</td>
</tr>
<tr>
<td>093-38-14</td>
<td>1:9</td>
<td>0.0137</td>
<td>0.4363</td>
</tr>
<tr>
<td>093-38-15</td>
<td>0:1</td>
<td>0.0000</td>
<td>0.4500</td>
</tr>
</tbody>
</table>

Table 3: Preparation conditions for amalgamations prepared as a series of rations between hydroquinone and bis-(N,N-dibutyl) terephthalamide.
**Figure 8:** Phase diagram plotting the mole percent of hydroquinone versus DBTA for each ratio against the dominant endothermic peaks in the DSC run on each ratio and showing the cocrystal as the maxima between two eutectic points.

**Ambient Oxidation of NCDs of Hydroquinone:** In order to develop a series of samples comparing the efficacy of hydroquinone stabilization by NCD at ambient conditions, samples were prepared as follows:

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Preparation method</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>093-10-1</td>
<td>DETA</td>
<td></td>
</tr>
<tr>
<td>093-10-2</td>
<td>Ground DETA, 4 hours</td>
<td></td>
</tr>
<tr>
<td>093-10-3</td>
<td>HQ</td>
<td></td>
</tr>
<tr>
<td>093-10-4</td>
<td>Ground HQ, 4 hours</td>
<td></td>
</tr>
<tr>
<td>093-10-5</td>
<td>Percipiting from Ethanol</td>
<td>1:1</td>
</tr>
<tr>
<td>093-10-6</td>
<td>No Grinding</td>
<td>1:1</td>
</tr>
<tr>
<td>093-10-7</td>
<td>10 min Grind</td>
<td>1:1</td>
</tr>
<tr>
<td>093-10-8</td>
<td>20 min Grind</td>
<td>1:1</td>
</tr>
<tr>
<td>093-10-9</td>
<td>30 min Grind</td>
<td>1:1</td>
</tr>
<tr>
<td>093-10-10</td>
<td>1 hour Grind</td>
<td>1:1</td>
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<tr>
<td>093-10-11</td>
<td>2 hour Grind</td>
<td>1:1</td>
</tr>
<tr>
<td>093-10-12</td>
<td>3 hour Grind</td>
<td>1:1</td>
</tr>
<tr>
<td>093-10-13</td>
<td>4 hour Grind</td>
<td>1:1</td>
</tr>
</tbody>
</table>

**Table 4:** Preparation conditions for 1:1 HQ:DETA samples for ambient oxidation studies.
Samples were then weighed out as 2.5 g increments and left in glass dishes at ambient temperature and humidity. The solids were spread across the bottom of each dish at similar thicknesses. Samples were then monitored by DSC, HPLC, NMR and solid-state densitometer. A GretagMacbeth D19 Densitometer was used to measure the solids. Each sample was tested three times and the values were averaged. Densitometers provide a measure of the density of color. This is a measure of the absorbance of light over a range of visible wavelengths compared to the control of the total absorbance of white. Thus in this data, an increased density of blackness corresponds to a more colored solid (Figure 6). This color change is most likely due to the formation of benzoquinone and the concurrent formation of the highly colored quinhydrone complex.

![Density of Blackness](image)

**Figure 9:** A colorimeter was used to record the color changes on the samples after 2.4 years of ambient oxidation. The results show that color change decreased with respect to increasing grind time.

**Accelerated Oxidation of Hydroquinone:** Samples of solid were spread across the bottom of 25 mL glass vial. The vials were sealed with parafilm and a glass pipette was positioned immediately over the solid. The pipette was attached via tygon tubing to an A2Z ozone generator. It was observed that a 1 g hydroquinone sample was converted upon exposure to ozone to the highly colored complex usually associated with benzoquinone. IR (Figure 10) and HPLC (Figure 11) were used to analyze a sample that had been exposed to 2 hours of ozone and confirmed that the sample was converted to benzoquinone.
**Figure 10:** IR showing hydroquinone (red) and the 1 g sample of hydroquinone ozone exposed to 2 hours of ozone (purple) resulting in the introduction of peaks associated with benzoquinone (green).

**Figure 11:** HPLC of hydroquinone sample treated with ozone. Peaks coincide with those for the hydroquinone (1.325) and benzoquinone (2.214) standards.

HPLC calibration curves for hydroquinone (Figure 12), benzoquinone (Figure 13) and bis-\(N,N\)-ethyl)terephthalamide (Figure 14) were prepared.

**Figure 12:** HPLC calibration curve for hydroquinone.

\[
y = 1E+06x + 30.146 \\
R^2 = 0.9999
\]
Samples of solid were spread across the bottom of 25 mL glass vial. The vials were sealed with parafilm and a glass pipette was positioned immediately over the solid. The pipette was attached via tygon tubing to an A2Z ozone generator. The calibration curves were used to calculate the percentage of benzoquinone present in a sample. A series of samples was 1g samples was exposed to ozone over the course of 2 hours. 25 mg samples were also tested (Figure 15).
Figure 15: Percent benzoquinone over the course of 2 hours of ozone exposure for 1 gram of hydroquinone. When the initial hydroquinone weight was reduced to 25 mg, the conversion increased to nearly 20% benzoquinone.

**Accelerated Oxidation of NCDs:** Ozonation was run on a series of cocrystals. Samples of 25 mg of solid were spread across the bottom of 25 mL glass vial. The vials were sealed with parafilm and a glass pipette was positioned immediately over the solid. The pipette was attached via tygon tubing to an A2Z ozone generator. Ozone was added for 20 consecutive minutes. Benzoquinone concentration was calculated and plotted (Figure 16). The conditions varied for the preparation of the cocrystal and showed that the unground material showed a similar level of oxidation of hydroquinone. More importantly, the material ground for 4 hours, corresponding to the cocrystal of 1:1 HQ to DETA, showed no conversion to benzoquinone. The cocrystal prepared from ethanol precipitation was also showed very limited oxidation by comparison to the pure hydroquinone and the unground mixture.
**Figure 16:** Comparison of benzoquinone in 1:1 HQ to DETA samples exposed to ozone. Material ground for 4 hours showed no conversion to benzoquinone. The cocrystal prepared from ethanol precipitation was also showed very limited oxidation by comparison to the pure hydroquinone and the unground mixture.

Ozonation was also run on series of 15 ratios of hydroquinone to bis-(N,N-diethyl)terephthalamide. Samples of 25 mg of solid were spread across the bottom of 25 mL glass vial. The vials were sealed with parafilm and a glass pipette was positioned immediately over the solid. The pipette was attached via tygon tubing to an A2Z ozone generator. Ozone was added for 20 consecutive minutes. Benzoquinone concentration was calculated and plotted. The resulting curve indicating the cocrystal regime prevented the oxidation of hydroquinone (**Figure 17**).
Figure 17: Percent benzoquinone produced from the ozonation of a series of 15 ratios of hydroquinone to bis-(N,N-diethyl) terephthalamide. The resulting curve indicating the cocrystal regime prevented the oxidation of hydroquinone.
5.2 Experimental Methods for Chapter 3

**Chemicals:** All materials were purchased from Sigma Aldrich with the exception of mushroom tyrosinase enzyme which was purchased from USB Corporation. The Sigma Aldrich materials were used as received except 3,4-dihydroxyphenylalanine (L-Dopa) which was milled by amalgamation for thirty minutes to a consistent particle size of 20 µm. Hair samples were purchased from Alinko Hair Company, New York, NY.

**Instrumentation:** Amalgamations were ground using a SPEX amalgamator, 5100 Mixer Mill. Solid state UV-Vis measurements were made via a Barralino solid UV-Vis probe for a Varian Carey 50 Spectrometer. Liquid cuvette UV-Vis measurements were run on a SpectraMax M5 Spectrometer and analyzed with the SoftMax Pro 6.2.1. IR measurements were made using a ThermoScientific Nicolette 6700 FT-IR with a Smart Golden Gate Diamond Attenuated Total Reflectance (ATR) accessory. The SEM employed was a JEOL instrument, JSM-6490LV. Both solid and liquid NMR scans were made using a JEOL NMR AS400. DSC was run on a TA Instruments Q200 in hermetically sealed T-Zero aluminum pans. TGA was run on a TA Instruments Q5000 in platinum high temperature pans.

**Preparation of Synthetic Eumelanin-Type Oligomers:** In round bottomed flask was combined 1g L-3,4-dihydroxyphenylalanine (L-Dopa) and 400 mL of deionized water. In a separate flask, a solution of 6.6 grams potassium hexacyanoferrate, 2.5g potassium hydrogen carbonate was dissolved in 60 mL DI water. This was added to the L-DOPA slurry via addition funnel. The solution turned red and became progressively darker toward black upon complete addition of the base solution. The solution was then left to stir overnight. The solution was quenched with 150mg of sodium dithionite and 10% HCl. Ethyl acetate was used to extract the product and the organic layers were collected, washed with brine and dried over sodium sulfate. The resulting black solid was analyzed by NMR.

**Initial Hair Dyeing Experiments:** Samples of pale yellow control hair of equal weight (0.5g) were weighed out and bundled by tape. Each bundle was then subjected to a different dying condition. In general, a solution was prepared and the dye was applied to the hair for overnight. The hair sample was then rinsed and left to air dry. To compare the application of premade polymer (027-93-5A) versus in situ polymerization (027-93-7B), solutions were prepared first by combining 1.1g of potassium hexacyanoferrate, 0.4g of potassium and 01.6g L-DOPA in 5 mL of DI water. This was left to stir overnight and then applied to the hair by soaking for a second night. For the in situ polymerization, the hair was soaked in 0.02 g L-DOPA and 2.5 mL water for a few moments. The same amounts of base and initiator were separately combined with 2.5 mL water and then added to the hair. Additional conditions were explored using the same method and amounts, but with variations in order or color former (Table 5). Samples were analyzed by visual comparison on a scale of 1-10 (Figure 18), UV-Vis with a solid probe (Figure 19) and SEM (Figure 20).
<table>
<thead>
<tr>
<th>Sample #</th>
<th>Hair Treatment</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair Control</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>027-93-3A</td>
<td>PHF</td>
<td>1</td>
</tr>
<tr>
<td>027-93-4A</td>
<td>PHF and KHCO\textsubscript{3}</td>
<td>2</td>
</tr>
<tr>
<td>027-93-5A</td>
<td>PHF, KHCO\textsubscript{3} and L-DOPA</td>
<td>6</td>
</tr>
<tr>
<td>027-93-6A</td>
<td>PHF, KHCO\textsubscript{3} and DHI</td>
<td>4</td>
</tr>
<tr>
<td>037-93-7A</td>
<td>L-DOPA in water</td>
<td>2</td>
</tr>
<tr>
<td>037-93-7B</td>
<td>L-DOPA soak followed by PHF and KHCO\textsubscript{3}</td>
<td>9</td>
</tr>
<tr>
<td>027-93-8A</td>
<td>L-DOPA and KHCO\textsubscript{3}</td>
<td>1</td>
</tr>
<tr>
<td>027-93-8B</td>
<td>L-DOPA and KHCO\textsubscript{3} soak followed by PHF and KHCO\textsubscript{3}</td>
<td>8</td>
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</tbody>
</table>

**Table 5**: Initial Soak Conditions and Results.
<table>
<thead>
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<th>Sample #</th>
<th>Hair Treatment</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hair Control</td>
<td>NA</td>
<td>1</td>
</tr>
<tr>
<td>3A</td>
<td>PHF</td>
<td>1</td>
</tr>
<tr>
<td>4A</td>
<td>PHF and KHCO₃</td>
<td>2</td>
</tr>
<tr>
<td>5A</td>
<td>Oxidized L-DOPA Applied to Hair: PHF, KHCO₃ and L-DOPA</td>
<td>6</td>
</tr>
<tr>
<td>6A</td>
<td>PHF, KHCO₃ and DHI</td>
<td>4</td>
</tr>
<tr>
<td>7A</td>
<td>L-DOPA in water</td>
<td>2</td>
</tr>
<tr>
<td>7B</td>
<td>In situ oxidation, L-DOPA soak followed by PHF and KHCO₃</td>
<td>9</td>
</tr>
<tr>
<td>8A</td>
<td>L-DOPA and KHCO₃</td>
<td>1</td>
</tr>
<tr>
<td>8B</td>
<td>L-DOPA and KHCO₃ soak followed by PHF and KHCO₃</td>
<td>8</td>
</tr>
</tbody>
</table>

**Figure 18:** Visual inspection and grading of the initial hair dye conditions indicated that L-DOPA soak on the hair followed by introduction of the initiator (*in situ* oxidation) resulted in the most effective hair color.
**Figure 19:** UV-Vis solid probe results showing significant color change with in situ oxidation of L-DOPA and oxidation with PHF and KHCO$_3$ (027-93-7B).

**Figure 20:** SEM images of hair samples and dyeing experiments with L-DOPA comparing application post oxidation and with in situ oxidation.

**Hair Dye Experiments Comparing Time and L-DOPA Concentrations:** Samples of pale yellow control hair of equal weight (0.5g) were weighed out and bundled by tape. Each bundle was then subjected to a different dying condition (*Table 6*). An initiator solution was prepared by dissolving 8.8g potassium hexacyanoferrate, 3.2 g potassium hydrogencarbonate in 40 mL of water. For each sample, dry L-DOPA was weighed and added to a petri dish. One mL of water was added to each dish. The hair sample was then added to the solution. One mL of the initiator solution was added to each dish. After the allotted time, the samples were removed, rinsed with
water and left to dry overnight. Samples were analyzed by visual comparison, UV-Vis with a solid probe (Figure 22) and SEM (Figure 23).

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Concentration</th>
<th>Concentration</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.05 g L-DOPA/mL H₂O</td>
<td>0.1 g L-DOPA/mL H₂O</td>
<td>0.25 g L-DOPA/mL H₂O</td>
</tr>
<tr>
<td>10</td>
<td>027-99-1A</td>
<td>027-97-1A</td>
<td>027-97-2A</td>
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<td>20</td>
<td>027-99-1B</td>
<td>027-97-1B</td>
<td>027-97-2B</td>
</tr>
<tr>
<td>30</td>
<td>027-99-1C</td>
<td>027-97-1C</td>
<td>027-97-2C</td>
</tr>
<tr>
<td>60</td>
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<td>027-97-2D</td>
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<td>027-97-1F</td>
<td>027-97-2F</td>
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<td>027-99-1H</td>
<td>027-97-1H</td>
<td>027-97-2H</td>
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</table>

Table 6: Conditions for dyeing comparing time and L-DOPA concentration

Figure 21: Results for dye experiments comparing time and L-DOPA concentration showing darkest
Figure 22: Solid State UV-Vis results for L-DOPA at differing concentration and time.
Figure 23: SEM results for differing concentrations of L-DOPA at 5 hours.

Hair Dye Experiments with Enzymatic Catalysis: Samples of pale yellow control hair of equal weight (0.5 g) were weighed out and bundled by tape. Each bundle was then subjected to a different dying condition. Two enzyme solutions were prepared: 8 mg of horseradish peroxidase was combined with 10mL of phosphate buffer solution (pH 7.4) and 4 mg mushroom tyrosinase. To initiate the horseradish peroxidase sample, a peroxide solution was prepared by diluting a 30% hydrogen peroxide solution 1:9 with water. For each sample, dry 0.05g L-DOPA was weighed and added to a petri dish. One mL of water was added to each dish. The hair sample was then added to the solution. For the tyrosinase samples, 1 mL of enzyme solution was added. For the horse radish peroxidase solutions, 0.5mL of the enzyme was added along with 0.5 mL of the hydrogen peroxide solution. One mL of the initiator solution was added to each dish. After the allotted time, the samples were removed, rinsed with water and left to dry overnight. Samples were analyzed by visual comparison, UV-Vis with a solid probe and SEM.

<table>
<thead>
<tr>
<th>Time (mins)</th>
<th>Initiator</th>
<th>Initiator</th>
</tr>
</thead>
<tbody>
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</tr>
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<td>027-99-2E</td>
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<tr>
<td>1440</td>
<td>027-99-2H</td>
<td>027-99-3H</td>
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</tbody>
</table>

Table 3: Enzyme catalyzed dyeing conditions
Figure 24: Comparison of enzyme catalysis results showing effective color formation with both enzymes, but improved with tyrosinase.
Figure 25: SEM results for enzymatic catalyzed coatings on hair showing effective coatings on hair fibers.

Comparison of Successive Dye Treatments using PHF and Tyrosinase: Samples of pale yellow control hair of equal weight (0.5g) were weighed out and bundled by tape. Each bundle was then subjected to a different dying condition. Two initiator solutions were prepared. 4 mg of mushroom tyrosinase was combined with 10mL of phosphate buffer solution to prepare the tyrosinase solution. To make the PHF solution, 1.6g KHCO$_3$ and 8.8g potassium hexacyanoferrate were dissolved in 20 mL of water. For each dye iteration, dry 0.05g L-DOPA was weighed and added to a petri dish. One mL of water was added to each dish. The hair sample was then added to the solution. One mL of the initiator solution was added to each dish and left to react for 10 minutes. The samples were removed, rinsed with water and samples were dried at room temperature between dye iterations. Samples were analyzed by visual comparison, UV-Vis with a solid probe (Figure 26) and SEM (Figure 27).
Figure 26: Repeat dye experiments showing that repeat tyrosinase dyeing (027-104-2, blue) resulted in better color than those with the repeat PHF catalyst dyes (027-104-1, green).
Effect of pH and Temperature on L-DOPA/Tyrosinase System: Samples were combined in glass vials equipped with a magnetic stir bar and caps. A stock tyrosinase solution was prepared from 0.0004g mushroom tyrosinase in 5 mL of DI water. 0.5 mL of stock tyrosinase solution was used for each vial. A sodium bicarbonate solution was prepared by dissolving 48g sodium bicarbonate in 500mL DI water. Materials were combined according to Table 4. The pH was assessed by pH strip. The vials were heated with stirring for one hour. The solids were filtered and dried under vacuum at room temperature overnight. The filtered weight was recorded and plotted (Figure 28). The dried solid was analyzed by Beckman Coulter particle size analyzer (Figure 29).

Figure 27: SEM images for successive dye experiments comparing PHF and Tyrosinase
<table>
<thead>
<tr>
<th>Sample #</th>
<th>Conc. of Tyrosinase (g/mL)</th>
<th>Rxn Time (hr)</th>
<th>T</th>
<th>pH</th>
<th>NaHCO₃ solution mL</th>
<th>water mL</th>
<th>Rxn Vessel</th>
<th>Starting wt L-DOPA (g)</th>
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</thead>
<tbody>
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<td>1</td>
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<td>2.5</td>
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<td>2.5</td>
<td>Vial</td>
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<td>0.2504</td>
</tr>
</tbody>
</table>

Table 4: Description of Sample Preparations for Studies on the Effect of pH and Temperature

![Changes in Filtered Wt vs Temp](image)

**Figure 28:** Measure of filterable material with changing conditions for the L-DOPA/tyrosine system. These results indicate a maximum fraction of water-soluble materials at pH 9 and 55°C.
Figure 29: Particle size analysis of effect of temperature and pH on L-DOPA oligomerization. The particle size results showed an increased size in the filtered material and distribution as the pH was increased from 7 to 9.

Stability Testing of Hair Color: Hair samples were prepared according to the standard procedure. Samples of pale yellow control hair of equal weight (1.0g) were weighed out and bundled by tape. Each bundle was then subjected to a different dying condition. An initiator solution was prepared by dissolving 0.0004g mushroom tyrosinase in 10 mL of DI water. For each sample, 0.1 g dry milled L-DOPA was weighed and added to a petri dish. One mL of water was added to each dish. The hair sample was then added to the solution and wetted. One mL of the initiator solution was added to each dish. Samples were then placed in the oven at 55°C for 120 minutes. After the allotted time, the samples were removed, rinsed with water and left to dry overnight. To assess the stability, samples were washed according to a simple method of successive washes in 45°C water. Prell shampoo was applied to the hair sample, massaged into the hair sample and rinsed until free of soap. The hair sample was then dried with an electric dryer for 5 minutes. This constituted a single wash and was repeated as necessary. Results were evaluated by visual inspection (Figures 30 & 31), colorimeter for a measure of lightness (Figure 32) and SEM (Figure 33).
**Figure 30:** Stability tests of hair samples at pH 7 and 55 °C. Results showed significant fading over the course of 30 washes at 45 °C.

**Figure 31:** Stability tests of hair samples at pH 9 and 55 °C. Results showed stability over the course of 30 washes at 45 °C

**Figure 32:** Calorimeter data testing the color stability by measure of lightness comparing samples dyed at pH 7 versus pH 9.
Figure 33: SEM images of hair dyed at pH 9 and 55°C with successive washes. Results showed no visual evidence of coating on the hair fiber and no significant change over the course of washes.

Characterization of Oxidized L-DOPA Product:

NMR analysis of the final dye product prepared at pH 9 and 55 °C was run, but the soluble fraction in both deuterated chloroform and dimethylsulfoxide showed only L-DOPA. The oxidation of L-DOPA was run in an NMR tube by first combining 0.0025 g L-DOPA, 0.0004 g tyrosinase, dissolved in 1 mL of deuterium oxide. 1 mL of sodium bicarbonate solution (96 mg/mL in deuterium oxide) was added. This solution was mixed to clear solution and 1 mL was pipetted into NMR tube. The tube was then heated in oil bath to 55 °C and NMR scans were taken at the starting point, 10 minutes, 40 minutes and 60 minutes. Each of the scans showed only L-DOPA.

Thermogravimetric analysis of L-DOPA comparing the solid products of reactions run at pHs 7, 8 and 9 at 35 °C, 45 °C, 55 °C (Figure 34). Samples were combined in glass vials equipped with a magnetic stir bar and caps. A stock tyrosinase solution was prepared from 0.0004 g mushroom tyrosinase in 5 mL of DI water. 0.5 mL of stock tyrosinase solution was used for each vial. A sodium bicarbonate solution was prepared by dissolving 48 g sodium bicarbonate in 500 mL DI water. Materials were combined according to Table 5. The pH was assessed by pH strip. The vials were heated with stirring for one hour. The solids were filtered and dried under vacuum at room temperature overnight. The dried solids were analyzed by TGA with scans run under nitrogen at a ramp rate of 20 °C/minute up to 1000 °C.
Table 5: Description of Sample Preparations for Studies on the Effect of pH and Temperature by TGA.

![Table showing sample preparations]

<table>
<thead>
<tr>
<th>Sample #</th>
<th>T (°C)</th>
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<th>NaHCO₃ solution</th>
<th>mL water</th>
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<td>2.5</td>
<td>2.5</td>
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<td>3A</td>
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<td>0</td>
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<tr>
<td>4A</td>
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<td>0</td>
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<td>7</td>
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<td>0</td>
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<tr>
<td>8A</td>
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<td>8</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>17A</td>
<td>55</td>
<td>9</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 34: Conditions and corresponding TGA’s showing minimal difference between the starting material, milled L-DOPA, versus products, supporting the idea that the product is a small oligomeric structure rather than a large polymer. A 2.5% greater decrease in weight % loss at 400°C was seen for solid collected from pH 9 and 55°C, indicate this as a set of conditions that produces a unique material.

DART analysis of the product did show evidence of the 5,6-dihydroxyindole as well as L-DOPA, amid a variety of additional unidentified peaks (Figure 35).
<table>
<thead>
<tr>
<th>Compound</th>
<th>hits</th>
<th>target</th>
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<th>diff</th>
<th>intensity</th>
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<td>2</td>
<td>4</td>
<td>6</td>
<td>27.2166</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 35:** DART analysis of the product of L-DOPA oxidized at pH 9 and heated at 55°C catalyzed by tyrosinase as indicated by conditions 027-133-17A in Figure 12.

Gel permeation chromatography (GPC) was used to compare pure L-DOPA to the product of L-DOPA oxidized at pH 9 and heated at 55°C catalyzed by tyrosinase as indicated by conditions 027-133-17A in Figure 36. These results showed little difference between the starting material and product, indicating that the product could be of very low molecular weight, possibly of only 1, 2, 3 or 4 repeat units of the indole. GPC was run using 0.05 g/mL sodium bicarbonate as a mobile phase.
Figure 36: GPC results showing little difference between the starting material and product, indicating that the product could be of very low molecular weight, possibly of only 1, 2, 3 or 4 repeat units of the indole.

**General Procedure for preparation of NCDs using L-DOPA and various coformers:** Into an SPEX amalgamator vial, the solid materials were weighed out according to the desired molar ratio for a total solids content of 500 mg (*Table 6*). The vial was equipped with a methylmethacrylate ball and ground for 90 minutes on the amalgator, in three 30 minute intervals run back to back. Initial coformer and molar ratios were as follows:

- **Blue:** L-DOPA control
- **Red:** Tyrosinase control
- **Green:** L Dopa polymer

![Chemical structures](image)
<table>
<thead>
<tr>
<th>Ratio L-DOPA:Coformer</th>
<th>B-DEPTA</th>
<th>L-Cysteine</th>
<th>L-Alanine</th>
<th>L-Arginine</th>
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<td>027-107-7</td>
<td>027-108-7</td>
<td>027-109-7</td>
</tr>
</tbody>
</table>

**Table 6:** Samples prepared by amalgamator for NCD studies.

**General Procedure for Dyeing Hair at pH 7 for 24 hours:**

Samples of pale yellow control hair of equal weight (0.5g) were weighed out and bundled by tape. Each bundle was then subjected to a different dying condition. In general, in a glass petri dish, each hair sample was treated with a mixture of 0.01 g of the potential NCD amalgamation and 1mL of water. The hair was then treated with 1 mL of a stock tyrosinase solution (0.0004g/mL PBS). Samples were then left overnight. The hair sample were then rinsed and left to air dry. Samples were analyzed by visual comparison (Figure 37), UV-Vis with a solid probe (Figure 38) and SEM (Figure 39).
**Figure 37:** Photographic images for hair dyed with amalgamations with bis-diethyldiethylterephalamide, alanine, and arginine.
Figure 38: Solid state UV-Vis results for hair dyed with amalgamations with bis-$(N, N$ diethyl)terephthalamide, alanine, and arginine.
Figure 39: SEM images showing the coatings on the outside of the hair fiber created by both the DETA and alanine based NCDs, but not evident in the arginine indicated possible incorporation of the dye into the hair.

UV-Vis Kinetics Experiments: Amalgamations were prepared using the SPEX amalgamator. Molar ratios were calculated such that each vial had a total solids content of 500 mg and combined in amalgamator vials. The vials were equipped with a methylmethacrylate ball. Samples were ground for 30 minutes. To test the kinetic effect of L-DOPA, 0.02 g of L-DOPA was added to a plastic UV-Vis cuvette and put in the pre-heated UV-Vis spectrometer at 60°C. A kinetic run was made with readings at 300 nm wavelength (predetermined by runs to determine ideal wavelengths) every 0.1 second over the course of one hour. These were plotted and the maximum rate of percent optical density per minute was calculated using the software SoftMax Pro 6.2.1. Each sample type was run three times and the average was plotted along with error bars. The samples referred to as grinds are samples in which the materials were combined and ground together on the amalgamator. Samples referred to as mixtures were ones in which the two materials were ground separately and then combined in the UV-Vis cuvette in appropriate quantities. This permitted a comparison of effects due to an NCD interaction (co-ground) and effects simply due to the presence of the co-former (mixture). The concentration of L-DOPA was maintained for each sample.
**Figure 40:** A comparison of the rate of color formation between L-DOPA and co-grinds versus mixtures of L-DOPA and both glutamine and arginine as co-formers.

Infrared (IR) spectroscopy was run using an ATR reflectance diamond anvil interface. IR of the parent compound showed the absence nor gain of a peak in 1:1 L-DOPA:Arginine sample or 1:2 L-DOPA:Arginine samples, as would be indicative of an NCD interaction (Figure 41).

**Figure 41:** IR comparison of IR of pure L-DOPA (blue) and pure arginine (green) versus the 1:1 molar L-DOPA:arginine (red) and the 1:2 molar L-DOPA:arginine (aqua). IRs of the amalgamations showed the absence nor gain of a peak in 1:1 L-DOPA:arginine sample or 1:2 L-DOPA:arginine samples, as would be indicative of an NCD interaction.

Solid state NMR showed no novel peaks evident in the 1:1 L-DOPA:arginine sample when compared to the pure parent compounds (Figure 42).
**Figure 42:** SS-NMR of 1:1 L-DOPA:arginine versus the parent compounds showing no novel peaks in the amalgamation.

A comparison by DSC of the parent compounds to the 1:1 L-DOPA to arginine showed only a broad melting peak slightly lower than the L-DOPA. This could simply be indicative of the broadening and depression associated with the inclusion of arginine as an impurity in the L-DOPA. The 1:2 L-DOPA:arginine scan showed evidence of a novel peak at 228°C, which is notably different from the melting points of pure L-DOPA (283°C) and pure arginine (241°C). However, the base line for this peak shows significant noise and melt before the peak and so this can be regarded as indicative, but not conclusive proof of the preparation of an NCD at 1:2 L-DOPA:arginine (**Figure 43**).
Figure 43: A comparison of the DSC scans for L-DOPA and arginine compared to the 1:1 and 1:2 molar ratio amalgamations. The 1:1 L-DOPA to arginine showed only a broad melting peak slightly lower than the L-DOPA, however the 1:2 L-DOPA:arginine showed evidence of a novel peak.
5.3 Experimental Methods for Chapter 4

**Instrumentation**: Amalgamations were ground using a SPEX amalgamator, 5100 Mixer Mill. Sped mixing was done using a Hauschild Speedmixer, Model # DAC 400 FVZ. DSC was run on a TA Instruments Q200 in hermetically sealed T-Zero aluminum pans. HPLC was run on a Agilent 1200 Series.

**Evaluation of peroxycacid degradation with heat by NMR**: In a glass vial, 25 mL sample of the peroxycacid containing formulation was heated. At each time point, a 100 µL aliquot was allowed to come to room temperature for one minute and then combined with 500 µL CDCl₃. NMR was immediately run. The methylene peaks of the peroxo acid were integrated against a total of integration of the methylene peaks of the peroxycacid plus the carboxylic acid (Figure 44). Samples were monitored for % benzoquinone at differing temperatures over time. Results were tabulated (Table 7) and graphed (Figure 45).

![Figure 44: Example of NMRs of peracid (PA) versus carboxylic acid (CA) with heating to 80°C at varying times.](image-url)
<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>% PA</th>
<th>%CA</th>
<th>Temp (°C)</th>
</tr>
</thead>
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<td>90%</td>
<td>80</td>
</tr>
</tbody>
</table>

**Table 7:** Degradation of peracid (PA) to carboxylic acid (CA) at varying times and temperatures as evaluated integration by NMRs.

**Figure 45:** Degradation studies of peracid in proprietary formulation with heat as determined by integration of NMR.
Evaluation of peroxyacid degradation with grinding by NMR: Aliquots of 0.5g were weighed into SPEX vials and equipped with methylmethacrylate balls. The samples were ground for the allotted time and then analyzed by NMR. A stock NMR solution was prepared with 20mL CDCl$_3$ and 0.4 mL of tertbutylbenzene as a reference standard. 0.05mg of sample was combined with 1mL of the NMR stock solution for each sample. The peracid (PA) and carboxylic acid (CA) methylene peaks were then measures against the standard’s methyl peak normalized as 1. Results were tabulated (Table 8) and graphed (Figure 46).

<table>
<thead>
<tr>
<th>Grinding Time (hours)</th>
<th>%PA</th>
<th>% CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>0.083</td>
<td>91</td>
<td>8</td>
</tr>
<tr>
<td>0.5</td>
<td>93</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>91</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>92</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>92</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 8: Evaluation of peracid (PA) degradation to carboxylic acid (CA) with grind time on the amalgamator.

Figure 46: Study of effect of grinding on amalgamator to insure peracid (PA) sample stability during NCD preparation.

Degradation Studies Comparing Coformers: For each coformer test, an initial NMR was run of dried PA to ensure potency. The ratio of PA to carboxylic acid (CA) in deuterated chloroform (CDCl$_3$) with a tertbutylbenzene standard was required to be 90:10 or greater. The solids total
for each amalgamation was kept at 500mg. Samples were ground on the amalgamator for 3 X 10 minute intervals, with cooling for five minutes in between each interval. NMR to confirm PA stability. An aliquot of 0.5 g PA or PA:coformer mixture was added to a speed mixer vial along with sufficient DI water to make a 20% solids solution. Samples were run on the speed mixer for a total of 7 minutes. An aliquot of 50 mg of each slurry was added to a test tube and combined with 1 mL of the deuterated chloroform with TBB. Solution was pipetted solution into an NMR tube and NMR was run immediately. The remaining slurry was transferred from the speed mix vial into tared glass sample vial for heating and weighed. Vials were placed in a holder in a pre-heated oil bath. At each time point, 50 mg of each slurry was transferred to a test tube and combined with 1 mL of the CDCl3 with TBB. Solution was pipetted solution into an NMR tube and NMR was run immediately. For samples in proprietary client formulation, the materials were run as above, but the formulation was substituted for the water. These experiments were used to identify two co-molecules which showed PA stabilization over time: adipic acid and glutaric acid (Figure 47).

![% PA in Aqueous Solids Dispersions vs Time](image)

**Figure 47**: Peracid concentration monitored in heated aqueous dispersions identifying two co-molecules that showed some PA stabilization over time: adipic acid and glutaric acid.

Stabilization of the adipic acid was confirmed by HPLC as well. This served to both validate the NMR method and reproduce the stabilization results (Figure 48).
Figure 48: Peracid concentration monitored in heated aqueous dispersions showing stabilization of the peracid by adipic acid as shown by both NMR and HPLC.

A series of degradation experiments were run comparing varying ratios of peracid ground with adipic acid showing that 1:1 peroxyacid to adipic acid to have the least degradation (Figure 49).

Figure 49: Degradation experiments comparing varying ratios of peracid showing that 1:1 peracid to adipic acid showed some reduction in degradation.

As might be anticipated, similar stabilization was seen with glutaric acid whose similar structure to adipic acid makes it a reasonable candidate (Figure 50).
Figure 50: Degradation experiments comparing varying ratios of peracid showing reduced degradation in samples that included glutaric acid.

The inherent insolubility of the peroxy acids provides them with some stability in pure water. However, the addition of any organic solvent leads to increased dissolution and subsequent degradation. In a subsequent set of studies, the pure peroxacid was tested in the product’s target formulation. This formulation contained ingredients that increased the dissolution of the peroxyacids (Figure 51).

Figure 51: Degradation studies with peroxyacid and adipic acid grinds in formulation showing some improvement in stability at the 1:1 and 1:2 ratios.
In another set of studies, a series of grinds were prepared at varying ratios of peracid to suberic acid (Figure 52). The grinds were then subjected to our standard accelerated degradation studies and evaluated for peracid degradation.

**Figure 52**: Degradation studies with peroxyacid and suberic acid in formulation showing a more rapid degradation of PA with decreasing PA concentration.

It can be seen suberic acid, despite its similar pKa provided no stabilization for the peracid. The differences seen here between the ratios can most readily be associated with the self-stabilization of the peracid. As the concentration of peracid is lowered, without the presence of a stabilizing factor, the peracid degrades more rapidly.

**Preparation of Phase Diagrams from DSC**: Phase diagrams were prepared to analyze any NCD behavior between the peracid and coformers. A series of 15 samples at differing ratios of PA and the three coformers: adipic acid (Table 9), glutaric acid (Table 10) and glutamic acid (Table 11).
Table 9: Preparation conditions for amalgamations prepared as a series of rations between PA and adipic acid.

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Ratio PA:adipic acid</th>
<th>g PA/0.45 g in vial</th>
<th>g adipic acid/0.45 g in vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>056-158-1</td>
<td>0:1</td>
<td>0.000</td>
<td>0.450</td>
</tr>
<tr>
<td>056-158-2</td>
<td>1:9</td>
<td>0.078</td>
<td>0.372</td>
</tr>
<tr>
<td>056-158-3</td>
<td>3:7</td>
<td>0.202</td>
<td>0.248</td>
</tr>
<tr>
<td>056-158-4</td>
<td>1:4</td>
<td>0.145</td>
<td>0.305</td>
</tr>
<tr>
<td>056-158-5</td>
<td>1:3</td>
<td>0.174</td>
<td>0.276</td>
</tr>
<tr>
<td>056-158-6</td>
<td>1:2</td>
<td>0.219</td>
<td>0.231</td>
</tr>
<tr>
<td>056-158-7</td>
<td>2:3</td>
<td>0.251</td>
<td>0.199</td>
</tr>
<tr>
<td>056-158-8</td>
<td>1:1</td>
<td>0.295</td>
<td>0.155</td>
</tr>
<tr>
<td>056-158-9</td>
<td>3:2</td>
<td>0.333</td>
<td>0.117</td>
</tr>
<tr>
<td>056-158-10</td>
<td>2:1</td>
<td>0.356</td>
<td>0.094</td>
</tr>
<tr>
<td>056-158-11</td>
<td>3:1</td>
<td>0.383</td>
<td>0.067</td>
</tr>
<tr>
<td>056-158-12</td>
<td>4:1</td>
<td>0.398</td>
<td>0.052</td>
</tr>
<tr>
<td>056-158-13</td>
<td>7:3</td>
<td>0.367</td>
<td>0.083</td>
</tr>
<tr>
<td>056-158-14</td>
<td>9:1</td>
<td>0.425</td>
<td>0.025</td>
</tr>
<tr>
<td>056-158-15</td>
<td>1:0</td>
<td>0.450</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 10: Preparation conditions for amalgamations prepared as a series of rations between PA and glutamic acid (Glu).

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Ratio PA:Glu</th>
<th>g PA/0.45 g in vial</th>
<th>g Glu/0.45 g in vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>056-172-1</td>
<td>0:1</td>
<td>0.000</td>
<td>0.450</td>
</tr>
<tr>
<td>056-172-2</td>
<td>1:9</td>
<td>0.078</td>
<td>0.372</td>
</tr>
<tr>
<td>056-172-3</td>
<td>3:7</td>
<td>0.201</td>
<td>0.249</td>
</tr>
<tr>
<td>056-172-4</td>
<td>1:4</td>
<td>0.144</td>
<td>0.306</td>
</tr>
<tr>
<td>056-172-5</td>
<td>1:3</td>
<td>0.174</td>
<td>0.276</td>
</tr>
<tr>
<td>056-172-6</td>
<td>1:2</td>
<td>0.218</td>
<td>0.232</td>
</tr>
<tr>
<td>056-172-7</td>
<td>2:3</td>
<td>0.251</td>
<td>0.199</td>
</tr>
<tr>
<td>056-172-8</td>
<td>1:1</td>
<td>0.294</td>
<td>0.156</td>
</tr>
<tr>
<td>056-172-9</td>
<td>3:2</td>
<td>0.332</td>
<td>0.118</td>
</tr>
<tr>
<td>056-172-10</td>
<td>2:1</td>
<td>0.356</td>
<td>0.094</td>
</tr>
<tr>
<td>056-172-11</td>
<td>3:1</td>
<td>0.382</td>
<td>0.068</td>
</tr>
<tr>
<td>056-172-12</td>
<td>4:1</td>
<td>0.397</td>
<td>0.053</td>
</tr>
<tr>
<td>056-172-13</td>
<td>7:3</td>
<td>0.367</td>
<td>0.083</td>
</tr>
<tr>
<td>056-172-14</td>
<td>9:1</td>
<td>0.425</td>
<td>0.025</td>
</tr>
<tr>
<td>056-172-15</td>
<td>1:0</td>
<td>0.450</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Table 11: Preparation conditions for amalgamations prepared as a series of rations between PA and glutaric acid.

The appropriate amount of material, as listed in Tables 9, 10 and 11, was added to a plastic amalgamator vial along with a methylmethacrylate grinding ball. Samples were ground on the amalgamator for 3 X 10 minute intervals, with cooling for five minutes in between each interval. DSC’s were run on each sample using a 25°C/minute ramp up to 250°C in hermectically sealed aluminum T-zero pans (Figures 53, 54 and 55). A phase diagram was prepared using this data by plotting the mole percent of each coformer versus PA for each ratio against the dominant endothermic peaks in the DSC run on each ratio (Figures 56, 57 & 58).

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Ratio PAP:Glutaric</th>
<th>g PAP/.45 g in vial</th>
<th>g Glutaric/.45 g in vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>056-173-1</td>
<td>0:1</td>
<td>0.000</td>
<td>0.450</td>
</tr>
<tr>
<td>056-173-2</td>
<td>1:9</td>
<td>0.085</td>
<td>0.365</td>
</tr>
<tr>
<td>056-173-3</td>
<td>3:7</td>
<td>0.213</td>
<td>0.237</td>
</tr>
<tr>
<td>056-173-4</td>
<td>1:4</td>
<td>0.155</td>
<td>0.295</td>
</tr>
<tr>
<td>056-173-5</td>
<td>1:3</td>
<td>0.185</td>
<td>0.265</td>
</tr>
<tr>
<td>056-173-6</td>
<td>1:2</td>
<td>0.230</td>
<td>0.220</td>
</tr>
<tr>
<td>056-173-7</td>
<td>2:3</td>
<td>0.262</td>
<td>0.188</td>
</tr>
<tr>
<td>056-173-8</td>
<td>1:1</td>
<td>0.305</td>
<td>0.145</td>
</tr>
<tr>
<td>056-173-9</td>
<td>3:2</td>
<td>0.342</td>
<td>0.108</td>
</tr>
<tr>
<td>056-173-10</td>
<td>2:1</td>
<td>0.363</td>
<td>0.087</td>
</tr>
<tr>
<td>056-173-11</td>
<td>3:1</td>
<td>0.388</td>
<td>0.062</td>
</tr>
<tr>
<td>056-173-12</td>
<td>4:1</td>
<td>0.402</td>
<td>0.048</td>
</tr>
<tr>
<td>056-173-13</td>
<td>7:3</td>
<td>0.374</td>
<td>0.076</td>
</tr>
<tr>
<td>056-173-14</td>
<td>9:1</td>
<td>0.427</td>
<td>0.023</td>
</tr>
<tr>
<td>056-173-15</td>
<td>1:0</td>
<td>0.450</td>
<td>0.000</td>
</tr>
</tbody>
</table>
Figure 53: DSCs of a series of ratios of peracid and adipic acid.
Figure 54: DSCs of a series of ratios of peracid and glutaric acid.
Figure 55: DSCs of a series of ratios of peracid and glutamic acid.

Figure 56: Phase diagram of adipic acid in peracid showing NCD interaction between the two solids.
Figure 57: Phase diagram of glutamic acid in peracid showing no NCD interaction between the two solids.

Figure 58: Phase diagram of glutaric acid in peracid showing NCD interaction between the two solids.