UNIVERSITY OF CINCINNATI

Date: August 13, 2006

I, Grettel Zamora-Estrada, hereby submit this work as a part of the requirements of the degree of:

Master of Science (M.S.)

in:

Pharmaceutical Sciences

It is entitled:
Partitioning of Perfume Raw Materials in Conditioning Shampoos using Gel Network Technology

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Partitioning of Perfume Raw Materials in Conditioning Shampoos using Gel Network Technology

by

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A dissertation proposal synopsis
Submitted in partial fulfillment
Of the requirements for
the degree of
M.S.
Pharmaceutical Sciences

University of Cincinnati
College of Pharmacy
Cincinnati, Ohio

July 23, 2006
ABSTRACT

Gel network technology in conditioning shampoo represents an advantage over traditional silicone 2-in-1 technology due to its main benefits: dry conditioning, wet feel and lower cost. The purpose of this study was to do a proof of principle investigation and to study the main factors that affected partitioning of PRMs into the gel network system shampoos and determine the effect that perfume incorporation had on the shampoo stability of the different formulations. Gel network premixes (literally a conditioner) were formulated then incorporated into a standard shampoo base. Changes in formulation of the gel network such as chain length of fatty alcohols and fatty alcohol ratios were done and its effect on stability and perfume migration studied. A technical accord with 25 PRMs with a very wide range of physical properties was used as a marker. Other perfume variables studied were hydrophobicity of the perfume, hydrophobically modified accords, and other user practices such as combing/wetting. The formulations were evaluated for stability using microscopy and differential scanning calorimetry. Compositional analysis was done using GC/MS and headspace analysis. Consumer acceptance was evaluated using sensory panel. The compositional analysis partition data was used in a QSAR model to predict future PRMs tendency to partition into the gel network. Three main conclusions were reached: 1) Hydrophobically modified accords partition favorably into the gel network, however, whether that translates into greater consumer benefit will need to be further tested. 2) PRMs that partition favorably into the gel network follow a structure-property relationship of lipophilicity and rigidity. 3) Changes in processing parameters influence the partitioning of PRMs into the gel network and can be stronger levers than formulations parameters for enhancing perfume bloom and longevity.
ACKNOWLEDGEMENTS

There were many people who contributed to my success during my graduate school years and I would like to recognize them. I feel God places people in your life so we learn from each other, and I have learned a lot from everyone in this list. Thanks!

Gerald Kasting Ph.D. I have always felt blessed to have Dr. Kasting as my major advisor. His breadth and wealth of scientific, professional and life knowledge has been a great source of support and strength for me. I feel I have learned a lot from him in every aspect and he has exceeded my expectations of what an advisor would do. Thanks for watching out for my best interest better than I could myself.

Randall Wickett, Ph.D., for instilling in me the spirit of critical thinking and questioning through classes, thesis committee, and daily interactions.

Eric Johnson, Ph.D. for providing guidance and coaching in the start of my journey as a personal care formulator, and his thoughtful comments and revisions on the thesis.

Kevin Labitzke, for opening my eyes to new ways to look at data, for his extensive contribution in making this project happen, endless revisions to ensure perfection, and teaching me about creating great synergies in a team.

Adel Sakr, Ph.D., for being a great mentor while I served as his lead teaching assistant in drug delivery lab.

Dave Stanton, Ph.D. for taking a lot of his time to introduce me to the exciting world of QSARs, manuscript revision, and explained patiently every single step and detail.

Bob Wells, Ph.D., for teaching me how to get things done the P&G way.

Michael Leblanc for helpful discussions on perfume technology.

The Procter & Gamble Company, and specifically to Jenni Hilvert, Ben Heath, Sarah Cooper, Virginia Hutchins, Bob Dykstra, JJ Zhang, Mike Niebauer, Denise Belanger, Bill Gore, Mary Lou Batt, Bill Canty, Terrance Fail, Shawn Mansfield, Chitra Laxmanan who all played a role in making this project happen in such a short timeframe.

The University of Cincinnati College of Pharmacy for providing me with a great environment to thrive on while pursuing my graduate studies and their financial support. Special thanks to Donna Taylor and Marcie Silver for helping me navigate through the paperwork.

The Estrada-Canizalez family, specially Mami Nilda, Ginny, Tathy, Myrtha, Noris, Tita, Javier and Ofelia, who even in distance, we are very close. To my mother, who instilled in me good values and love for learning. I owe her all my inquisitiveness, and the relentless discipline to
search for answers and solving the “why”.  Thanks for your unwavering support during this endeavour.

My friends Amit, Elaine, Greg, Rachna and my old friends Memo, Leiza, Freddy, Alex, Maia, and Lisa who always push me to be the very best version of myself. To Bob Agee for being such a “positive” role model in my life.

My fellow cosmetic scientists students Hemali, Varsha, Arjun, Fair, Lola, Kelly for their support and teamwork during my tenure at UC.

Last, but not least, God, for giving me the gift of life.
# TABLE OF CONTENTS

Cover Page .......................... i
Title Page ............................ ii
Abstract .............................. iii
Acknowledgements .................. v
Table of Contents .................... 1
List of Figures ....................... 3
List of Tables ....................... 5
List of Symbols ...................... 6

## 1. BACKGROUND

1.1 Significance of Research Problem
1.2 Hair Structure and Basics
1.3 History of Shampoo
1.4 A Basic Shampoo Introduction
1.5 Shampoo Formulation Basics
1.6 Fragrances
1.7 Fragrance Formulation Basics
1.8 Gel Network Technology

## 2. HYPOTHESIS AND SPECIFIC AIMS

2.1 Hypothesis
2.2 Specific Aims

## 3. MATERIALS AND METHODS

3.1 Materials
3.2 Methods
   3.2.a Formulation
   3.2.b Microscopy
   3.2.c Differential Scanning Calorimetry
   3.2.d Perfume Compositional Analysis
   3.2.e Sensory Evaluation
   3.2.f Headspace Analysis
   3.2.g Partition Modeling-Quantitative Structure Activity Relationship

## 4. RESULTS AND DISCUSSION

4.1 Gel Network Premix Characterization
   4.1.a Microscopy-Basic Formulation
   4.1.b Differential Scanning Calorimetry-Basic Formulation
   4.1.c Reproducibility Studies
   4.1.d Perfume Processing Time Studies
4.1.e Perfume Processing Temperature Studies
4.1.f Formulation Variables Effect on Gel Network Premix

4.2 Shampoo Characterization
4.2.a Microscopy-Basic Formulation
4.2.b Differential Scanning Calorimetry-Basic Formulation
4.2.c Formulation Variables Effect on Final Shampoo

4.3 Perfume Analysis
4.3.a Basic Formulation
4.3.b Equilibrium Study
4.3.c Formulation Variables Effect on Gel Network Partitioning

4.4 Sensory Evaluation
4.4.a Effect of Gel Network Incorporation in Shampoo Chassis
4.4.b Effect of Perfume Hydrophobicity, Combing and Moisture Release
4.4.c Effect of Hydrophobically Modified Fragrance

4.5 Headspace Analysis
4.5.a Perfume Bloom-0-Hr
4.5.b Perfume Longevity-3-Hr

4.6 Mathematical Modeling-Quantitative Structure Activity Relationship

5. CONCLUSIONS AND FUTURE WORK

5.1.a Conclusions
5.2.b Future Work

6. BIBLIOGRAPHY
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 1.1</td>
<td>Shampoos for normal hair: Aussie normal hair formula and Aveda rosemary mint shampoo for normal hair</td>
<td>9</td>
</tr>
<tr>
<td>Figure 1.2</td>
<td>Shampoos for oily hair: Aubrey Organics for oily hair and Pert Plus</td>
<td>10</td>
</tr>
<tr>
<td>Figure 1.3</td>
<td>Shampoos for dry hair: Dove and Infusion 23</td>
<td>11</td>
</tr>
<tr>
<td>Figure 1.4</td>
<td>Shampoos for damaged hair: Herbal Essence hydrating shampoo and Garnier Fructis shampoo for damaged hair</td>
<td>12</td>
</tr>
<tr>
<td>Figure 1.5</td>
<td>Conditioning shampoo for long hair: Pantene extra straight and Pantene hydrating curls conditioning shampoos</td>
<td>13</td>
</tr>
<tr>
<td>Figure 1.6</td>
<td>Extrait perfumes: Channel 5 and Versace Blonde</td>
<td>17</td>
</tr>
<tr>
<td>Figure 1.7</td>
<td>Eau de Parfum: Angel by Thierry Mugler and Lovely by Sarah Jessica Parker</td>
<td>18</td>
</tr>
<tr>
<td>Figure 1.8</td>
<td>Eau de cologne: Pierre Cardin and original eau de cologne</td>
<td>19</td>
</tr>
<tr>
<td>Figure 1.9</td>
<td>Eau de toilette: Hugo Boss and Isabelle Armani</td>
<td>20</td>
</tr>
<tr>
<td>Figure 1.10</td>
<td>Compositional makeup of gel network premix</td>
<td>24</td>
</tr>
<tr>
<td>Figure 1.11</td>
<td>Gel network system-Bilayers of alternating fatty alcohols/surfactants and water</td>
<td>25</td>
</tr>
<tr>
<td>Figure 2.1</td>
<td>Visual representation of hypothesis-PRMs incorporated in gel network premix</td>
<td>27</td>
</tr>
<tr>
<td>Figure 2.2</td>
<td>Visual representation of shampoos with different order of addition for PRMs</td>
<td>28</td>
</tr>
<tr>
<td>Figure 2.3</td>
<td>Simplified partition model-Equilibrium representation</td>
<td>30</td>
</tr>
<tr>
<td>Figure 2.4</td>
<td>Simplified partition model-Preferred partitioning into the gel network phase representation</td>
<td>31</td>
</tr>
<tr>
<td>Figure 3.1</td>
<td>Schematic of a shampoo tube after centrifugation with different layers: gel network, EGDS and shampoo</td>
<td>37</td>
</tr>
<tr>
<td>Figure 4.1</td>
<td>Light micrograph of the gel network premix for formulation B</td>
<td>45</td>
</tr>
<tr>
<td>Figure 4.2</td>
<td>Light micrograph of the gel network premixes a) no perfumes-formulation A and b) perfumes-formulation B</td>
<td>46</td>
</tr>
<tr>
<td>Figure 4.3</td>
<td>Representative thermogram-Perfume addition on gel network premix effect of phase transition temperature of gel network structure</td>
<td>49</td>
</tr>
<tr>
<td>Figure 4.4</td>
<td>Solubilization effect of temperature on perfume addition in gel network premix- a) Cold -50 °C premix and b) hot -74 °C premix</td>
<td>52</td>
</tr>
<tr>
<td>Figure 4.5</td>
<td>Thermograms of effect of fatty alcohol chain length</td>
<td>55</td>
</tr>
</tbody>
</table>
and ratio on phase transition temperature a) $C_{18}OH-C_{16}OH$ 3:1 b) $C_{18}OH-C_{16}OH$ 1:3 c) $C_{18}OH-C_{22}OH$ 3:1 and d) $C_{18}OH-C_{22}OH$ 1:3

Figure 4.6 Full shampoo light micrograph: crossed polar

Figure 4.7 Thermograms for Full Shampoo Product: a) direct mix b) premix c) shampoo base with no gel network structure and 0.7% fragrance level and d) Shampoo chassis with no gel network and zero fragrance level.

Figure 4.8 Thermogram of the effect of fatty alcohol chain length and ratios variation in phase transition temperature in full shampoo for direct mix of perfume a) $C_{18}OH-C_{16}OH$ 3:1 b) $C_{18}OH-C_{16}OH$ 1:3 c) $C_{18}OH-C_{22}OH$ 3:1 and d) $C_{18}OH-C_{22}OH$ 1:3

Figure 4.9 Proof of concept-PRM partitioning data for shampoo and gel network phases following centrifugation-Division by top, mid and base notes

Figure 4.10 PRM partitioning data for gel network phase graphed as a function of clogP

Figure 4.11 Partitioning of PRMs as a function of time.

Figure 4.12 Perfume analysis-Effect on gel network PRM partitioning data of formulation variables such as fatty alcohol chain lengths, ratio and order of addition

Figure 4.13 Perfume analysis-Replication of unusual feature for C18OH/C22OH 3:1 ratio formulation.

Figure 4.14 Sensory results-Effect of gel network and shampoo chassis in olfactory response

Figure 4.15 Sensory results-Effect of gel hydrophobicity, combing and moisture in perfume olfactory response

Figure 4.16 Sensory results-Effect of hydrophobically modified perfumes in perfume olfactory response

Figure 4.17 Headspace comparison results on hair switches treated with shampoo-0 hr

Figure 4.18 Headspace comparison results on hair switches treated with shampoo-3 hr

Figure 4.19 PRMs partitioning data-Plot of fitted vs. experimental values

Figure 4.20 Sample PRMs classified by increasing hydrophobicity and rigidity (Courtesy of Dave Stanton-P&G Pharmaceuticals)
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Basic formula of a simplified shampoo</td>
<td>15</td>
</tr>
<tr>
<td>Table 1.2</td>
<td>Technical accord-Representative PRMs and physical properties</td>
<td>23</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Standard formulas for gel network premixes a) Zero fragrance b) 2.57% fragrance</td>
<td>44</td>
</tr>
<tr>
<td>Table 4.2</td>
<td>Pure raw materials melting points as determined by DSC</td>
<td>47</td>
</tr>
<tr>
<td>Table 4.3</td>
<td>Phase transition temperature for standard gel network (11% active (\text{C}<em>{16}\text{OH}/\text{C}</em>{18}\text{OH}) in a ratio of 1.8 to 1.0-89% water) under different PRM addition conditions</td>
<td>48</td>
</tr>
<tr>
<td>Table 4.4</td>
<td>Design of Experiment for Formulation Variables</td>
<td>53</td>
</tr>
<tr>
<td>Table 4.5</td>
<td>Effect on phase transition temperature of formulation and processing variables-Gel network premix</td>
<td>55</td>
</tr>
<tr>
<td>Table 4.6</td>
<td>Perfume order of addition effect of shampoo on PTT-direct and premix</td>
<td>58</td>
</tr>
<tr>
<td>Table 4.7</td>
<td>Effect of formulation variables on phase transitions temperature-full shampoo</td>
<td>61</td>
</tr>
</tbody>
</table>
## LIST OF SYMBOLS/ABBREVIATIONS

<table>
<thead>
<tr>
<th>SYMBOLS</th>
<th>DEFINITION</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;16&lt;/sub&gt;OH</td>
<td>Cetyl alcohol</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;OH</td>
<td>Stearyl alcohol</td>
</tr>
<tr>
<td>C&lt;sub&gt;22&lt;/sub&gt;OH</td>
<td>Behenyl alcohol</td>
</tr>
<tr>
<td>clogP</td>
<td>Octanol water partition coefficient</td>
</tr>
<tr>
<td>DM</td>
<td>Direct Mix-Refers to mode of addition of PRMs to final shampoo formulation</td>
</tr>
<tr>
<td>DOE</td>
<td>Design of experiment</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminotetraacetic acid</td>
</tr>
<tr>
<td>EGDS</td>
<td>Ethylene glycol distearate</td>
</tr>
<tr>
<td>ELD</td>
<td>Equilibrated lamellar dispersion</td>
</tr>
<tr>
<td>Form</td>
<td>Formulation</td>
</tr>
<tr>
<td>GC/MS</td>
<td>Gas Chromatograph/Mass Spectrometer</td>
</tr>
<tr>
<td>GN</td>
<td>Gel network</td>
</tr>
<tr>
<td>Ko/w</td>
<td>Octanol water partition coefficient</td>
</tr>
<tr>
<td>MSD</td>
<td>Mass Selective Detector</td>
</tr>
<tr>
<td>PLS</td>
<td>Partial Least Squares-Regression Method</td>
</tr>
<tr>
<td>PRMs</td>
<td>Perfumery Raw Materials or Perfume Raw Materials</td>
</tr>
<tr>
<td>Q&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Cross validated correlation coefficient</td>
</tr>
<tr>
<td>qs</td>
<td>Quantum sufficit</td>
</tr>
<tr>
<td>R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>Sw</td>
<td>Water solubility</td>
</tr>
</tbody>
</table>
1. BACKGROUND

1.1 Significance of Research Problem

The expectations of consumers from their personal care products have greatly increased over time with multifunctional products being the standard. Currently, most shampoos are expected to have some degree of conditioning, even if a full regime with a two-step conditioning is the norm. Traditional conditioning shampoo technologies use silicone as the main conditioning agent during dry combing. The cost of custom silicones with specific functional moieties represents an actual challenge for the formulator. Customers nowadays also show a strong tendency to prefer natural products that restore original compounds present in the hair. Gel network technology aims to provide a solution by using coacervate technology (Ertel 2000, Huisinga 2004, Lochhead 2001) to deposit materials such as fatty alcohols and silicones while simultaneously reducing the silicone level. Silicone at high concentrations has the tendency to stay on the hair and build up—creating an unpleasant film in the hair or scalp. From a formulation standpoint, gel networks provide both a dry and wet conditioning benefit (that traditional dimethicone cannot alone provide) at a fraction of the cost. Fragrance traditionally has been treated as an agent that is added to the formulation towards the end. In this project, we aim to show that perfume addition can be crucial in modifying the final shampoo formulation, and that upstream product design work will ensure that all different variables are taken into account for enhancing perfume bloom and longevity in our gel network shampoo prototypes.

1.2 Hair Structure and Basics

Hair is mainly made of protein. The diameter of a human hair ranges from about 18 µm to 180 µm. The structure of a hair shaft is made of three main layers, concentric to each other:
the medulla, the cortex and the cuticle. The medulla is the innermost hair layer. The cortex, in the middle, contains the pigment and can be modified to an extent via the chemical hair processes such as bleaching, coloring, perming and straightening. The inner cortex contains spindle-shaped cells and has scale shaped layers. Within each cortical cell, a large amount of fibrils run parallel to the fiber axis. The cuticle, on the outside, is the layer that is exposed to everyday use and abuse. The cuticle is composed of tiny scales that overlap. Human hair typically has 6-8 layers of cuticle. Ideally, cuticles should be whole (no breakage) and lie flat providing a smooth and shiny look when reflecting light. Cuticles provide mechanical strength of the hair fiber.

1.3 History of Shampoo

Shampoo is relatively a new invention—up until 1930s mankind used soap for hair cleansing. The drawbacks with using soap are its irritation potential and incompatibility with hard water, resulting in buildup. The first synthetic shampoo detergent was introduced in the 1930s and current shampoo technology with silicone as the main conditioning agent was developed during the 1960s (Barrel, 2001).

1.4 A Basic Shampoo Introduction

Shampoos are personal care products that are primarily used for hair cleansing of the sebum and dirt in the scalp and hair respectively. The most common way to classify shampoos is by the consumer demographic needs: normal hair, oily hair, dry hair, damaged hair, and color treated hair. Conditioning shampoos—the type of shampoo being formulated in this project—are an intersection of products used for dry, damaged, color-treated and long hair. In other words,
except for normal or oily hair, all the other end users will need a conditioning shampoo to reach the desired state of silky, flowing, clean, and healthy looking hair.

Normal hair users have virgin and/or relatively healthy hair, and they minimize the treatment of their hair with chemicals. Shampoos are formulated with lauryl sulfate surfactants which provide good cleaning and minimal conditioning.

Figure 1.1: Shampoos for normal hair: Aussie normal hair formula and Aveda rosemary mint shampoo for normal hair
Oily hair is characteristic of users that have a moderate to medium sebum production so the hair and scalp presents the problem of not always being clean. In order to address this situation, these shampoos are formulated with either lauryl sulfates or sulfosuccinates that provide excellent cleaning (which is required) and minimal conditioning.

Figure 1.2: Shampoos for oily hair: Aubrey Organics for oily hair and Pert Plus
Dry hair formulation provides mild cleaning properties while providing very good conditioning with agents such as quaternary ammonium compounds, fatty alcohols, fatty acids, or dimethicones. The lack of cleaning power and strength could result in an accumulation of the conditioning agent in the hair and scalp, creating an unpleasant end result for the consumer.

Figure 1.3: Shampoos for dry hair: Dove and Infusion 23
Damaged hair can be caused by either chemical or physical changes. Chemical treatments include permanent color, bleaching agents, permanent waving or straighteners. Physical treatments comprise overcleansing, excess use of heating irons, and even vigorous brushing or daily combing. These shampoos are similar in formulation to the dry formulas, using conditioning and restorative agents.

Figure 1.4: Shampoos for damaged hair: Herbal Essence hydrating shampoo and Garnier Fructis shampoo for damaged hair
Long hair represents a challenge in terms of damage due to weathering, phenomena in which the scales of the cuticle are decreased. Long hair users commonly use conditioning shampoos due to its various properties.

Figure 1.5: Conditioning shampoo for long hair: Pantene extra straight and Pantene hydrating curls conditioning shampoos
Conditioning shampoos are sometimes known as stand alone or 1-step shampoos, because a conditioner does not need to be applied after its application (Hossel 2000). This labeling may be misleading, as shampoos for dry or damaged hair can be considered conditioning shampoos too. Also, due to the high level of consumer expectations, the majority of shampoos in the market have conditioning properties. Currently, conditioning shampoos have several claims regarding their mechanisms of action, including (but not limited to) the following:

- decreasing static electricity
- improving manageability
- increasing shine
- improving flexibility of the hair

The basic mechanisms of actions on which the proof of claims are based on include: since the hair shaft is negatively charged, conditioners deposit a positively charged compound neutralizing the charge and minimizing frizz. The manageability claim is obtained by decreasing the friction between hair shafts, smoothing the cuticle surface by filling the spaces between the cuticular scales (Ruetsch 2003). More shine is obtained by making the surface smoother with the quaternary compounds, so that it reflects more light which appears as shine to the consumer. The flexibility of the hair is due to conditioning agents that leave a thin film of conditioner on the hair.
1.5 Shampoo Formulation Basics

A basic simplified shampoo formula follows in Table 1.1 (Barrel, 2001):

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Function</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preservative</td>
<td>Microbial stability</td>
<td>1-3</td>
</tr>
<tr>
<td>Color</td>
<td>Holistic and sensorial benefits</td>
<td>1-3</td>
</tr>
<tr>
<td>Fragrance</td>
<td>Holistic and sensorial benefits</td>
<td>1-3</td>
</tr>
<tr>
<td>Thickener/Auxiliaries</td>
<td>Appearance/stability/product claims</td>
<td>0-10</td>
</tr>
<tr>
<td>Cosurfactant</td>
<td>Cleansing, lather, mildness</td>
<td>3-5</td>
</tr>
<tr>
<td>Basic surfactant</td>
<td>Cleansing and lather</td>
<td>10-15</td>
</tr>
<tr>
<td>Water</td>
<td>Handling</td>
<td>q.s. 100</td>
</tr>
</tbody>
</table>

Table 1.1: Basic formula of a simplified shampoo

The list of ingredients for a shampoo formula can be quite extensive: surfactants, foaming agents, thickeners, chelators, conditioners, pH adjusters, specialty additives, fragrance and preservatives (Rushton, 2000).

Surfactants or detergents are the primary ingredient as they are responsible for sebum and dirt removal, i.e., cleansing. Surfactant use is a balancing act, as too much cleansing can result in dull hair that is frizzy and difficult to comb but too little use may mean that we fail to clean the hair thoroughly (which is the “raison d’etre” for shampoos). The main mechanism of action for detergents is reducing the surface tension at interfaces, therefore promoting mixing of different
phases. Examples of common surfactants are the anionics lauryl sulfates, laureth sulfates, and the betaines. The psychological component of abundant and long-lasting lather—which is equated by the consumer as proof of cleansing—is an everpresent challenge which foaming agents address.

Thickeners are used to increase the viscosity of a shampoo to provide the desired texture, pourability, and thickness.

Opacifiers give a specific look and special effects to the shampoo appearance in the bottle and during usage that delights the consumer. They work by changing the distribution of light absorbed in the surface. They make the formula opaque in the bottle and give a pearlescent look.

Conditioning agents, such as quaternary compounds, fatty alcohols, and silicones are added to improve hair performance and cosmetic appearance (Schueller 1995).

Preservatives, such as methylchloroisothiazolinone and sodium benzoate, are substances that inhibit or slow down the growth of microorganisms.

Chelators, such as ethylenediaminetetraacetic acid (EDTA), form complexes with metal ions to avoid stability issues or interactions.

1.6 Fragrances

A perfume or fragrance is a mixture of essential oils, aroma compounds, fixatives, and solvents to improve the smell of the human body, objects, and living spaces. The amount and type of solvent mixed with the fragrance oil dictates whether a perfume is considered a perfume extract, eau de parfum, eau de toilette, or eau de cologne (Secondini 1990).
An extrait contains 15-45% perfume content and is a concentrate. It is the purest form of scented product and the priciest. It is also labeled *perfume* or *extrait perfume*, or *extract*.

Figure 1.6: Extrait perfumes: Channel 5 and Versace Blonde
An eau de parfum, containing 7-15% pure perfume content, is the most popular and common form of perfume and provides a long-lasting fragrance. Its literal translation from French into English is “perfumed water”.

Figure 1.7: Eau de Parfum: Angel by Thierry Mugler and Lovely by Sarah Jessica Parker
An eau de cologne contains 2-5% perfume total. Even though sometimes eau de cologne is used as a synonym for eau de toilette, they vary in the content and eau de cologne has its origins in a light, citrus, fresh fragrance mix made popular by Napoleon.

![Image of eau de cologne and original eau de cologne document]

Figure 1.8: Eau de cologne: Pierre Cardin and original eau de cologne
An eau de toilette contains 3-8% perfume content. Its original purpose was to be used as a body splash to wake people up in the morning. Its scent is lighter so it does not become overpowering unlike some of the other perfume forms.

Figure 1.9: Eau de toilette: Hugo Boss and Calgon Marshmallow mist spray

In shampoos and other personal care products, fragrances are an ingredient that is added at very small levels, usually in the range of 0-5% depending on the overall design of the product. In this sense, the level of fragrance in haircare products is similar to the level of fragrance present in a eau de toilette or eau de cologne, the lighter perfume forms.

Commonly fragrances represent a final element that is added to the formulation or listed as “other ingredients”. Up until the 1940s, fragrance was mainly an ingredient that formulators just added until the final composition of a 100% was reached. However, perfumes are a key ingredient of holistic design, specially in the cosmetic and personal care industry. Perfume raw materials have inherent characteristics and are not inert; they can react and affect the stability of the product (Kanei 2005).
1.7 Fragrance Formulation Basics

A fragrance is a blend of raw materials which can have different origins: animal, vegetable or mineral. The components of a fragrance are a combination of base notes, middle notes and top notes.

A note is the impression of a single smell on the olfactory sense, or it refers to one of the three different periods in the overall process of fragrance diffusion (top, middle, and base). The term was borrowed from the musical nomenclature.

A top note is the impression a fragrance first leaves when it is first smelled or applied to the substrate, and is characterized by the most volatile perfume or perfumery raw materials (PRM) in a fragrance. These scents are perceived immediately on application, and form the first impression of a perfume. They are commonly the assertive or sharp notes. Some of the physical properties of top notes are strong in scent, very volatile, and high in vapor pressure. Common top notes are citrus, aldehydes, eucalyptus, and ginger.

Middle (or heart) notes are PRMs with lower volatility than the top notes that make up the main blend of a fragrance. The middle notes may take ten to twenty minutes to develop, and can be described as the scent of a perfume that emerges after the top notes dissipate—they are more mellow and rounded smells. Heart notes may appear from two minutes to one hour after perfume application. Some common heart notes are lavender and rose scents.

Top notes along with heart notes are described as the head notes, while the body of the fragrance make-up the base notes. Top notes are responsible for the perfume bloom of fragrance. The base and middle notes provide the perfume longevity as they do not flash off as easily.
Base notes are the scent of a perfume that appears after departure of the top notes. Together with the base and middle notes they constitute the main theme of a perfume bringing depth to the fragrance. Perfumery raw materials classified on the base notes are described as rich and deep, may take as long as 30 minutes after perfume application and dry-down to develop, and enhance the strength of the head of the fragrance. Examples of base notes are: Musk, vetiver resinoid plants scents.

Formulators usually follow the perfume triangle when making perfumes. This is a modern trend, as in the past the triangle was upside down. The main approach is to add a smaller quantity of top notes which evaporate sooner, enhancing and preserving the scent by using the not-so-volatile middle and base notes.

A perfume accord is a blend of two or more smells that produces a third very distinct smell—different from the original individual entities. An accord can be a simple mixture of two materials, or it may contain an extensive list of materials. The key factor is that all materials are balanced with each other so no one PRM can be singled out. In this case, we used a technical accord that had twenty five different PRMs. Its purpose was to serve as a modeling tool to study the effect of partitioning in the different shampoo phases for a wide range of perfume raw materials (PRMs) with different physical properties, listed in Table 1.2, such as: lipophilicity, water solubility, volatilities, molecular structures, and molecular weights. This technical tool was used as a marker to predict future new raw materials. The molecular weight of the PRMs ranged from 128-258 Da and their lipophilicity value (log P) ranged from 1.12 for the most hydrophilic PRM to 6.30 for galaxolide, the most lipophilic PRM. The smell of the technical accord was a strong pungent odor, and not one note of the individual PRMs could be individually identified.
<table>
<thead>
<tr>
<th>PRM</th>
<th>log Octanol-Water Partition Coefficient-log $P$</th>
<th>Molecular Weight-Mw (Da)</th>
<th>Water Solubility-Sw (g/L)</th>
<th>Kovats Index-Ki</th>
<th>Vapor Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heliotropin</td>
<td>1.12</td>
<td>150.13</td>
<td>8.32E-03</td>
<td>1401</td>
<td>6.90E-03</td>
</tr>
<tr>
<td>Tripal/Ligustrual 1</td>
<td>2.27</td>
<td>138.21</td>
<td>5.13E-02</td>
<td>1149.7</td>
<td>3.29E-01</td>
</tr>
<tr>
<td>Dimethyl Phenyl Ethyl Carbinol</td>
<td>2.44</td>
<td>164.24</td>
<td>2.88E-02</td>
<td>1338.2</td>
<td>6.03E-03</td>
</tr>
<tr>
<td>Octanal</td>
<td>2.89</td>
<td>128.22</td>
<td>3.63E-03</td>
<td>1021.1</td>
<td>1.48E+00</td>
</tr>
<tr>
<td>Citronellyl Nitrile</td>
<td>3.44</td>
<td>151.25</td>
<td>1.51E-03</td>
<td>1096</td>
<td>4.90E-02</td>
</tr>
<tr>
<td>Rose Oxide</td>
<td>3.48</td>
<td>154.25</td>
<td>6.17E-03</td>
<td>1168.4</td>
<td>4.45E-01</td>
</tr>
<tr>
<td>dl-Menthy Acetate</td>
<td>3.56</td>
<td>198.31</td>
<td>5.13E-04</td>
<td>1337.6</td>
<td>4.92E-02</td>
</tr>
<tr>
<td>iso-Butyl Quinoline Major</td>
<td>3.58</td>
<td>185.27</td>
<td>1.41E-03</td>
<td>1493.9</td>
<td>7.72E-04</td>
</tr>
<tr>
<td>Phenyl Ethyl Benzoate</td>
<td>3.59</td>
<td>226.28</td>
<td>2.00E-04</td>
<td>1832.8</td>
<td>7.45E-05</td>
</tr>
<tr>
<td>Linalyl Propionate</td>
<td>3.91</td>
<td>210.32</td>
<td>3.89E-03</td>
<td>1314.8</td>
<td>2.50E-02</td>
</tr>
<tr>
<td>cis-Hexenyl Salicylate</td>
<td>3.97</td>
<td>220.27</td>
<td>1.62E-03</td>
<td>1721</td>
<td>5.88E-05</td>
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<tr>
<td>d-Limonene</td>
<td>4.01</td>
<td>136.24</td>
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<td>1067</td>
<td>1.55E+00</td>
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<tr>
<td>para-Cymene</td>
<td>4.08</td>
<td>134.22</td>
<td>1.95E-04</td>
<td>1086.7</td>
<td>1.33E+00</td>
</tr>
<tr>
<td>Lilial</td>
<td>4.14</td>
<td>204.31</td>
<td>5.50E-05</td>
<td>1559.6</td>
<td>2.72E-03</td>
</tr>
<tr>
<td>Myrcene</td>
<td>4.21</td>
<td>136.24</td>
<td>9.12E-05</td>
<td>952.18</td>
<td>2.45E+00</td>
</tr>
<tr>
<td>alpha-Pinene 2</td>
<td>4.26</td>
<td>136.24</td>
<td>5.37E-05</td>
<td>1051</td>
<td>3.04E+00</td>
</tr>
<tr>
<td>Methyl Ionone</td>
<td>4.34</td>
<td>206.24</td>
<td>2.82E-03</td>
<td>1458.3</td>
<td>5.29E-03</td>
</tr>
<tr>
<td>Veloutone</td>
<td>4.61</td>
<td>196.33</td>
<td>1.32E-03</td>
<td>1420.7</td>
<td>2.61E-02</td>
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<tr>
<td>Galaxolide</td>
<td>6.30</td>
<td>258.41</td>
<td>1.41E-06</td>
<td>1925.5</td>
<td>4.56E-05</td>
</tr>
</tbody>
</table>

Table 1.2: Technical accord-Representative PRMs and physical properties

Some other important concepts that are important in perfume formulation are explained:

The Odor Detection Treshold (ODT) is the concentration at which a smell can be identified in the surrounding atmosphere. A low ODT perfume has a higher concentration of top notes and base notes that can be easily identified and smelled by the consumer—as they can be detected by the human nose at very low concentrations.

A Hydrophobically Modified Accord is an accord that has been modified to include perfumery raw materials that are hydrophobic or have a tendency to partition into lipid phases.
1.8 Gel Network Technology

Previously, we discussed one of the main technologies for conditioning shampoos that relied on anionic surfactants and cationic conditioners which in the past led to problems such as charge complexation. The use of silicone in its various forms (dimethicone in small particle size or volatile silicones) is the most common conditioning methodology used—however we can run into problems with buildup of the silicone as a layer and inhibit sebum removal (Rushton, 1994).

Gel network technology is frequently used in creams and ointments as the main mechanism for performance enhancement and delivery of actives. Conditioners for haircare products have gel network structures. In our conditioning shampoo systems, the overall application consists of a conditioner embedded in a shampoo. One of the great cost advantages of the gel network form is the high percentage of water (greater than 80 %)—as shown in Figure 1.10—present in the mixture with the fatty alcohols and the surfactant or co-surfactant.

![Figure 1.10: Compositional makeup of gel network premix](image-url)
For the gel network premix, a mixture of the components was heated up and raised above the chain melt temperatures of the solids. The mixture was kept at a high temperature for an extended time period, and a mixed liquid crystal phase is formed as the surfactant and water penetrated the fatty alcohol phase. After a selected time period, the batch was left to cool below its chain melt temperature—which allowed the chains to crystallize in a lamellar phase with alternating bilayers of water and fatty alcohol/surfactant. The colloidal gel structure formed has two distinct regions as shown in Figure 1.11, a hydrophilic gel portion (interlamellar water layer), and a lipophilic gel portion (fatty alcohol and surfactant bilayers). This cooled premix is later incorporated into a previously mixed shampoo quench.

Figure 1.11. Gel network system-Bilayers of alternating fatty alcohols/surfactants and water
Several changes occur to the colloidal structure once the gel network premix is incorporated in the shampoo. This incorporation indicated the formation of a new structure, the equilibrated lamellar dispersion (ELD). From previous compositional analysis, it has been determined that the surfactant migrates from the shampoo chassis into the gel network creating an equilibrated lamellar dispersion (Wells 2006). This lamellar dispersion has different properties than the original gel network premix. For example, the phase transition temperature is reduced roughly half the value it had on the premix alone, indicative of a fundamentally different structure. The interlayer spacing between the fatty alcohol bilayers decreases once the premix is incorporated into the shampoo-indicating that the premix interacts with other materials present in the shampoo chassis along with the salt and surfactants. The short range order, measured by wide angle x-ray diffraction, stays approximately the same, which denotes a uniform packing of the fatty alcohol and surfactant in the bilayers.

The deposition of the ELD is a coacervate based phenomenon where the cationic polymer complexes with the anionic surfactant upon dilution of the shampoo, thus entrapping the gel network. Both conditioning actives, the fatty alcohols and silicone, are deposited via coacervate technology onto the hair to deliver conditioning benefits.
2. HYPOTHESIS AND SPECIFIC AIMS

2.1 Hypothesis

We hypothesize as follows:

“Deposition and/or release of perfume raw materials (PRMs) to and from hair can be enhanced by incorporating them into the gel network”.

Perfume Raw Material

Figure 2.1: Visual representation of hypothesis-PRMs incorporated in gel network premix
Another way to represent the perfume incorporation is shown in Figure 2.2. There were two distinct modes of incorporation of the perfumes into the gel network premix. On the left, a visual is shown of the gel network premix, in which the perfume is added directly to the gel network premix prior to shampoo incorporation. An ideal representation of the occurring phenomena is shown in Figure 2.2a: the perfume remains in the gel network premix after shampoo incorporation to be later deposited by coacervate technology onto the hair. On the other end of the spectrum—the separate mix (direct) case—both the perfume and the gel network premix are added concurrently as shown in Figure 2.2c. In reality, we expect to reach an equilibrium state as represented Figure 2.2b figure below, similar to the other components in the gel network shampoo system.

<table>
<thead>
<tr>
<th>a) Gel Network and Perfume Premix</th>
<th>b) Gel Network and Perfume Equilibrated</th>
<th>c) Gel Network and Shampoo Direct Add (Separate Add)</th>
</tr>
</thead>
</table>

Figure 2.2: Visual representation of shampoos with different order of addition for perfume
Furthermore, when understanding which PRMs prefer to partition into the gel network phase for subsequent hair deposition, a simplified partition model that would form the basis for a Quantitative Structure Activity Relationship Model was created. This is a very simplified model, since due to the diversity of the PRMs in the technical accord, each will have its individual tendency to migrate into different regions due to the variation in physical properties. At equilibrium, the system will reach a point where the activity (concentration) is the same in both phases (shampoo and gel network). If we were to separate the shampoo into two phases, the semi-solid gel network phase and the viscous liquid shampoo phase—if the PRM has no distinct preference for partitioning—it will have the same composition in each phase, as shown on the 0.7% perfume control diagram.
Figure 2-3: Simplified partition model-Equilibrium representation
On the other hand, suppose we were to separate the shampoo into two phases: the semi-solid gel network phase and the viscous liquid shampoo phase. If the PRM partitions favorably into the gel network phase (which is our desired output for further depositing the perfume into the hair), the composition of the PRM will be higher in the gel network layer than in the shampoo layer relative to the shampoo control (which is the uncentrifuged sample).

Figure 2-4: Simplified partition model-Preferred partitioning into the gel network phase representation
2.2 Specific Aims

The specific aims of this project were as follows:

**Aim 1- Formulation and Characterization**
- To formulate shampoo gel network premixes and full shampoo batches under different composition ratios and components, processing parameters, and different fragrance perfumery raw materials while maintaining the level of fragrance constant.
- To characterize how fragrance addition to the gel network shampoo alters such formulations via microscopy (visual solubility), differential scanning calorimetry (melt transition temperature) and perfume partition analysis in both the gel network premix and the full shampoo formulation.

**Aim 2 -Sensory Evaluation**
- To test selected shampoo formulations and assess olfactory differences between prototypes containing gel network technology with different formulation variables in order to obtain an understanding of plausible consumer perception and impact of formulation changes on sensory evaluations.

**Aim 3 -Prediction QSAR Modeling**
- To create a model that predicts which PRMS have a preference to partition into the gel network phase to be further deposited by coacervate technology onto the hair.
3. MATERIALS AND METHODS

3.1 Materials

The materials used (all obtained internally from the Procter & Gamble Company) were: distilled water, sodium laureth sulfate, sodium lauryl sulfate, cocamidopropylbetaine (CAPB), ammonium lauryl sulfate, dimethicone, sodium chloride, disodium ethylenediaminetetraacetic acid (EDTA), sodium benzoate, citric acid, sodium citrate, methylchloroisothiazolinone/ methylisothiazolinone (Kathon®), ethylene glycol disteareate (EGDS), cetyl alcohol (C16OH), behenyl alcohol (C22OH), stearyl alcohol (C18OH), and fragrances.

The technical accord contained the following 25 PRMs at a 4% percentage each to make a total of a 100%:
alpha pinene, cis-3-hexenyl salicylate, ethyl acetate, exaltolide, heliotropin, indole, ionone gamma methyl, iso butyl quinoline, linalyl propionate, maltol, methyl isobutenyltetrahydropyran, myrcene, octylaldehyde, P.t.bucinal, p-cymene, p-hydroxy phenyl butanone, phenyl ethyl dimethyl carbinol, ligustral, veloutone, heliotropin diethyl acetal, citronellyl nitrile, menthyl acetate, phenyl ethyl benzoate, hexamethylindanopyran, and orange terpenes.
3.2 Methods

3.2.a Formulation

The containers of choice for the formulation of the gel networks were 200mL or 2L stainless steel beakers for small or large batches respectively. Water (88%) was mixed in the container to a temperature above the fatty alcohol and surfactant melting point. It was heated in an IKA temperature control hot plate IKA (Rec control viscosity safety control) heated stirring mixer with a stainless steel temperature probe. Overhead mixers (IKA Eurostar Power control-vis) with custom mixer blades appropriate for the viscosity of the premix were used to appropriately blend. After mixing the water, the surfactant (3%) and fatty alcohols mixture (8%) were added and mixed for twenty-five minutes allowing the materials to fully melt. Citric acid was added to achieve the target pH of 5.5. After this final mixture, the fragrance was added at a 0.7 % level and mixed for five minutes. The beaker was covered with aluminum foil and/or Saran wrap to minimize evaporation. After 30 minutes, the premix was milled with a homogeneizer for 30 seconds and then further mixed with the overhead mixer while cooling to room temperature for a period of three hours. The premixes were later incorporated at a 27% level into a pre-mixed shampoo base. The shampoo incorporation was done using high speed mixer (Hauschild SpeedMixer DAC 400 FVZ) set at 1500 RPM for 60 seconds in batches of 300 grams. The container of choice for shampoo formulation was a plastic polyethylene (PE) jar.
3.2.b Microscopy

Optical microscopy was performed using a microscope with a digital camera attached for both the gel network premixes and the final shampoo formulation. Each sample was prepared on a microscope slide, covered with a microscope cover and viewed at 40X with a Nomarski optics microscope. Both bright field and crossed polar images were taken. In the bright field mode, the qualities assessed were homogeneity, presence of vesicles or lamellar sheet structures, and presence or absence of solubilized perfume droplets. In the crossed polar mode, the presence of maltese crosses or birefringence was the key observation.
3.2.c Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry was used to investigate the thermal properties of the lamellar gel phase formed in the shampoo. A 50 mg sample was placed in a stainless steel high volume DSC pan and compared against the reference pan with the calorimeter (TA Instruments Q100 DSC). The heating range was 50°C to 90°C and the heating rate was 3°C/minute. Nitrogen flow was used. The data were analyzed using TA Instruments Universal Analysis Software. A Mettler microbalance was used for weighing the samples.
3.2.d Shampoo Partitioning-Perfume Compositional Analysis

The first step in the perfume analysis was centrifugation (Beckman Coulter Optima L-series ultracentrifuge). 40 ml samples were taken from the neat product and carefully placed in 39mL centrifuge tubes slowly carefully avoiding bubbles and void spaces. Samples were spun at 28,000 RPM (141,000 g-force) for 15 hours using a SW 28 swinging bucket rotor (for a horizontal uniform separation of the layers). After centrifugation, three distinct layers were present: diffuse gel network on top, EGDS layer on the middle, and shampoo layer on the bottom. The diffuse gel network and shampoo layer were isolated very carefully with a spatula and withdrawn with a 20 ml syringe respectively.

![Schematic of a shampoo tube after centrifugation](image)

Figure 3.1: Schematic of a shampoo tube after centrifugation with different layers: gel network, EGDS and shampoo

Two gram aliquots of each layer were combined with six grams of pentane for extraction. The treated aliquots were shaken for 20 minutes, then centrifuged (IEC HN-SII) at 2,000 RPM (100,000 g-force) to enhance separation.
Aliquots of the pentane layer (containing the perfume) were analyzed. A 20 uL sample was injected into the Gas Chromatograph/Mass Spectrometer (GC/MS) and the peaks obtained.

Perfume compositional analysis was performed using GC/MS, a method in which we separate the individual components of a mixture. The details for the instruments used are listed:

- Instrument Agilent 6890 Gas Chromatograph
- Capillary Column-Agilent DB5 (a relatively non-polar stationary phase)
  - Length-60 meters
  - 0.25 mm ID and 1 um stationary film thickness
- Detector-Agilent 5973 Mass Selective Detector (MSD)
- Detector Temperature: 280°C
- Injection Port Temperature: 50°C at inject ramped to 280°C following solvent vent mode
- Splitless injection
- Helium carrier gas at 1.2mL/min

The main principle behind GC/MS is that each component chemical has a different affinity for the column stationary phase thus being eluted at a different timeline. To increase sensitivity, a large volume injection-20 microliters-was utilized. In the mass spectrometer, the eluted chemical was separated into smaller components via electron ionization. The fragments were then detected and identified by their unique compound spectra.
3.2.e Sensory Evaluation

The full shampoo formulations were evaluated by a trained panel of 30 subjects. The panelists went through extensive training prior to tests to differentiate intensity of odor. Odor anchors in the rooms assisted the graders. There was an initial 0-hr grading, a 3-hr grading, a 5-hr grading, a 24-hr grading and a 25-hr combing point after evaluation. The hair swatches were 20 g hair swatch units, which were treated with 6 grams of shampoo per swatch. There were three blanks per experiment setup and the switches were run in triplicate each. Each hair swatch was treated independently and hung on the hair rack before starting treatment of the next hair switch. Half of the shampoo sample (3 cc) was applied down the hair swatch with a syringe. The hair swatch was lathered for forty seconds using the milking technique. The hair was rinsed for fifteen seconds, flipped over and the process repeated on the other side of the hair. The hair swatches were then gently picked to remove tangles (minimize appearance), shaken three times from side to side to minimize tangles, and the excess water blotted with paper towels. This process was repeated with each individual hair swatch prior to the start of the next. After the rack had been set up, expert odor graders assessed the odor intensity and character at the predetermined time points.
3.2.f Headspace Analysis

Ten gram hair switches were treated with three grams of shampoo, ensuring the hair switches were handled with the same treatment (milking method) as in the sensory evaluation. The treatment was applied both to the controls and the treatment samples, and the number of samples executed was four for each. The treatment for the hair switches was shampoo with gel network, while the treatment for the controls was the shampoo without the gel network. Both formulations had the same 0.7% level of perfume. The hair switches were placed in a 250 mL headspace jar and allowed to equilibrate for two hours. Two different time points were measured: the initial time point and the three-hour time point to further compare with the sensory evaluation results. The samples from the air were collected using a dynamic headspace collection protocol (at a rate of 30 mL/min for a 10-minute time period) where the perfumes were absorbed in a Tenax trap while helium gas was flowing in and out of the jar. After the resin entraps the perfume, the Tenax trap is thermally desorbed onto the GC and the same protocol for compositional quantification as in section 3.2.d is followed.
3.2.g  Partition Modeling-Quantitative Structure Activity Relationship

The training data set selected was the technical accord with the twenty-five different perfumery raw materials (PRMs). The physical properties of the PRMs were obtained from different databases. Molecular weights were obtained from Chemfinder. Vapor pressures at 25°C were obtained using the EPIWIN® software based on previous research from Syracuse Research Corporation. Other key properties such as water solubility (Sw) and octanol-water partition coefficient (Ko/w) were estimated using the property-prediction software ChemSilico®. Since not all the twenty-five PRMs could be detected by our analytical method, only the detected PRMs were included in the model calculations. Based on correlation of existing compositional data, a decision was made to use the 8-day equilibrated gel network and shampoo uptake data of PRMs data for building the model. The structures of the PRMs were entered into the software Sybyl® and stored in a database. Initial 3D atomic coordinates for each structure were generated using the software Concord®. Conformational analysis was then performed in Spartan® using molecular mechanics. The final conformation of the structures was then entered into the software ADAPT®. A very wide range of descriptors was generated: geometric, electronic, topological, and hybrid-shape descriptors. Initial models were generated in ADAPT® using the optimization methods of genetic algorithm or simulated annealing method for descriptor selection. Models of interest were further analyzed with Minitab® projection of latent structures (PLS) function. A final quantitative structure activity relationship equation was generated and validated via the “leave-one-out-method” and a cross-validated R² value (Q²) was obtained. After the regression equation was obtained, it was further applied to a new database to predict the activity of perfume raw materials and assess which PRMs would be most likely to provide
the desired steric and physical characteristics for gel network partitioning/uptake.
4. RESULTS and DISCUSSION

4.1 Gel Network Premix Characterization

The first step in formulation was the creation of the gel network premix, literally a conditioner. For this purpose, the optimal settings for incorporation of fragrance into gel network premixes were investigated. Standard gel network (GN) premixes were made using the procedure detailed in section 3.2 with water, stearyl alcohol (C18OH), cetyl alcohol (C16OH), behenyl alcohol (C22OH), sodium laureth sulfate, fragrance, citric acid and methylchloroisothiazolinone/methylisothiazolinone. The standard gel network premix (PM) was 11% active (22% sodium laureth sulfate). The % activity refers to the amount of solids and was held constant at 11% in all experiments. Of the 11% total activity, the composition breakdown was 22% surfactant and 78% fatty alcohols. The ratio of fatty alcohols was optimized in previous studies and kept at 1.8 parts of C18OH to 1.0 parts of C16OH. Table 4.1 describes the composition of the standard basic formulations. Formulation A was the standard without perfume to be used as a control (Table 4.1.a) and formulation B (Table 4.1.b) was a basic formulation with 2.57% fragrance level (which is equivalent to 0.7% fragrance in the final shampoo formulation when the gel network premix is incorporated into the shampoo at 27%). Except for the perfume addition, both formulas were processed simultaneously and exactly in the same manner for a total of 30 minutes process time period and a 3-hr cooling down period. Both formulation A and B were characterized via the same exact methods: microscopy and differential scanning calorimetry (DSC) as described in section 4.1.i. Compositional analysis was not performed on the gel network premix because it would not be representative of the final end product, the shampoo.
<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Wt %</th>
<th>Ingredient</th>
<th>Wt %</th>
</tr>
</thead>
<tbody>
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<td>QS 100</td>
<td>Water</td>
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</tr>
<tr>
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<tr>
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<td>Sodium laureth sulfate (28% active)</td>
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<td>Fragrance</td>
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<td>2.57</td>
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</tbody>
</table>

Table 4.1. Standard formulas for gel network premixes a) Zero fragrance b) 2.57% fragrance

4.1.a Microscopy-Basic Formulations

Optical microscopy was used to obtain visual information about the phases present in our basic formulations A and B. The results are shown in the form of micrographs obtained by the digital camera in Figure 4.1. The gel network in the premix is represented by hexagonal shaped three dimensional structures shown in Figure 4.1.a. It was observed that some perfumes droplets were embedded and surrounded by the gel network itself. Gel network structure was also confirmed via maltese crosseses in the crossed polar mode.
The light micrograph (Fig 4.1) show a portion of unsolubilized drops that are perfume -it can be hypothesized that somehow the PRMs are being pushed out of the crystallized gel network structures after they reach a maximum solubility of the gel network phase. Another feasible explanation for the phenomenon is that out of the twenty-five different PRMs-those with a lower affinity for the gel network phase will not solubilize as well as the PRMs that are more lipophilic (Labows 1997).

Figure 4.1: Light micrograph of the gel network premix for formulation B

It was observed that not all the perfume solubilized into the gel network, as seen in the bright field image. In figure 4-2, we can see the distinct difference between the gel network premixes that have perfume and the standard gel network premixes with no perfume incorporated.
Formulation A, in figure 4-2a, shows a more uniform surface in the bright field mode and the presence of maltese crosseses in the cross polar mode. Formulation B, on the other hand, shows a more grainy surface with unsolubilized oil droplets surrounded by the gel network (Fig. 4.2). Formulation B also shows maltese crosseses in the cross polar mode.

<table>
<thead>
<tr>
<th>BRIGHT FIELD</th>
<th>Formulation</th>
<th>CROSSED POLAR</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Form A" /></td>
<td>a) Form A-NO PERF</td>
<td><img src="image" alt="Form A" /></td>
</tr>
<tr>
<td>a) i</td>
<td></td>
<td>a) ii</td>
</tr>
<tr>
<td><img src="image" alt="Form B" /></td>
<td>b) Form B-2.6% PERF</td>
<td><img src="image" alt="Form B" /></td>
</tr>
<tr>
<td>b) i</td>
<td></td>
<td>b) ii</td>
</tr>
</tbody>
</table>

Figure 4.2: Light micrographs of gel network premixes a) no perfume-formulation A and b) perfume-formulation B
4.1.b Differential Scanning Calorimetry—Basic Formulation

First, the melting points for the pure fatty alcohols were measured by thermal analysis. As expected, the melting point increased with increasing chain length. Sharp single endotherms were measured for the pure raw materials and the values summarized in Table 4.2. The measured values obtained showed some variation when compared to literature values due to raw material impurities.

<table>
<thead>
<tr>
<th>Carbon Chain Length (ROH)</th>
<th>Name</th>
<th>Measured Melting Point (°C)</th>
<th>Literature Value (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C₁₆OH</td>
<td>Cetyl alcohol</td>
<td>51</td>
<td>56</td>
</tr>
<tr>
<td>C₁₈OH</td>
<td>Stearyl alcohol</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>C₂₂OH</td>
<td>Behenyl alcohol</td>
<td>73</td>
<td>71</td>
</tr>
</tbody>
</table>

Table 4.2: Pure raw materials melting points as determined by DSC

The effect of perfume addition on the gel network premixes was investigated by performing thermal analysis on our two basic formulations A and B. Representative results are shown in Figure 4.4 and Table 4.3.
<table>
<thead>
<tr>
<th>Fragrance Addition</th>
<th>Phase Transition Temperature PTT (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Processing Method</td>
<td>Standard Gel Network 11% active (22% Sodium Laureth 3 Sulfate 8% C\textsubscript{18}OH/C\textsubscript{16}OH in a ratio of 1.8 to 1.0 ratio, 3% surfactant), 89% water</td>
</tr>
<tr>
<td>No Perfume-Formulation A</td>
<td>62.41</td>
</tr>
<tr>
<td>Perfume-Formulation B</td>
<td>55.58</td>
</tr>
</tbody>
</table>

Table 4.3: Phase transition temperature for standard gel network (11% active (C\textsubscript{16}OH/C\textsubscript{18}OH in a ratio of 1.8 to 1.0-89% water) under different perfume addition conditions
Figure 4.3: Representative thermogram-Perfume addition on gel network premix effect of phase transition temperature of gel network structure

In the thermograms, the phase transition temperature (where the frozen gel network structure melts) for the premix was present as a sharp endotherm between the melting point of the individual components. A depression of 7-10 °C in the phase transition temperature was observed in the premixes following perfume incorporation indicating a decrease in thermal stability of the gel network premix upon perfume addition.
This method of characterizing gel network system melt transition temperature had been used previously (Ribeiro 2004, De Vringer 1987, and Fukushima 1983). By adding the perfume, we incorporate the perfume within the fatty alcohol and surfactant bilayers.

4.1.c Reproducibility Studies
Reproducibility studies were conducted on three batches (for both formulation A and B) with assessment via microscopy and DSC. When available, informal rheology measures were also obtained. The relative standard deviation (RSD) in each case was less than 5%, showing there was good reproducibility of the overall method.

4.1.d Processing Time Factor
The effect of perfume processing time in formulation B was studied due to the inherent volatility of these ingredients. Different processing times for the perfume technical accord incorporated at 74°C were studied. The heating processing time for the premix, prior to milling and cooling periods, was 30 minutes total. For the first portion (without perfume) of the hot processing, the gel network premix was mixed to allow for swelling of the fatty alcohols and formation of the bilayers. The heating processing times for perfume incorporation into the gel network premix were varied from 5 minutes to 20 minutes (5, 10, 15, and 20 mins), while keeping all the other formulation and processing variables constant. When comparing the different formulations, no significant difference was observed via microscopy data, and DSC (melt transition temperatures showed a narrow variation 55-56°C.)
Based on these results, the final heating process time was set at 25 minutes of gel network mixing (without the perfume) followed by five minutes of perfume processing into the gel network. This overall procedure mimics pilot plant processing times.

4.1.e Perfume Processing Temperature Studies

The effect of temperature (T) for perfume processing in formulation B was investigated. For safety purposes, traditional processing systems incorporate fragrances at lower temperatures such as 50°C to avoid flashing off of high notes. However, that temperature may not represent the optimal temperature for perfume solubilization in the gel network so further research was conducted. In this set of experiments, the technical accord was added at both 74°C (high T) and 50°C (low T). The resulting products were assessed by microscopy and DSC. Figure 4.4 shows micrographs for both temperature addition conditions.
While there was no significant difference in the DSC endotherms, it was observed in the micrographs (see Figure 4.4) that not all perfumes were incorporated into the gel network at the lower temperature. The hot processing image (Fig 4.4.b) has less perfume droplets than the cold processing image (Fig 4.4.a). These droplets represent fragrances that have not been completely solubilized in the gel network phase. From this pilot trial, it was confirmed that perfume addition at a higher temperature yielded greater perfume incorporation into the gel network bilayers.
4.1.f  Formulation Variables Effect on Gel Network Premix

The effect of simultaneous variables, both compositional and processing, on our formulations was studied with a D-optimal multifactorial design. The responses measured for the formulation were microscopy, DSC, and perfume analysis in different layers after centrifugation. The factors varied, as outlined in Table 4.4, were: fatty alcohol chain length, fatty alcohol ratio, and order of addition of perfume (premix vs. direct mix). The data was analyzed using JMP® statistical software. Due to constraint resources and for simplicity purposes, the design of experiment (DOE) included only binary mixtures (vertexes and the corners of the design space).

<table>
<thead>
<tr>
<th>Factors</th>
<th>Level 1</th>
<th>Level 2</th>
<th>Level 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fatty Alcohol Chain Length</td>
<td>$C_{16}OH$</td>
<td>$C_{18}OH$</td>
<td>$C_{22}OH$</td>
</tr>
<tr>
<td>Fatty Alcohol Ratio</td>
<td>1:3</td>
<td>1:1</td>
<td>3:1</td>
</tr>
<tr>
<td>Point of Perfume Incorporation</td>
<td>Premix</td>
<td>Direct Mix</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.4: Design of Experiment for Formulation Variables

The melt transition temperatures for the gel network premixes were determined via DSC for the formulations from the DOE. A summary of the results are shown in Table 4.5 and representative thermograms for the gel network premixes with perfume incorporated are shown in Figure 4.6.
<table>
<thead>
<tr>
<th>Fatty Alcohol Binary Mixture</th>
<th>Ratio of Fatty Alcohols</th>
<th>Phase Transition Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Direct Mix-Modified Formulation A</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;OH- C&lt;sub&gt;16&lt;/sub&gt;OH</td>
<td>3:1</td>
<td>64.34</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;OH-C&lt;sub&gt;16&lt;/sub&gt;OH</td>
<td>1:3</td>
<td>61.65</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;OH-C&lt;sub&gt;22&lt;/sub&gt;OH</td>
<td>3:1</td>
<td>66.55</td>
</tr>
<tr>
<td>C&lt;sub&gt;18&lt;/sub&gt;OH-C&lt;sub&gt;22&lt;/sub&gt;OH</td>
<td>1:3</td>
<td>66.50</td>
</tr>
</tbody>
</table>

Table 4.5: Effect on phase transition temperature of formulation and processing variables-Gel network premix
When evaluating the variable order of addition of the perfume, it was observed that varying the fatty alcohol chain length and their respective ratio yielded a difference of 3-8 °C for the phase transition temperature between the premixes with no perfume and the premixes with perfume. This is consistent with previous DSC data from Figure 4.4 when the basic formulations A and B were compared. The endotherms in the premixes were a direct function of composition, chain length of the fatty alcohols, and the individual raw material melting point.
4.2 Shampoo Characterization

4.2.a Microscopy

The gel network premixes (or conditioners) were then incorporated into previous shampoo bases and assessed via microscopy. The brightfield and crossed polar micrograph are shown in Figure 4.6.

Figure 4.6a: Full shampoo light micrograph: brightfield
Figure 4.6b: Full shampoo light micrograph: crossed polar

Figure 4.6.a shows that a new structure is formed, different from the three dimensional structure observed in the gel network premix. This new structure, the equilibrated lamellar dispersion or ELD (Wells 2003, 2006) is visible as a different crystalline formation in the micrographs. In figure 4.6b, two main features were observed: the equilibrated lamellar structure along with the EGDS crystals. The presence of maltese crosseses in Fig. 4.6b confirms the presence of gel network structure which upon incorporation in the shampoo base undergo a series of transformations covered in Section 1.7. The elongated needles visible in the micrograph represent the EGDS crystalline structures.
4.2.b Differential Scanning Calorimetry-Basic Formulation

The variable of perfume incorporation mode into the final shampoo was studied. The two modes were premix and direct mix. In the premix mode, the fragrance was incorporated into the gel network premix (formulation B), let equilibrate overnight and further incorporated into the shampoo base. In the direct mix mode, the gel network premix did not have any fragrance content (formulation A) and the fragrance was incorporated simultaneously with the gel network premix into the shampoo base. The PTT for both systems was measured and representative results are shown in Table 4.6 and Figure 4.7.

<table>
<thead>
<tr>
<th>Perfume Order of Addition Variable</th>
<th>Phase Transition Temperature (° C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct Mix</td>
<td>36.42</td>
</tr>
<tr>
<td>Premix</td>
<td>35.26</td>
</tr>
</tbody>
</table>

Table 4.6: Perfume order of addition effect of shampoo on phase transition temperature-direct mix and premix
Figure 4.7: Thermograms for Full Shampoo Product: a) direct mix b) premix c) shampoo base with no gel network structure and 0.7% fragrance level and d) Shampoo chassis with no gel network and zero fragrance level.
In the full shampoo, the phase transition temperature (temperature at which the frozen gel network structure melts) dropped to about 38°C upon shampoo incorporation. The reason for the shift is the migration of the surfactants from the shampoo chassis into the gel network premix, creating the ELD. The thermogram (Fig. 4.7a and 4.7b) for a full shampoo containing gel network shows two distinct peaks. The first peak at 34-45°C represents the ELD—the “new form” or structure of the gel network. The next peak at 68°C represents the ethylene glycol distearate (EGDS) melting point, which is also present in the thermograms in Fig. 4.7c and d, which are shampoo bases without gel network.

It was observed that there was no significant difference in the phase transition temperature between premix and direct mix shampoo. Based on this data, it was hypothesized that the system reached an equilibrium and the perfume raw materials partitioned into or out of the gel network phase (fatty alcohol/surfactant bilayers) based on their respective physical properties. This data is consistent with microscopy visual assessments. In micrographs, when comparing the premix and the direct mix (form A and B respectively) in the shampoo, no significant difference was visible via microscopy, suggesting the system reached an equilibrium point.

4.2.c Formulation Variables Effect on Final Shampoo-Design of Experiment-Characterization by DSC

In analogy to the DOE performed in Section 4.1.f, the effect of formulation variables such as fatty alcohol chain length, fatty alcohol ratios, and the order of addition of perfume in the final shampoo formulation was studied.
The responses studied were phase transition temperature at which frozen gel network structure melts via DSC, and partitioning of PRMs via compositional analysis. The latter will be covered in the subsequent section 4.3. A summary of the results is shown in Table 4.6 and representative thermograms are shown in Figure 4.8a-d.

<table>
<thead>
<tr>
<th>Formulation Changes and Ratios</th>
<th>Direct Mix (Perfume Addition)</th>
<th>Premixed (Perfume Addition)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{18}OH-C_{16}OH 3:1</td>
<td>36.48</td>
<td>35.77</td>
</tr>
<tr>
<td>C_{18}OH-C_{16}OH 1:3</td>
<td>34.80</td>
<td>34.28</td>
</tr>
<tr>
<td>C_{18}OH-C_{22}OH 3:1</td>
<td>38.62</td>
<td><strong>46.38</strong></td>
</tr>
<tr>
<td>C_{18}OH-C_{22}OH 1:3</td>
<td>53.43</td>
<td><strong>41.22</strong></td>
</tr>
</tbody>
</table>

Table 4.7: Effect of formulation variables on phase transitions temperature-full shampoo
Figure 4.8: Thermogram of the effect of fatty alcohol chain length and ratios variation in phase transition temperature in full shampoo for direct mix of perfume  a) C\textsubscript{18}OH-C\textsubscript{16}OH 3:1 b) C\textsubscript{18}OH-C\textsubscript{16}OH 1:3 c) C\textsubscript{18}OH-C\textsubscript{22}OH 3:1 and d) C\textsubscript{18}OH-C\textsubscript{22}OH 1:3
Even though we had previously seen a difference earlier in the PTT between incorporating perfume in the premix by itself, for the full shampoo it was observed that PTT for the ELD formed was not statistically significant between the different addition points of perfume. This indicates that the system eventually achieves an equilibrium point where the perfume is embedded in the bilayer structure to an extent, and in the shampoo layer to a different extent. It was observed that varying the ratios of the fatty alcohols and its chain length, the endotherms varied as a linear function of the individual pure raw material melting points and composition. However, two unusual features were observed in one of the thermograms. The PTT for the formulation \( \text{C}_{18}\text{OH}-\text{C}_{22}\text{OH} \) with a 3:1 ratio was higher than the PTT for the formulation with a ratio of \( \text{C}_{18}\text{OH}-\text{C}_{22}\text{OH} \) 1:3 ratio (this formulation in theory should have a higher PTT due to a higher percentage of \( \text{C}_{22}\text{OH} \) which has a higher melting point). Also, the formulation \( \text{C}_{18}\text{OH}-\text{C}_{22}\text{OH} \) with a 1:3 ratio showed a depression in the PTT (41.22°C) when compared to an equivalent shampoo. It was determined that the processing conditions were different for the \( \text{C}_{22}\text{OH} \) set—in both cases a modified heating profile had been used in which the mixture had been heated to a temperature of 74°C-the melting temperature of the longest fatty alcohol- to ensure the material fully melting, yet the temperature was not high enough to fully melt the fatty alcohol resulting in portions of unhydrated or unswollen fatty alcohol in the gel network systems. In summary, a different non-equilibrium and non-homogeneous ELD was obtained by using the modified processing conditions in the longer chain length fatty alcohol formulations.
4.3 Perfume Analysis

4.3.a Basic Formulation

In order to further understand the impact of formulation changes on perfume bloom and longevity, perfume compositional analysis to quantify the partitioning of the PRMs into individual layers of shampoo neat product was performed (after centrifugation) via GC/MS. The reproducibility of the method was examined and determined to have a relative standard deviation of less than 10% (n=4). In this manner, the partitioning of the technical accord with the wide array of PRMs in the gel network and shampoo layer was studied. The data are plotted (y-axis) as a ratio of the percent composition of perfume present on the layer selected (gel network or shampoo) ratio to a 0.7% perfume matrix control (gel network or shampoo respectively). The x-axis shows the different PRMs in the technical accord. As explained in section 2.1, a PRM that prefers to partition in the gel network will have a percent composition over 100 % when compared to the specific control. Figure 4.9 shows the results of partitioning of the PRMs into the different phases (gel network and shampoo) following centrifugation.
Based on the partitioning data from Figure 4.9, there was no strong tendency for any particular group of notes (top, mid, or base) to partition into the gel network layer based on vapor pressure or relative volatility.
Subsequently, the gel network data was analyzed as a function of clogP, which is indicative of the hydrophobicity of a molecule. The results for partitioning of the PRMs into the gel network phase are shown in Figure 4.10.

Figure 4.10: PRM partitioning data for gel network phase graphed as a function of clogP

Increasing clogP of a PRM led to an increase in partitioning (uptake) into the gel network phase. As a result of these findings, hydrophobically modified accords were employed in further tests including headspace analysis and sensory evaluations. The hypothesis tested was “The more hydrophobic the PRM, the higher partitioning into the gel network”.

66
4.3.b Equilibrium Study

An equilibrium study was done to understand how our gel network system changed over time. The system was studied at 2, 8 and 17 days. The results are shown in Figure 4.11.

Figure 4.11. Partitioning of PRMs as a function of time.

Statistical analysis of the data was done using the statistical software Minitab. It was found out that the 2-day data was well correlated with the 8 and 17-day data. The 8-day data was predictive of the 17-day data, so, at a minimum, 8-days was considered the equilibrium point.
for the system (although the 2-days data was considered sufficient based on statistical analysis). The correlation coefficient $R^2$ in each case was greater than 80%.

4.3.c Formulation Variables Effect on Gel Network Partitioning-Design of Experiment

Next, the effect of the formulation variables fatty alcohol chain length, fatty alcohol ratio, and the order of addition of perfume in the shampoo was studied following the experimental design in Table 4.4. The compositional analysis results for the formulations evaluated are shown in Figure 4.12.

Figure 4.12: Perfume analysis-Effect on gel network PRM partitioning data of formulation variables such as fatty alcohol chain lengths, ratio and order of addition
It was found in most cases, that there was no statistically significant difference in PRM’s uptake for those PRMs that partitioned favorably into the shampoo layer (clogP < 3.5). A similar trend was observed for those PRMs that favored the lipid phase (clogP > 3.5)—there were variations among the different variables (order of addition, fatty alcohol chain length, fatty alcohol ratios), yet a question still remains about whether these differences will translate into a sensory difference for the consumer. The formulation most differentiated from the rest was the one having a 3:1 C\textsubscript{18}OH to C\textsubscript{22}OH fatty alcohol ratio. This formulation had on average four times as much perfume in the lipid as the other formulations. This sample was visually different than the rest following centrifugation: a grainy layer of fatty alcohol was noticeable on top of the always present gel network layer. This phenomenon was attributed to the modified processing procedure at lower temperature. We hypothesize that some of the fatty alcohol was not fully hydrated, thereby creating a non-homogenous gel network that had not achieved equilibrium state in which the PRMs resided in the unhydrated fatty alcohol layer.
Consequently, the next step was to understand whether we could repeat the unusual results obtained with the modified process by heating the system at a lower temperature for the standard period of time. Replicates were generated for the binary mixture of C$_{18}$OH/C$_{22}$OH with a 3:1 ratio. The results are shown in Figure 4.13.

Figure 4.13: Perfume analysis-Replication of unusual feature for C$_{18}$OH/C$_{22}$OH 3:1 ratio formulation.
The composition of perfume in new batches made with both the standard and modified process along with a one-week-old sample of the unusual formulation was measured. Based on the results obtained (Figure 4.13), the modified process was not reproducible. However, from a feasibility standpoint, it was demonstrated in this experiment that enhanced perfume partitioning of the PRMs using gel network technology is viable. It was evident from the data obtained with the one-week-old sample from the unusual observation that migration of the perfume raw materials from the gel network layer to the shampoo layer had occurred.
4.4 Sensory Evaluation

4.4.a Gel Network Effect on Shampoo Chassis

The olfactory response to different formulations was assessed at different time points by means of a sensory panel. The response measured was fragrance intensity with a scale from 0-6. The first factors evaluated were a) the formulation variable of adding a gel network to the shampoo and b) the order of addition of the perfume (premix and direct mix). In this experiment, the same fragrance, Shaolin®, was added at a constant level (0.7%) to different shampoo formulations having identical shampoo chassis based on coacervate technology. As mentioned, the hypothesis tested was whether a) the presence or absence of gel network for conditioning, and b) the timing of the addition point of the fragrance into the shampoo created a difference in fragrance intensity perceived. Fragrance intensity ratings at different time points for the different shampoos are shown in Figure 4.14.
Initially, both the shampoo with the premix and the shampoo without the gel network were rated significantly higher than the shampoo where the perfume had been incorporated directly with the gel network. For both the 3-hr and the 24-hr time points, the formulation’s fragrance intensity ranking was not statistically different.
After 24 hours and extra combing, both prototypes containing gel network were rated significantly higher than the shampoo without the gel network. We hypothesize that combing releases the perfume from the gel network system by mechanical force.

4.4.b Effect of PRMs Hydrophobicity, Combing and Moisture

The effect of PRMs hydrophobicity on perceived fragrance intensity was examined using the sensory panel, whom evaluated fragrance intensity as the response. Three different fragrances in order of increasing hydrophobicity (clogP) were used: Forever Barcelona ® (high), Shaolin® (average), and Fresh Floral ® (low). Forever Barcelona ® had a citrus character, Shaolin had a fruity floral character and Fresh Floral character was self-explanatory. Three shampoo formulations were made with a constant level (0.7%) of fragrance incorporated into a gel network premix (11% active [22% sodium laureth sulfate] 2.57% fragrance C18OH/C16OH 1.8:1 ratio). The treatment for the fragrances was consistent with the previous ones, however, a slight modification of the protocol was done at 25 hours—hair switches were rewet to assess how moisture release affects perceived fragrance intensity. The intensity rankings at different time points are shown in Figure 4.15.
The perfumes with low and average clogP were not statistically different from each other at any time point. However, in all cases, the hydrophobically modified accord (Forever Barcelona) showed a statistically significant improvement in fragrance intensity level over the fragrances that were not as hydrophobic. It was observed that after moisture addition, there was no statistically significant difference between Shaolin and Forever Barcelona, but both had significantly higher ratings in perfume intensity than the least lipophilic fragrance. The moisture effect was explained by water interacting with the PRMs to form new non-covalent bonds to replace the existing bonds (Dykstra, 2005). From this data, we conclude that the higher the degree of hydrophobicity of a fragrance incorporated into a gel network, the greater the perceived fragrance intensity level.
At the same time, another factor to consider is the fragrance effect—we do not know if the higher preference for a specific fragrance was due to the sensory preference of the panelist, i.e. the possibility that panelists simply preferred one fragrance to the others (due to their different character) and ranked it higher (Dalton 2004, Lawless 1999).

4.4.c Effect of Hydrophobically Modified Fragrance

The compositional analysis from section 4.2.c has shown that PRMs with a clog P greater than 3.5 partitioned favorably into the gel network phase. We next compared in a sensory panel the effect of a hydrophobically modified perfume (Forever Barcelona ®) incorporated into a shampoo with and without gel networks. Figure 4.16 shows the results for fragrance intensity as a function of hydrophobicity. Similarly to the previous section, all formulation variables were kept constant.
Initially when still wet, both shampoos had a similar sensory impact as shown in Figure 4.16. However the shampoo with gel network had a lower intensity rating at 3-hr, 24-hr and 25-hr (after combing). Based on these results, two conclusions can be drawn: either the gel network was suppressing the perfume volatility since a large amount of the hydrophobically modified accord partitions favorably into the gel network phase, or the sample without gel network retained a higher amount of fragrance. This phenomena is counterintuitive with the previous results obtained in section 4.3.
4.5   Headspace Analysis

4.5.a  Bloom-Initial Time Point

Headspace analysis is an in vitro technique to sample the air above the hair switches-simulating in laboratory settings the sensory panel environment. Headspace analysis was performed on hair switches treated with shampoo at the initial time period (0-hr). The hair switches were treated with the same protocol as those on the sensory panel to minimize variation. The results (Figure 4.17) are plotted as percent composition on the ordinate as a function of decreasing volatility of the PRMs in the abscissa.

![Graph showing Headspace comparison results on hair switches treated with shampoo-0-hr](image)

Figure 4.17: Headspace comparison results on hair switches treated with shampoo-0-hr
It was observed that the system is initially rich in the most volatile PRMs, which shows a composition greater than 100% relative to the control. This phenomenon mimics the results of the sensory data—where the top notes are the most impactful in the beginning of the diffusion process.

4.5.b Perfume Longevity-3-Hour Time Point

A similar analysis was performed at the 3-hr time point as shown in Figure 4.18.

![Figure 4.18: Headspace comparison results on hair switches treated with shampoo-3 hr](image)

At the 3-hr time period, it was observed that the headspace composition was lower in the top notes region. This data was consistent with sensory evaluations.
The relationship between partitioning of a perfumery raw material in the gel network and its chemical structure was studied and its basics elucidated. The objective was to predict in silico whether a material will partition in the gel network. First, the option of creating a model for the partitioning of a PRM to be related to some of its macroscopic physical properties such as molecular weight, clog and vapor pressure was evaluated, however, no clear definite correlation was obtained. Consequently, attention was focused on a more detailed analysis of how differences between the molecular structures of PRMs affect the observed differences in their partitioning behavior. The property modeled was the 8-day perfume equilibrium concentrations obtained from perfume compositional analysis. A set of 170 descriptors including solvent accessible surface area and volume, charged partial surface area, hydrogen bonding, molecular connectivity, molar refraction, fragment and geometric descriptors, and carbon types descriptors were computed for each PRM in the training set. Subsequent model development yielded the following equation relating the logarithm of the partitioning of the PRMs to three unique representative measures of molecular structure (descriptors):

\[
\text{Log } K (\text{GN}) = -1.31 + 0.890 \text{ FPSA-1} + 1.12 \text{ V6CH} + 0.131 \text{ NDB} \quad \text{Equation 1}
\]

The predictive equation (equation 1) was correlated with the experimental values as shown in Fig. 4.19 and a correlation coefficient $R^2$ of 0.91 was obtained. The equation was validated by the leave-one-out method and the predictive coefficient $Q^2=0.86$ was obtained.
The term FPSA-1 is the type-1 fractional positive surface area, one of the general class of descriptors called charged partial surface area or CPSA descriptors (Stanton 1990). This descriptor combines solvent–accessible surface area and atomic partial charges from the molecule while providing a measurement of polar intermolecular interactions and hydrophobic interactions.
The term V6CH, or valence corrected sixth order chain, is a molecular connectivity descriptor (Kier 1976, 1986). It accounts for molecular structure and gives information such as molecular size, molecular shape, and degree of branching. This is a valence corrected descriptor that has embedded mathematical calculation corrections for atom types and bond types.

The term NDB is a fragment descriptor. It represents the simple count of double bonds in the structure of the molecule.

Based on these descriptors, it is clear that the major influence in the partitioning coefficient is done by the main structural property relationship (SPR) of hydrophobicity of the molecular surface and rigidity of the molecule. In other words, the more hydrophobic a molecule is, and the more rigid a molecule is, the greater the degree to which it will partition into the gel network phase. This SPR trend is illustrated in Figure 4.20.

Figure 4.20: Sample PRMs classified by increasing hydrophobicity and rigidity (Courtesy of Dave Stanton-P&G Pharmaceuticals)
Based on the correlation, the more hydrophobic and the more rigid a molecule is, the higher the tendency it will have to partition in the gel network. Also, if materials have a similar hydrophobicity, the more rigid material will have a greater tendency to partition into the gel network. For example, we have four of the PRMs (heliotropine, alpha-pinene, lilial and veloutone) aligned in the order increasing hydrophobicity and rigidity in the figure below. Heliotropine is the least hydrophobic and is the one that partitions the least in the gel network structure, even though is a rigid structure. The ideal PRM would be a material such as alpha-pinene which has a hydrophobic surface area paired with a very rigid structure. Veloutone has a higher hydrophobic surface area than lilial, therefore it has a higher partition coefficient into the gel network phase. The applications of equation 1 are wide: this model can then be used to predict partitioning in the gel network of new perfume raw materials and other oil soluble actives.
1. CONCLUSIONS AND FUTURE WORK

5.1 Conclusions

The purpose of this work was to develop an improved understanding on the role of PRMs and other oil soluble actives in partitioning and thermal stability when incorporated into gel network shampoo systems.

First, the optimal process parameters to enhance perfume incorporation were studied. It was found that temperature had a direct relationship with perfume incorporation into the gel network system and was the main factor for perfume longevity while factors such as perfume incorporation heating time were secondary. From the experimental design involving different formulation variables such as fatty alcohol length and ratio were varied, it was learned that processing conditions such as energy of mixing, level of dispersion, homogeneization time, preparation time, cooling mode, and order of addition influence favorably the partitioning of the PRMs while enhancing perfume longevity as well. From our findings, we conclude that processing variables played a major role in enhancing perfume composition while formulation variables had a lesser role. An example of this phenomena was the unusual feature we saw with the modified process that increased the amount of perfume in the gel network layer, establishing the achievability of perfume bloom and longevity with this technology.

Hydrophobically-modified perfumes partitioned more favorably into the gel network systems than fragrances with lower average lipophilicity. The higher the octanol-water partition coefficient of the individual PRM, the greater the tendency to partition into the gel network and further be developed for future delivery applications.

The effect that gel network technology on shampoos had on fragrance intensity perception was studied. The results of the sensory panels raised several questions. We observed
that hydrophobically modified perfumes enhance fragrance intensity yet a point is reached at which if the hydrophobically modified fragrance is too hydrophobic, a suppression effect occurs. The implications of this research in terms of perfume design indicate that hydrophobicity is a key variable when formulating the desired fragrance. Combing and rewetting the hair were behaviors that also enhanced fragrance intensity by affecting the release of the PRMs into the air. The headspace work mirrored the sensory data.

It was elucidated from the QSAR model that hydrophobicity and rigidity are key predictive indicators of partitioning of PRMs into the gel network systems. The more hydrophobic or rigid a molecule is, it was concluded that it will partition to a favorable degree into the gel network. It is important to note that since this perfume accord was used as a marker-this knowledge can be translated to other oil soluble actives for enhanced delivery using gel network technology.
6.2 Future Work

This research has served as a proof-of-principle study to identify the main variables (hydrophobicity, rigidity of molecule, formulation variables, and process parameters) that affect perfume longevity in shampoos containing gel network technology. Further work needs to be done to elucidate the role of hydrophobically modified accords and whether a controlled release problem is being faced. Future directions are as follows:

- Conduct new studies with different process parameters such as temperature, time and order of addition to evaluate optimal incorporation conditions for fragrances into gel networks.
- Validate the partition QSAR model with a new full fragrance or a new technical accord developed based on the SPR properties learnings.
- Develop a new physical properties or QSAR-based mathematical model to predict perfume release in environment using headspace GC/MS data, and perfume deposition data obtained from new studies (Kasting 2001).
- Measure perfume deposition on hair to estimate whether partitioning into the gel network translates into greater hair deposition of perfume thus increasing perfume longevity.
- Evaluate selected prototypes in a consumer test to confirm whether the sensory results guidelines obtained translate into differences noticeable by the consumer.
2. **BIBLIOGRAPHY**


