Part 1
GENERAL TOPICS

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Lip Biophysical Properties and Characterization Methods for Long-Wear Lipsticks

Rebecca Barresi and I-Chien Liao*

L'Oreal Research and Innovation
Clark, NJ, USA

Abstract

The lips are two pliable and mobile muscular folds that surround the oral cavity and assist in a variety of functions which enable both human survival and communication. In addition to their functional purpose, the lips are frequently cosmetically enhanced, in terms of color and gloss, through the application of lipsticks. Understanding both the biophysical and surface properties of lips is crucial for the successful formulation and development of lipsticks, particularly those with long-wear or transfer-resistant claims. Various *in vitro* methods provide a clear insight as to the stability, physical properties, and visual characteristics of a lipstick formulation, but a correlation between such results and consumer use experience is still lacking.

This book chapter provides an overview of the lip anatomy and lip surface properties, which can be used to provide a general knowledge for lipstick formulation. While much is known in regards to the structure and functionality of the lip tissue, obtaining further knowledge can assist in the development of superior performing lipstick formulations. Classic *in vitro* test methods for long-wear lipsticks, in addition to such test results correlations with consumer sensory testing, are also discussed. It is important to note that although there is an abundance of *in vitro* methods available in order to characterize long-wear and transfer-resistant lipsticks, there is still a definite need to develop new methods that better correlate with consumer experience. Existing methods have the potential to be further improved with new knowledge of lip properties and use of better test substrates to illustrate the benefits of new product innovations.

**Corresponding author*: ichien.Liao@rd.loreal.com

K.L. Mittal and H.S. Bui (eds.) Surface Science and Adhesion in Cosmetics, (3–34) © 2021
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Keywords: Lip anatomy, lip biophysical properties, lip surface properties, long-wear lipstick, *in vitro* evaluation, consumer sensory testing

1.1 Introduction

Lipsticks have been an integral part of cosmetics since the dawn of civilization. The first man-made lipstick, which consisted of black kohl, was made famous during the ancient Egyptian period as part of Cleopatra's makeup routine. Lipsticks went through a period of low popularity during the European Middle Ages, but returned to glory during the reign of Queen Elizabeth I. During the Second World War, the use of lipsticks had not only made women feel more feminine, but rather red lipstick was seen as a symbol of patriotism and defiance of difficult times during the war. The basis of modern lipstick was invented by chemist Abu al-Qasim al-Zahrawi during the Islamic Golden Age and became a product of commercialization in late 19th century, thanks to industrial advancements. Given the long history of lipstick, consumers have developed clear expectations in regards to performance, appearance, and use experience [1]. The obvious immediate requirement is that the lipsticks should contain no toxic components and irritants. Exposure to potential irritants from lipstick is mainly by swallowing, such as after a consumer licks their lips. Currently, color additives must have FDA approval for its intended use, as many can contain traces of lead as an impurity. Following an investigation effort in 2007, the FDA determined that up to 10 parts per million (ppm) of lead in lipsticks would not pose a health risk.

The long-wear lipstick market can be classified into four key categories, each of which has its own benefits and appeals to consumers: long-wear, gloss, lasting lip gloss, and lip care. Long-wear lipstick, which was also advertised as transfer-resistant, was introduced in the cosmetics market by Shiseido in 1986 as a solution to problems associated with wear and movement experienced by a majority of lipstick users [2]. Functionally, lipsticks are expected to bring instant gratification in regards to the user's appearance, regardless of style and color. To accomplish this, an ideal lipstick is expected to be non-drying, provide sun protection, and have great wear, color, and shine. Wear of lipsticks shall be mentioned further throughout the chapter and can hereby be formally defined as the user's experience as a whole, consumer perception of performance and comfort, and formulation lastingness. Lipsticks should also be easy to apply on the lips and leave a thin film deposit. Early iterations of lipstick technology did not withstand the challenges associated with consumer use and wear, which,

in turn, left the consumers desiring a formulation that was able to weather their lifestyle without reduced performance or need for re-application. As a result, long-wear lipstick, substantiating claims of non-transfer, lasting color, and no smearing/smudging, was introduced to the public in the form of a two-part kit. In this kit, the first layer, a pigmented lipstick, was intended to deposit color on the lips and was to be covered by a secondary overcoat containing film-forming polymers [3]. It is important to note the key difference between lipsticks described as long-wear versus transfer-resistant. Long-wear lipstick refers to the coloring remaining the same or visually consistent from the time of application over a period of time [4]. Transfer-resistance relates to the ability of the product not to be transferred onto a secondary surface upon contact. This would relate to the formulation being removed from the consumer's lips after touching another surface, such as a glass. However, most of the time these terms are used interchangeably with consumers.

A key challenge in lipstick development is attaining an acceptable adhesion to the skin and semi-mucous areas around the lip region. After application, products can tend to migrate into cracks in the lip due to poor adhesion, creating an uneven coating and coloring over time [5]. Efforts to alleviate this problem resulted in the use of a pressure-sensitive silicone resin with MQ units (trimethyl endcap and four-way branch point units). This MQ resin, as seen in Figure 1.1, is commonly used to boost lip adherence, but the material itself is brittle, thus requiring a plasticizer in order to allow for film formation [2, 3]. This plasticizer contributes to the formation of a pliable film. Interactions with other components of the formulation, such as pigments, solvents, and fillers, also enhance product adhesion to the skin [2]. Most long-wear cosmetics utilize MQ resin technology because its surface free energy is similar to that of the human skin [2]. Skin surface free energy is a key factor for a cosmetic's wettability, adsorption, and adhesion [6]. This key characteristic is also what permits the technology to allow for better wear and adhesion to the consumer's lips. In formulations of products like Revlon ColorStay and CoverGirl, the MQ resin is used in conjunction with poly(dimethylsiloxane) (PDMS) gum to enhance the tack of the final product [2]. Additionally, initial versions of these non-transfer technologies utilized a volatile solvent with low viscosity PDMS or high viscosity gum in order to deposit a non-transferrable film upon solvent evaporation [2]. L'Oreal further developed the non-transfer MQ resin technology by introducing the use of a thermoplastic elastomer as a replacement for the PDMS gum and oil, in turn enhancing the product durability and adhesion to the lip [2]. This was incorporated into Maybelline Superstay 24 and L'Oreal Infallible lipsticks,

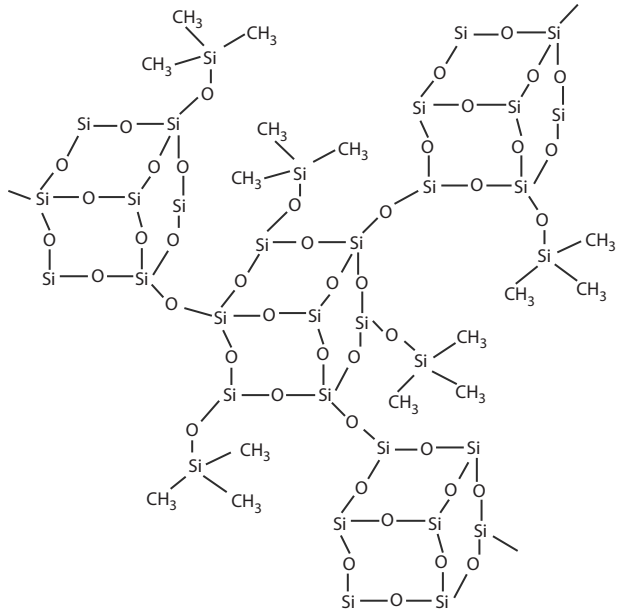


Figure 1.1 Structure of the MQ resin used to enhance formulation adhesion [2].

permitting a wider range of motion of the lips and longer contact with a secondary surface, such as a drinking glass, without sacrificing the durability and appearance of the formulation [2].

The use of the MQ resin created quite a tradeoff for formulators: while the MQ resin improves long-wear qualities, it also created issues in terms of application, feel, and shine/gloss capability [4]. The MQ resin also contributes to dryness experienced by consumers post application; this is because the silicone derivative in combination with other lipstick ingredients would result in a dry and tightening effect on the lips [7]. The enhanced adhesion to the skin is beneficial in terms of product appearance, durability and consumer experience, but time and time again consumers have criticized the long-wear formulations due to their drying tendency and discomfort. With no hydrating property, application of the formulation to the lips stratum corneum, which is also relatively low in water content, reduces consumer comfort [2]. This effect is also caused by the film itself: a continuous film applies stress on the skin's keratinous layer which, in turn, translates to the discomfort experienced by the user [8]. A secondary top layer was introduced to the two-step kit in order to help address consumer complaints in regards to durability and performance [2]. A second benefit to the two-step application was the freedom for the consumer to decide

what appearance they would like. Rather than solely having a matte option, a shiny topcoat could be used to achieve a satin or sheer effect. Although beneficial, there are limitations to this technology; in order to provide an acceptable level of shine and appearance, the topcoat may need to be re-applied throughout the course of the day and, therefore, cannot truly be classified as long-wearing [4]. At this point, consumers needed to make a tradeoff between appearance and comfort versus the need for constant re-application to achieve the desired shine. This further drove the need for a simple application method that met consumer product requirements through formulation optimization.

A second issue raised by formulations incorporating the MQ resin related to the pigment appearance. Although the resin had enhanced lipstick water repellence, the color was found to run once applied to the lips. This is because the MQ resin was soluble in the same phase as the pigments within the formulation, causing the formation of a discontinuous film that allowed the color to run due to detached pigments [3]. To alleviate this issue, the oil within the formulation was replaced with a volatile oil. Not only did this help the color remain in place, but it introduced an additional benefit of enhanced oil resistance [3]. Even with these formulation improvements, consumers still desired a simple, single-step application. Modern long-wear formulations include a complex silicone, which provides the lipstick's transfer-resistant characteristics, and a volatile oil which contributes towards ease of spreading during application and evaporates to leave behind the benefits of the silicone [3]. From this point on, continuous adjustments to the long-wear lipstick formulation have been made to enhance product performance and perception.

As the long-wear lipstick formulation was being optimized, the consumer need for a single-step application with varying gloss levels arose. The current trend leaned towards glossy, which signified a youthful and healthy appearance. In order to meet this consumer need, the "silicone plus" polymer, a product from 3M known for being a water-resistant film-former with good bioadhesion, was incorporated, which brought shine to the lipstick while also providing long-wear benefits [3]. These tweaks to the long-wear lipstick formulation enabled better performance experienced by the consumer and brought the industry to where products on the market are today. Currently, there are a number of products on the market with claims of long-wear benefits that last between eight and twenty-four hours. Some examples of such products can be seen in Figure 1.2.

Further tweaks to formulations have been made to bring about additional benefits to the consumer. Guerlain Paris, a cosmetics company, introduced a long-lasting lipstick under their KissKiss brand in 1995 that



Figure 1.2 Commercially available examples of long-wear lipsticks.

incorporated ceramides and a sun filter [9]. In doing so, the lipstick not only was long-lasting and transfer-resistant, but also introduced additional benefits of moisturizing from the ceramides and protection from the sun. After this point, companies such as Ellen Betrix and Lancôme began incorporating ceramides in their long-lasting lipstick portfolio. In theory, the ceramides help strengthen the skin and decrease the water loss experienced by the skin [9]; therefore, the lip would not be left feeling as dry, like the results of earlier formulation iterations. This was demonstrated through a clinical study evaluating the impact of different concentrations of ceramides on lipstick wear. After a thirty minute application time, it was concluded that wear improved as the amount of ceramides in the formulation increased [9].

Historically, the development of lipstick formulations has been conducted without a comprehensive understanding of the biophysical properties of the lips. Compared to the knowledge base for skin, there is limited existing literature on the properties of lips. With the development of new and sophisticated analytical tools, gaining a strong understanding of lip surface and biophysical properties can provide key information towards the future innovation of lipstick and lip care products. Towards that goal, this book chapter has two main objectives: to provide an overview of biophysical properties of the lips and to provide a review of conventional lipstick evaluation methods.

1.2 Overview of Lip Anatomy & Lip Surface Properties

1.2.1 Lip Anatomy and Biophysical Properties

The lips are two pliable and mobile muscular folds which surround the oral cavity, and act functionally to support food intake, articulation, facial

expression as well as being an important tactile organ [10–14]. The upper lip (Labium superioris) extends from the base of the nose to the nasolabial folds, while the lower lip (Labium inferioris) lies between the mouth and labiomental groove (Figure 1.3a) [15, 16]. The interface between the outer versus inner surface of the lip is defined as the vermilion border, where, histologically, the transition between the keratinized vs. the non-keratinized epithelium can be visualized. The two paramedian elevations of the vermilion of the upper lip are defined as the Cupid's Bow [15, 16]. The two raised vertical columns form the philtrum, which is a midline depression located between the paramedian elevations of the vermilion and the columella. The lips have three distinct surfaces: outer surface, edge, and inner surface. The outer surface of the lip is composed of stratified squamous keratinized epithelium (Figure 1.3b) while the inner surface of the lip is composed of stratified squamous non-keratinized epithelium. The inner surfaces are lubricated with saliva to facilitate chewing and swallowing, while the keratinized outer surface of the lip serves to protect against friction, drying, and potential microbial invasion.

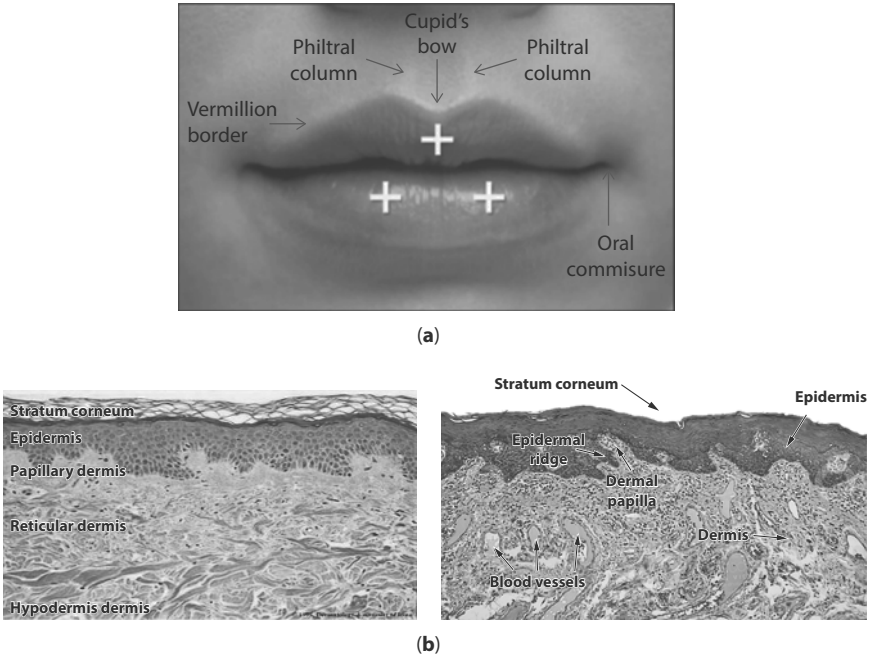


Figure 1.3 (a) The anatomical landmarks of the lips [15] (b) Histological sections of human skin (left) and lip (right) as stained by H&E [66].

The tissue structure of lip skin is illustrated in Figure 1.3b. The tissue of lip skin is made of epidermis, subcutaneous tissue, orbicularis oris muscle, and mucosa [17]. Compared to the human skin (Figure 1.3b, left), the keratinized epithelium of the lips is much thinner (3-5 cell layers averaging to only a few micrometers in thickness in the lip versus 16-20 cell layers averaging to 10-20 micrometers for the skin). In terms of the extracellular matrix content, both the skin and the lips contain similar levels of epithelial glycogen, elastin and collagen [17]. The lips, however, are richer in blood vessel networks, contain many sensory nerve endings from the mandibular and maxillary nerves and show absence of hair, sebaceous glands, and sweat glands [10]. These histological features of the lips provide the unique tissue properties that can be perceived. The thin keratinized epithelium is part of the reason why the lips are more susceptible to drying and chapping, as the reduced barrier layer makes the lips more susceptible to water loss. The rich vascular network, combined with the thinness of epidermis and the lack of pigments, is what makes the lips appear red. The lips receive blood supply from external carotid arteries [10, 18, 19]. The facial artery branches into an inferior and a superior labial artery, which courses beneath the orbicularis oris muscle. The superficial artery reach the skin of the upper lips and form a vascular plexus [18]. The lower lip receives blood supply from three labial arteries: the inferior labial artery, horizontal and vertical labiomental arteries [18]. Under extremely cold temperatures, the blood vessels in the lips will undergo vasoconstriction as well as having reduced level of oxygen, thus turning the lips purple. Similarly, the sensory nerve endings in the lips can make the lips sensitive to touch and temperature. The upper lip receives sensory innervations from the infraorbital nerve, while the lower lips receive its sensory innervation from the mandibular nerves. The buccal nerve, a sensory branch of the mandibular nerve, provides innervation to the entire cheek mucosa and skin around the mouth [20]. In the context of makeup, the sensitive lip surface is what allows the consumers to perceive tightness and drying after the application of lipsticks.

A 2,023-subject study on the soft tissue 3D landmarks around the lips was conducted using a Three-Dimensional Facial Morphometry system and provided valuable insight into the development process of the lips [21]. This study investigated the differences in lip dimensions (linear distance and ratio as well as area and volume of vermilion) as a function of gender and aging. Their measurements, taken from subjects aging from 6-7 years old all the way through adulthood, illustrated that male subjects have on average higher lip volume, greater vermilion area, and greater lip heights thorough all ages [21]. Linear distance in girls has similar dimension as

measured in adults, while in the boys in the 13-14 year old age group reported a period where a large year to year increase was observed [21].

The motions of the lips are controlled by the orbicularis oris muscle, which subdivides into pars marginalis and pars peripheralis [10, 11, 13, 16, 17] The pars marginalis consists of the muscle fiber group lodged within the vermilion zone of the lips, while the pars peripheralis is located in the cutaneous lip. When the mouth is closed, the orbicularis oris is tonically contracted; while in motions such as kissing and whistling, the orbicularis oris muscle undergoes active contraction. During chewing, four other muscle groups (masseter, temporalis, medial pterygoid and lateral pterygoid) are responsible for the adduction of the jaw. Many of the biophysical properties of the lips have been reported by Fogel and Stranc in a 500-subject study representing different genders and age groups [22]. In this study, the lips of the subjects were evaluated for intercommissural distance, perioral tissue strength & elasticity and lip sensitivity. Logically, the age and gender of the subjects have profound effects on the biophysical properties of lips. The soft tissue gape and oral aperture of the lips generally peak in the age group of 16-30 and have consistent reducing trends in older age groups [22]. In 2011, Sjogreen *et al.* used 3D imaging systems to capture the mobility of the lips for their subject at rest and during open mouth smile and lip puckering [23]. Not surprisingly, the lips are subjected to very large range of mechanical motions. During open mouth smile, the lips experience 34% extension over rest, while during lip puckering the lips experience 37% contraction over rest. Using a pommeter, Fogel and Stranc were able to measure the strength of the orbicularis oris muscle of the subjects and reported higher strength values in age groups ranging from 16-30 to 46-60, while subjects of advanced age (61-75) had much reduced value of muscle strength [22]. The mechanical properties of the lips were measured using a device called LASTIC (Light Aspiration device for *in vivo* Soft TIssue Characterization) which functioned very similarly to a cutometer [24]. In this study, Luboz *et al.* reported that the measured Young's modulus of the lips ranged from 18 to 46 kPa [24]. The measured Young's modulus values of the lips are on par with the measured value of skin from the cheek, cheekbone and forehead [24]. Such biophysical understanding of the lip motion can be very important information for designing lipstick products that have ideal comfort and durability.

1.2.2 Surface Properties of the Lips

In order to understand the surface properties of the lips, it is essential to understand the process of barrier assembly in the keratinized epithelium. This process is controlled by the differentiating epithelial cells in a tightly

regulated manner in order to form a functional physical barrier and to maintain skin and lip epithelium [25, 26]. In the process of barrier formation, epidermal cells accumulate lamellar bodies containing keratins, ω-hydroxy-ceramides, free fatty acids, cholesterol, and other barrier lipids. At a late stage of differentiation, these organized extracellular lipids assemble into orderly lamellae forming what is defined as cornified cell envelope. Cornified envelope consists mostly of loricin (85%), small proline rich (SPR) protein, involucrin, keratins, desmoplakin, and filaggrin [25]. Keratins are crosslinked to cornified envelope at the final stage in the lipid lamellae, which has the vital mechanical and water permeability barrier functions for skin homeostasis. Since keratins are the main cytoskeletal component of keratinized epithelium, the differences in the types of keratins found in the keratinized vs. non-keratinized epithelium also suggest significant functional differences (Figure 1.4). In the keratinized epithelium layers, keratins K5 and K14 are expressed in the basal layer, while K19 is expressed in the non-keratinized epithelium [27]. In the differentiating suprabasal layers, the keratinized epithelium expresses keratins K1 and K10, while K4 and K13 are expressed mostly in non-keratinized epithelium. In K1 and K10 deficient mice, the missing keratins in the cornified envelope maturation process induced significant inflammation, hyperkeratosis, and skin fragility, thus suggesting the functional need for these keratins in skin homeostasis [28, 29].

Using a double immunostaining technique with Nile Red and anti-Involucrin, Hirao and coworkers were able to shed light into the maturation level of cornified envelopes at various locations on the body, including

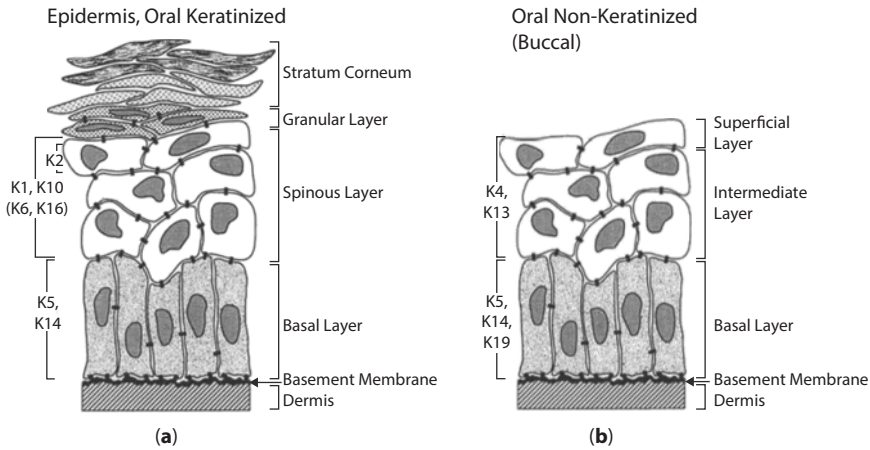


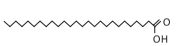
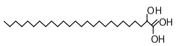
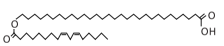
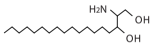
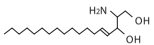
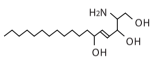
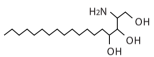
Figure 1.4 Keratin assembly in the different layers of keratinized vs. non-keratinized oral epidermis [27].

the lips [30, 31]. This method utilized stratum corneum obtained by tape stripping, followed by double labeling with Nile Red and anti-Involucrin. The immature cornified envelope was double labeled with the hydrophobic Nile Red dye and anti-Involucrin, while the mature cornified envelope was labeled with Nile Red only. In this work, the maturation process of cornified envelope was illustrated by sequential tape stripping, where they illustrated both the presence of immature cornified envelope on the 25th tape strip and the transition towards more mature cornified envelope in the first tape strip [30]. They went on studying different locations of the body to demonstrate potential regional differences in the cornified envelopes. Immature cornified envelopes were discovered at the forehead and lips, but not in the trunk and extremities [30]. Hirao *et al.* hypothesized that the cornified envelope maturity is correlated with the barrier function, where locations (such as lips) with more immature cornified envelope also have higher trans-epidermal water loss [30].

The difference in functional properties between the lips and the cheeks was illustrated by Kobayashi and Tagami where they measured transepidermal water loss (TEWL), conductance, size of corneocytes, blood flow and surface skin temperature in over three hundred volunteers [32]. They reported significantly higher TEWL (33 vs. 11 $g/m^2/h$), lower conductance (61 vs. 163 μS), larger and more parakeratotic corneocytes (824 vs. 776 μm^2), higher blood flow (16 vs. 7 mL/min*g) and higher surface temperature (33 vs. 31 °C) on lips vs. on cheeks. The reported values not only verified the relationship of the poor barrier function of the lips with the physiological makeup of the lip tissue, but it also illustrated the need for lip moisturizing products, especially in dry and cold environments.

The ceramide profile can be used to shed light on the relationship of barrier function and surface properties for both the skin and lips. Ceramides, which consist of 30-40% of stratum corneum lipids by weight, have an integral role in barrier homeostasis [33, 34]. To date, there are over 300 ceramide species that have been identified on the human stratum corneum, and they can be loosely classified into 12 groups based on their hydroxylation patterns in the sphingoid bases and fatty acid moieties [34–37]. There are 4 main classes of sphingoid bases: Dihydrosphingosine (dS), sphingosine (S), 6-hydroxy sphingosine (H) and phytosphingosine (P) [33, 34]. In terms of the fatty acid moieties, there are 3 general classes: non-hydroxy fatty acid (N), α-hydroxy fatty acid (A) and esterified ω-hydroxy fatty acid (EO) [33]. These fatty acid moieties and sphingoid bases combine to make up the different ceramide classifications such as CER [NP], CER [EOS] and CER [NS]. The different ceramides containing different sphingoid bases and fatty acid moieties are generalized in Table 1.1.

Table 1.1 Structure and nomenclature of human stratum corneum ceramides [33].

Fatty acid / Sphingoid	**Non-hydroxy fatty acid [N]** 	**α-hydroxy fatty acid [A]** 	**Esterified ω-hydroxy fatty acid [EO]** 
Dihydrosphingosine [DS] 	CER [NDS]	CER [ADS]	CER [EODS]
Sphingosine [S] 	CER [NS]	CER [AS]	CER [EOS]
6-Hydroxysphingosine [H] 	CER [NH]	CER [AH]	CER [EOH]
Phytosphingosine [P] 	CER [NP]	CER [AP]	CER [EOP]

There are 4 main classes of sphingoid bases: Dihydrosphingosine (DS), sphingosine (S), 6-hydroxy sphingosine (H) and phytosphingosine (P) [33, 34]. In terms of the fatty acid moieties, there are 3 general classes: non-hydroxy fatty acid (N), α-hydroxy fatty acid (A) and esterified ω-hydroxy fatty acid (EO) [33]. These fatty acid moieties and sphingoid bases combine to make up the different ceramide classifications such as CER [NP], CER [EOS] and CER [NS].

To illustrate the body regional difference and seasonal impact on the ceramide profile, Ishikawa *et al.* conducted a one-year study on ten Japanese subjects with normal skin. In this study, their research team measured TEWL and capacitance, as well as taking stratum corneum samples to quantify the different ceramide groups from 10 different locations of the body [33]. Their findings suggested that lips, palm and fingers have least amounts of measurable total ceramides, while forehead, lower legs, upper arms, and buttocks have the most [33]. Lips, along with the palm, fingers, and cheeks showed lower ratio of CER[NP], CER[EOS], CER[EOH] and CER[EOP] and a higher ratio of CER[NS] and CER[AS] [33]. Furthermore, amongst all body locations, lips were found to have the lowest carbon numbers in CER[NS] and the highest level in C34-CER[NS] [33]. These findings are very interesting, as ceramides with a shorter free fatty acid chain length and an increase in C34 ceramide and unsaturated fatty acids have been identified in the stratum corneum of atopic dermatitis patients [38, 39]. Similarly to the findings by Kobayashi *et al.*, Ishikawa *et al.* also found lips as one of the anatomic regions with the highest TEWL values while having relatively average capacitance values [32, 33]. When examining the correlation between TEWL and the ceramides from different body locations, Ishikawa *et al.* observed an inverse relationship between TEWL and CER[NS], CER[NP] and CER[NDS] [33]. Conversely, a positive relationship between TEWL and CER[AS] was found [33]. Interestingly, seasonal variations have very little impact on the TEWL, total ceramide and CER composition on the lips; however, an increase in capacitance value was shown in the summer from the spring [33]. This work was followed by another publication in 2016, which studied the relationship between lip roughness and ceramide profile in an attempt to clarify the biological contributing factors to lip chapping (Figure 1.5) [40]. In the work conducted by Tamura *et al.*, CER content, TEWL, and capacitance were measured from forty-one Japanese subjects with different classifications of lip roughness [40]. In their work, an inverse correlation between lip roughness and capacitance was found, while TEWL showed no significant trend between the different lip roughness scores [40]. Lip roughness also correlated negatively, albeit with relatively low correlation coefficients, with the level and average carbon number of CER[NH] and CER[NP] [40]. Hikima *et al.* investigated the lip surface corneocytes and desquamation regulating proteinase of normal and chapped lips and found that reduced levels of cathepsin D activity tended to be a factor for chapped lips [41]. In this work, apricot extracts were applied topically to chapped lips, resulting in a decerease in lip roughness accompanied by an increase of cathepsin D activity [41]. The effect of season was investigated by Gubanova and Caisey



Figure 1.5 Lip roughness score [40].

where they followed the lip surface properties of 20 women in Moscow between April and September [16]. In the study, lip surface properties were evaluated by examining four key criteria: dryness, cracking, scaling, and pleating. Not surprisingly, dryness, cracking and scaling were found to be more significant in April and May compared to July and August, while pleating was less visible in July than in September [16]. In a separate study, Gubanova and Caisey investigated the effects of lip balm application using the same criteria for a four-week study during December in Irkutsk [16]. The application of lip balm showed significant improvement in the drying, scaling, and pleating scores of the lips over the four-week period [16].

Thus far, this book chapter has provided an overview of the lip anatomy and the surface properties of lip skin. Similarly to the skin, with age there are dynamic changes that can occur to the lips as part of the multifactorial aging process of the face. The topic of face aging is well documented [42–49] and is not the focus of this book chapter; however, it is important to understand the process of lip aging in the context of facial aging. Around the nasolabial and labiomental crease areas, aging subjects experience subcutaneous fat atrophy, changes in bone support in the pyriform area, significant loss of collagen and elastin fibers, and changes to the platysma and orbicularis oris muscle [16, 46, 49, 50]. These changes in the tissue structure contribute significantly to the development of bitterness fold (lines from the corner of the mouth down to the chin), sagging jowls, shriveled skin, and double chin around the chin and neck areas [49]. Changes to the lip volume and thickness have been elucidated by a number of research teams, but most impressively by Ramaut *et al*. where they conducted magnetic resonance imaging on the upper lips of 200 Caucasian subjects [51]. In their study, they segregated the population by gender (100 men and 100 women) and by age (20-30 and 65-80 years old) and evaluated for lip length, lip thickness, nasolabial tissue thickness and volume [51]. The older population reported to have significantly greater lip length, thinner lip thickness and lesser lip volume [51]. As for the comparison between genders, the female population had thinner lips

and lesser lip volume compared to the males amongst the younger population; however, this difference was not as obvious in the older population [51]. Injection of hyaluronic acid based dermal fillers, botulinum toxin, fractional laser resurfacing and autologous fat transfer are amongst the most popular methods to reverse the signs of aging [52, 53]. However, these dermatological procedures still pose varying health risk to aging consumers. This opens an important opportunity for the cosmetics industry to offer an alternative solution to aging consumers or consumers seeking lip plumping innovations.

1.3 *In Vitro* Evaluation Methods for Lipsticks

Throughout the development of a lipstick product, *in vitro* performance analysis is conducted in order to develop and optimize the best-performing product to be launched in market. Typically, both standardized and product-specific characterization tests are conducted in order to properly rank formulations and identify product weaknesses. Formulation characterization tests can be conducted by applying the formulation onto lips, skin, artificial skin, or glass, depending on the test method being used. This section shall provide a brief overview as to the types of tests available and used in the development of lipstick formulations, more specifically for long-wear lipstick formulations.

1.3.1 Stability Testing of Lipstick Formulations

It is important to note that the priority in lipstick development is product stability. Not only does stability impact product performance, but it can also directly influence consumer perception of the brand. Stability testing is typically conducted for different time periods at varying temperatures at which samples are observed. Common observations may include lipstick sweating, in which the oil within the formulation forms visible droplets on the exterior of the formulation, in addition to discoloration, crystallization, separation, or cracking. A Design of Experiment can be utilized during lipstick formulation stages in order to identify the best optimized formulation that passes stability testing. Currently, there is no *in vitro* method to predict a formulation performance in regards to stability. Therefore, utilizing a Design of Experiment is the best way to identify the optimal formulation within a particular batch.

Stability testing is conducted by placing formulation samples in a controlled environment for a specified period of time in order to relate to shelf-life and consumer environments in which the finished product may

be stored. This indicates what effects different temperatures and humidity levels might have on the product following a certain time frame. Initial stability tests are carried out in a 25°C chamber and samples are observed for signs of degradation or separation at particular time points [54]. According to a patent issued to L'Oreal, inspections for this initial stability testing are done at 24 hours, 3 days, 1 week, 2 weeks, 4 weeks, and 8 weeks, and the sample is observed for phase separation, bending, sweating, and other physical conditions [54]. Once a formulation passes this stage, a second stability test is conducted for 8 weeks in varying temperature chambers in addition to a freeze-thaw condition. Such temperatures disclosed in a patent issued to L'Oreal include 37°C, 40°C, 45°C, and 50°C [54].

Stability testing of long-wear lipstick formulations is especially important due its volatile solvent component. Isododecane, which has a flashpoint of 45°C, constitutes nearly half of the volatile solvents in both the liquid and stick forms of long-wear lipstick. Therefore, the stability readout at 45°C is crucial in identifying and optimizing potential formulations. In the liquid format, should the volatile solvent flash off and fail stability, there would be an increase in viscosity; meanwhile, the stick would become dry. The packaging design and seal of such formulations is also tested through stability to help identify any loss of volatile solvent over time. Should a formulation pass these stability tests, further formulation characterization will be conducted through the use of *in vitro* test methods and consumer studies.

1.3.2 Physical Properties of Lipstick Formulations

1.3.2.1 Lipstick Hardness and Deposition

Lipstick hardness helps provide some insight as to the stability of lipstick for throughout the product lifecycle, from manufacturing to transportation, use and eventual end of use [55]. Hardness can be measured according to two common ASTM methods: ASTM D-1321 and ASTM D-937. These methods utilize needle penetration and cone penetration, respectively, in order to calculate formulation firmness in a quantifiable measure [56, 57]. Previous studies have indicated the use of a texture analyzer using a 500N load cell, needle penetration of 3.6 mm, velocity of 1 mm s^{-1}, and a trigger force of 0.05N [58]. Although this method is able to provide some quantitative data, it lacks in its relation to product performance when in use by consumers. Human panels are typically used in order to subjectively assess the residual left on the lips and product feel after application. Because this value is subjective, it is subject to change and result in reproducibility

issues, making it even more difficult to correlate lipstick hardness with customer satisfaction and performance. In efforts to alleviate this issue, a method and apparatus has been developed to better correlate hardness and deposition to user experience. This method studies different deposition rates of semisolid lipsticks when applied to a substrate under controlled conditions and relates the drag and deposition to consumer experience [59]. It is claimed that the results from this method relate to the consumers 'mouth feel' through the relation of consumer preference in terms of lipstick coverage with the deposition accomplished by the invented apparatus [59]. As a result, this creates an *in vitro* method which better correlates with results from human panels.

Hardness testing is only conducted for formulations that are in the stick format. In long-wear lipstick formulations, the isododecane acts as a good solvent for polyethylene wax; this wax is used to help soften the stick and is difficult to crystallize. These long-wear lipstick sticks, such as Revlon ColorStay and Maybelline SuperStay Ink Crayon Lipstick, are made slim to keep the stick softer without compromising ease of application and preventing breakage of the stick. Should a stick be wide and kept soft, it would not be able to support the stress of application and would be easy to break. Additionally, sticks with a larger diameter enable more evaporation of the volatile solvent due to its larger surface area, making the formulation tacky. In the hardness testing described above, a slim stick should have a readout of 70-100 gForce hardness, making it ideal to deposit a coating onto the lips that is not too thick.

1.3.2.2 Lipstick Wear Assays

1.3.2.2.1 Adsorption by Contact Angle Measurements

Adsorption testing of lipsticks provides an understanding of how oil and saliva adsorb onto the lipstick film over a period of time by looking at the spreading and wetting on the formulation sample. Such testing requires the use of a tensiometer, which is a machine used to measure the surface tension of liquids by dropping the liquid of interest (in this case olive oil or saliva) onto the film surface in the form of a sessile drop. This provides us with an idea of how resistant to oil or liquid the formulation may be; if there is a low value for the contact angle (Θ), the film surface is considered to be highly wetting. When the drop placed is water and the formulation has a contact angle greater than 90°, it is hydrophobic; a value less than 90° indicates the formulation has an affinity with water and can be characterized as hydrophilic. A contact angle less than 90° when testing with oil indicates the formulation is lipophilic and has an affinity with silicone.

1.3.2.2.2 Rub Test with Oil and Water

This test method is commonly used in order to determine a lipstick's durability in regards to oils and water. Should a consumer eat greasy foods, the lipstick will become tacky or removed from the lips. In this case, the oil in the greasy food essentially acts as a make-up remover and breaks down the formulation. While resistance to grease is improved through the use of the MQ resin, products on the market today are not yet producing satisfactory outcomes when interacting with such oils. *In vitro* tests can be used to help predict the formulation resistance to such oils. Lipstick formulation is drawn down with 25 micrometer draw down bar on BIOSKIN, which is a urethane elastomer that is made to resemble human skin[60]. This substrate is commonly used to evaluate cosmetics due to its similarity, in terms of resiliency and feel, to human skin. The drawn down sample is left to dry at 37°C and 35%RH. In order to test oil resistance and waterproof qualities, a droplet with a controlled quantity of oil and water is then placed on the sample for ten minutes; after this point, the sample is manually wiped with a Kimwipe fifteen times, and a visual inspection is made in regards to the quality of the sample after wiping. The formulation passes the assay if there is no visual removal of the formulation after wiping with the Kimwipe. Figure 1.6 (left) shows what is considered to be a good and a bad sample after the test is completed. This method correlates to the challenges associated with eating and drinking in regards to lipstick durability. Long-wear lipstick formulations should have no removal after being wiped as no formulation should transfer.

1.3.2.2.3 Adhesion with Scotch® Tape

To determine the adhesion property of the formulation, the wear assay can also be completed with a Scotch tape, in which the tape is placed on

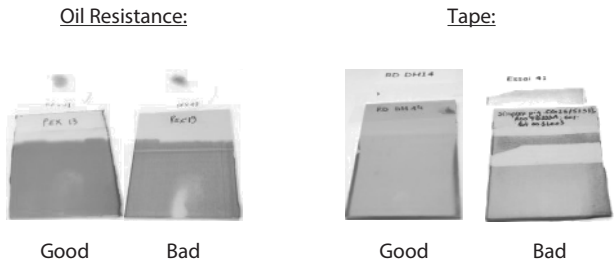


Figure 1.6 Wear assay results indicating satisfactory (left) and unsatisfactory (right) resistance to oil and formulation adhesion (tape test).

top of the sample and removed at a 90° angle. A similar visual assessment is made to analyze the quality of the sample. These visual inspections are then translated to a ranked scale. Figure 1.6 (right) shows what is considered to be a good and a bad sample after the test is completed. The long-wear lipstick formula would ideally have a higher adhesion to the substrate rather than to the tape, preventing any formulation from being removed.

1.3.2.2.4 Adhesion Testing

Adhesion testing measures the adherence quality to a specific substrate after formulation application. This indicates how well a formulation will remain on a consumer's lips over a period of time. The formulation is applied onto the BIOSKIN surface and left to dry. ASTM crosshatch tape is applied onto the film surface and removed at a 180° angle [54]. The amount of film removed with the tape is rated on a scale from 1-3, where 1 indicates no removal, 2 is some removal, and 3 is severe removal [54]. This scale relates back to how formulations are rated in consumer sensory testing. There are other means to measure the film adhesion; rather than using the ASTM crosshatch tape, two pieces of BIOSKIN can be used in contact with the formulation located in between. One piece of BIOSKIN can be peeled off either manually and the adhesion is evaluated by the ratings described above. Alternatively, a texture analyzer can be used to peel apart the BIOSKIN in order to quantify the force needed for removal.

1.3.2.3 Lipstick Cohesion Test

This method utilizes lipstick formulation drawn down on the BIOSKIN substrate using a 25μm or 75μm drawdown bar. The sample dries for about one to two hours at 37°C and 35%RH. Once dry, the BIOSKIN substrate is stretched twice manually in both the X and Y directions. A visual assessment of the sample for cracking is made.

Figure 1.7 demonstrates what would classify as performing well with the stretch test (left), as compared to that which performed poorly (right). This test is used to mimic consumer experience through the movement of their lips to determine if the deposited film would result in cracks and other undesirable visual effects. Such movements may include talking, yawning, and smiling which could result in the lipstick wearing off. Cracking is rated on a scale from 1 to 5 depending on the severity of crack formations that were observed.

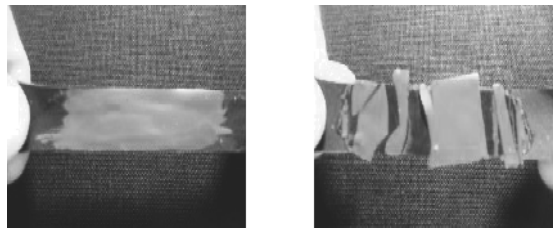


Figure 1.7 Lipstick stretch test results indicating no cracking (left) and severe cracking (right) after manual stretching.

1.3.2.4 Tack Testing

Tack is used to identify the quality of the formulation after application to a substrate and relates to what the consumer may feel in regards to stickiness either after application or after eating oily foods that may cause the formulation to degrade, in turn causing the formulation to run off or have a tacky feel. This method uses the Texture Analyzer and calculates the removal force of a 1/2" stainless steel ball probe after contact with the dried formulation [54]. The maximum force detected is considered to be the tack. This measurement is conducted five times on a sample and averaged in order to find the average tack force for the sample. This test can also be conducted after exposure to olive oil and saliva to determine the impact on the formulation, relating to product performance after eating or after the consumer licks her lips. Long-wear lipsticks that have a wide stick have a higher tack force as a result of higher evaporation of the volatile solvent.

1.3.2.5 Thermal Analysis of Lipsticks

Differential Scanning Calorimetry (DSC) measures the melting of the fats and oils in the sample to provide an idea of product performance [61]. This technique, referred to as fingerprinting, provides information regarding product wear and performance as a direct relation to peak shape, size, and temperature after being run on a single furnace, heat flux DSC [61]. We can look at the signature peaks from the DSC readout to help characterize the type of wax and expected consumer sensory. This method is ideal for long-wear sticks as the isododecane helps keep the stick soft and acts as a solvent for the different waxes in the formulation. Typically, liquid format long-wear lipsticks are not preferred for this method as there is a very small amount of wax in the formulation, making it hard to fingerprint.

A sample profile can be seen in Figure 1.8. In this experiment, 30mg of samples for lipstick formulations A, B, C, and D were placed in hermetically sealed aluminum pans and were ramped from -100°C to 100°C at a rate of 2°C/minute to obtain the full DSC profile of the sample [61]. The figure demonstrates that there are melting peaks between 25°C to 50°C, indicating that the samples will spread on warm surfaces, such as the lips [61]. The location of the peaks can help to identify the type of wax used in the formulation; for example, in Formulation D in Figure 1.8, we can conclude that different waxes that have a higher melting temperature and must be harder compared to Formulations A-C due to the location and intensity of the peaks. Peaks located at higher temperature indicate that the formulation may be more difficult to apply to the lip due to its lower temperature, but once applied will stay longer [61].

This provides further insight into the application process experienced by consumers. Softer formulations will melt on the lip during application but will still be hard enough to resist snapping when used; this provides a positive sensory experience for the consumer.

1.3.3 Visual Properties of Lipstick Formulations

1.3.3.1 Transfer Resistance Test

The transfer resistance test, also known as the "kiss" test, is a commonly used evaluation method in order to determine the transfer of product from the lip after lipstick application. This provides an indication as to what may be experienced by consumers in terms of transferring color to undesired places such as glassware, clothing, silverware, or other lips [3]. Not only

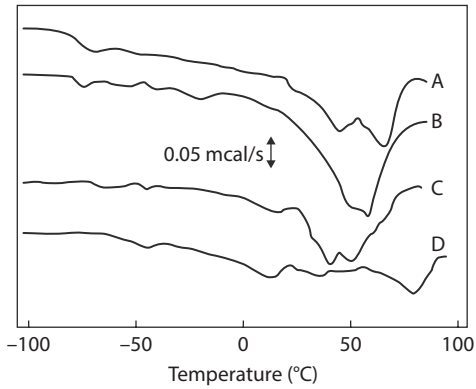


Figure 1.8 DSC profiles of lipstick formulation samples A-D [61].

does this issue result in color appearing in unwanted locations, but it also ruins the appearance of the consumer's lips, resulting in an uneven coating of the lipstick. This method is an effective way to rank the transfer-resistant ability of long-wear lipsticks.

The formulation is applied to a keratinous material, such as the human lips, and left to dry for about fifteen minutes. After this time, another substrate such as skin, artificial skin, lips, or glass is kissed. The amount of formulation transferred to the substrate is reviewed and ranked on a scale from little or no transfer to complete transfer. Should the formulation be transfer-resistant, then a majority will remain on the lips [4].

Figure 1.9 demonstrates the results from the kiss test by a sensory panel. In this study, lipstick was applied to a consumer panel in order to confirm the lastingness of the lipstick being investigated; after drying, four prints were made on a piece of white paper [58]. As seen in Figure 1.9, Formulation A had a much larger transfer amount compared to Formulations B and C; therefore, one could conclude that Formulation A is not as transfer-resistant.

Such studies help identify key differences between formulations and the impact these components have on durability, transfer, and lastingness. For example, the study above indicates the impact that the wax, butter, and copolymer type have on performance [58]. Not only does this information help formulators further optimize their compositions, but it also helps in regards to comparisons with competitor products. This method is a relatively quick and efficient way to help identify transfer in ideal conditions, without the influence of water or oil.

1.3.3.2 Gloss Measurement

Currently, most single-step long-wear lipsticks have a matte finish simply due to formulation components, but fashion and beauty trends may result in the consumer desiring other options for lipstick appearance; for example, some view glossy lips as an indication of health or youthfulness. During the formulation process, the level of gloss can be measured using a glossmeter, which measures the reflection from a surface by shooting a beam of light (the incident light) at a specified angle. The reflected light is then measured and the ratio of reflected light to incident light is taken as a measure of gloss.

In using this meter, the formulation is spread between fifty to five hundred micrometers in thickness on either a Leneta contrast card or BYK Opacity Chart and left to dry for twenty-four hours at room temperature [4]. Once dry, the sample is measured at 60° on a white background using

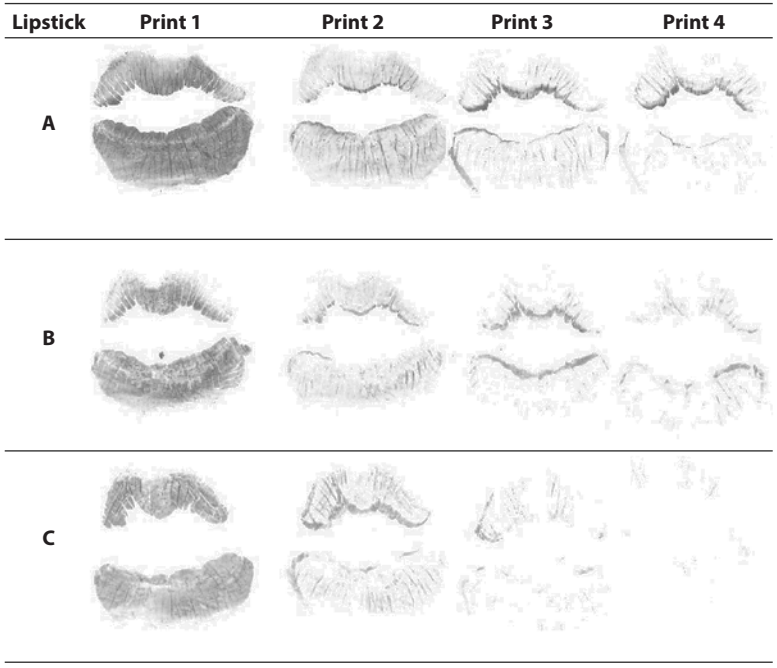


Figure 1.9 Comparison of 3 lipsticks demonstrating high formulation transfer (A) and low formulation transfer (C). Each panelist kissed the white paper four consecutive times without reapplication, as seen through prints 1-4 [58].

a BYK Gardner glossmeter [4]. This measurement is conducted three times and averaged. In the event that the measured value obtained at 60° is too large, therefore meaning the sample is very glossy, the angle is adjusted to 20° in order to allow for an accurate reading. This analysis can also be completed after the application of olive oil or saliva to determine the impact on formulation appearance.

1.4 Relation of *In Vitro* Analysis with Consumer Sensory Testing

Sensory tests are commonly used in a wide variety of fields, especially in the cosmetics field, in order to gain a better understanding as to the product's performance and perception made by the consumer. In doing so, the product under development can be further improved and optimized whilst

being compared to similar competitor products. Sensory testing can be conducted in three different methods: discriminative, affective, or descriptive [58]. Discriminative testing is typically used to screen for a detectable difference between two products, whether of the same brand or that of a competitor. Affective testing determines the consumer's acceptance or preference towards a particular product. Descriptive testing provides more detail in regards to characteristics of a product. While each of these methods provides useful information to formulators, each is restricted in terms of information provided or ease of analysis.

Currently, there is limited information available relating data obtained from the *in vitro* characterization of lipsticks directly to that observed during consumer sensory tests. Typically, products are evaluated and then passed on for consumer testing. These tests aim to target specific characteristics of the formulation which may correlate with quantitative measurements made in a laboratory setting. While this is useful for relating the quantitative characteristics described by consumers, it does not quite indicate if the product will perform well in terms of the consumer actually liking the product. Consumer sensory tests are also restricted in the sense that only three to four formulations can be tested at a time due to the limited availability of panelists, time restrictions, and cost; therefore, it is imperative that the best rated lipsticks in laboratory screenings are selected.

The sensory evaluation is typically conducted in a controlled environment, where the temperature, humidity, and lighting can be regulated. Panels may be untrained or trained, depending on the scope of the study being carried out. A reliable sensory panel is necessary when investigating in order to allow for study reproducibility and consistency. Typically, this can be achieved by establishing an agreement between panelists regarding terms, definitions, and characteristic descriptions prior to product use; this allows for members of the panel to be consistent with their grading and ensures that each panel is analyzing fairly. A new approach for enhancing panel reliability has been described by Latreille *et al.;* this utilizes a mixed linear model which tests the panel's reliability through various criteria [62]. This model measures both an individual's reliability in addition to the group as a whole by looking at discrimination ability, repeatability, and agreement/disagreement using contrasts [62]. Though this method provides an accurate representation as to the panel's reliability and reproducibility, it is restricted in the sense that it only works well for small panel groups with few products to evaluate; this is because as more products are added, results become much more complex [62]. Larger panels with more products are recommended to use other means to evaluate panel

performance in relation to consistency of descriptors between products [62]. In short, it is imperative to be able to obtain repeatable and dependable results from such *in vitro* studies, as these help shape the evolution of a product's formulation. These results also help scientists in translating findings from *in vitro* tests to product performance in panels, thus making it even more imperative that the findings are accurate.

In order to properly assign scores to lipstick products, studies typically include multiple sessions that help users precisely define key characteristics and develop sensory language to prevent any misinterpretations. For example, a study conducted by Yap and Aminah conducted five separate sessions with the panelists; these are shown in Table 1.2 [63].

These panels are typically controlled in a few different aspects, the first being the environment in which the test is being conducted. Secondly, the desired key outcomes or findings are used to shape panelist questions and discussion points. For example, a panel of 50 lipstick users was used to compare transfer-resistant lipstick formulations with that of the user's regular lipstick through two different tests [64]. The first test evaluated the transfer of the lipstick 30 minutes after application; panelists patted their lips with a white cloth and were asked if an acceptable amount was removed from the process, where 98% said yes [64]. Panelists were also asked if the amount was significant (to which 94% stated insignificant) and asked to compare to regular lipsticks (to which 92% stated the amount was less) [64]. The second test followed the same suit as the prior one, but after the 30-minute drying period, panelists were asked to drink water. 100% stated an acceptable amount stayed on the lips, where 98% said the amount removed was insignificant and 96% claimed the amount removed was less than regular lipstick [64].

Table 1.2 Summary of session objectives in sensory panels [63].

Session	Objective
Session 1	Development of a list of characteristics based on personal perception of the product.
Session 2	Defining key terms that translate to sensory attributes.
Session 3	Development and use of scoring system for formulations.
Session 4	Repeat session 3 for consistency.
Session 5	

A study conducted by Rafferty *et al*. investigated the ability to predict lipstick sensory products through the use of *in vitro* testing. Here, 12 different lipstick formulations were studied in regards to sensory perceptions (spreadability, stickiness, opacity, and gloss) with a panel of nine users and in regards to *in vitro* performance parameters (friction test, application test, tack test, and color development retention test) [65]. From statistical modelling, it was found that the *in vitro* analysis correlated well with findings from the sensory panel; more specifically, results from the friction test alone were relatable to sensory panel findings in regards to spreadability, stickiness, opacity, and gloss [65]. This is because the friction tests were able to separate the products as creamy, waxy, oily, or sticky from which then explained the adhesion and cohesive properties of each lipstick sample [65].

Ultimately, there is a tradeoff that needs to be made by consumers in regards to achieving the 'perfect' long-wear lipstick that is durable and comfortable. Most long-wear lipsticks with claims of 12+ hours of wear tend to be a two-step application process which may be inconvenient for a particular customer base who is always on the go. If a consumer prefers for the product to rank higher in terms of comfort, then the lastingness of the product may suffer. This is because long-wear lipsticks that rank high in comfort typically have a gloss component that introduces moisture to the lip, reducing the tightening and dry sensation experienced by the wearer. These glosses, however, are more likely to be transferred from the lip. Products with better durability tend to be matte films which may feel dry to the consumer.

1.5 Summary

Although an abundance of methods may be used to help characterize long-wear and transfer-resistant lipsticks, there is still a need to find a better way to correlate *in vitro* findings with consumer experience. In doing so, the cost and time associated with running consumer panels can be saved and allow for a more agile and successful development process of lipsticks. From the overview of lip surface biophysical properties, there are significant lessons about how lipstick products may interact with the lip surface. Surface properties, such as the ceramide profile of the lip, provide important fundamental knowledge about lip care products aimed at restoration of lip barrier function, while also meeting challenges associated with consumer comfort. Lip biophysical properties, like Young's modulus and lip extension/contraction during motion, are important considerations for lipstick products that enhance product durability and acceptance. Existing

test methods, such as adhesion tests, can be utilized to achieve these goals and act as a better predictive model through the use of appropriate substrates. Factoring in the mechanical properties of both the keratinous layer of the lips and the formulation would assist in enhancing the adhesion whilst also ensuring consumer comfort. Additionally, further investigation into the chemistry of a ceramide based surface will help optimize formulations while opening the possibility to incorporate additional benefits. In turn, these developments will be able to provide further insight into current lipstick formulations whilst also elucidating the benefits of new product innovations.

Acknowledgements

A special thank you to Jody Ebanks and Frances San George for assisting with technical knowledge and to Qian Zheng and Charbel Bouez for their continued guidance and support.

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