MICROENCAPSULATION OF RETINYL PALMITATE BY MELT DISPERSION FOR COSMETIC APPLICATION

by

ADITI NANDY

(Under the Direction of Suraj Sharma)

ABSTRACT

Retinyl palmitate was encapsulated in wax by melt dispersion and microcapsules were characterized for size, loading capacity, encapsulation efficiency, and antioxidant activity. Results were analyzed statistically to understand the effect of process variables i.e., type of wax, theoretical loading capacity, surface concentration and stirring speed. Shelf life and kinetic release of the developed microcapsules were evaluated by measuring actual loading capacity over time. The transfer of particles from non-woven facial wipe to a skin-like surface was also investigated. The method was effective to produce microcapsules with mean size as small as 28 µm. The encapsulation efficiency ranged from 60% to 80%. Theoretical loading capacity and surfactant percentage were the most significant factors to control loading and size of microcapsules. Retention of core content in particles stored as dispersion for 1 month and 21.7% transfer to skin-like fabric showed potential of providing skin-care properties by means of capsule loaded textile substrate.

INDEX WORDS: Microencapsulation, melt dispersion, cosmeceuticals, vitamin A, retinyl palmitate, anti-aging.

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DEDICATION

This thesis is dedicated to my parents, whose unconditional love and support have been the greatest strength for me. Thanks to my fiancée and friends, whose immense support have made this journey possible.

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CHAPTER 1

INTRODUCTION

Cosmetic industry nowadays is in constant search for innovation and improvement in cosmetic formulations due to increased demand of consumers for products with multiple benefits (Carvalho et al., 2016, Arora et al., 2012). Research has been going to on to deliver active ingredients to skin such as vitamins, essential oils, plant extracts, peptides, sugar amines, ceramides, hydroxy acids or other skin-treating substances (Bissett, 2009). Active contents are incorporated into cosmetics by means of carriers such as cream, gel, liquids or sometimes a textile substrate. Versatile cosmetic products are coming to the market in various formulations including cleanser, exfoliators, toners, serums, facials wipes and masks. As wearable technology is on the rise, likewise, wearable cosmetic products are also getting attention. These specialized products have similar results as pharmaceutical drugs, but they are produced and marketed as cosmetics and usually sold over-the-counter. Therefore, the terms such as cosmeceuticals and cosmetotextiles are introduced. Due to the immense popularity of such products among consumers, extensive research on active ingredients and their application have become more important than ever. Vitamin A and its derivatives have great importance in the cosmeceutical industry, because they act as an antioxidant as well as cell regulators, hence improve the skin texture by stimulating collagen production and reducing skin damage (Ganceviciene et al., 2012). As most biologically active ingredients including vitamin A are susceptible to environmental factors, researchers adopt microencapsulation as one of the major techniques to develop cosmeceuticals.

Active ingredients such as essential oils, plant extracts, vitamins and other antioxidants are being encapsulated for cosmeceutical application. However, there is still need for extensive studies on characterization and statistical analysis of process parameters for microparticles developed to cosmetic use. Casanova and Santos, in their review on encapsulation of different active ingredients for topical application (2016), emphasized on the importance of exploring new materials and methods as well as optimizing existing encapsulation methods in order to improve cosmetic formulations.

In this study, we investigated a novel approach i.e. melt dispersion to microencapsulate retinyl palmitate (an ester derivative of vitamin A), which has not been explored before for this particular application, possibly because the process involves heating and melting of encapsulation materials. However, waxes have been previously used to encapsulate bioactive compound, including heat-sensitive ones (Bodmeier et al., 1992). Natural waxes such as beeswax as shell can also provide anti-inflammatory, healing and skin softening properties, whereas carnauba wax helps provide protective skin barrier. Nonetheless, using natural ingredients can provide benefits without incorporating auxiliary solvents or chemicals that can have adverse side effects on sensitive skin. We employed oil-in-water emulsion system, which is one of the most frequently used cosmetic formulations because of its non-greasy properties and also cost effectiveness (Barel et al., 2014). Retinyl palmitate-loaded wax microcapsules can break upon the pressure of rubbing action and can facilitate penetration of active ingredient into the skin.

Objectives of the study:

1) To microencapsulate retinyl palmitate in waxes through melt dispersion technique;
    - investigate the effect of process variables such as type of wax, theoretical loading, surfactant % and stirring speed, by characterizing the produced microcapsules in terms of size and morphology, loading capacity, encapsulation efficiency, and antioxidant activity
2) To evaluate characteristics of developed microcapsules for cosmetic application;
    - investigate the shelf life of developed microcapsules
    - conduct kinetic release study
    - examine transfer of particles from substrate to skin-like material
3) To explore potential application and encourage future studies.

Outline of the study

This thesis is broken down into six chapters, while the first two chapters containing the introduction and literature review provide the readers the background of the study. In the third chapter, we explore the feasibility of melt dispersion method to produce microcapsules containing retinyl palmitate and investigate the process variables in order to determine significant control points to achieve desired size and efficiency of the microcapsules. In the fourth chapter, we discuss the evaluation of shelf life, kinetic release and simulated transfer study of the microparticles to understand the potentials of RP-loaded microparticles for cosmetic application. The fifth chapter focuses on various cosmetic applications that the microcapsules can be used for and important future studies. Finally chapter six concludes with overall accomplishments and outcomes of this thesis.

CHAPTER 2

LITERATURE REVIEW

Cosmeceuticals and cosmetotextiles:

The term ‘cosmeceutical’ was first introduced by Dr. Albert M. Kligman, for products containing active ingredients that can neither be called merely cosmetics, nor pharmaceutical drugs (Manela-Azulay and Bagatin, 2009). He explained the extensive demand of such products as a result of aging baby boomers’ fascination for youth and addressed them as ‘antiaging generation’ (Kligman, 2000). Cosmeceuticals signify one of the most promising sectors of the skin-care market, particularly for products that are intended to treat or inhibit the aging of the skin (Oliveira, 2014). According to Zion market research (2016), the global market revenue of anti-aging products is predicted to be over USD 216 billion by 2021 (figure 2.1).

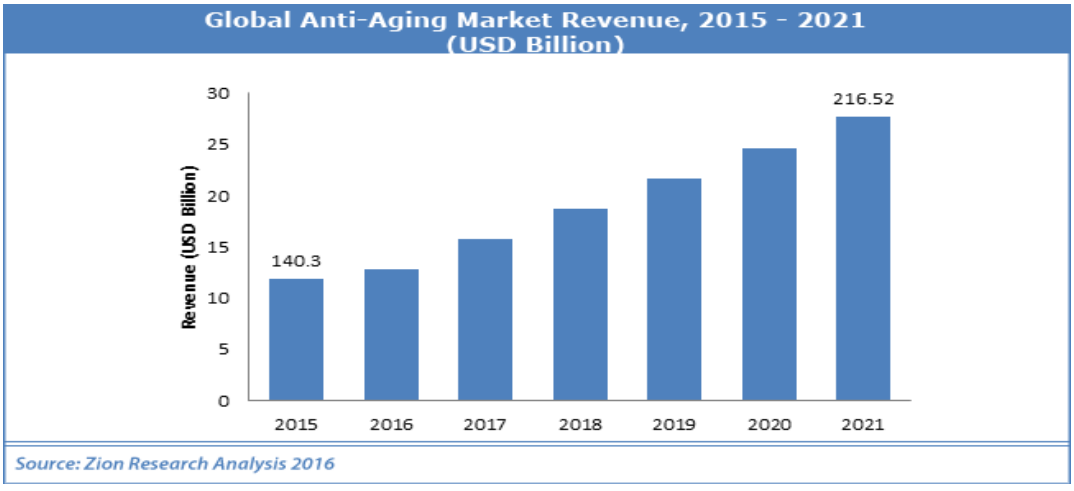

Figure 2.1: Global anti-aging market revenue by Zion market research (2016)

Another report by Orbis research (2018) forecasts that the global cosmeceuticals market has been growing at a CAGR of 9.38% during the period 2018-2023 and is expected to reach to a value of USD 80.36 billion by the end of the forecast period.

From the perspective of textiles, it is estimated that approximately 80% of textiles will be technical or functionalized in the next twenty years (Upadhyay, 2016). Today, cosmetic textiles, being a category of innovative textile materials, is also considered as technical textiles. Cosmetotextiles can be expressed as the fusion of textile substrate containing cosmetic properties that can impart functionality by releasing the active ingredient over time (Singh et al., 2011, Ömeroğullari Başyiği et al., 2018). Among the delivery vehicles of various topically administered cosmetics, textile-based substrates have their benefits due to flexibility and ease of application (Petrusic and Koncar, 2016). Moreover, the open permeable structure as well as large surface area make textile structure an ideal support for topical application (Nierstrasz, 2007).

Transdermal delivery of active ingredients to skin

Human skin protects the body from external environment through its barrier function. Topical delivery of an active ingredient requires the molecule to permeate through this skin barrier. Major part of this skin barrier is due to thin outer layer of the epidermis called 'stratum corneum' (Scheuplein and Blank, 1971). The stratum corneum consists of corneocytes, surrounded by extracellular lipid layers, often called the 'bricks and mortar' structure (figure 2.2) (Prausnitz et al., 2012). Because of this oriented layers of lipids, excessive loss of water from the body is prevented. Consequently, it also restricts the permeation of external drugs. The stratum corneum is highly lipophilic whereas the inner layer of epidermis and dermis gradually become more hydrophilic. Thus, delivery of topically administered drugs become challenging and should have

enough lipophilicity to overcome this barrier and reach to the targeted layer of the skin.(Casanova and Santos, 2016).

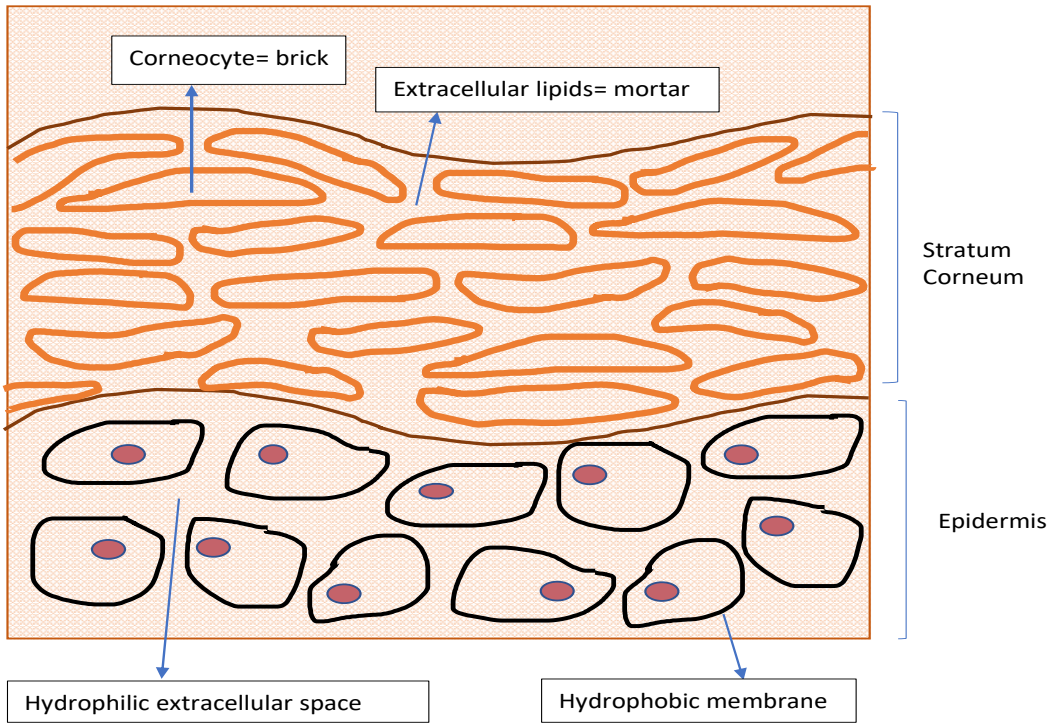

Figure 2.2: Skin barrier of stratum corneum (brick and mortar structure)

Adapted from: Prausnitz et al. (2012)

Retinoids for topical treatment of skin

Retinoids are chemical compounds of vitamin A, which include retinoic acid, retinal, retinol and retinol derivatives. They are widely recognized to address skin concerns such as acne, rosacea, pigmentation and symptoms of photoaging (Baumann, 2009). Retinoic acid has been well researched and found to be effective as topical treatment for photoaging, hyperpigmentation, wrinkles and dry skin (Kligman et al., 1984, Connor and Lowe, 1985, Elias and Williams, 1985, Haas and Amdt, 1986).

Extra-cellular matrix (ECM) proteins such as fibrillar collagens contribute to skin repair and regeneration (Watt and Fujiwara, 2011), whereas matrix-metalloproteinase (MMP) is liable for skin degradation (Fisher et al., 1999). Retinoic acid prevents and treats photo-aging not only by increasing ECM deposition but also by decreasing synthesis of MMP via inhibiting the activator complex of MMP, named AP-1 (Fisher and Voorhees, 1998). Thus, upon treatment with retinoids, the photoaged skin achieves a visibly improved texture and smoothness. Bradley et al. (2015), in their review on over-the-counter anti-ageing topical agents discussed the mechanism of action of all-trans retinoic acid (t-RA) on the skin, which is shown in figure 2.3 .

However, many patients suffer from retinoid dermatitis causing dryness, redness, burning sensation and flaking of skin as side effects of aggressive reaction to retinoic acid (Barel et al., 2014). Therefore, researchers have been studying retinol and its derivatives for cosmetic application to impart the benefits by minimizing the irritation on skin (Mordon et al., 2004, Bertin et al., 2008, Watson et al., 2008, Song et al., 1999).

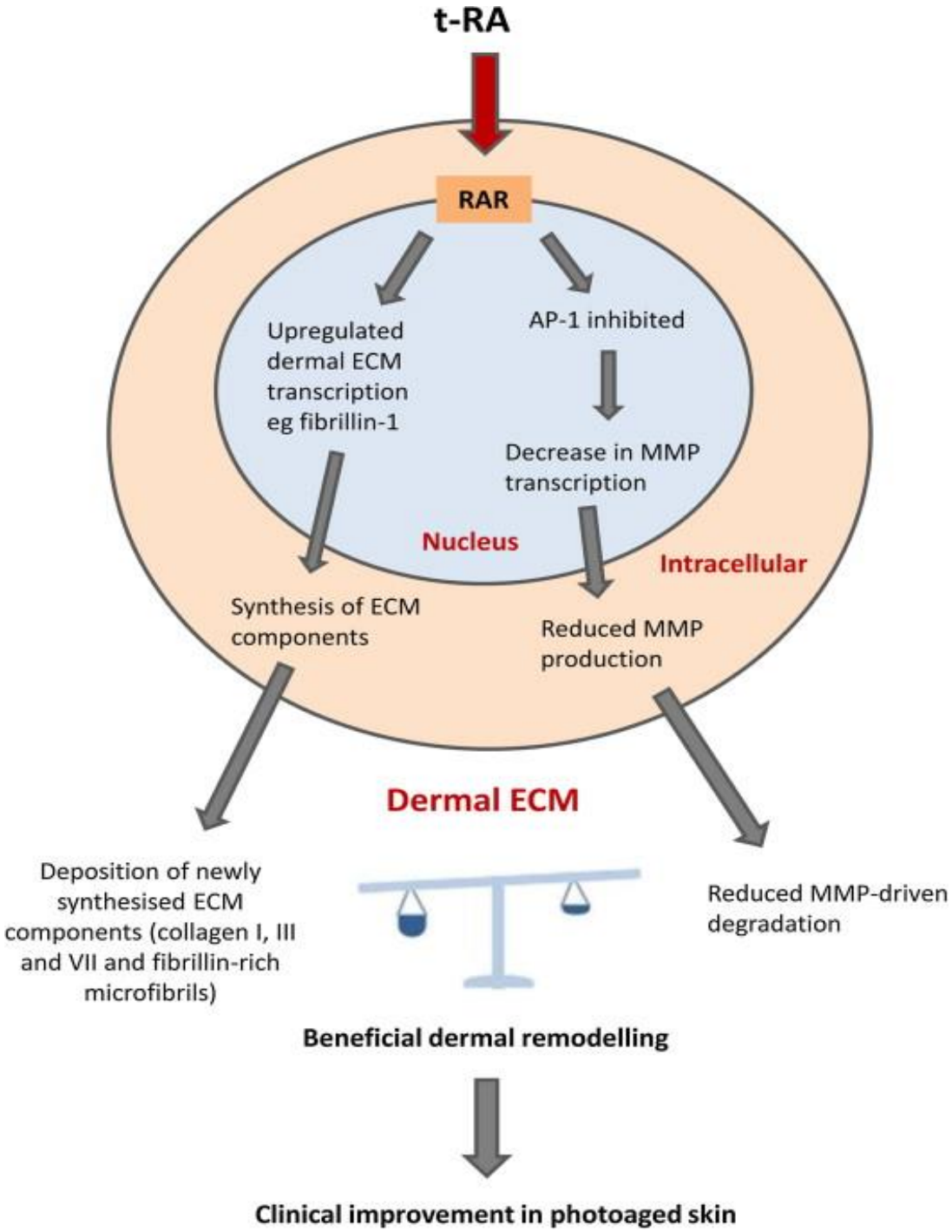

Figure 2.3 : Action of retinoic acid on skin

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Retinyl palmitate and its effect on skin

Retinyl palmitate (RP) ($C_{36}H_{60}O_2$), is a stable lipophilic ester of retinol and palmitic acid. Retinol, retinal and their derivatives need to convert into a biologically active form i.e., retinoic acid after being topically absorbed by the skin (Kurlandsky et al., 1994). RP converts to retinol being catalyzed by enzymes e.g., esterase within the skin, and then to active retinoic acid (RA) through oxidative processes (Boerman and Napoli, 1996, Lupo, 2001, Oliveira, 2014). A schematic of this mechanism is shown in figure 2.4. As a result, RP has a milder and slower reaction on the skin compared to retinol.

Many studies revealed that topically applied retinoids including retinyl palmitate are effective in skin penetration, percutaneous absorption, metabolization to retinol and retinoic acid, and skin treatment. (Boehnlein et al., 1994, Bailly et al., 1998, Antille et al., 2003, Abdulmajed and Heard, 2004, Kang et al., 1995, Duell et al., 1996, Tsunoda and Takabayashi, 1995, Vahlquist, 1999). A study by Counts et al. (1988) showed that topical administration of RP in rats for 14 days resulted in an epidermal thickening with enhanced protein and collagen stimulation. In another study, an increased epidermal thickness was observed in human skin as well (Duell et al., 1997). Antille et al. (2003) reported that RP can inhibit UVB-induced erythema in human skin.

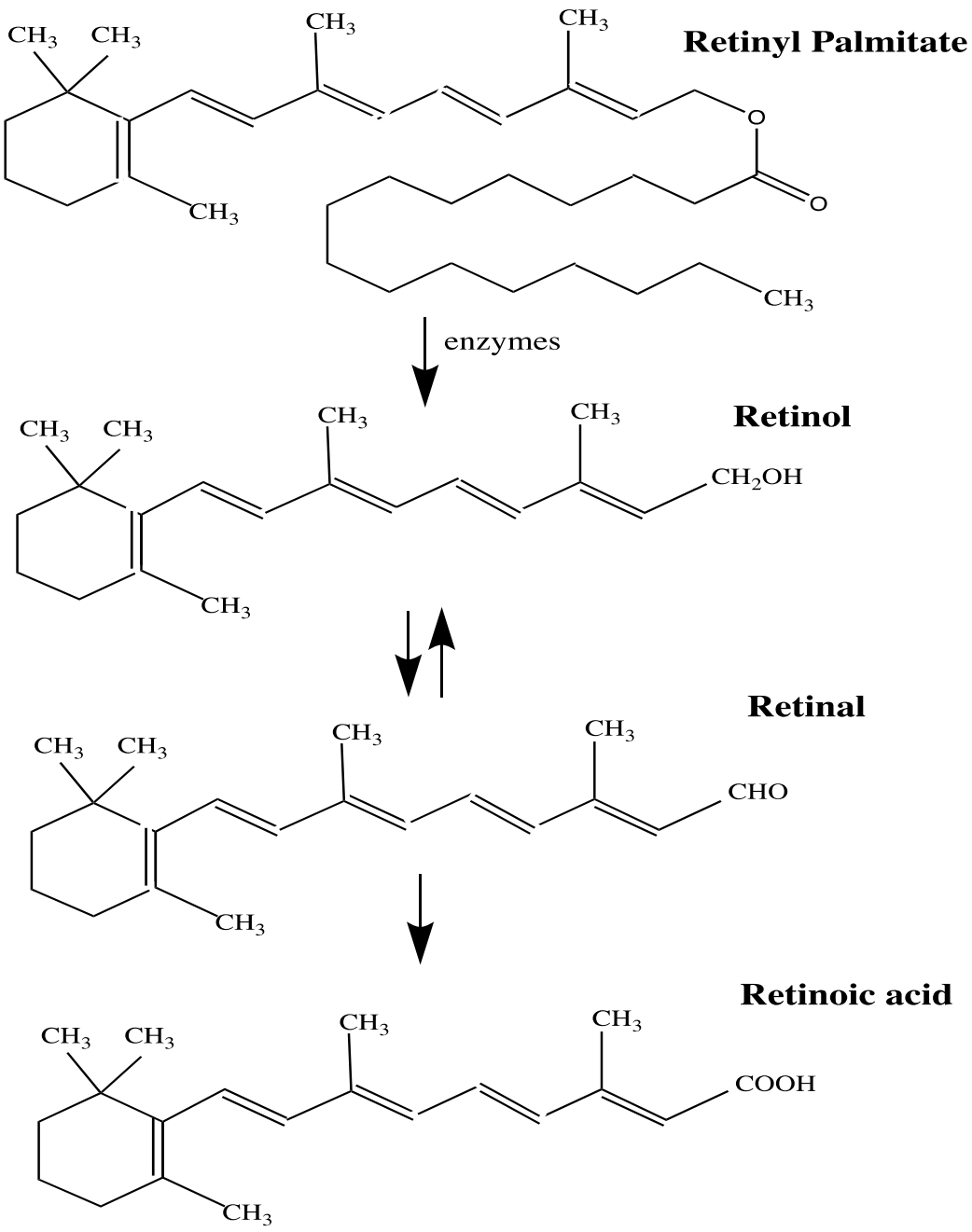


Figure 2.4 : Conversion of retinyl palmitate to active retinoic acid

Adapted from: Goncalves et al. (2016)

Microencapsulation technology and its application in cosmeceuticals and cosmetotextiles

Active ingredients including retinyl palmitate are vulnerable to environmental factors such light, heat, pH, moisture, oxidation and chemical reactions. Moreover, RP has poor aqueous solubility and it can be incompatible in other ingredients in cosmetic formulation. Encapsulation technology increases the stability of active ingredients by providing a protective shell and releasing the core substance when triggered by different factors such as external pressure, dissolution, enzymatic degradation, abrasion, heat etc. (Jyothi et al., 2012) Microencapsulation is defined as a technology of encasing solids, liquids, or gaseous substances in small capsules with size range of sub-micrometer to several millimeters that can release their contents at controlled rates during application (Benita, 2005, Mishra, 2016). By providing a physio-chemical barrier, the encapsulation system not only serves the purpose of preventing incompatibility but also prevents the side effect of overdose of an active component by controlling delivery (Gonnet et al., 2010).

A wide range of industry such as pharmaceuticals, food, chemicals, printing, and cosmetics have embraced encapsulation technology. There are various microencapsulation methods, however the selection of suitable method largely depends on the specific application and properties of the material being encapsulated. One of the widely used method is solvent evaporation/ extraction (Barroso et al., 2014, Koo et al., 2008, Ito et al., 2013, Giri et al., 2013, McCall and Sirianni, 2013, Kim et al., 2010). In this method, the active content is dissolved, dispersed or emulsified into a polymer solution containing organic volatile solvents such as dichloromethane, chloroform or ethyl acetate, followed by their emulsification into an external aqueous or oil phase (Deshmukh et al., 2016, Mishra, 2016). Resulting microcapsules are formed by the evaporation/extraction of the solvent. Coacervation method involves the phase separation of two immiscible liquids triggered by change in temperature, pH or addition of salt, resulting in one dense coacervate phase (droplets) and another dilute colloidal phase. (Salaün, 2016, Martins et al.,

2009, Xiao et al., 2014). Ionic gelation is one of the simplest methods, where the active agent is entrapped by a biopolymeric gel network such as alginate or chitosan. (Mishra, 2016, Yoksan et al., 2010, Jimtaisong and Saewan, 2014). In order to produce atomized, dry particle, spray drying is often used where emulsion containing the ingredient and carrier is homogenized and fed into a hot spray dryer (Yin and Yates, 2009, Harris et al., 2011). Other means of encapsulation includes desolvation ((Duclairoir et al., 2002, Banjare and Ghillare, 2012), thin film hydration (Bhalerao and Raje Harshal, 2003), high pressure homogenization (Shigeta et al., 2004), coprecipitation (Yang et al., 2003) etc. Thus active ingredients such as essential oils, plant extracts, vitamins and other antioxidants have been encapsulated for cosmeceutical application.

Microcapsules can be classified into three types according to the basic morphology of how the core material is distributed within the system: mononuclear, polynuclear, and matrix form (Mishra, 2016), as illustrated in figure 2.5.

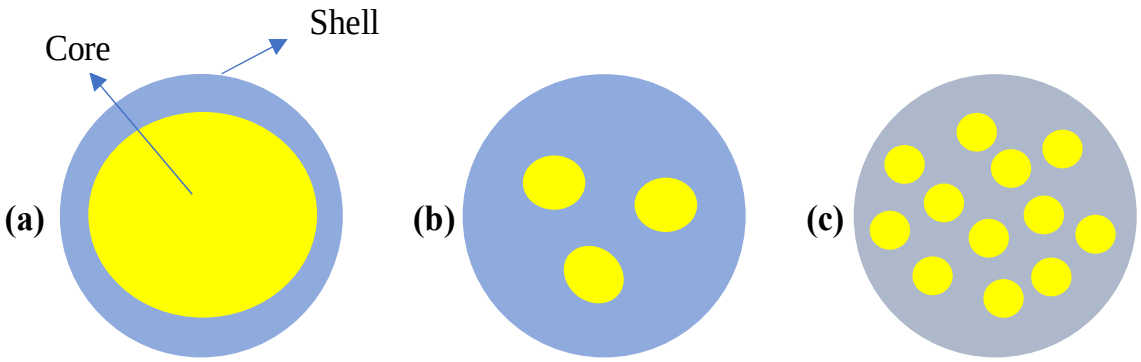


Figure 2.5 : Types of microcapsules- a) mononuclear b) polynuclear c) matrix

A mononuclear or core-shell type has a single core with a shell around it. For example, interfacial polymerization technique can produce capsules with core-shell morphology (Fu and Hu, 2017). Polynuclear capsules have multiple cores within the shell. Yourdkhani et al (2017)

produced polylactide microcapsules containing grape seed extract (GSE) using a combination of double emulsion and solvent evaporation techniques, where polynuclear morphology was observed. Matrix capsules have their core material homogeneously or heterogeneously distributed throughout or within the shell material. Particles produced through melt dispersion of waxes produce non-homogeneous, matrix capsules, however, may also consist of hollow-shell morphology (Djordjević et al., 2015).

At the beginning of 1990s, several commercial applications of microencapsulation in textiles came into appearance, particularly for dyeing, printing and medical uses. (Nelson, 1991). In the course of time, significant research has been conducted in textile industry based on microencapsulation. Fabrics with durable fragrances were developed with fragrance-loaded microcapsules adhered by resinous binder (Ono, 1990). Fourrey (2001) developed thermo-regulatory vehicle seats by incorporating microcapsules of phase change materials. Leskovšek (2005) reviewed use of microencapsulation in medical and hygienic products, including textiles containing microencapsulated curative substances and essential oils.

Several studies have investigated the application of microencapsulation in cosmetic textiles. Yamato *et al.* (1993) formulated treating liquids containing microcapsules of skin-care substances and their incorporation into textile structure. A fabric coating composition was developed by Zuckerman (2001) that contained microspheres of energy absorbing phase change materials. Chang (2005) developed a process of low temperature plasma treatment of fabric that improves the adhesion of natural oil essence microcapsules within the fabric. Aroma-therapeutic textile with fragrance-loaded cyclodextrin inclusion compound was prepared by Wang and Chen (2005). Koenig (2007) formulated a cleansing composition with microencapsulated delivery vehicle comprised of active agents that can be introduced into wet wipes. Another study developed

vitamin C-loaded gelatin microcapsules using emulsion hardening process that can be grafted into textiles to impart skin-care benefits (Cheng et al., 2009). Alonso (2016) reported the preparation of polyamide cosmetotextile comprising of gallic acid (GA)-loaded poly-ε- caprolactone (PCL) microspheres to impart antioxidant effect to skin.

Microencapsulation of retinoids

Previously vitamin A has been microencapsulated in various methods including spray drying, spray cooling, coacervation (phase separation), emulsion system, liposomes, solid lipid nanoparticles, and inclusion complexation (Goncalves et al., 2016). For cosmetic formulations, retinoids has been reported to be successfully encapsulated in albumin by emulsion method (Torrado et al., 1992), in glyceryl behenate solid lipid nanoparticles by melt solidification (Jenning et al., 2000), in tetraethyl orthosilicate by sol-gel encapsulation (Lee et al., 2001), in chitosan nanoparticles using solvent evaporation (Kim et al., 2006), and in maltodextrin /modified starches using spray drying method (Gangurde and Amin, 2017).

Microencapsulation by melt dispersion

The fundamental principle of melt dispersion method is based on the atomization of a molten matrix (melting point ranging from 32ºC to 85ºC) in finely dispersed microdroplets that contain the active ingredient, followed by solidification to provide powder-like microparticles. (Djordjević et al., 2015).

As an oil-in-water emulsion system is used, the oil phase is dispersed in the bulk continuous phase to make droplets of oil surrounded by water. Surfactants reduces the interfacial tension, sheathes the droplets and stabilizes the droplet shape by minimizing the surface area as

well as surface energy (Mokhatab et al., 2018). As a result, spherical droplets are formed. A shear force such as mixing is required break up the droplets structure into smaller droplets and prevent their coalescence. When the dispersion is cooled, the melted wax in the oil phase get solidified, entrapping the functional ingredient in their matrix.

There have been limited studies to evaluate the effect of different process variables, however, some important process variables for this method includes, temperature, surfactant concentration, agitation speed, type of lipid, lipid crystallinity and hydrophilicity, chronological order of events and cooling rate of the emulsion ((Milanovic et al., 2011, Djordjević et al., 2015). Melt dispersion is an inexpensive and convenient method that can produce free-flowing particles with size as small as 45 µm (Mishra, 2016). Such small particles can be multipurpose in the cosmetic formulation as well as easy to handle and incorporate into fibrous textile materials. Although this method requires application of heat and can be perceived as unsuitable for heat-sensitive compounds, if the active substance is exposed to heat for a minimal time, this can be an effective method considering microcapsules' size, shape, loading capacity, and encapsulation efficiency it can provide.

Waxes as encapsulation material

Waxes are widely used carriers in melt-based techniques that can act as release retardants of encapsulated active ingredients. There are many advantages of waxes including their stability in different pH and moisture levels, insolubility in water, non-toxicity and superior ease of handling (Kamble et al., 2004, Milanovic et al., 2010). Rheology of waxes also make them suitable for encapsulation. Most waxes are pliable at room temperature without cracking, whereas their plate-like crystal region make them efficient barrier materials to reduce the diffusion of water, ions

or organic molecules of low molecular weight (Donhowe and Fennema, 1993, Schreiber and Riederer, 1996, Milanovic et al., 2010).

Release mechanism of active content from the microcapsule:

The mechanism of controlled release of active ingredients can be broadly categorized into two types: physical and chemical. The physical mechanism of controlled release include diffusion of the active substance through polymer matrix, degradation or dissolution of the polymer layer, osmotic pressure or erosion of shell material (Acharya and Park, 2006, Singh et al., 2010). On the contrary, chemical mechanism involves alteration of active molecules (Petrusic and Koncar, 2016).

Diffusion mostly involves penetration of the dissolution medium through the shell to allow seepage by dissolving the core, or permeation of core content from shell matrix. Dissolution mechanism applies to shell materials that are soluble in the dissolution fluid. Osmosis happens in semi-permeable shells, where release of active content is driven by osmotic pressure difference (Veronese et al., 1999). Erosion of shell or coating material may happen due to pH, enzymatic reaction or hydrolysis. ((Singh et al., 2010, Lakshmi et al., 2003).

In case of waxy materials as matrix components, the most significant mechanisms of release are diffusion of active core through the matrix and erosion of wax matrix through ester hydrolysis reaction (Figure 2.6) (Djordjević et al., 2015, Jogunola et al., 2011).

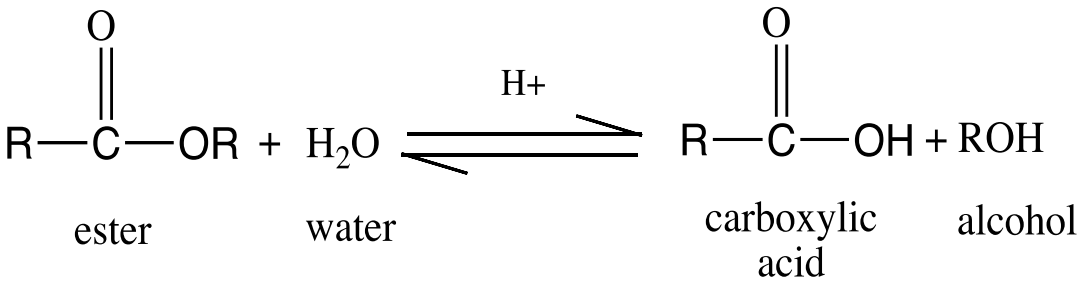


Fig 2.6: Ester hydrolysis reaction

The long chain esters in the wax hydrolyzes via autocatalytic reaction, resulting in increased porosity in the wax matrix as well as mass loss of the active content (Kheradmandnia et al., 2010). Kinetic release studies are performed in order to understand the release rate of active ingredients in dissolution medium and also to better understand the storage stability.

CHAPTER 3

MICROENCAPSULATION OF RETINYL PALMITATE BY MELT DISPERSION FOR COSMETIC APPLICATION[1]

[1] Aditi Nandy, Eliza Lee, Abhyuday Mandal, Raha Saremi, and Suraj Sharma. To be submitted to Journal of Microencapsulation.

Abstract

Retinyl palmitate was encapsulated by melt dispersion technique and the effect of process parameters on capsule properties was studied in the perspective of cosmeceuticals and capsule-loaded cosmetic textile application. The acquired microparticles were characterized for size using image analysis; loading capacity and encapsulation efficiency using ultraviolet-visible spectroscopy; antioxidant activity through DPPH (2,2-diphenyl-1-picrylhydrazyl) assay; and the results were analyzed through statistical analysis to understand the effect of process variables such as type of wax, theoretical loading capacity, surface concentration and stirring speed. Melt dispersion method by using waxes as shell material was effective to produce microcapsules with a spherical shape and mean size as small as 28 µm. The encapsulation efficiency ranged from 60% to 80%. Theoretical loading capacity and surfactant ratio were found to be the most significant factors to control the actual loading capacity and size of microcapsules. This paper discusses the importance of vitamin A palmitate in the present cosmeceutical industry, cosmetic research based on microencapsulation technology, and a study on microencapsulation of retinyl palmitate by melt dispersion method in this prospect.

Introduction

Cosmeceuticals i.e., cosmetic products that can impart beneficial results for human body signify one of the most promising sectors of the skin-care market, particularly for products that are intended to treat or inhibit the aging of the skin (Oliveira, 2014). Cosmeceuticals have similar results as pharmaceutical drugs, but they are produced and marketed as cosmetics and usually sold over-the-counter. According to Zion market research (2016), the global market revenue of anti-aging products is predicted to be over USD 216 billion by 2021. Another report by Orbis research (2018) forecasts that the global cosmeceuticals market has been growing at a CAGR of 9.38% during the period 2018-2023 and is expected to reach USD 80.36 billion by the end of the forecast period. In this respect, vitamin A and its derivatives have great importance in the cosmeceuticals industry because they act as antioxidants as well as cell regulators, hence improve the skin texture by stimulating collagen production and reducing skin damage (Ganceviciene et al., 2012)

Bradley et al. (2015), in their review on over-the-counter anti-ageing topical agents discussed the mechanism of action of all-trans retinoic acid (t-RA) on the skin, which is shown in figure 2.3. Extra-cellular matrix (ECM) proteins such as fibrillar collagens contribute to skin repair and regeneration (Watt and Fujiwara, 2011), whereas matrix-metalloproteinase (MMP) is liable for skin degradation (Fisher et al., 1999). Retinoic acid prevents and treats photo-aging not only by increasing ECM deposition but also by decreasing synthesis of MMP via inhibiting the activator complex of MMP, named AP-1 (Fisher and Voorhees, 1998). Thus, upon treatment with retinoids, the photoaged skin achieves a visibly improved texture and smoothness.

Retinyl palmitate (RP) ($C_{36}H_{60}O_2$), is a stable lipophilic ester of retinol and palmitic acid. Although pure retinol is more effective in anti-aging than its derivatives, it has adverse effects such as burning, redness and, peeling off the skin. On the contrary, RP has a mild and slow reaction on

the skin. After being topically applied onto the skin, RP needs to be converted to retinol catalyzed by enzymes within the skin, and then to active retinoic acid through oxidative processes (Boerman and Napoli, 1996, Lupo, 2001, Oliveira, 2014). A schematic of this mechanism is shown in figure 2.4. However, there is evidence of the effectiveness of RP as antiaging in previous studies. A study by Counts et al. (1988) showed that topical administration of RP in rats for 14 days resulted in an epidermal thickening with enhanced protein and collagen stimulation. In another comparison study (Duell et al., 1997), an increased epidermal thickness was observed in human skin as well.

From the perspective of textiles, it is estimated that approximately 80% of textiles will be technical or functionalized in the next twenty years (Upadhyay, 2016). Today, cosmetic textiles, being a category of innovative textile materials, is also considered as technical textiles. A standard definition by French textile & apparel industry standardization office (Bureau National des Industries du Textile et de l'Habillement) goes by: "Cosmetic textiles are textile articles which contain a substance or preparation designed to be released over the long term onto different, superficial areas of the human body, in particular, the epidermis, and claiming one or more specific properties, such as cleansing, perfume, figure enhancement, skin protection, maintenance or anti-odor"(Saini and Manideep, 2017). Cosmetotextiles can be expressed as a fusion of textile substrate with cosmetic properties that can impart functionality to skin by releasing the active ingredient. (Singh et al., 2011, Ömeroğullari Başyiği et al., 2018). Cosmetic ingredients can be from herbal, animal derivatives or synthetic and inorganic sources (Yılmaz, 2014).

Nowadays, many of the cosmetics, as well as personal care products, contain ingredients that are biologically active, hence are unstable and vulnerable to environmental factors such as temperature, light, pH, and oxidation (Casanova and Santos, 2016). Microencapsulation technology increases the stability of such ingredients by providing a protective shell, and releasing

the core substance when triggered by different factors such as external pressure, dissolution, enzymatic degradation, abrasion and heat (Jyothi et al., 2012). Microencapsulation is defined as a technology of encasing solids, liquids, or gaseous substances in small capsules with size range of sub-micrometer to several millimeters that can release their contents at controlled rates during application (Benita, 2005, Mishra, 2016).

There are various microencapsulation methods. However, the selection of suitable method largely depends on the specific application and properties of the material being encapsulated. One of the widely used method is solvent evaporation/ extraction (Barroso et al., 2014, Koo et al., 2008, Ito et al., 2013, Giri et al., 2013, McCall and Sirianni, 2013, Kim et al., 2010). In this method, the active content is dissolved, dispersed or emulsified into a polymer solution containing organic volatile solvents such as dichloromethane, chloroform or ethyl acetate, followed by their emulsification into an external aqueous or oil phase (Deshmukh et al., 2016, Mishra, 2016). Resulting microcapsules are formed by the evaporation/extraction of the solvent. Coacervation method involves the phase separation of two immiscible liquids triggered by change in temperature, pH or addition of salt, resulting in one dense coacervate phase (droplets) and another dilute colloidal phase. (Salaün, 2016, Martins et al., 2009, Xiao et al., 2014). Ionic gelation is one of the simplest methods, where the active agent is entrapped by a biopolymeric gel network such as alginate or chitosan. (Mishra, 2016, Yoksan et al., 2010, Jimtaisong and Saewan, 2014). In order to produce atomized, dry particle, spray drying is often used where emulsion containing the ingredient and carrier is homogenized and fed into a hot spray dryer (Yin and Yates, 2009, Harris et al., 2011). Other means of encapsulation includes desolvation ((Duclairoir et al., 2002, Banjare and Ghillare, 2012), thin film hydration (Bhalerao and Raje Harshal, 2003), high pressure homogenization (Shigeta et al., 2004), coprecipitation (Yang et al., 2003) etc. Thus active

ingredients such as essential oils, plant extracts, vitamins and other antioxidants have been encapsulated for cosmeceutical application. Previously retinol and RP have been encapsulated in Glyceryl behenate SLN by melt solidification (Jenning et al., 2000), in tetraethyl orthosilicate by sol-gel encapsulation (Lee et al., 2001), and in chitosan using solvent evaporation (Kim et al., 2006).

In this study, we investigated a novel approach i.e. melt dispersion to microencapsulate RP. Melt dispersion is an inexpensive and convenient method that can produce free-flowing particles with size as small as 45 µm (Djordjević et al., 2015). The fundamental principle of melt dispersion method is based on the atomization of a molten matrix such as waxes (melting point ranging from 32ºC to 85ºC) in finely dispersed microdroplets that contain the active ingredient, followed by solidification to provide powder-like microparticles (Djordjević et al., 2015). As an oil-in-water emulsion system is used, the oil phase is dispersed in the bulk continuous phase to make droplets of oil surrounded by water. Surfactants reduces the interfacial tension, sheathes the droplets and stabilizes the droplet shape by minimizing the surface area as well as surface energy (Mokhatab et al., 2018). As a result, spherical droplets are formed. A shear force such as mixing is required to break up the droplets structure into smaller droplets and prevent their coalescence. When the dispersion is cooled, the melted wax in the oil phase get solidified, entrapping retinyl palmitate in the matrix.

Small particles can be multipurpose in the cosmetic formulation as well as easy to handle and incorporate into fibrous substrates. The particle size for cosmetic formulation depends on the type of application. For example, facial scrubs or exfoliators, the particles size should range from 75-200 µm, whereas 200- 700 µm would be suitable for body scrubs (Morice, 2016). On the other hand, for cosmetic textile application such as non-woven wipes, the capsule size should range from

1 μm to about 100 μm based on the pore size of the substrate (Varona and Wright, 2005). The present aims to determine the significant process variables to control the size of RP-loaded microparticles for cosmetic application.

Melt dispersion method has not been explored before to microencapsulate retinoids, possibly because the process involves heating and melting of encapsulation materials. However, waxes have been previously used to encapsulate bioactive compound, including heat-sensitive ones (Bodmeier et al., 1992). Furthermore, natural waxes such as beeswax or carnauba wax can impart additional skin benefits, without incorporating auxiliary solvents or chemicals that can have a side effect on sensitive skin.

Microcapsules can be classified into three types according to the basic morphology of how the core material is distributed within the system: mononuclear, polynuclear, and matrix form (Mishra, 2016), as illustrated in figure 2.5. A mononuclear or core-shell type has a single core with a shell around it. Polynuclear capsules have multiple cores within the shell. Matrix capsules have their core material homogeneously or heterogeneously distributed throughout or within the shell material. Particles produced through melt dispersion of waxes mostly produce non-homogeneous, matrix microcapsules, however, may also consist of hollow-shell morphology (Djordjević et al., 2015).

Research on microencapsulation often involves the investigation of multiple variables with a limited amount of data. Traditionally, two-level fractional factorial experiments are used for such screening designs. In this study, we used three levels to have better flexibility. More importantly, the coding of variables is used by factorial experiments, which does not make any distinctions between actual levels of factors. This essentially restricts us from using actual factor levels in the analysis. Moreover, in factorial designs, some terms are often found fully confounded.

Consequently, one may not be able to distinguish between the effects of certain factors and interactions. Nonetheless, they require a greater number of experiments to understand the main effects thoroughly. Definitive screening design can provide estimates of main effects being unbiased by any second-order effect (Jones and Nachtsheim, 2011). This design also requires a smaller number of time-consuming experiments to extract the same information as those from regular factorial or fractional factorial designs. Therefore, definitive screening designs are known to be well suited for such situations.

The overall objective of this study was to microencapsulate retinyl palmitate through melt dispersion; statistically investigate the effect of process variables such as type of wax, theoretical loading, surfactant % and stirring speed; and characterize the produced microcapsules in terms of size and morphology, loading capacity, encapsulation efficiency, and antioxidant activity.

Materials and Methods

Materials

Refined, white, beeswax pearls were purchased from Bulk Apothecary (Aurora, OH). Pure, granular paraffin wax; refined, yellow carnauba wax and retinyl palmitate (vitamin A) of 1.7 M.I.U./g. were purchased from Fisher Scientific USA (Pittsburg, PA). Tween™ 20 (Fisher BioReagents™; Pittsburg, PA), Span 85 (Sigma-Aldrich, St. Louis, MO) and Ethanol (Decon Labs, Inc., King of Prussia, PA) were used as received.

Preparation of microcapsules

The method of preparing RP-loaded wax microcapsules by melt dispersion was developed from another study that encapsulated ethyl vanillin in carnauba wax (Milanovic et al., 2011). We modified the procedure based on the surfactants used, melting temperature of the materials and the

levels of process variables studied. A schematic of the process of preparation is shown in figure 3.1.

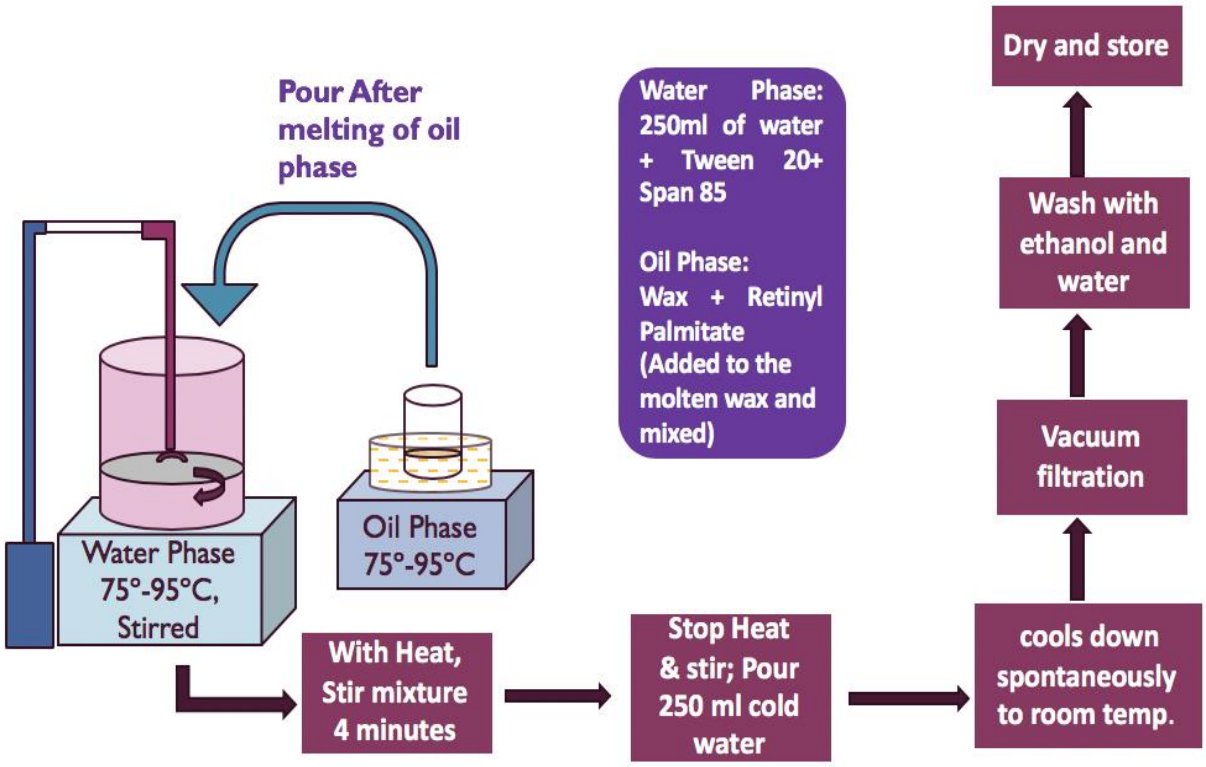


Figure 3.1: Schematic of microencapsulation by melt dispersion

A vessel was filled with DI water and emulsifiers (a mixture of tween 20/span 85) and was heated 10˚C higher than the melting temperature of the wax. The emulsion mixture was stirred with a mechanical mixer. Simultaneously, a pre-weighed amount of wax (depending on theoretical loading selected) was completely melted in a water bath. The melting temperature of beeswax and paraffin wax are close to 65ºC, whereas carnauba wax has a melting temperature around 86ºC. Henceforth, the heating temperature was 75ºC for beeswax and paraffin wax, while carnauba wax requires 95ºC. RP was added into the molten wax just before the mixture was poured to the vessel containing the emulsion. In this way, it was possible to ensure minimal exposure of the RP to heat. The emulsion was stirred with heat for 4 minutes. Then the heating was stopped, and cold DI water

(at 2˚C-5˚C) was poured into the resulting dispersion in order to cool it down and to enable solidification of wax droplets. The dispersion with produced particles was cooled down spontaneously to the room temperature. Then the dispersion was vacuum filtered to collect solid wax particles and washed with ethanol and DI water to remove residual surfactants and superficial RP. Finally, the resultant particles were dried and stored in a petri dish.

Amount of wax, RP, and surfactant were calculated based on the theoretical loading percentage and surfactant percentage according to the design of the experiment. The nature of emulsion, i.e. oil in water or water in oil helps decide the selection of emulsifiers in order to attain a desired hydrophilic-lipophilic balance (HLB), a concept initially described by Griffin (1949) and later modified by Davies (1957). As Tweens (polyethoxylated sorbitan esters) are more water soluble (HLB>10) whereas Spans (sorbitan esters) are more oil soluble (HLB<9), often a blend of both are used.

For measuring how much of emulsifier (A) to blend with emulsifier (B) value of X, equation (1) and (2) were used:

$$\%A = \frac{(X - HLBb) \times 100}{HLBa - HLBb} \qquad (1)$$

$$\%B = 100\% - \%A \qquad (2)$$

Where: X=desired HLB value (For Beeswax and Paraffin wax, 10 and Carnauba wax, 12),

A= Tween 20, B= Span 85, HLB_a = HLB of Tween 20, HLB_b= HLB of Span 85.

Statistical analysis of effect of process variables on microcapsule properties

A three-level definitive screening design was applied to assess the effect of four important factors: type of wax, theoretical loading capacity, emulsifier concentration, and stirring speed. The response variables we looked into were the actual loading capacity%, encapsulation efficiency (%), antioxidant activity (%), and mean size of produced microcapsules. We conducted nine experiments based on the design of experiment, as mentioned in Table 1.

Table 1: Design of experiment with varying process parameters

Experiment	A	B	C	D	Factors	Levels
1	1	0	1	-1	A= Type of wax B= Theoretical Loading(%) C= Surfactant (%) D= Speed (rpm)	For A: 1=Beeswax, 0= Paraffin, -1= Carnauba For B: 1=25%, 0=15%, -1=10 For C: 1=2%, 0=1%, -1=0% For D: 1=280, 0=230, -1=180
2	0	-1	1	1		
3	0	1	-1	-1		
4	-1	0	-1	1		
5	0	0	0	0		
6	-1	1	1	0		
7	1	1	0	1		
8	-1	-1	0	-1		
9	1	-1	-1	0		

We used three types of wax, i.e. beeswax, paraffin wax, and carnauba wax in order to compare their performance as a shell material.

As fraction of the active compound in the mixture is an important factor that influences the characteristics of the particles, we investigated three levels of the theoretical loading capacity in this study: 10%, 15%, and 25%. We did not choose a higher level because a higher loading percentage could compromise the wall barrier as well as the stability of the microcapsules. As a response, encapsulation efficiency of more than 50% was considered to be acceptable that can enable upscaling (Gavory et al., 2014).

The levels chosen for evaluating the effect of surfactant percentage were 0%, 1%, and 2%, in order to compare the morphology of particle produced with no surfactant and gradually with higher ratios of surfactant.

The speed levels chosen were within the range of a simple mechanical mixer we used in the laboratory scale, which were 230, 250, and 280 rpm. Previous studies (Barakat and Yassin, 2006, Gowda and Shivakumar, 2007, Milanovic et al., 2011) on such process parameters investigated the effect of stirring speed on particle size at a much higher range (~1000 rpm). We studied a lower range of stirring speed in order to compare their effectiveness and estimate optimum speed level for successful encapsulation with much less consumption of energy.

We fitted an Analysis of Variance (ANOVA) model to analyze the data, with wax-type as a qualitative factor and others as quantitative factors, where actual levels of factors were used. Their significance was tested with F-test using R Core Team (2019).

Characterization

Differential Scanning Calorimetry (DSC) analysis: DSC of the waxes were carried out by using Mettler Toledo DSC821e instrument, where an standard empty aluminium pan was used as the reference. Samples were scanned from 25ºC to 100ºC under N_2 atmosphere with a heating rate of

10ºC/min, and then reversely cooled at the same rate and range of temperature. All samples were prepared with weights between 2.0 and 10.0 mg.

Thermogravimetric analysis (TGA): In order to understand the stability of microcapsule in during storage and transportation , thermal stability of the three waxes were assessed with TA Instruments Discovery TGA-MS by scanning the samples from 25ºC to 600ºC under N_2 atmosphere at a heating rate of 10ºC/ min. Samples were prepared with weights between 12.0 and 25 mg.

Size and Morphology: We observed the shape, morphology, and size distribution of the produced microcapsules by using FEI Teneo scanning electron microscope and Image-J 1.51s digital image analyzer. Samples were vacuum coated with a gold film after mounting them on copper stubs and then surface morphology was examined using an FEI Teneo SEM.

The particle size distribution for particles produced by experiments with varying parameters was also determined from diameters of 100 particles measured by Image-J, with the exception of experiment 3 (count =8) , experiment 4 (count =40), experiment 5 (count =80) and experiment 9 (count m=50) because of irregular shape or large size of the resultant capsules.

Loading Capacity and Encapsulation Efficiency: We characterized the particles based on loading capacity and encapsulation efficiency. Loading capacity was defined as the ratio of the weight of RP to the weight of total encapsulation material for shell and core, expressed in percentage. Expected loading capacity was calculated by taking into account the amount of RP initially used for production. Actual loading capacity provided the actual amount of RP loaded, excluding the amount lost during the process due to evaporation as well as solidification with wax in the

container wall. Encapsulation efficiency was defined as the ratio of actual loading capacity to expected loading capacity, expressed in percentage. Hence, these terms can be expressed by equation (3), (4), and (5):

$$\text{Expected LC\%} = \frac{M_0}{M_0 + M_p} \text{ x } 100 \qquad (3)$$

$$\text{Actual LC\%} = \frac{M_e}{M_e + M_p} \times 100 \qquad (4)$$

$$\text{EE\%} = \frac{\text{Actual LC\%}}{\text{Expected LC}} \times 100 \qquad (5)$$

Where, M_0 = initial weight of Vitamin A; M_e = weight of Vitamin A encapsulated, M_p= amount of wall material used.

Pure ethanol containing a fixed amount of microparticles was heated to the melting temperature of wax to release all the content of the capsules. Then the system was stirred for 1 hour without heat. The extractant was filtered using the syringe, and the volume was recorded. All samples were measured in triplicates. The supernatant was diluted with ethanol to an appropriate concentration. The dilution solution for each sample was replaced in quartz cuvette containing ethanol in each respective test to achieve UV-Vis (Ultraviolet-visible spectroscopy) absorbance spectrum. The concentration was determined by using the absorbance at 327 nm and a concentration curve of known concentrations of the RP.

Antioxidant activity (DPPH assay): Evaluation of antioxidant activity was carried out by assay of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, DPPH is a free radical that becomes a stable diamagnetic molecule by accepting an electron or hydrogen radical. As a result, a decrease in absorbance at 517 nm should be observed.

In our experiments, 100 µl of sample or control (ethanol) was added to 3.9 mL of DPPH solution (ethanol; 60uM, 25mg/L). We measured absorbance at 517 nm, using UV spectrophotometer at the time after 1 hour of shaking in the dark. We used the following equation to determine Antioxidant activity (AA%):

$$AA\% = \frac{A_0 - A_1}{A_0} \times 100 \qquad (6)$$

Where: A_0 is the blank (contains only ethanol), and A_1 is the sample.

Results and discussion

Thermal properties of waxes

Study of the thermal properties of waxes using DSC was conducted to verify the melting and crystallization temperature of the waxes, which helped to decide the processing temperature during preparation and characterization of microcapsules. The thermal decomposition of waxes were evaluated by TGA in order to compare the thermal stability of the three waxes. Besides, the stability of the waxes during the storage and transportation of cosmetic products could also be understood by this study.

Beeswax is an insect wax secreted from honeybees, consisting mainly of fatty acid esters (65%), hydrocarbons (23%), free acids (12%) and free alcohols (1%) (Tulloch, 1970). In the DSC curve (figure 3.2), beeswax showed its melting peak (T_m) around 65.84ºC and crystallization peak (T_c) at around 57.5ºC. Paraffin wax, being derived from petroleum source and composed of mostly hydrocarbons (Himran et al., 1994), showed T_m at around 64.73ºC and T_c at around 54.83ºC, which are lower than the T_m and T_c of beeswax, respectively.

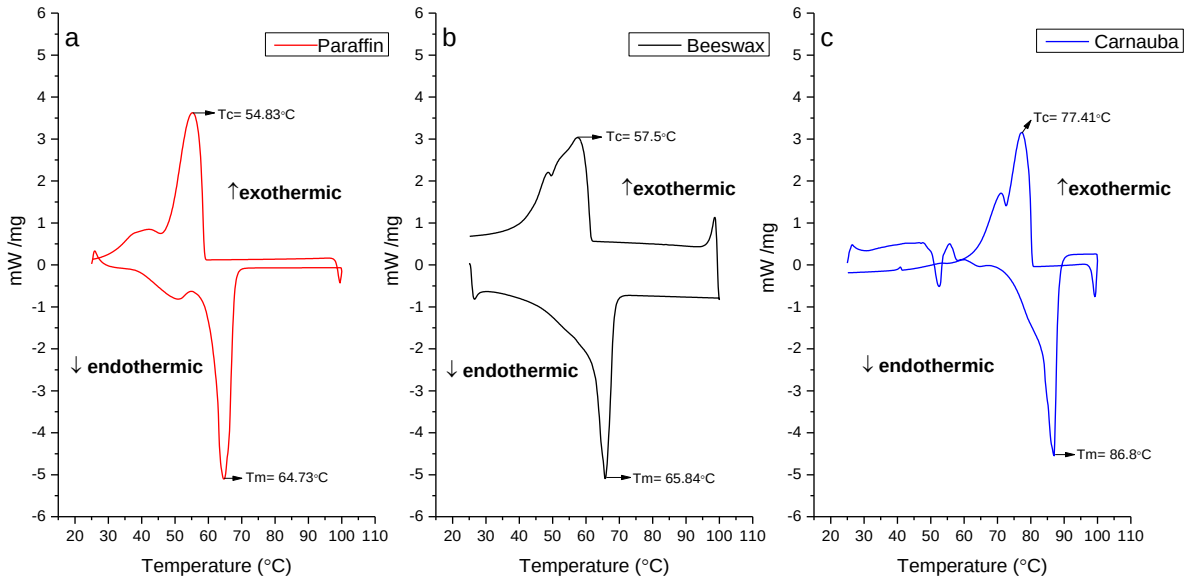


Figure 3.2: DSC of (a) paraffin wax (b) bees wax and (c) carnauba wax

Carnauba wax is a hard wax derived from Brazilian palm leaves, usually with a high content of aliphatic esters (38-40%), diesters of fatty acids (30-34%), a small number of fatty alcohols (10-12%), acids, hydrocarbons, etc. (Vandenburg and Wilder, 1970). The T_m of carnauba wax was found at 86.8ºC whereas the crystallization peak was at 77.41ºC. Therefore, carnauba wax exhibited the highest melting and crystallization temperature. These results were consistent with the results obtained by Ruguo et al (2011) for thermal properties of these waxes.

From the TGA and DTG (first derivative of TG) curves (Figure 3.3), the beeswax sample showed initial decomposition around 312.08°C, and after 465ºC the sample was completely decomposed. The highest rate of decomposition with respect to temperature is at 375.01ºC. Similarly, the onset of decomposition of paraffin wax was at 264.83ºC, highest rate of decomposition being at 318.41ºC. In the case of carnauba wax, onset of decomposition was at 381ºC, while the highest rate of decomposition was at 425.69ºC. Therefore, among the three

waxes, carnauba wax was found to be the most thermally stable, whereas paraffin wax had the least thermal stability.

In a review by Turton and Cheng (2007), it was reported that the stable temperature for incorporating active substances into melt-solidified matrices should be in the range of 30ºC- 200ºC and the range of melting temperature should be narrow . All results obtained from DSC and TGA for the melting temperature and thermal stability comply with these requirements.

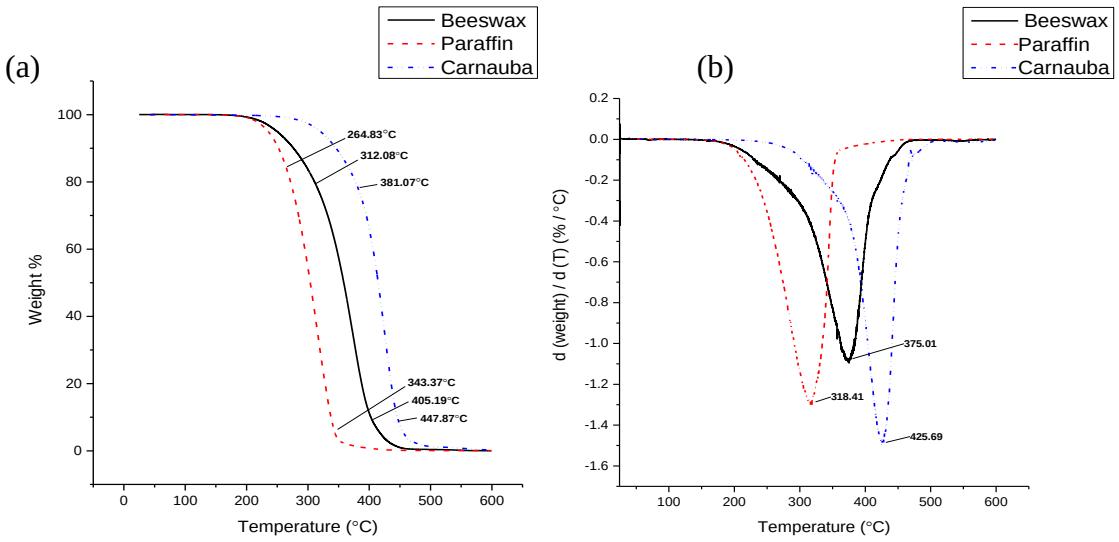


Figure 3.3: (a) TGA and (b) DTG of beeswax, paraffin wax & carnauba wax

According to a review on the cooling process and congealing (Turton and Cheng, 2007), the carriers for incorporation of active substance into melt-solidified matrices should be stable in the range of 30ºC- 200ºC and should have a narrow range of melting point. All results obtained from DSC and TGA for the melting temperature and thermal stability comply with these requirements.

Effect of process variables on microcapsule properties

For the four response variables, i.e., actual loading capacity, encapsulation efficiency, antioxidant activity, and mean particle size, the results of nine experiments are reported in Table 2.

Table 2: Result of experiment with varying process parameter*

	Process Variables				**Response Variables**			
Experiments	***A*** ***Type of Wax***	***B*** ***Theoretical Loading (%)***	***C*** ***Surfactant (%)***	***D*** ***Stirring Speed (rpm)***	***Mean Actual LC± SD (%)***	***Mean EE± SD (%)***	***Mean AA±SD (%)***	***Mean size ± SD (µm)***
1	1	0	1	-1	11.01 ± 0.45	73.40 ± 2.98	6.36 ± 0.61	94 ± 43
2	0	-1	1	1	7.65 ± 0.39	76.60 ± 3.95	5.9 ± 1.00	69 ± 31
3	0	1	-1	-1	17.33 ± 1.25	69.26 ± 5	20.39 ± 0.64	464 ± 318
4	-1	0	-1	1	12.34 ± 0.94	82.17 ± 6.27	5.17± 0.88	213 ± 167
5	0	0	0	0	12.13± 1.54	80.77 ± 10.26	4.1± 2.44	124 ±44
6	-1	1	1	0	17.63 ± 0.78	70.52 ± 3.14	6.27 ± 1.63	28 ± 20
7	1	1	0	1	19.8 ± 1.18	79.22 ± 4.7	10.70 ± 1.25	86 ± 42
8	-1	-1	0	-1	7.79 ± 0.16	77.53 ± 1.81	15.8 ± 3.19	36 ± 24
9	1	-1	-1	0	6.06 ± 0.18	60.18 ± 1.76	10.49 ± 3.26	162 ±124

*Note: LC= Loading capacity, EE= Encapsulation efficiency, AA= Antioxidant activity and SD= Standard deviation (Levels of the process variables are described in Table 1)

Loading Capacity and Encapsulation Efficiency

We scanned the supernatant of extracted microcapsules with UV-Vis spectrophotometry from 190 nm to 1100 nm. For both beeswax and carnauba wax as shell materials, the wavelength of maximum absorbance, λ_{max} was observed approximately at 327 nm, which is the peak wavelength for RP (Appendix B). Therefore, it became evident that RP remained unchanged throughout the production and extraction process, and no other species were formed in the process.

The encapsulation efficiency was more than 60% for all of the experiments. Statistically, the efficiency was not affected by the type of wax, theoretical loading%, surfactant%, and stirring speed. However, the actual loading capacity was significantly affected by the theoretical loading capacity, as expected (p-value= 0.00232, α=1%). From the applied statistical model (Appendix A), it can be predicted that for one unit increase in theoretical loading (%), the actual loading will increase by 0.72 %. The coefficient of determination i.e. , R^2 value of this model is 0.97, that means 97% of the variance in actual loading capacity (%) can be explained by the theoretical loading (%).

Within an optimum level of theoretical loading capacity, the active oil phase can be entrapped within the molten wax matrix to form microcapsules. Conversely, at a much higher level of theoretical loading, the amount of wall material would not be sufficient to encapsulate the core material, hence would practically result in unstable particles with poor actual loading. Barakat and Yassin (2006) attempted a theoretical loading of 50% to encapsulate carbamazepine using molten Precifac® ATO 5 (PRF), which led to aggregated and fragile particles, making them unsuitable for the purpose of drug delivery.

Antioxidant activity (DPPH assay)

In all of the experiments, the particles retained their antioxidant activity. The results of antioxidant activity varied in each experiment without a definite pattern; therefore, statistically, none of the process variables came out to be significant ($p>0.1$). However, the assay of DPPH free radical scavenging could be affected by the actual loading of RP in particles and solvent interactions. Further research needs to be completed in order to understand how antioxidant activity is affected throughout the process of production and extraction.

Size and Morphology

The morphology, size, and shape of the microcapsules produced with varying process parameters were analyzed from the SEM images of the particles, as illustrated in figure 3.4 and figure 3.5. The particles produced without any surfactant, irrespective to other factors, did not have definite size and shape and they often coalesced into large mass (Figure: 3.4 c, 3.4 d, 3.4 i and 3.5c, 3.5d, 3.5i). Consequently, the mean size is quite large for such particles, i.e., greater than 160 µm. (Table 2). A similar result was found in a study with ibuprofen drug microparticles using carnauba wax (Bodmeier et al., 1992). On the other hand, with the presence of surfactant, all of the rest of the experiments could produce particles with spherical shape and small size in micron scale. The reason behind this phenomenon is the role of surfactant in the lowering of interfacial tension and the partition of particles. The presence of surfactants induces a repulsive force among the droplets, hence prevents their agglomeration and promotes stabilization. The surface of the microcapsules appeared to be rough and porous, which might contribute to the leaching of the core over time.

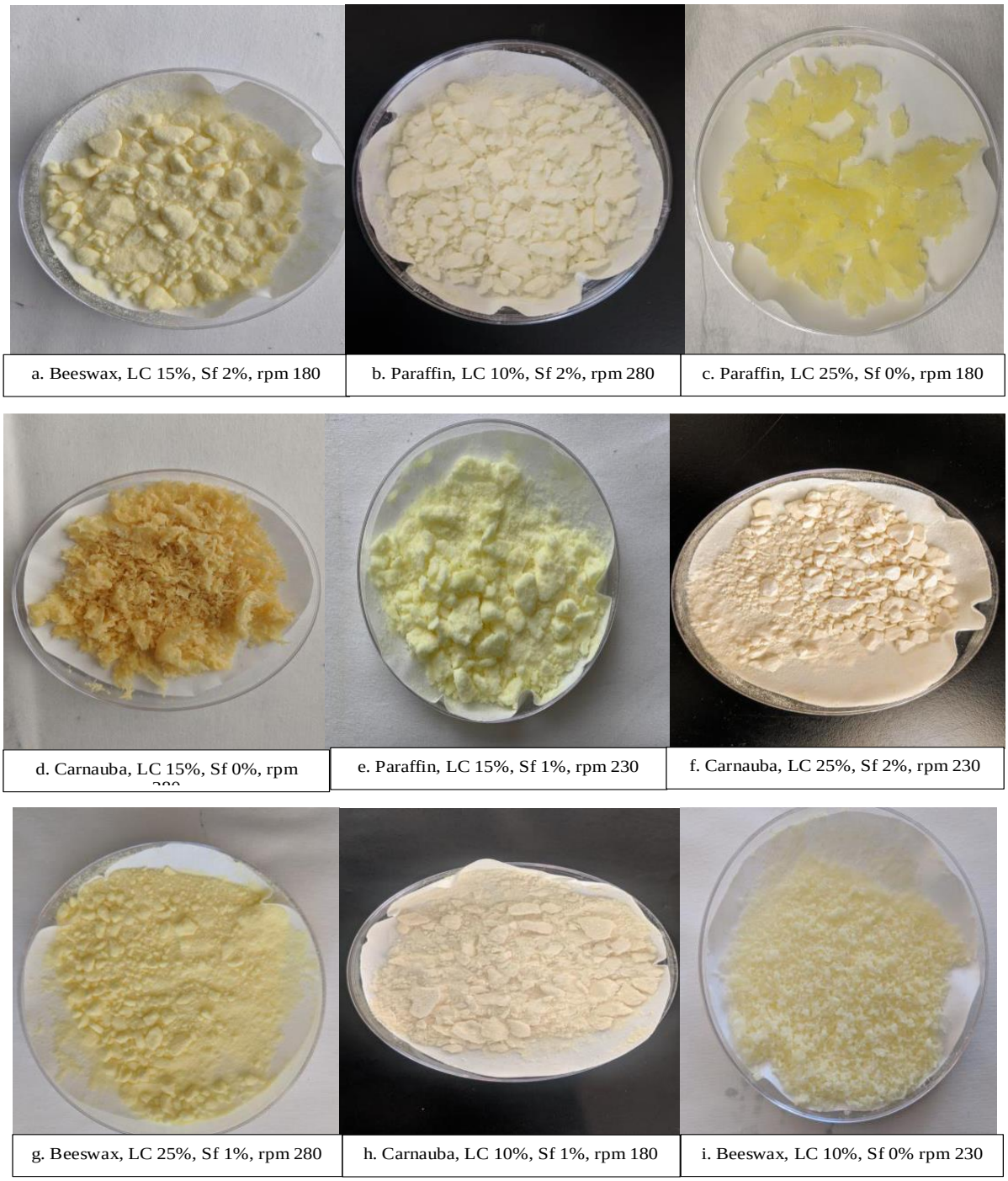
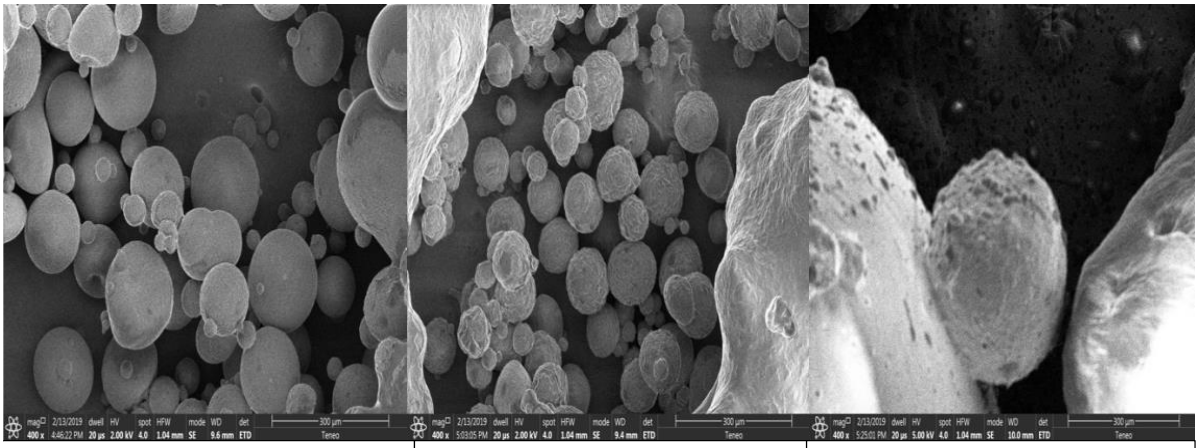
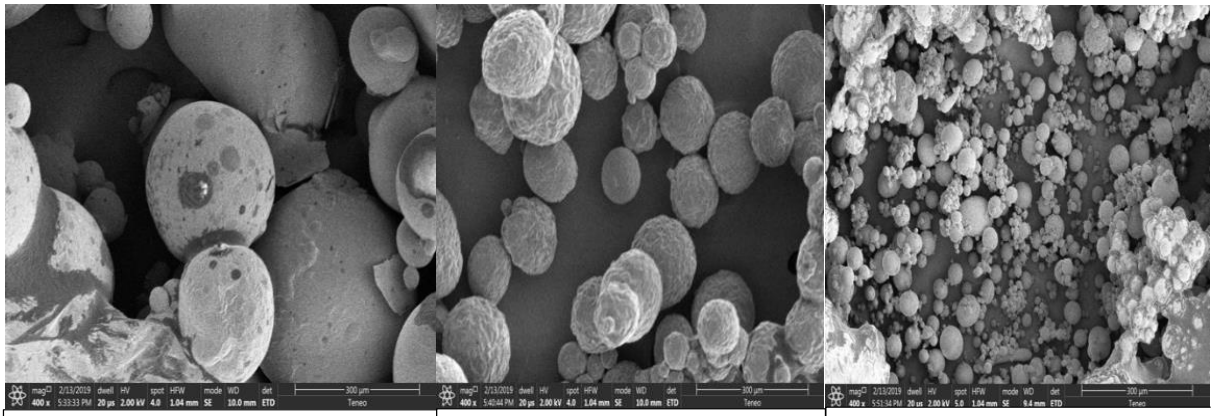


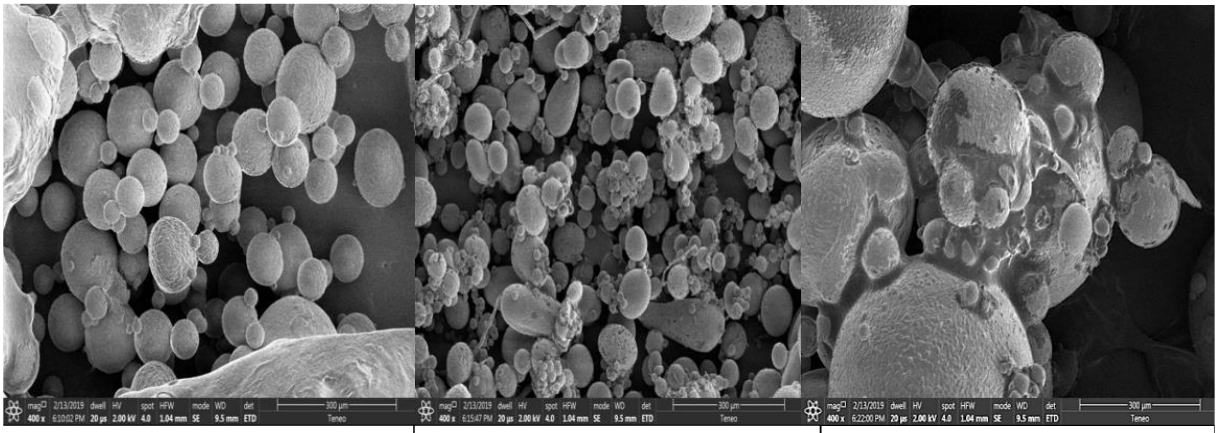
Figure 3.4: Images of microcapsules with varying process parameters



a. Beeswax, LC 15%, Sf 2%, rpm 180	b. Paraffin, LC 10%, Sf 2%, rpm 280	c. Paraffin, LC 25%, Sf 0%, rpm 180



d. Carnauba, LC 15%, Sf 0%, rpm 280	e. Paraffin, LC 15%, Sf 1%, rpm 230	f. Carnauba, LC 25%, Sf 2%, rpm 230



g. Beeswax, LC 25%, Sf 1%, rpm 280	h. Carnauba, LC 10%, Sf 1%, rpm 180	i. Beeswax, LC 10%, Sf 0% rpm 230

Figure 3.5: Morphology of produced microcapsules with varying process parameters

(400x magnification)

In the statistical analysis, it was found that the amount of surfactant significantly affects the size of microcapsules ($p= 0.0573$, $\alpha=10\%$), supporting the result from image analysis. This result is similar to another study (Ahlin et al., 1998), where solid lipid nanoparticles were prepared by the melt-emulsification process, and optimum surfactant concentration was found to be around 2-3%. From our applied statistical model (Appendix A), it can be predicted that for one unit increase in surfactant (%), the mean size of particles will decrease by 107.85 µm. The R^2 value of this model is 0.48 that means 48% of the variance in mean size can be predicted from the amount of surfactant (%). Here R^2 is not very high , which is consistent with the fact that the regression coefficient corresponding to surfactant is significant only at level alpha = 10% but not at 5%. The reason for this is due to the very large mean and standard deviation of size observed for experiment 3. In the absence of surfactant, agglomerated mass was produced instead of free flowing particles. As a result, this value acted as an outlier and affected the overall correlation between surfactant (%) and mean size.

The size distributions obtained for the experiments mostly showed wide distribution (Figure 3.6). Experiment 1, 6, and 9 showed right-skewed distribution, suggesting a small number of large particles. Experiment 2 and 8 showed approximately bell-shaped distribution with heavy tail (suggesting both small and large values are existing in large number) and thin tail (suggesting less values in the tails). The distribution for experiment 3 showed a mulimodal distribution, where some capsules were relatively small (150-400 µm) while some were very big (800-1200 µm). In the size distribution for experiment 4, a combination of two unimodal distributions were observed.. Experiment 5 showed a bimodal distribution, having two distinct modes between 100-150 µm. Particles formed from experiment 7 had approximately an uniform distribution. Overall, the distributions indicate that the produced particles are mostly inhomogeneous and non-uniform in

size, which is also evident with the high standard deviation of the mean size of particles. Djordjević et al. (2015) described the size of particles prepared by melt dispersion as non-uniform, corroborating with our results for most of the experiments. However, the most uniform distribution was achieved for experiment 7, where the 25% theoretical loading, 1% surfactant and stirring speed of 280 rpm were used as process parameter for producing beeswax microcapsules with size 20-140 µm.

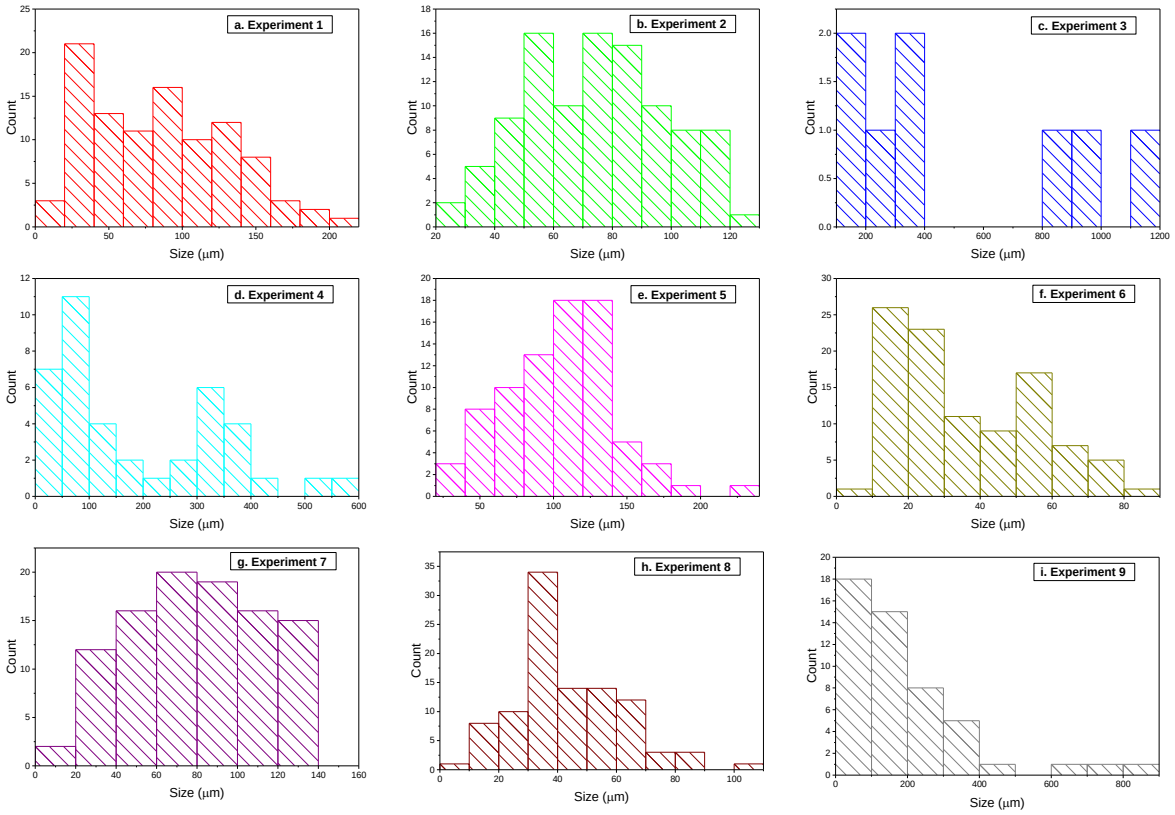


Figure 3.6: Size distribution of particles

Type of wax did not have a significant effect on the response variables ($p > 0.1$), despite the differences in their chemical composition. However, particles with carnauba wax as shell

material appeared to have smoother surface morphology (figure 3.5) , compared to those with beeswax and paraffin wax.

Stirring speed (within low-speed range ~300 rpm) showed no significant effect on the responses, including the size of particles ($p>0.1$). This result is unusual according to existing literature, specifically because higher speed should impart more force to break interfacial forces, resulting in smaller particles. Barakat and Yassin (2006) found a gradual decrease in average size when stirring speed was elevated from 400 rpm to 800 rpm. A similar result was found for a speed range of 900-100 rpm by Gowda and Shivakumar (2007) and 1000-1500 rpm by Milanovic et al. (2011). However, a plausible explanation for the outcome in our study can be that due to the low range of the levels of speed, the interaction with high surfactant concentration could overcome the limitation of low speed (such as 180 rpm). On the contrary, a comparatively higher speed (such as 280 rpm), being still in the low-speed range (<300 rpm), could not overcome the effect of zero surfactant concentration, that is, a high interfacial force. Hence, it can be concluded that with appropriate surfactant concentration, it is possible to attain good efficiency (>70%) with a mean particle size as small as 35µm even at the low speed level (Table 2) .

Conclusion

This study demonstrated that RP can be successfully encapsulated in wax matrix by using melt dispersion method. Theoretical loading capacity and surfactant% came out to be prominent factors affecting the actual loading capacity and mean size of particles, respectively. We concluded through the statistical analysis that the highest theoretical loading (25%) and highest surfactant (2%) in the study would provide us a high actual loading, hence high encapsulation efficiency and also a small mean size of the particles. In other words, these two factors can play a significant role

in controlling the loading and size of the capsules, based on the intended application such as cosmetic exfoliating scrubs, facial wipes, etc. By using melt dispersion technology, encapsulation efficiency of 60%-80% was achievable, where particle size distribution had high variation, ranging around 30µm- 300µm.

In today's world, when cosmeceuticals such as anti-aging products have become so popular and well accepted among the consumers, it is important to regulate the formulations of such products and systematize their characterization methods through scientific experiments. In this study, we could successfully encapsulate RP by melt dispersion method with small particle size and determined the significant process parameters by statistically analyzing the properties of particles. Further study is required to evaluate the shelf life, kinetic release mechanism over time, and application on substrates. Thus, this research makes a foundation not only to develop innovative cosmeceuticals but also will contribute to encouraging further empirical studies in the field of cosmeceuticals and cosmetotextiles.

Acknowledgment

This work was supported by the AATCC Foundation Student Research Support Grant 2019.

Declaration of interest

The authors declare no conflict of interest.

CHAPTER 4

EVALUATION OF STABILITY AND APPLICABILITY OF RETINYL PALMITATE LOADED BEESWAX MICROCAPSULES FOR COSMETIC USE[2]

[2] Aditi Nandy, Raha Saremi, Eliza Lee, Abhyuday Mandal, and Suraj Sharma. To be submitted to Journal of Microencapsulation.

Abstract

Retinyl palmitate was successfully encapsulated by melt dispersion using waxes as shell materials. In this paper, the study of evaluating the shelf life and kinetic release of the developed microcapsules was conducted by measuring actual loading capacity over a period of time using spectroscopic analysis. The transfer percentage of particles from non-woven facial wipes to skin-like surfaces was also investigated by simulating the rubbing mechanism with a robotic transfer replicator. Although particles stored as powder form under room temperature showed only 8 days of shelf-life, particles stored as dispersion in refrigerator maintained 60% of the theoretical loading capacity after 1 month. Kinetic release profile of the particle in ethanol with shaking at 100 rpm and 37 2ºC showed an initial burst in the first half an hour, followed by sustained release. 98% of the retinyl palmitate content released within 4 hours. Particles incorporated into wet non-woven wipes showed approximately 22% transfer to skin-like fabric, indicating potential of providing skin care properties by means of capsule loaded textile substrates.

Introduction

In cosmetic science and technology, retinoids are widely recognized to address skin concerns such as acne, rosacea, pigmentation and symptoms of photoaging (Baumann, 2009). Retinoids are chemical compounds of vitamin A, which include retinoic acid, retinal, retinol and retinol derivatives. Retinoic acid has been well researched and found to be effective as topical treatment for photoaging, hyperpigmentation, wrinkles and dry skin (Kligman et al., 1984, Connor and Lowe, 1985, Elias and Williams, 1985, Haas and Amdt, 1986). However, many patients suffer from retinoid dermatitis as side effect of aggressive reaction of retinoic acid (Barel et al., 2014). Therefore, researchers have been studying retinol and its derivatives for cosmetic application to impart the benefits by minimizing the irritation on skin (Mordon et al., 2004, Bertin et al., 2008, Watson et al., 2008, Song et al., 1999). After being topically absorbed by the skin, retinol, retinal and their derivatives need to enzymatically convert into a biologically active form i.e., retinoic acid through oxidative processes (Figure 2.4) (Kurlandsky et al., 1994).

Many studies revealed that topically applied retinoids including retinyl palmitate (a lipophilic, ester derivative of retinol) are effective in skin penetration, percutaneous absorption, metabolization to retinol and retinoic acid, and skin treatment. (Boehnlein et al., 1994, Bailly et al., 1998, Antille et al., 2003, Abdulmajed and Heard, 2004, Kang et al., 1995, Duell et al., 1996, Tsunoda and Takabayashi, 1995, Vahlquist, 1999). Still, instability has been a challenge to incorporate retinoids into cosmetics due to oxidation of retinol over time and sensitivity to heat and light (Clarys and Barel, 2009, Andersson et al., 1999). Microencapsulation can solve this problem by protecting active ingredients from reactive compounds in formulations as well as releasing them when applied on to the skin (Hawkins et al., 2005). Retinoids has been reported to be successfully encapsulated in albumin by emulsion method (Torrado et al., 1992), in glyceryl

behenate using solid lipid nanoparticles (Jenning et al., 2000), in chitosan using ultrasonication (Kim et al., 2006), and in maltodextrin /modified starches using spray drying method (Gangurde and Amin, 2017) , specifically intended for cosmetic formulations.

In order to assess the quality of topical products containing active substances, the testing includes assay, content uniformity analysis, impurities, pH, water content, preservative content, particle size etc. (Ueda et al., 2009). Another study (Gangurde and Amin, 2017) described separation of oil/water phases, change in color, consistency of formulation and development of unpleasant odor and active content percentage as parameters of the stability study of vitamin A palmitate microcapsules. In our study, we evaluated the visual change in color and retention of retinyl palmitate content to understand the stability, hence shelf life of produced microcapsules.

In vitro kinetic release studies are performed in order to understand the release rate of active ingredients in the body and also to better understand the storage stability. The mechanism of controlled release of active ingredients can be broadly categorized into two types: physical and chemical. According to Acharya and Park (2006), the physical mechanisms may involve diffusion of the active substance through polymer matrix, degradation or dissolution of the polymer layer, osmotic pressure or use of ion exchange in the instance of ionized drugs. On the contrary, chemical mechanism involves alteration of active molecules (Petrusic and Koncar, 2016). In case of waxy materials as matrix components, the most significant mechanisms of release are diffusion of active core through the matrix and erosion of wax matrix through ester hydrolysis reaction (Djordjević et al., 2015).

Topically applied active ingredients are often incorporated into a carrier such as creams, gels or textile substrates to ensure targeted trans-dermal delivery. Textile-based substrates as delivery vehicle have their benefits due to flexibility and ease of application (Petrusic and Koncar,

2016). Moreover, the open permeable structure as well as large surface area make textile structure an ideal support for topical delivery of active ingredients (Nierstrasz, 2007). Therefore, we aim to explore non-woven facial wipes as means to incorporate microcapsules containing retinoids and evaluate the transfer of microparticles from substrate to skin-like material.

In our previous study, we successfully encapsulated retinyl palmitate using waxes as shell material. The overall objective of our present study is to evaluate the shelf life and kinetic release of the developed microparticles by measuring the loaded content of retinyl palmitate over time and also to investigate simulated transfer of microparticles from wet non-woven substrate to skin-like fabric by using robotic transfer replicator.

Materials and methods

Materials

Refined, white, beeswax pearls and retinyl palmitate (vitamin A) of 1.7 M.I.U./g. were purchased from Bulk Apothecary (Aurora, OH) and Fisher Scientific USA (Pittsburg, PA) respectively. Ethanol was obtained from Decon Labs, Inc., (King of Prussia, PA). Pampers aqua pure™ nonwoven wipes were purchased from Kroger (Athens, GA), while compression fabric (warp knit : 77% nylon and 23% spandex) was obtained from the Marena Group (Lawrenceville, GA).

Microencapsulation of retinyl palmitate by melt dispersion

In our previous study, we microencapsulated retinyl palmitate (RP) by melt dispersion technique and investigated the effect of process variables on the produced microcapsules. An oil-in-water emulsion method was applied, where water with surfactants was heated and stirred by

overhead stirrer and molten mixture of wax and RP was dispersed in the emulsion. Pouring ice-cold water enabled solidification of wax droplets, resulting in free-flowing particles upon filtration and drying (figure 3.1).

We investigated the effects of four process variables at three levels such as different theoretical loading capacity (10%, 15%, 25%), types of wax (beeswax, carnauba wax, paraffin wax), emulsifier concentrations (0%, 1%, 2%) and stirring speeds (180, 230, 280 rpm), using a design of experiment named definitive screening method. The characterization results were compared across all variables to investigate the efficacy in terms of size and morphology using scanning electron microscope (SEM), actual loading capacity and encapsulation efficiency using ultraviolet-visible spectrophotometry, and antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

In the statistical analysis of that study, theoretical loading capacity and surfactant% were found be the most significant factors that affected the actual loading capacity and mean size of particles, respectively. Therefore, we were able to determine that the highest theoretical loading (25%) and highest surfactant (2%) selected in that study can provide us high actual loading with small mean size of the particles. Encapsulation efficiency of 60%-80% was achievable, where particle size distribution ranged from 30µm- 300µm, with high variation among them.

Although we investigated three waxes: beeswax, carnauba wax and paraffin wax in order to compare their performance as shell material, there was no significant difference found among their effects on loading capacity, encapsulation efficiency, antioxidant activity or mean size of particles.

We decided to conduct further study selecting beeswax as shell material because of its natural skin-care benefits as well as operational convenience due to low melting point (65ºC).

Beeswax has anti-inflammatory and antimicrobial properties, suitable for topical treatment (Al-Waili, 2003, Gans et al., 1986). Besides, beeswax is also efficient to improve the barrier function of the skin (Teichmann et al., 2006). Theoretical loading of 25% and 2% surfactant were chosen based on the result obtained from our previous study. We selected 280 rpm (highest level chosen in our previous study) as stirring speed to facilitate dispersion of the oil-in-water emulsion and formation of small size particles.

Thermal characterization by Differential Scanning Calorimetry (DSC)

Thermal analysis of the beeswax, retinyl palmitate (RP) and RP-loaded beeswax microcapsules were carried out by using Mettler Toledo DSC821e instrument, where a standard empty aluminium pan was used as the reference. The weight of sample were within 2-9 milligrams and Samples were scanned from 25ºC to 100ºC under N_2 atmosphere with a heating rate of 10ºC/min.

Shelf life study

After preparing the microcapsules with 25% theoretical loading, we looked into the shelf life of microcapsules by measuring the actual loading percentage i.e., the content of retinyl palmitate in a fixed amount of capsules over a period of time. 0.1 g of microcapsules were extracted by heating in 20 ml of ethanol solution and the concentration of supernatant aliquots were measured at 327 nm by ultraviolet-visible spectrophotometer. The amount of retinyl palmitate was determined from a standard curve of known concentrations.

We evaluated the shelf life of the beeswax microcapsules with approximately 71% encapsulation efficiency stored in powder form in a enclosed petri dish, when they were filtered

and dried before storing in room temperature; and also in dispersed form (approximately 75% encapsulation efficiency), when the particles were stored as dispersion inside dark vials in refrigerator and a portion was filtered on each day of measurement (Day 1, Day 4, Day 8, Day 15 and Day 31). We also investigated the shelf life of carnauba wax particles (approximately 64% encapsulation efficiency) in powder form to compare with that of beeswax to examine if a harder wax like carnauba wax can provide better shelf life.

Kinetic Release study

We conducted an in vitro kinetic release study similar to prior literature (Borodina et al., 2011, Gangurde and Amin, 2017), with some modification based on particle content, solvent type and machine parameters.. The retinyl palmitate release profile from 3g of suspended particles (approximately 77% encapsulation efficiency) was examined in 600 ml of pure ethanol. The study was performed in a New Brunswick C24 incubator shaker (figure 4.1) with a speed of 100 rpm and temperature set at 37± 2 ºC. Supernatant aliquots of 2 mL were withdrawn and replaced by fresh medium at appropriate time intervals (1 min, 5 min, 15 min, 30 min, 1 hour, 2 hour, 4hour). The supernatants containing dissolved retinyl palmitate were diluted and analyzed by UV-vis spectroscopy at 327 nm. The results were compared with a standard to calculate the vitamin A concentration and to evaluate the release ratio. All experiments were conducted in duplicates.

Figure 4.1: Kinetic release study of microparticles suspended in ethanol (using New Brunswick C24 incubator shaker at 100 rpm and 37 ± 2 ºC)

Simulated transfer study from textile substrate to skin

We used a robotic transfer replicator (figure 4.2) to simulate the transfer of microparticles from a non-woven wipe to skin and evaluate the transfer percentage, by means of a similar method as described by Yu et al. (2018). 1 g of microparticle were spread as evenly as possible by a spatula over a nonwoven wipe containing 99% water that acted as a donor surface with a diameter of 133 mm. The receptor material was a compression fabric, i.e., a warp knit with composition of 77% nylon/23% spandex. This fabric was chosen because Yu et al. (2018) revealed that this fabric matched the human skin best, in terms of transferring particulates. The receptor fabric was attached to an aluminum nose piece with the help of O-ring made of rubber. After the activation of the replicator, the nose piece descended the receptor fabric onto the donor surface and rubbed the

receptor against the donor by executing a certain number of motions (imitating a hourglass pattern) under a constant pressure maintained by programmed hydraulic system. After performing this rub cycle, the nose piece was raised and delivered onto a glass jar containing 20 ml of ethanol. The fabric was released into ethanol and shaken vigorously followed by sonication for 2 hours, so that all the particle content is released into ethanol. Then aliquots were removed for assay in ultraviolet-visible spectrophotometer to measure the content of retinyl palmitate. Finally, amount of transfer of retinyl palmitate was calculated in percentage. The experiment was performed in duplicates.

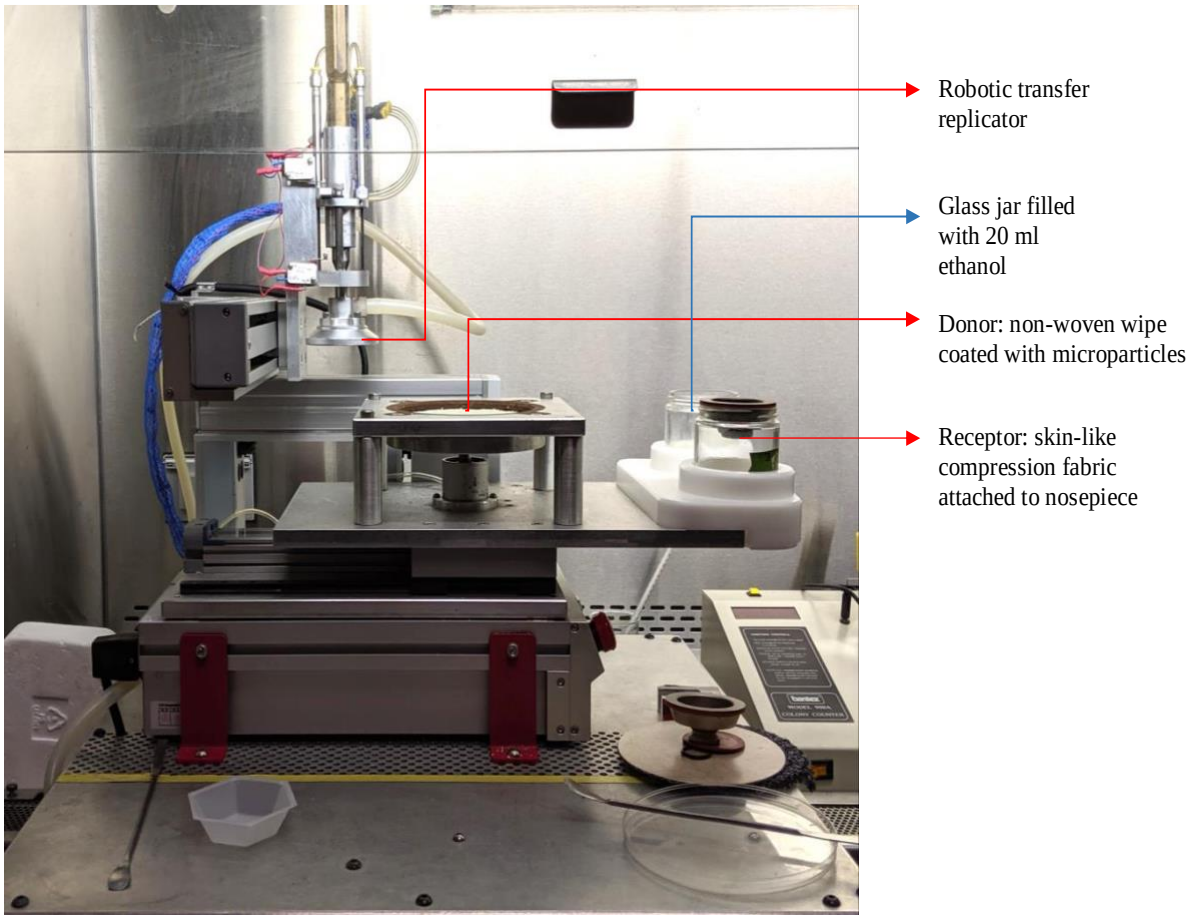


Figure 4.2: Robotic transfer replicator for simulating transfer of microparticles from non-woven wipe to skin-like fabric

Result and discussion

Thermal Analysis

Differential scanning calorimetry (DSC) scans of bees wax, retinyl palmitate , and beeswax microcapsules with 25% theoretical loading capacity are shown in figure 4.3. In the thermogram of retinyl palmitate, a sharp endothermic peak is observed at 34.33 °C, which corresponds to its melting point. However, it is observed that the microcapsules show no endotherms corresponding to the melting point of retinyl palmitate. This implies that retinyl palmitate dissolved within the matrix of beeswax when the temperature reached its melting point (Carlotti et al., 2005). This observation was consistent with the result found by Milanovic et al.(2010) for encapsulated ethyl vanillin in carnauba wax.

Untreated beeswax and RP-loaded beeswax microcapsules show their melting peaks at 65.67 °C and 63°C, respectively. The slight decrease in the melting point of particle should be due to the small particles with greater specific surface area, which requires less heat flow to melt than that of larger crystals of wax (Pople and Singh, 2006).

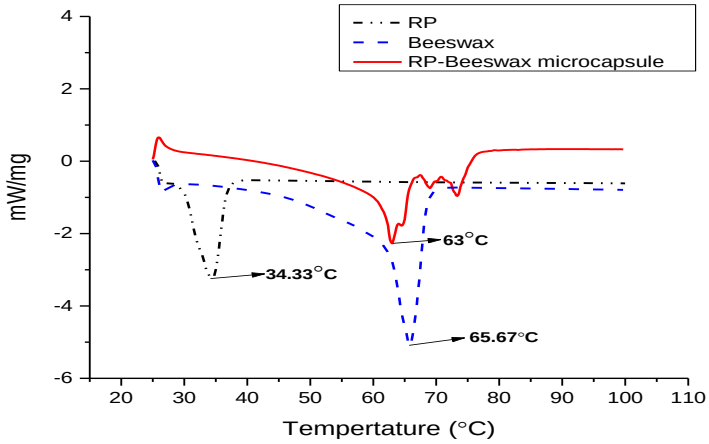


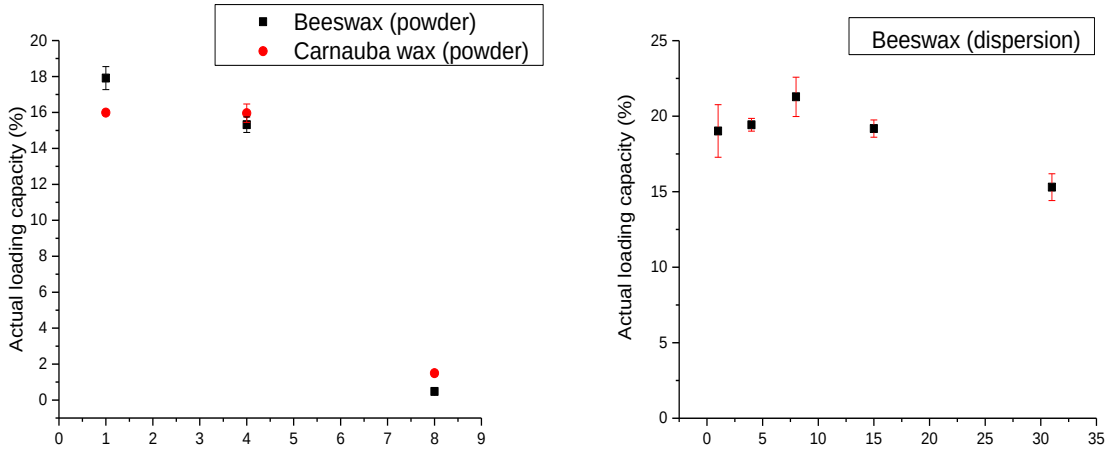
Figure 4.3: DSC thermogram of untreated beeswax, RP and RP-loaded beeswax microcapsules

Untreated beeswax and RP-loaded beeswax microcapsules show their melting peaks at 65.67 °C and 63°C, respectively. The slight decrease in the melting point of particle should be due

to the small particle size with greater surface area, less heat flow is required than that of larger crystals to melt (Pople and Singh, 2006).

Shelf Life

Figure 4.4 shows the comparative shelf life study of the particles in 3 conditions (a) beeswax-shell in powder form stored under room temperature (b) carnauba wax in powder form stored under room temperature (c) bees-wax shell, stored in dispersion in refrigerator.



(a) (b)

Figure 4.4: Shelf life study of (a) RP loaded beeswax and carnauba wax microparticles stored as powder under room temperature (b) RP loaded beeswax microparticles stored as a dispersion in refrigerator

When the particles were evaluated in powder form under room temperature, in both cases of beeswax and carnauba wax as shell materials, the particles lost their active content within 8 days. This phenomenon can be attributed to the diffusion of retinyl palmitate through the wax shell. The high compatibility between lipophilic, low molecular weight active substances with wax is the major cause of diffusion (Mishra, 2016). Diffusion can be accelerated in small sized particles due

to availability of larger contact areas as well as due to pores existing in the shell matrix (Deasy, 1984).

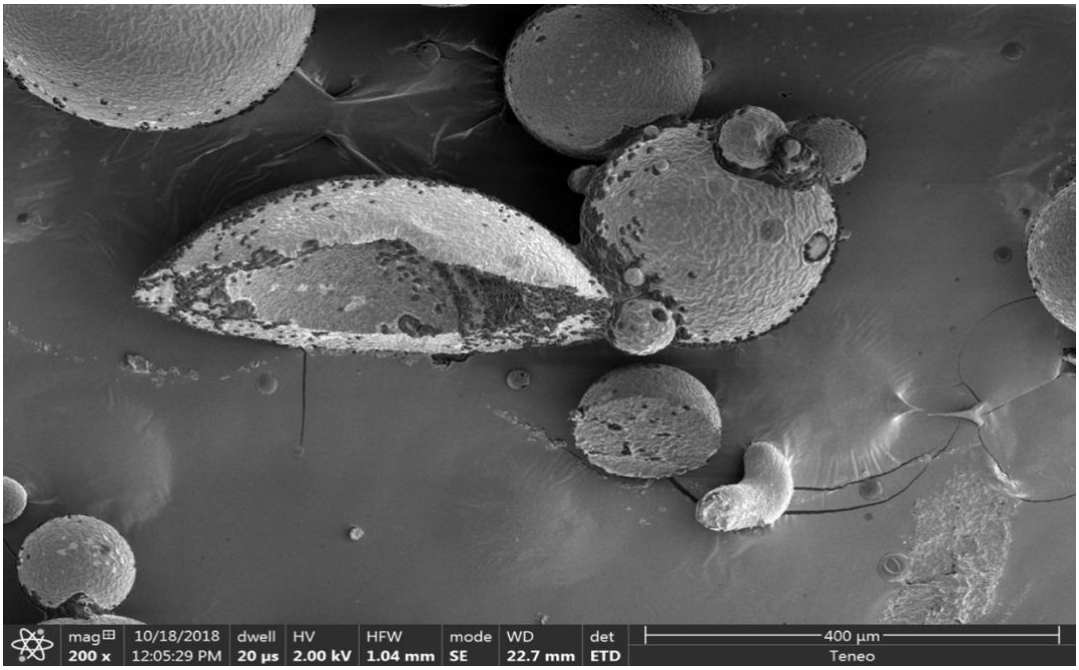


Figure 4.5: SEM image of RP-loaded beeswax microcapsules (cut) shows both hollow shell-core and matrix morphology

Djordjević et al.(2015) described internal structure of particles produced by melt dispersion with wax shell to be non-homogeneous with matrix or hollow-shell morphology. From the electron microscopy of cut microcapsules (figure 4.5) , we found evidence that the capsules are mostly matrix type, with a few with hollow morphology, where the size is comparatively bigger. Therefore the core retinyl palmitate is distributed within the shell. By the course of time, the core

content comes up to the surface and diffuse through the shell. From figure 4.6 a, the gradual change in color of beeswax microcapsules supports this claim. The particles stored as powder form appear to be bright yellow after the retinyl palmitate diffuses to the surfaces and they turn white (beeswax) when almost all of core content leaches out. For carnauba wax (figure 4.6 b), this change is not visually perceivable due to inherent pale yellow color of the wax.

On the other hand, when the particles were stored in dispersed aqueous emulsion in refrigerator, they retained the core material and showed no significant decrease until the 15^{th} day. The variability in size distribution of different batches of filtered particles may account for the slight increase observed in actual loading capacity. After 30 days, a decrease in loading was observed, which can be explained by ester hydrolysis of the beeswax while stored in aqueous emulsion resulting in release of the content (Kheradmandnia et al., 2010). The filtered particles did not show significant visual difference in color (Figure 4.6c).

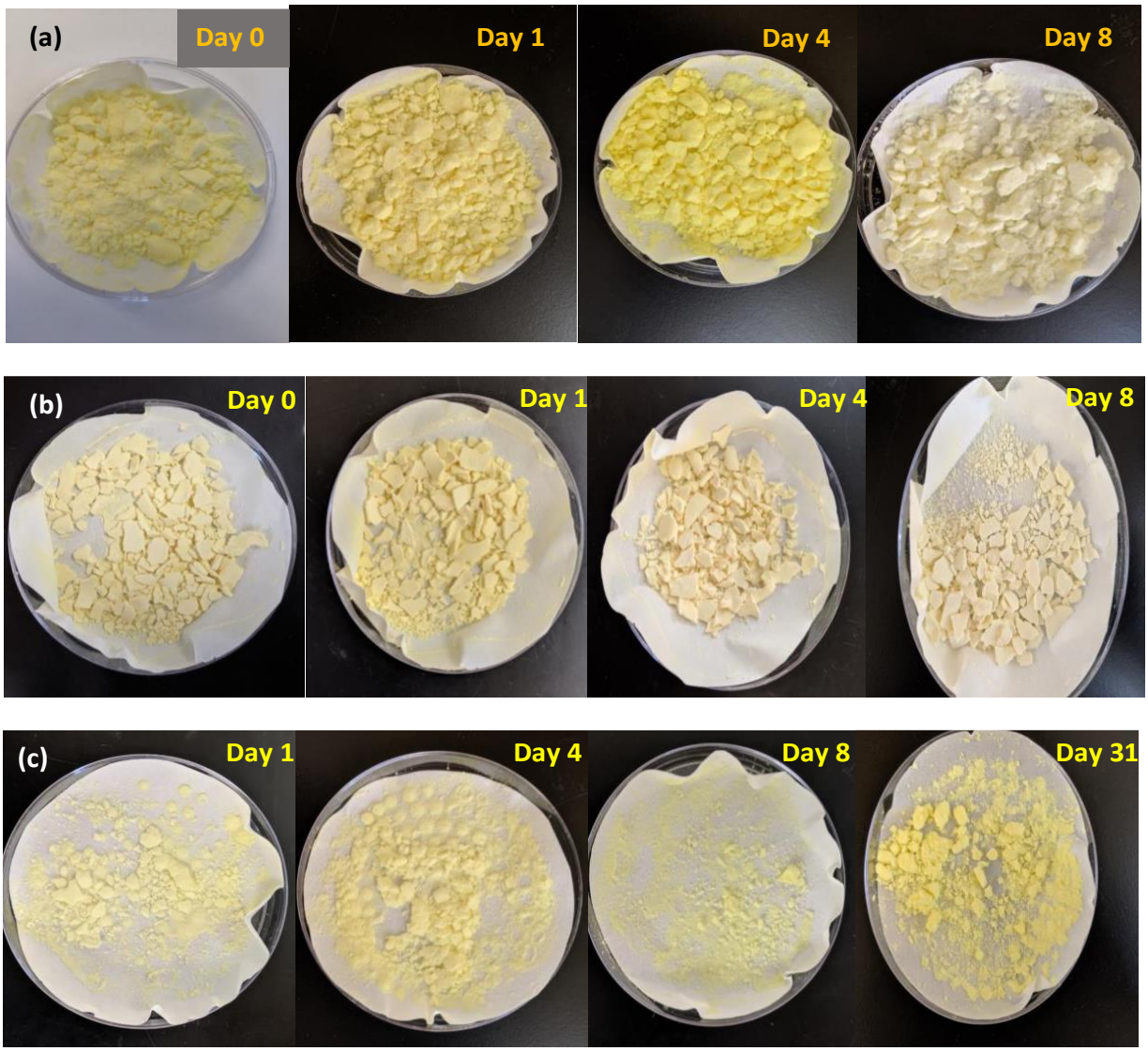


Figure 4.6: a) beeswax microparticles and b) carnauba wax microparticles c) beeswax microparticles stored in dispersion, containing 25% theoretical loading of retinyl palmitate (Note: untreated beeswax was white and carnauba wax was pale yellow in color)

Kinetic Release study

The release profile (figure 4.7) of retinyl palmitate-beeswax microcapsule was showed an initial burst followed by a slower release of the vitamin entrapped inside the beeswax matrix. Due to the initial burst effect, 7% of the retinyl palmitate released at the first minute, leading to around 55% release in the first half an hour. After the burst effect, the release profile showed a sustained release over time. Within 4 hours, approximately 98% of retinyl palmitate was released. Similar pattern of release was found by Kheradmandnia et al. (2010) from ketoprofen-loaded solid lipid nanoparticles incorporated in matrix of beeswax-carnauba wax mixture. Zigoneanu et al. (2008) described the phenomenon of such initial burst as the result of cumulative effect of diffusion of core through the matrix, penetration of dissolution medium into the particle and degradation of the shell matrix. As retinyl palmitate is soluble in ethanol, this explanation is mostly agreeable to our result. Permeation of ethanol through the pores of the shell matrix and simultaneous diffusion of retinyl palmitate through the matrix facilitated the fast dissolution of the vitamin into ethanol.

From this result, we can understand that alcohol based cosmetic formulations will not be stable over time as the core content would be released in the carrier substrate during storage period, making RP susceptible to oxidation and degradation. On the contrary, as we already observed in the shelf life study, an aqueous medium prevents the active content to release from the capsule because of having no affinity to the lipophilic content. As a result, a water-based formulation would be suitable to contain the particles for cosmetic application.

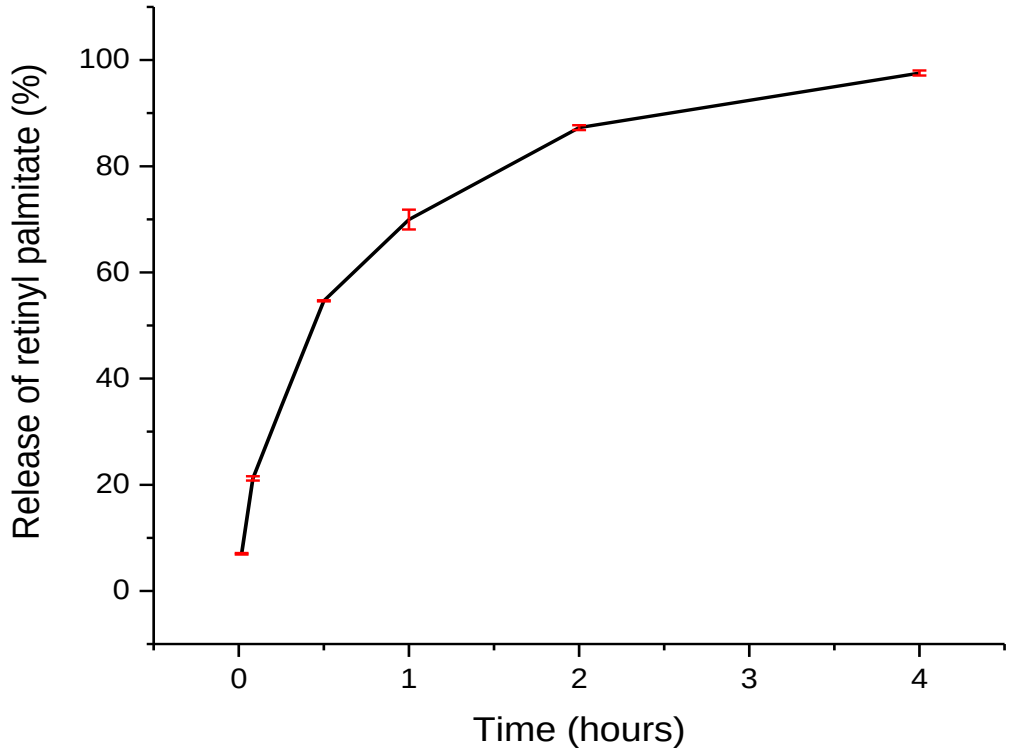


Figure 4.7: Kinetic release profile of RP-loaded beeswax particles suspended in ethanol

Simulated transfer study from textile substrate to skin

From the transfer study, we found that 21.7± 0.02 % of retinyl palmitate was transferred to the receptor material from the donor surface of wet non-woven wipe after the rubbing cycle. Although this amount may vary depending on encapsulation efficiency, method of particle incorporation and the amount of particle incorporated , this study demonstrates the potential of using such particles into facial wipes to impart skin-care properties. Considering the approved doses of retinoids, absorption and conversion rate of retinyl palmitate to retinoic acid within the skin, a proper formulation has to be developed in further study.

Conclusion

The ongoing demand for functional cosmetics has encouraged the cosmetic industry as well as researchers to explore novel delivery methods and test for efficacy of the products. This study contributes to the study of stability, release profile and potentiality of incorporating retinyl palmitate-beeswax microcapsules as a mean to transfer active ingredient to skin. From this study, it was determined that ethanol would accelerate the release of the content, whether water-based formulations would preserve the stability of the capsules better than powdered form. Further study on release in different mediums and storage conditions will help develop innovative cosmeceutical and cosmetotextile products containing retinyl palmitate.

Acknowledgment

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Declaration of interest

The authors declare no conflict of interest

CHAPTER FIVE

APPLICATION OF RETINYL PALMITATE LOADED MICROCAPSULES AND FUTURE STUDIES

Application

The use of disposable non-woven moisturizing facial wipes and sheet masks have been growing in the recent years. They are soaked with water based, oil-based or emulsion-based lotion or serum. As we showed in our study, retinyl palmitate-loaded microcapsules can be incorporated into water-based non-woven wipes to impart functionality. However, in case of sheet masks, a suitable formulation and system would be required to trigger release of active ingredient as use of sheet masks usually do not involve a rubbing action.

Microcapsules can be incorporated into textile substrate by means of coating, impregnation or immersion, spraying or printing (Bojana and Marica, 2016). Figure 5.1 shows a non-woven facial wipe coated with retinyl palmitate loaded beeswax microcapsules using a micro-coater. This implies that even distribution of microparticles is possible by spreading them with suitable method.

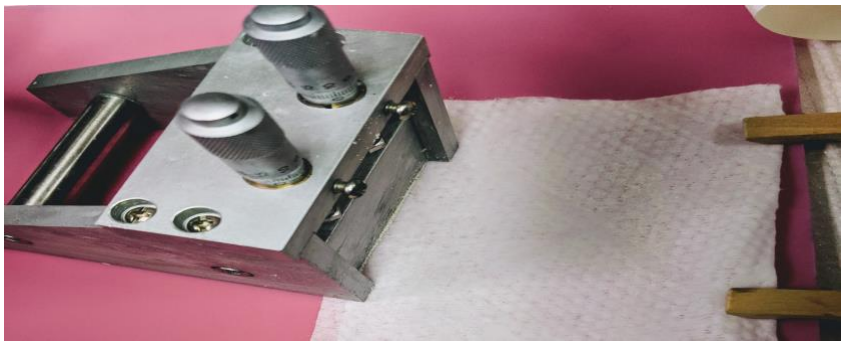

Figure 5.1: Micro-coating of particles on a facial wipe

Another potential application can be facial scrub agents, which are effective to exfoliate skin by getting rid of superficial layer of dead skin and dirt through mechanical abrasion i.e., rubbing or massaging. These scrub agents can originate from either natural substances e.g., clay, seeds, fragments of vegetable shells or chemically manufactured beads e.g. polyethylene or styrene (Zocchi, 2009). Chemical synthesis and disposal of plastic microbeads are certainly not environment friendly, rather they impose a threat to the aquatic habitats (Rochman et al., 2015). Vitamin loaded wax particles can be a great natural alternative which not only can provide enough texture to scrub the dead skin, and also can impart benefits of the vitamin as well as natural wax during massaging. For this particular cosmetic application, the preparation process can be customized by decreasing the surfactant amount to produce larger size of particles. Beeswax, carnauba wax or a combination of such natural waxes can be used to encapsulate the active ingredient and formulate facial scrubs.

Retinyl palmitate can also be incorporated into acne patches, creams or sunscreens. Although there has been a speculation based on a 2010 report by Environmental Working Group on sunscreen containing retinyl palmitate that they might be photocarcinogenic, a study by Wang et al. (2010) disapproved this claim. They stressed on the clinical evidence of retinyl palmitate being beneficial to skin and rather being chemo-preventive. Retinyl palmitate has poor solubility in glycerol ($C_3H_8O_3$), which is also used topically for improving elasticity, barrier repair and hydration of skin (Fluhr et al., 2008). Therefore, glycerol-based system can be further studied for cosmetic formulations.

Other vitamins can also be microencapsulated by the similar procedure. However, long chain esters existing in waxes are susceptible to ester hydrolysis catalyzed by acids , which may induce active release affecting long-term stability (Kheradmandnia et al., 2010). Therefore, a

hydrophilic active ingredient e.g., vitamin C might not be a suitable candidate for water-based system as the release rate would be faster than those with lipophilicity.

Future Studies

In our study, the shelf life of beeswax microcapsules as powdered form was not satisfactory (8 days) . Bodmeier et al. (1992), in their study on process variables for preparation of ibuprofen loaded- wax microparticles, deducted cooling rate to be an important factor as it could affect the crystallinity of wax. Whether the wax crystallize in hexagonal system or in denser orthorhombic system can influence the permeability, because hexagonally-packed molecules would have more rotational freedom due to increased volume(Small, 1986). On the other hand, waxes crystallized in an orthorhombic system would make them harder and less malleable, decreasing their permeability (Donhowe and Fennema, 1993).

If the cooling mechanism of the resulting microparticles are not thermodynamically favourable, the active ingredient recrystallizes during storage that affects its dissolution (Keen et al., 2013). Therefore, in order to improve the shelf life, cooling rate during solidification can be further studied as a process parameter. Moreover, as kinetics of recrystallization is a temperature-dependent phenomenon, storage temperature can also be taken into consideration to evaluate its effect on the stability of microcapsules over time.

Another important study should be regarding the antioxidant activity of the prepared microcapsules. In our study, no significant effect of the four process variables on the antioxidant activity was found. A kinetic study of the antioxidant activity should be carried out to better understand the variation in antioxidant activity. Moreover, method of evaluation should also be well-studied, addressing solvent interaction, ambient conditions etc.

Furthermore, suitable method of coating should be investigated to coat textile substrates with vitamin A palmitate microcapsules. Although we used micro-coating or direct application of the particles onto the substrate, they are not effective means of large scale industrial application. Hence, immersion or spray coating can be investigated in future studies.

Diffusion of RP from microcapsules in other mediums such as oil, silicon and common cosmetic ingredients should be tested to have proper understand of suitable cosmetic formulation. Nonetheless, appropriate dosage of the microcapsules should also be identified for any cosmetic formulation , agreeing to the recommended dosage for topical application.

Finally, study can be extended to evaluate the actual result through human subjects, who can use the formulated prototype of product for a short period of time and results can be evaluated from their feedback as well as effect of skin.

CHAPTER SIX

CONCLUSION

Functional cosmetic products has become an ever-growing market in recent years and the industry is becoming competitive to supply superior cosmetic benefits to keep pace with the demand of consumers. Hence, both improved technology and cost reduction become important to produce novel cosmetic products. In our study, we employed an inexpensive technology i.e., microencapsulation by melt dispersion, which can successfully encapsulate retinyl palmitate with desired small particle size and encapsulation efficiency. This study also addressed significant process parameters by statistically analyzing the properties of particles. Evaluating the shelf life, kinetic release mechanism over time, and transfer study of particles from non-woven substrate to skin showed potential to develop cosmeceutical product containing retinyl palmitate. Future study can be carried out for further investigation into processing and storage conditions in order to improve the shelf life of particles. Besides, research on preparing cosmetic formulation and exploring suitable method to incorporate microparticles to textile substrate will also enrich this study. Nonetheless, encapsulation of other active ingredients with this method can also to investigated. Thus, this research makes a foundation not only to develop innovative cosmeceuticals but also will contribute to encouraging further empirical studies in the field of cosmeceuticals and cosmetotextiles.

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APPENDICES

Appendix A: Output of statistical analysis

Fitted model for actual loading:

We fit a model with wax, Theoretical loading, surfactant and speed as predictor variables for the actual loading and the ANOVA output table is given as follows:

	Df	Sum Sq	Mean Sq	F value	p-values
factor(Wax)	2	0.14	0.07	0.036	0.96516
Loading	**1**	**184.17**	**184.17**	**94.181**	**0.00232**
Surfactant	1	0.05	0.05	0.027	0.88053
Speed	1	2.24	2.24	1.144	0.36329
Residuals	3	5.87	1.96		

Only theoretical loading comes out to be significant. Hence, the following model can be used for prediction.

$$\text{Predicted value} = -2.7 + 0.7 \text{ Loading}, R^2 = 0.97$$

When we fitted a similar model to efficiency and antioxidant activity, we did not find any significant effect.

Fitted model for efficiency:

```
> g = lm(y2~factor(Wax)+Loading+Surfactant+Speed)
> summary(g)$r.squared
[1] 0.3193093
```

> summary(aov(g))

	Df	Sum Sq	Mean Sq	F value	p-values
factor(Wax)	2	56.40	28.20	0.324	0.746
Loading	1	0.02	0.02	0.000	0.990
Surfactant	1	13.23	13.23	0.152	0.723
Speed	1	52.81	52.81	0.607	0.493
Residuals	3	261.05	87.02		

Fitted model for antioxidant activity:

> g = lm(y3~factor(Wax)+Loading+Surfactant+Speed)

> summary(g)$r.squared

[1] 0.5764284

> summary(aov(g))

	Df	SumSq	Mean Sq	F value	p-values
factor(Wax)	2	2.01	1.00	0.029	0.971
Loading	1	14.27	14.27	0.418	0.564
Surfactant	1	51.16	51.16	1.498	0.308
Speed	1	71.97	71.97	2.108	0.242
Residuals	3	102.44	34.15		

***Fitted model for antioxidant size**:*

	Df	Sum Sq	Mean Sq	F value	p-values
factor(Wax)	2	27646	13823	1.792	0.3076
Loading	1	15311	15311	1.985	0.2536
Surfactant	**1**	**69785**	**69785**	**9.047**	**0.0573**
Speed	1	8528	8528	1.106	0.3703
Residuals	3	23142	7714		

Only surfactant comes out to be significant, that too at level $\alpha = 10\%$, and hence the following model can be used for prediction.

Fitted model for Size

$$\text{Predicted value} = 249.5 - 107.9 \text{ Surfactant}, R^2 = 0.48$$

Summary Recommendation

Significant Factors	Actual Loading	Size
Theoretical Loading	High value will increase the response	
Surfactant		High value will decrease the response

Appendix B: Absorption spectra of retinyl palmitate

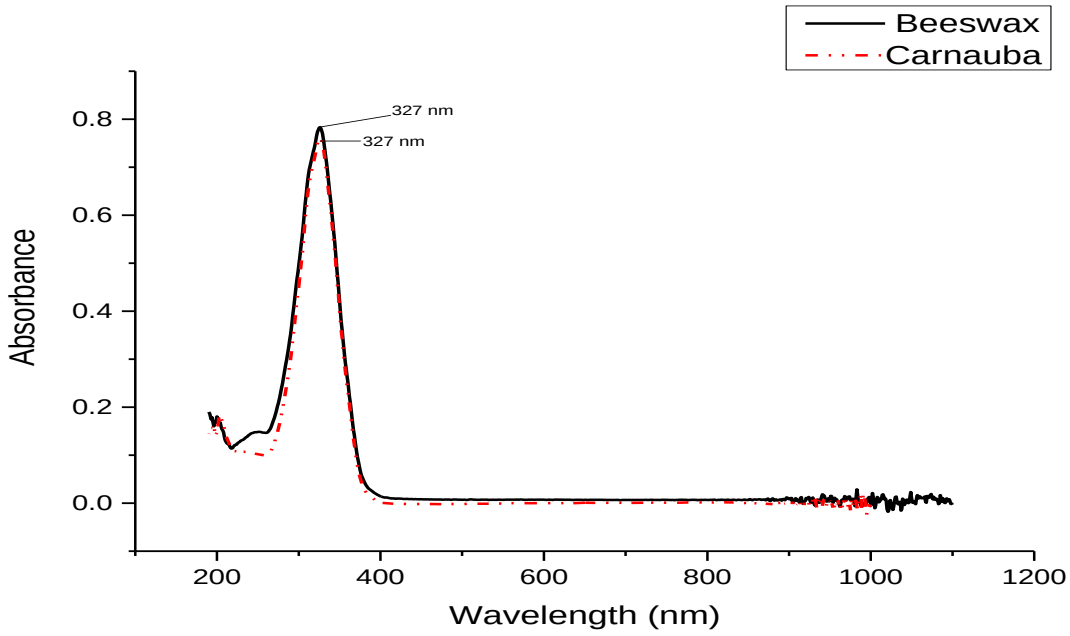

Figure B.1 : Vitamin A palmitate showing peak close to 327 nm