Technological use of beeswax for obtaining organic products, non-toxic for the human being

Aprovechamiento tecnológico de la cera de abeja para la obtención de productos orgánicos, no tóxicos para el ser humano

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Abstract

An ointment is a semisolid preparation consisting of a single external phase in which liquid or solid substance(s) can be dispersed. In the case of beeswax ointments, there are several studies that confirm their antibiotic and healing power over injuries, especially in those caused by burns. Beeswax can be used also as an oil phase for the production of soaps by saponification. Soaps that contain beeswax have many advantages: they solidify quickly, provide detergency and cleaning properties, give a solid and pleasant texture and their aroma can be improved by adding natural essences. In this work, dermocosmetic ointments and soaps based on beeswax and sesame oil were formulated implementing the “Reproducing an Innovation Environment in the Classroom” (RAIS) strategy at the Chemical Engineering School at Universidad de Los Andes. Beeswax purification procedures were applied and sesame oil - beeswax formulations were obtained. In this sense, soaps were manufactured at the same concentrations as the ointments were formulated. Studies of pH, foamability and formulation scans were made to determine interfacial properties of the soaps produced.

Keywords: beeswax, dermocosmetic ointment, soap, foam, formulation

Resumen

Un ungüento es una preparación semisólida que consiste en una sola fase externa como base en la que se pueden dispersar sustancias líquidas o sólidas. En el caso de los ungüentos de cera de abejas, existen varios estudios que confirman su poder antibiótico y curativo sobre las lesiones, especialmente en aquellas causadas por quemaduras. La cera de abejas se puede utilizar también como una fase oleosa para la producción de jabones por saponificación. Los jabones que contienen cera de abejas tienen muchas ventajas: se solidifican rápidamente, proporcionan propiedades de limpieza y detergencia, dan una textura sólida y agradable y su aroma se puede mejorar agregando esencias naturales. En este trabajo, se formularon ungüentos dermocosméticos y jabones a base de cera de abeja y aceite de sésamo, utilizando la estrategia “Reproducción de un Ambiente de Innovación en el Salón de Clase” (RAIS) en la Escuela de Ingeniería Química de la Universidad de Los Andes. Se aplicaron procedimientos de purificación de cera de abejas y se fabricaron formulaciones de aceite de sésamo y cera de abeja. En este sentido, los jabones se fabricaron con las mismas concentraciones con las que se formularon los ungüentos. Se realizaron estudios de pH, espumabilidad y barridos de formulación para determinar las propiedades interfaciales de los jabones producidos.

Palabras Clave:cera de abeja, ungüento dermocosmético, jabón, espuma, formulación

1 Introduction

The value of a dermocosmetic product is determined not only by how well it fulfills the support or care for the symptoms of skin condition it was intended for, but also by the sensory experience that it gives a consumer as it is applied. That sensory experience depends mainly on the nature, quantity and quality of the components it is made of. Usually, the functional ingredients - those inactive ingredients that are used as a vehicle and determines the texture and appearance of the product, e.g. cream, ointment, lotion, or gel - contains water and oil, which could be natural, synthetic/mineral or a mix. The most commonly used natural oils are vegetable oils from fruits, nuts and seeds, but also natural waxes can be found in the formulation, less frequently.

Beeswax is one of these natural waxes that have been used as a support ingredient in cosmetic and pharmaceutical formulation. Although it has well known healing properties, it still remains as a secondary and poorly valued product, particularly, in South American countries apiarian production. In Venezuela, the apiarian activity can be traced back to the use of stingless bees in pre-Columbian times. Then, with the arrival of the Spaniards in the 16th century, honey bees (*Apismelliferaiberian* and *A. m. mellifera*) were introduced, which were joined afterwards by others breeds such as *A. m. ligustica* and *A. m. scutellata*. Along the years, honey has been the main product from the apiarian farmers, being beeswax a secondary product, mainly used for the regular renewal procedure of the hives. Nowadays, the cosmetic and pharmaceutical use of beeswax is found at the level of small laboratories and small businesses.

In order to contribute to a scientific knowledge of the use of natural beeswax in dermocosmetic products, we studied the preparation of both an ointment base and a soap with beeswax and sesame oil as oily components. The investigation examines the different physical characteristics of the ointment base obtained, from mixes of beeswax and sesame oil, and the different dermocosmetic products that can be prepared from those bases. It is also studied the incorporation of beeswax in a soap formulation, and the surfactant characteristic parameter for a soap containing beeswax is reported for the first time. Sesame oil is a liquid oil while beeswax is a solid fat, so the mix of this two different oily components results in new final physical characteristics. The addition of different amounts of beeswax can produce a broad range of texture and appearance changes in the ointment base, which allows to obtain dermocosmetic products with different uses and applications. On the other hand, the incorporation of beeswax in a sesame oil soap formulation allows to take advantage of the beneficial properties of the beeswax.

This investigation was carried out by the students of the Industrial Chemistry Laboratory course at the Chemical Engineering School, Universidad de Los Andes, Venezuela. The twenty (20) students' laboratory class engaged in a project-base learning strategy called RAIS *"Reproduction of an Innovation Environment in the Classroom"* which has been applied for the author in similar learning contexts and is published elsewhere(Marquez y col., 2016, Marquez y col., 2017,Tolosay col., 2018). RAIS is a strategy based on *learning by doing* education theory, which was framed in an entrepreneurship environment. In this case, students are allowed to design, formulate and produce the ointment and soap products that comply with both the learning objectives of the course and their own need or interest.

2 Literaturereview

2.1 Beeswax

The term "wax" refers to mixtures of different compounds such as: esters, long-chain hydrocarbons, ketones, among others, which form materials with high melting points and great water resistance. There are waxes of animal, vegetable and microorganism origin (Flaherty 1971).

Beeswax is a fat produced by bees to build their honeycombs. The bees secrete the wax in the form of small rounded scales in the four ventral glands that are in the lower part of the abdomen, and it is synthesized as a reduction of sugars of food origin. The phases of the beeswax making process are: bees eat honey and sugars (6 carbons molecules) are absorbed in the intestine. From there, they pass to the interior of their body, where they are transformed into small fragments (2 carbons). Then, in the wax glands, they recombine differently to form, on the one hand, the fatty acids and hydrocarbons (between 18 and 36 carbons), and on the other, the wax esters and alcohols (between 24 and 33 carbons). The mixture of these products is what is known as beeswax (Tulloch 1970, 1980, Blomquisty col., 1980,Bogdanov 2004).

2.2 Uses and applications

Beeswax has played an important role in history and popular tradition for many years. Historically, beeswax has been used for candles manufacture; it also was used in letter envelopes seals, sculpture making, and to seal coffins, among other applications. Currently, due to beeswax characteristics, properties and benefits, it is used in both handcrafted and industrial products (Ahnert 2015).

Industry uses beeswax as an insulating and hydrophobic component of numerous products. For example, it is used in electrical cables to isolate copper from moisture, electronic circuits, to protect leather, in the preparation of varnishes, inks, matches and protective waxes for cuttings (Kester ycol., 1989, Hepburn 2012).

Beeswax goes into the composition of ointments and creams, as a fat base and as a thickener. The major use in this field is depilating wax, which is a mixture of beeswax and resins. Beeswax has anti-inflammatory and healing properties, which is one of the reasons why it is widely used in cosmetic and pharmaceutical products (Al-Waili2003,Fratiniy col., 2016). It is used to cover sewing cords in shoe production, paperboards, and even in some cultures to produce dried meat. It is also used in the manufacture of shoe polishes and creams, to protect cans from acidic attack from fruit juices and other corrosive agents. In jewelry and sculpture modeling it is used to make models for pieces, due to its malleability (Tulloch 1980, Mladenoska 2012). Beeswax in some Asia and Africa countries is used to create batik fabrics, and in the manufacture of small metal ornaments by means of the molten wax method (FAO 2005). Currently, companies such as Stockmar and Filana use beeswax to make wax crayons (Stockmar 2018a, Filana 2018), additionally, candelilla wax have been proposed in beeswax crayon formulations (Gaytan 2005). Stockmar also manufactures modeling beeswax (Stockmar 2018b).

2.3 Beeswax physical properties

Beeswax is an inert material with high plasticity at a relatively low temperature (around 32ºC) (Hepburn, 2012). Its melting point is not constant, since the composition varies slightly depending on its origin. Typical values are between (62 °C to 65 °C) (Gaillard y col., 2011). Its relative density at 15 ºC is reported between 0.958 g/cm^3 to 0.970 g/cm^3, while its thermal conductivity is approximately 0.25 W/m.K (Morgan y col., 2002). It is also known that the viscosity at 100 °C is less than 20 mPa. The boiling point is unknown and it has a flash point at temperatures higher than 180 °C (Buchwald y col., 2008).

2.4 Pharmaceutical properties

Sterols present in beeswax are therapeutically useful compounds that are effective in lowering cholesterol levels. The incorporation of sterols to different foods may be convenient (Mellema 2008).

In cosmetology it is used for delicate skin care, especially when it is dry. It cleans the epidermis, softens and nourishes the dermis, thus preventing skin aging. Products that contain beeswax soften the skin. White wax normally enters the composition of nourishing, astringent, cleansing creams and skin masks.

The therapeutic properties of beeswax were already known in antiquity. Avicenna in his famous "Canon of medicine" cites a number of medicine formulas, whose composition includes beeswax. Archeological evidence of beeswax ointments have been found from as early as the 16th century (Baeteny col., 2010).

Nowadays, beeswax continues to occupy a prominent place in medicine preparations (Fratiniy col., 2016). According to Pharmacopoeia, plasters, ointments and creams should be prepared in pharmacies with a beeswax base. The white wax is included in the composition of creams, astringents, cleaning, whitening, as well as facial masks. In the United States, the chewing gum (combs wax) is attributed to have certain useful properties, among others, to activate the secretion of saliva and gastric juice, as well as to eliminate dental stones and reduce nicotine concentrations in smokers (Valega 2008). Recently, beeswax has been used for encapsulation of drugs and flavors (Ranjha y col., 2010,Fabray col., 2009).

2.5 Beeswax chemical composition

Beeswax comprises at least 284 different compounds, of which 21 major compounds represent 56% of the total composition of the wax. The average composition of beeswax is presented in Table 1 (Tulloch 1971).

Table 1. Beeswax composition

Components	Percentage (%)
Hydrocarbons	14
Monoesters	35
Diesters	14
Triesters	3
Hydroxy monoesters	4
Hydroxy polyester	8
Monoester acids	1
Polyester acids	2
Free fatty acids	12
Unidentified material	7

2.6 Beeswax purification

Beeswax, as found in combs, is yellow and has a particular smell similar to honey. Its purification is carried out through several procedures reported in the literature.

The purification procedure consists in melting beeswax in a water bath at a temperature higher than 60 ºC, and then it is bleachedthrough a variety of methods, among them: exposition to the sun (Midgley 1993), through diatomaceous earths and activated carbon (Orantes2012), or with sulfuric acid (Valega 2008). The molten beeswax is then poured on a vessel and partially submerged in temperate water,while slowly mixing, and the impurities are scraped off the surface. The purified beeswax is white and translucent and has thin edges.

2.7 Beeswax formulation for creams and ointments

In general, to obtain a dermocosmetic cream, the components of each phase must be mixed separately at a temperature close to 60 ºC, then incorporate one phase in the other under mixing, cool and homogenize. However, for ointments, the preparation is simpler, as a single phase is prepared. The procedure consists basically in melting the beeswax at a temperature higher than 65 ºC, and adding the formulation components.

In this sense, the cream or ointment components must be chosen according to the objective that is pursued with the application on the skin. Thus, beeswax can be used as a component in moisturizing creams, for burns, stretch marks, wrinkles, cellulite, lip balms and even for sunscreen formulations (Salisbury y col., 1954, Herrero 2000, Remiro 2000, Goncalves, Miñana 2011,Lombardero 2011).

3 Experimental

3.1 Beeswax purification

Beeswax was purchased from ApicolaAndina, Venezuela, and purified afterwards. For this purpose small beeswax pieces are cut and disposed in a container which is submerged in a thermostatted bath to completely melt it. Once the wax is completely liquid, which occurs at an approximate temperature of 82ºC, all the impurities, which will be floating on the surface of the liquid or sedimented in the bottom, should be removed with the help of a spatula. At the end of the process, beeswax should be placed in easy-to-mold containers to solidify, and then impurities are scraped with the help of a knife, until the beeswax is completely clean.

3.2 Sesame oil – beeswax ointment formulation

The already purified beeswax is weighed to prepare ointments with different compositions 5, 10, 20, 30 and 35% beeswax content (correspondingly 95, 90, 80, 70 and 65% sesame oil); each ointment is obtained per duplicate. The amount of beeswax is measured, completing it with sesame oil (extra virgin, Elite, Venezuela) in a graduated cylinder until a total of 40 g of ointment is obtained. The beeswax and sesame oil mixture is heated to 60 ºC and stirred with a IKA stirrer (Eurostar model) at 250 rpm per 10 min. Then it is left to cool at ambient temperature under continuous stirring.

3.3 Physical characteristics of beeswax ointments

A sensory analysis of each ointment sample is carried out and the following aspects are evaluated:

- Oiliness on the skin: oil residue that is left over the skin after the ointment is applied.
- Spreading: ease to spread and ointment capable of flowing easily on the skin surface.
- Odor or scent: aroma of the ointment.
- Color: user confidence when observing the color of the ointment.
- Appearance: granules or impurities observation in the ointment.
- Consistency: homogeneous or heterogeneous.
- Taste: acceptable or not.

3.4 Production of sesame oil - beeswax soap

50 g of purified beeswax are used for each formulation. Sodium hydroxide is from IQE (Venezuela). A saturated NaCl (Riedel de Häen, Germany) solution is used to precipitate the soap. The filter paper is weighted and the sample placed in a glass container, using the same percentages of beeswax and sesame oil used in ointments formulation. A solution of sodium hydroxide is prepared for 70% saponification (0.134 g NaOH/g sesame oil and 0.067 g NaOH/g beeswax), with a water/oil relationship of 345 g of water per 1000 g of total oil phase. The reaction is carried out in a thermostatted bath, maintained at a constant temperature of 70 °C. The mixture is constantly stirred to keep it homogeneous until the formation of a viscous solution with foam. To know if the reaction is over, a small sample of the mixture is taken and added to water, if the sample does not dissolve, the stirring under heating should continue. Then the soap paste is packed into an easy-to-mold container to shape it, after 3 days the paste is removed and stored in a paper bag for up to 28 days. pH of the soap is measured to determine its value as time proceeds.

3.5 Foamability tests

1% soap solution and distilled water are used. Foamability is measured through the Ross-Miles method (ASTM D 1173-53). This is a standardized method to measure the foaming capacity from a surfactant solution by measuring the height of the foam produced. 250 ml of a 1% soap solution are prepared and heated to 120 °F; then 50 ml of the same solution is added to the bottom of a glass cylinder 1 m high and 5 cm in diameter which is thermostatted at 120 °F, in the same way the solution is placed inside a funnel (200 mL). Subsequently, 1 meter height must be measured between the cylinder solution and the funnel nozzle. Then the nozzle is placed in the direction of the cylinder and the valve of the cylinder is completely opened. At the exact moment when the valve opens, the chronometer starts. Initial intervals of 30 seconds are used to take measurements, after the height changes are not very noticeable the interval is increased to 1 minute and finally 15 minutes. Height variations should be reported as well as time variations. The solution forms foam when it falls on the solution at the bottom of the cylinder. The maximum height reached (Hmax) represents the foamability of the solution, the latter being measured between the top of the generated foam and the height corresponding to the 250 ml of the solution at the bottom of the cylinder, that is, when the foam has disappeared completely. The uncertainty of the measurements is mainly related to the difficulty of reading the height of the foam, and corresponds to the appreciation of the graduated cylinder.

3.6 Formulation scan

Soap solution, NaCl solution, kerosene, n-pentanol and distilled water are used to perform a formulation scan between 2 and 6% NaCl, according to the standard procedure (Salagery col., 1982). In a graduated cylinder are added the following: NaCl solution (in the amount necessary for 3%, 4%, 4.5%, 5%, 6%, 7% NaCl), Milli-Q water, soap solution (1%), kerosene (WOR = 1) and n-pentanol (5%). The test tube is shaken gently and allowed to pre-equilibrate for 24 h. Afterwards, the contents of the tube are emptied into a beaker and continuously mixed with a magnetic stirrer for about 1 min. Then, it is transferred again to the test tube and the chronometer immediately begins, measurements of the volume of the aqueous phase separated against time are made.

4 Results and discussion

4.1 Beeswax purification

The results for beeswax purification are presented in table 2, all are above 80% yield, with an average of 88.1%.

Table 2. Beexwax purification

Sample	1	2	3	4	5
Initial weight (g)	297.1	322.6	300.0	300.1	295.3
Purified weight (g)	267.2	260.0	268.0	272.1	265.7
%Yield	89.9	80.6	89.4	90.6	90.0

4.2 Sesame oil – beeswax ointment formulation

In Table 3, the amount of beeswax and sesame oil used for each ointment formulation is presented.

The physical characteristics of the ointments were the following:

- 5% beeswax ointment: very oily, spreadable, beige color, buttery odor, very fluid.
- 10% beeswax ointment: opaque yellow, vegetable oil odor, very oily, and slightly viscous.
- 20% beeswax ointment: yellowish color, medium viscosity, very good absorption and spreading and unpleasant odor.
- 30% beeswax ointment: thicker ointment, difficult to adsorb on the skin
- 35% beeswax ointment: ointment consistent, good aspect, smell of honey and good spreading ability.

Table 3. Composition of sesame oil - beeswax ointment bases

% beeswax in ointment	Beeswax weight (g)	Sesame oil weight (g)	Total weight ointment (g)
5	2.0	38.0	40.0
10	4.0	36.1	40.1
20	8.0	32.0	40.0
30	12.0	28.1	40.1
35	14.1	26.0	40.1

In the elaboration of ointments based on beeswax, a survey was carried out to perform a sensory analysis of the different products obtained. Table 4 reflects the average results of this analysis, which was performed on a total of 20 subjects, where 0 is the lowest value and 5 is the highest one.

Table 4. Sensory analysis results for the ointments at different beeswax concentrations.

% beeswax	Oiliness	Spreading ability	Odor	Color	Appearance	Consistency	Taste
5	3.7	4.1	1.8	3.9	3.7	4.1	3.1
10	4.3	4.2	1.3	1.0	2.3	4.3	2.7
20	3.0	4.3	4.7	2.7	3.3	4.0	3.7
35	1.3	4.7	3.7	2.7	3.0	4.7	3.0

The 35% beeswax ointment gave a better acceptance among the respondents. Depending on the percentage of beeswax with which each of the ointment is formulated, a specific use can be obtained i.e. lip balm, dermocosmetic ointment and massage formulation, according to its beeswax content (high, medium and low, respectively). When working with higher concentrations of beeswax, a solid-like ointment was obtained, since more solids are found suspended in the oil and the mixture obtained is much denser, while at a lower concentration, a fluid ointment was obtained.

4.3 Production of sesame oil - beeswax soap

In Table 5 the quantities of beeswax, sesame oil, NaOH solution and water used to obtain each soap are depicted. Figure 1 shows pH measurement of the soaps obtained. A final pH between 8 and 9 was reached in 10 days, being this time necessary to reach equilibrium in the saponification reaction at 25 ºC. The 20 and 35% beeswax soaps reached low pH values rapidly; however, it is not obvious to evaluate the role of the changes of the oil phase composition to explain this behavior.

Table 5. Raw materials for sesame oil - beeswax soap making

Beeswax %	Beeswax weight (g)	Sesame oil weight (g)	NaOH 70% weight (g)	Water weight (g)
10	4.0	36.0	3.5	13.8
20	8.0	32.0	3.4	13.8
25	10.1	30.2	3.2	13.8
30	12.0	28.1	3.2	13.8
35	14.0	26.0	3.1	13.9

pH decreases progressively over a long period of time in the 10, 25 and 30% beeswax soaps, beginning with a high pH = 14, down to a range of pH = 8-9. This decrease in pH is due to the fact that over time, the remaining NaOH reacts with the fatty oils, causing the decrease thereof.

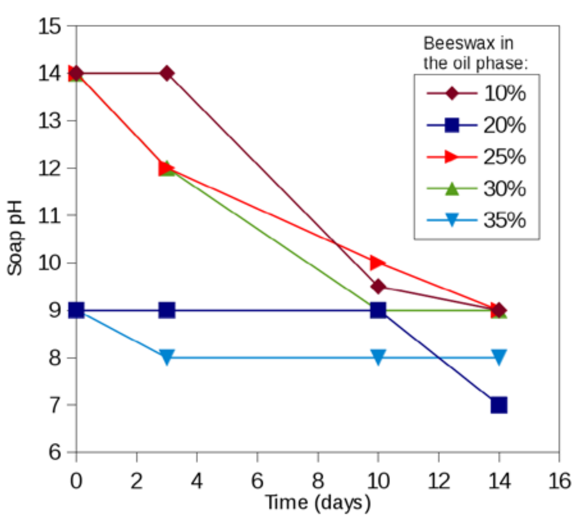

Figure 1. pH of sesame oil - beeswax soaps against time for 10, 20, 25, 30 and 35% beeswax in the oil phase (correspondingly 90, 80, 75, 70 and 65% sesame oil).

Comparing the final pH results with those of commercial soaps, referenced in the Venezuelan Dermatology journal, such as Palmolive (pH = 9.99), Dove (pH = 7.39), Safeguard (pH = 10.23), Protex (pH = 9.6) (D'Santiago and de Marcano 1996), among others, it is found that beeswax is a viable and effective raw material to obtain soap when combined with sesame oil.

4.4 Soapfoamability study

Foamability, measured with the Ross-Miles method, was similar in all formulations, with an average of Hmax = 20.4 ± 2.1 cm. This foamability value is comparable to other anionic surfactants (Rosen 2004).

Figure 2 depicts foam stability as the lifetime to break 50% of the foam of each of the soap formulations. Longer foam stabilities (46 and 47 minutes) were found for the 10% - 30% soaps. On the other hand, for the 35% beeswax soap, a lifetime of only 10.5 minutes was observed, yielding the lowest stability value. This probably is due to the presence of beeswax particles in the soap, which can break the foam.

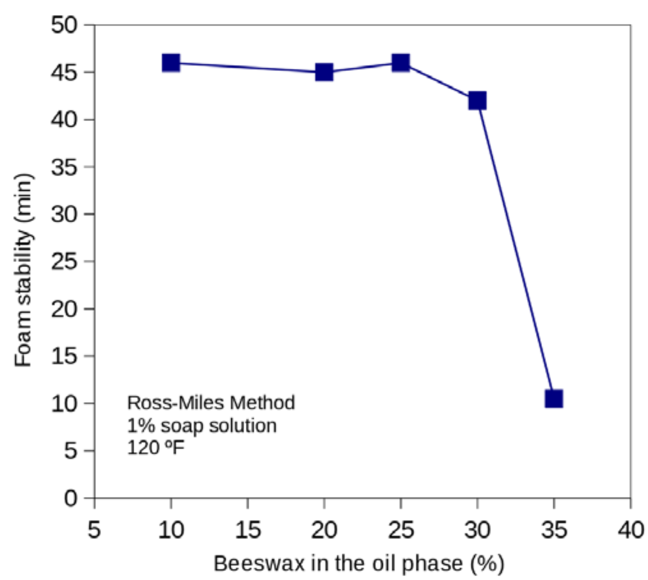

Figure 2. Foam stability vs. % beeswax in the oil phase

4.5 Soap formulation scan

Salinity scans for soap products (sodium carboxylate surfactants) were carried out from 2 to 6 % NaCl for the soap (1%)-n-pentanol (5%)-kerosene-brine system. Two formulations were studied, one with a low (20%) and the other with a high (35%) beeswax content. Emulsion stability against salinity shows a minimum where optimum formulation is found. In the present study, the time for 50% volume separation of the aqueous phase was taken as the lifetime of emulsions. It has been shown previously that the minimum is found at the same salinity no matter the phase measured aqueous or oil phase (Marquez y col., 2018a, Marquez y col., 2018b).

HLD equation (1) (Salagery col., 1979,Salagery col., 2013) can be expressed for this system as:

$$HLD = \ln S + \sigma - k\ EACN + f(A) - a_t \Delta T \quad (1)$$

Sodium carboxylate soaps are very hydrophilic surfactants, and kerosene has an equivalent alkane chain number EACN = 8.5. a_t = 0.01 and k = 0.10 for carboxylate surfactants. Temperature in the present experiments is 25 ºC, i.e. equation (1) can be expressed as:

$$HLD = \ln S + \sigma - 0.10 \times 8.5 + f(A) - 0.01\ \Delta T \quad (2)$$

Salinity to reach optimum formulation, for this kind of very hydrophilic surfactant, in a system without alcohol is very high. In this system a 5 % n-pentanol (with an f(A) = 2.3) is used to reach optimum formulation at relatively low 3.5-4 % salinity (Celis y col., 2008,Marquez y col., 2016b).

In Figure 3, the lifetimes for 50% of coalesced volume vs. salinity for formulation scans are shown. A minimum in emulsion stability is found, where optimum formulation i.e. HLD = 0, is attained. According to equation (2), and using the optimum salinities shown in figure 3 (3.5 and 4 % NaCl), the surfactant characteristic parameters were σ = -3.1 and -3.2 for the 20 and 35 % beeswax soaps, respectively.

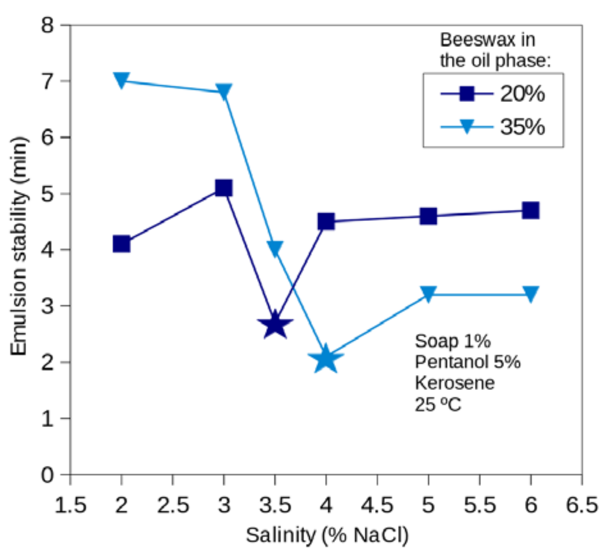

Figure 3. Emulsion stability vs. aqueous phase salinity

5 Conclusions

Soaps and dermocosmetic ointments from natural products such as beeswax and sesame oil were obtained, with an experimental procedure that allows attaining organic products, nontoxic for the human being. Different formulations of dermocosmetic ointments were developed. At 35% beeswax in the oil phase the ointment has consistency similar to cocoa butter, therefore it can be used as a lip balm, due to the healing properties and nature of beeswax. At concentrations close to 15% beeswax the product obtained is less viscous, so it can be used for massages. Likewise at concentrations between 20-30% beeswax, the dermocosmetic ointment is slightly solid, thus this formulation could be used in applications for scars and burns treatment. It was determined in sensory analysis surveys that the ointment of 20 and 35 % beeswax, respectively, are the most suitable for dermatological use. Foam stability studies indicate that beeswax soap solutions have high foamability; although at 35% beeswax the foam is unstable, probably due to the presence of beeswax particles. By means of a formulation scan, optimum formulation was found, which is in the range of 3.5 to 4% NaCl for the soap-kerosene-n-pentanol-brine system, being the soap a highly hydrophilic surfactant with a surfactant characteristic parameter value σ = -3.1 and -3.2, similar to sodium dodecyl sulfate, σ = -3.0.

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