Faculdade de Engenharia da Universidade do Porto

Improving Skin Conductivity to Small Ions for Enhanced Biosignal Monitoring

Ana Sofia Nascimento Sarmento

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Orientador: Prof. Dr. José Carlos Fonseca

Coorientador: Prof. Dr. Maria Helena Amaral

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Presidente Prof. Doutor José Alberto Peixoto Machado da Silva
Professor Associado do Departamento de Engenharia Eletrotécnica e de Computadores da FEUP - U.Porto

Prof. Doutor José Carlos Magalhães Duque da Fonseca
Professor Auxiliar do Departamento de Engenharia Metalúrgica e de Materiais - FEUP - U. Porto

Prof.ª Doutora Ana Catarina da Cruz Rodrigues da Silva
Professora da Faculdade de Farmácia da U. Porto

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Autor - Ana Sofia Nascimento Sarmento

Faculdade de Engenharia da Universidade do Porto

Abstract

Electroencephalography (EEG) is a widely used brain imaging technique. The Ag/AgCl gel-based electrode is the current gold standard for signal acquisition. However, this type of electrodes raises some problems, such as the fact that its use is time consuming, dirties the hair, causes discomfort and possible allergies, due to the use of the gel. Therefore, a cleaner and quicker measurement can be achieved using dry electrodes, which do not need any gel, yet there are still some problems correlated with this type of electrodes. The main problem regarding dry electrodes is the strong movement artefact susceptibility, which is much stronger than in wet electrodes.

Thus, to overcome this hurdle a solution is proposed in the form of a hydrating emulsion, since artefact generation is related with the skin dry state. The main objective of this emulsion is the hydration of the skin scalp, which causes a decrease in the skin-electrode impedance by creating ionic paths for electrical conduction, without having the disadvantages of commercials sticky gels. The developed emulsion contains an electrolyte and components with *stratrum corneum* (SC) moisturizing properties. The emulsion was characterized from the point of view of its chemical and physical properties, namely viscosity, pH, emulsion droplet size, etc. The emulsion effectiveness was also tested *in-vivo* in terms of transepidermal water loss (TEWL) and hydration properties. Impedance studies were carried out *in-vitro* with mouse skin. Afterwards, the skin was analysed using FTIR. Additional, *in-vivo* studies were carried out consisting in measuring the impedance in the forearm.

The developed emulsion accomplished the main requirements of reducing skin impedance, by hydrating the skin, and the presence of sodium chloride allows to create an ionic path. In addition, the emulsion hydrates the skin rapidly, as high hydration values and low skin impedance were achieved after just 15 minutes. These values were also maintained for at least four hours. Moreover, no volunteer presented any irritation or discomfort during the use of the emulsion.

Resumo

Eletroencefalografia (EEG) é uma técnica de imagiologia cerebral extensamente utilizada. Os elétrodos à base de gel Ag/AgCl, são atualmente os elétrodos mais utilizados para aquisição de sinal. No entanto, este tipo de elétrodo está relacionado com alguns problemas, como o facto de ter um tempo de preparação longo, bem como causa desconforto e possíveis alergias, devido à utilização de um gel condutor. Como tal, uma medição mais rápida e limpa pode ser alcançada usando elétrodos secos, que não necessitam da aplicação do gel. Contudo, ainda existem alguns problemas relacionados com este tipo de elétrodos. Um dos principais problemas dos elétrodos secos é que estão mais suscetíveis a artefactos de movimento do que os elétrodos do tipo húmido.

Assim, para ultrapassar este obstáculo, é proposta uma solução sob a forma de uma loção hidratante, pois a geração de artefactos está relacionada com o estado de hidratação da pele. Logo, o principal objetivo da loção é hidratar o couro cabeludo, o que vai provocar uma diminuição na impedância pele-elétrodo. Isto vai criar um caminho iónico que vai permitir que ocorra condução elétrica, sem as desvantagens dos géis comerciais. A loção desenvolvida é uma emulsão contendo eletrólitos, e componentes com propriedades hidratantes para o *stratum corneum*. As propriedades químicas e físicas da loção foram testadas, como a viscosidade, pH, tamanho das partículas da emulsão, etc. Para além disso, a loção também foi testada *in-vivo* em termos da perda transepidérmica de água e de hidratação. Estudos de impedância foram realizados *in-vitro* em pele de rato. Após, a pele foi analisada usando FTIR. Além disso, estudos *in-vivo de* impedância da pele foram efetuados no antebraço.

A loção desenvolvida cumpre os requisitos principais de reduzir a impedância da pele, ao hidratar a pele, e a presença de cloreto de sódio permite que se crie um caminho iónico entre o elétrodo e a pele. Adicionalmente, a loção hidrata rapidamente, sendo que altos valores de hidratação e baixos valores de impedância são conseguidos apenas após 15 minutos da aplicação. Estes valores mantêm-se ao longo de pelo menos quatro horas. Por fim, nenhum voluntário apresentou irritação ou desconforto durante o uso da loção.

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Abbreviations, Acronym and Symbols

List of abbreviations and acronyms

AC	Alternating Current
DC	Direct Current
EIS	Electrochemical Impedance Spectroscopy
EEG	Electroencephalography
FEUP	Faculty of Engineering of the University of Porto
FFUP	Faculty of Pharmacy of the University of Porto
FTIR	Fourier Transform-Infrared Spectroscopy
HPMC	Hydroxypropyl Methylcellulose
NMF	Natural Moisturizing Factor
O/W	Oil-In-Water Emulsion
PBS	Phosphate Buffer Saline
SC	*Stratum Corneum*
SLS	Sodium Lauryl Sulfate
TEWL	Transepidermal Water Loss
UV	Ultraviolet
W/O	Water-In-Oil Emulsion

1. Introduction

1.1. Electroencephalography

Electroencephalography (EEG) is the study of the brain's electrical activity, in other words, is a technique that records the oscillations of the brain electric potentials [1]. This technique is used to analyse how the brain operates, relating with human performance and how certain cognitive tasks influence brain activity [2]. It may also be used to study how the brain functions in real time situations, since it has the ability to register normal and abnormal electric activity of the brain [2], [3].

EEG is a common technique used in various research fields, such as psychology, medicine and biology [4]. Additionally, it is less expensive than other brain imaging techniques, examination time is reduced, and it is a non-invasive and painless technique that can be performed multiple times, both in adults and children [3]. The recording of the brain's natural electrical activity is made using several electrodes placed over the scalp and the measurement can last from 20 minutes to several days [5].

1.1.1. Physiology related to EEG

The measurement of brain activity is possible due to the fact that the human brain contains 10^{11} neurons, the main cell of the nervous system [6]. Neurons are excited by action potentials that create an impulse that is conducted from cell to cell until it reaches its destination [6]. Consequently, neurons communicate by electrical currents and the summation of all the excitatory effects, that come from multiple cells at the same time, will lead to the action potential, which can be measured with an electroencephalography equipment [6], [4]. This impulse is propagated by changes in ions concentrations through the membranes of nerve cells, leading ultimately to a potential difference [6].

The encephalogram is the result of the EEG, in which brain patterns are displayed in the form of waves [3]. Accordingly, brain activity is differentiated by the amplitude and frequencies of the EEG signal [4].

Therefore, in an EEG exam there are multiple stages where stimuli may or may not be introduced [4]. When there is not a stimulus, spontaneous EEG is recorded and when there are external stimuli, averaged evoked potentials will be verified, since they are correlated with certain sensory stimuli such as constant light flashes, auditory sounds, etc [4].

1.1.2 Uses of EEG

As already referred, EEG can be used in multiple areas, but more specifically in medicine it has many uses, being a central clinical tool for following, treating and diagnosing brain pathologies [7]. To illustrate, EEG can be used in epileptic patients, being epilepsy a chronic disease in which unexpected and unpredictable seizures occur and can lead to health problems and abnormal lifestyles [8]. This analysis is possible due to the fact that epileptic seizures have characteristic wave patterns that are unalike the normal EEG signal, being these patterns high-voltage waves [3], [9].The atypical patterns are the principle for the diagnosis of any of the possible diseases that can be analysed using EEG technology [3].

Moreover, EEG can be used to trace areas of damage after head injury, strokes and brain tumours, to monitor patients in coma or brain death [3], [7].

Electroencephalography can be used in a continuous way, being widely used in hospitals since it provides the possibility to assess causes of delirium, coma, epileptic activity in patients with neurological and non-neurological illnesses [10]. Moreover, the use of continuous EEG boosts the detection of undetected neurological illnesses in 10% to 67%, presenting higher detection results than routine EEG, due to occult seizures [10].

Recently, EEG portable equipment has been introduced that can be connected for example to a smartphone, as is the case of McKenzie *et al* study, in which the equipment can be used in epileptic subjects [11]. Another possible application is monitoring of athletes [12], [13].

1.1.3. EEG Structural Units

EEG machines have some essential structural units. Each of these units has a specific function [14]. Therefore, an EEG machine comprises an electrode board, an electrode selector, an amplifier, filters and in some cases and outside the circuit, a conductive media [3], [14]. Most of the EEG machines are multichannel instruments, containing multiple channels that can record at the same time the electrical activity from numerous pairs of electrodes [14]. The recorded signal will be acquired in digital form, where it can be visualized and stored in real time through a computer [15]. The focus will mainly be in the electrodes, since they are one of the main components that characterize EEG.

1.1.3.1. Electrodes

The body electric conductivity comprises ions as charge carriers [16]. Therefore, to understand bioelectric signals there is the need to interact with these ionic charges carriers, in which the interactions will lead to transduction of ionic currents into electronic currents that are read by electronic instruments [16]. The transduction is made by the electrodes that are essentially electrical conductors [16]. Electrodes can have multiple shapes and can be built from different materials, mainly metals [17]. For the recording to be precise, there shouldn't be distortions of the signal at the interface [17]. However, this is impossible due to several reasons that will be listed later [17]. Nevertheless, a proper functioning of the electrodes is essential for the acquisition of high quality data [3].

There are multiple types of electrodes with diverse characteristics [3], [15]. Some of the examples are reusable disc electrodes, that can be made of gold, silver, stainless steel; headbands and electrodes caps, and finally saline based [3],[15]. The headbands, electrode caps and reusable disc electrodes are the most used [14]. One of the reasons, is the fact that a lower electrode impedance is possible [14]. In any case, even though there are a significant variety of different electrodes, the classic electrodes have a fundamental component that is the metal-electrolyte interface [14], [18]. Accordingly, the electrode is a metal and the electrolyte can be a conducting solution, namely in the form of a gel or paste [14]. This electrolytic gel/paste makes the bridge between the scalp and the electrode, enabling the ionic-to-electronic signal transduction [14].

1.1.3.1.1. Electrodes Caps

In order to perform an electroencephalography exam a multichannel montage is necessary, being easier to use electrode caps, which contain multiple electrodes already assembled in the right positions - Figure 1.1 [3]. Caps are faster and easier to apply to the head of a subject, when comparing with the placement of individual electrodes [19]. At the same time, caps eliminate some mechanical issues correlated with usage of individual electrodes, since the placement is more accurate and the electrode/scalp coupling more reliable than the individual electrodes [19]. Furthermore, these caps can have different number of electrodes, existing caps of 16, 32, 64 electrodes, etc, being also made in different sizes to accommodate the variety of head sizes [20].

Figure 1.1 - Electrode Cap [21].

1.1.3.2. Amplifiers

EEG machines include amplifiers, which magnify the EEG voltage [14]. This is essential to make the EEG signal compatible with certain devices, as displays, recorders and A/D converters [3].

Accordingly, to prevent distortion and receive the wide dynamic range of the voltage fluctuations, since the recorded signal has a particular bandwidth, amplifiers can change amplification or "gain" [14], [18]. Gain is the ratio of output signal to input signal [3]. Generally, gain can be referred as sensitivity [14].

1.1.3.3. Wet electrodes

Wet electrodes are the current EEG standard, being the ones used for clinical EEG and in hospitals [3]. They are often presented as a metal-disk electrode with a hole in the centre, through which the electrolyte gel is introduced [3]. This is the reason why they are named wet electrodes, since there is the need of an interface between skin and the electrode [3]. These particular electrodes can be constructed using different metals, such as gold, tin or silver [3].

The most commonly used are the silver-silver chloride (Ag/AgCl) electrodes [14]. This specific electrode, when in contact with a solution of NaCl undergo the following chemical processes: [17], [22]

$$Ag \rightleftharpoons Ag^{+} + e^{-} , \qquad (1.1)$$

$$NaCl \rightleftharpoons Na^{+} + Cl^{-}, \qquad (1.2)$$

$$Ag^{+} + Cl^{-} \rightleftharpoons AgCl \quad , \ (1.3)$$

In the end, there is an electrical balance between the three equations [14].

These electrodes have been for many years the gold standard in EEG, owing to the low impedance skin contact, non-polarizability and low noise [23]. This effect is partially due to the gel that is added [23]. What this gel does is providing the conditions for equilibria 1.1 and 1.3 to take place and hydrating the *stratum corneum* that, as it is going to be demonstrated ahead, is the uttermost layer of the skin and displays a high impedance when it is dry [23]. Therefore, an ionic path is created between the metal of the electrode and the skin below the first layer, making the transduction of ionic current into electric current a lot easier [23]. All in all, the skin-electrode impedance is lower with the gel [3], [23]. Additionally, the gel is a viscous liquid almost like a hydrogel that grants flexibility and good contact, during movement, between the skin and electrode [23].

On the other hand, there are many disadvantages related with wet electrodes. The main problems relate to the gel and how the skin is treated for gel application. For instance, the skin is

sometimes rubbed with alcohol or with a harsh gel for part of the SC to be removed, which is uncomfortable and eventually painful to the patient [3], [23], [24]. Moreover, when using Ag/AgCl electrodes, all the spaces, between the electrodes and the skin, have to be carefully filled with the conductive gel [3], [23]. This process is time consuming, and it has to be carried out by specialized personnel [23]. Furthermore, if the electrodes have to be positioned in a sensitive area, for instance near the eye, preparation should be even more careful [25]. Additionally, as exam time goes by, signal degradation starts to appear (after 2-4h), meaning that skin-electrode contact impedance starts to increase, since the gel begins to dry, and more gel must be applied [23], [24], [12], [26]. At the end of the EEG exam, all electrodes need to be removed, which is once again a time consuming process, that can be even more difficult in the case of glued electrodes, being uncomfortable to the patient [25], [23]. Moreover, the metal used in the electrode might have impurities, or the surface can be contaminated, thus cleaning and efficient storage is needed [14]. Due to all this problems, correlated with wet electrodes, its application is unsuitable for other environments beside the lab environment [12].

1.1.4. Dry electrodes

A class of electrodes that have been gaining momentum is the dry electrodes, which are gel free electrodes [26]. These electrodes consist of a conductive interface that doesn't use the gel to couple the electrode with the skin [26]. The material used in the electrode has some requirements that have to be met, namely the material may be a conductor or an insulator, being at the same time inert to sweat and disinfection agents [27].

There are two central categories of dry electrodes, contact and non-contact electrodes [23]. The non-contact electrodes are capacitively connected to the skin, meaning there is not the need for direct contact between the skin and the electrode surface [28].

On the other hand, contact electrodes can be of different types, shapes and can also use different principles. One possible contact electrode has microscale needles that penetrate the skin, being part of the invasive electrodes [23].

These other direct contact electrodes are easier to produce and normally cheaper, being as well, non-invasive [1]. These electrodes can be reused more times than the needle electrodes, since in the later the needles easily break [1]. Following, there are some examples of this type of electrodes. One of the possible shapes for the electronic conductor is a comb-like conductive polymer elastomers, used by Mullen *et al* - Figure 1.2A [26], [29]. Correspondingly, the electrodes have flexible prongs that can go through the hair and contact the scalp [29]. They are made of a flexible polymer coated with a conductive layer of Ag/AgCl [29].

Dry electrodes can also be flexible metal-coated bristles developed by Grozea *et al* - Figure 1.2B [26], [30]. The electrodes extremities are flexible conductive bristles made of silver-coated polymer, which reduces the discomfort sometimes caused by dry electrodes [30].

Another electrode design is an inert metallic tips, as it is the case of the electrode designed by Liao *et al*, which is a needle like electrode that contacts with the scalp without piercing it - Figure 1.2C [26], [31]. This specific electrode is made of gold, and has a mechanism that makes the needles flexible, therefore they fit the scalp interface well [31]. Fiedler *et al*, also designed a dry electrode, in which the ends are shaped as 24 conic pins with circular tops, enabling the passage through the hair layer - Figure 1.2D [12]. The material used is polyurethane, which is easy to modulate making production simple, and it is coated with silver [12].

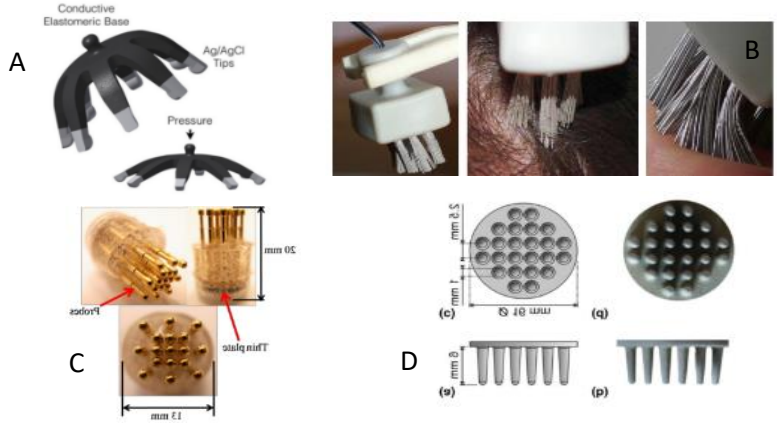


Figure 1.2 - Different types of design for dry electrodes. A - Design of electrode developed by Mullen *et al* [29]; B - Proposal of Grozea *et al* [30]; C - Design of Liao *et al* [31]; D - Design of Fiedler *el al* [12].

Furthermore, dry electrodes are associated with many advantages as cleanliness, the setup is quick and user friendly [26]. Dry electrodes can also be self-applied, since there is not the need of an expert to insert the conductive gel [26]. So, they can be used for real-world application [26]. Yet, without the use of an electrolyte the electrode-skin impedance is higher [26].

Moreover, dry electrodes can be particularly rigid, not conforming totally to the surface head, which in return, causes unstable electrode-skin interface [26]. Additionally, motion artefacts disturb harshly signal recording in these electrodes [32], [33]. This is because the electrodes slip from its location [32], [33]. In addition, most of times there are hairs between the skin and the electrode that reduce the contact area [32], [33]. Dry electrodes can be more uncomfortable to wear since almost all the electrodes are made of solid metal [33].

1.1.5. EEG Artefacts

An artefact in EEG is any recorded electrical potential that does not come from the brain [17]. There are four main sources of artefacts, namely the EEG equipment, external electrical interference, electrodes and the patient [17].

External interferences, should be well attenuated in a properly projected laboratory. This means that the laboratory has to be tested for electrostatic, electromagnetic and radio-frequency fields [17].

Electrode interferences are mainly due to the interface between the electrode and the patient [17]. An example is when a subject moves, it will cause changes in the contact resistance that translates in electrical noise [17].

The artefacts that come from the patient can be for example the muscle potentials that will also be recorded [17]. One of the most recurrent artefacts is related with eye movement, but there are also artefacts caused by the pulsation of an artery and even sweat [3], [17]. Another aspect, and the one without a proper solution, is the impedance caused by the skin [14]. Impedance is the resistance of an electrical circuit or component, to an alternating current (AC) [34]. The skin impedance varies with body location and it also depends in the state of hydration of the skin [35]. Wet electrodes present a smaller impedance than dry electrodes, due to the use of the electrolyte that hydrates and creates an ionic path, as it was already described [23]. This is a remarkable difference, since in wet electrodes the impedance is in the order of few kOhms and, generally, in dry electrodes it reaches hundreds of kOhms, due to the inexistence of pre-treatment in the skin [26]. Furthermore, the magnitude and stability of electrode-skin impedance is one of the aspects that determines the quality of EEG signals [26].

As for interferences caused by the EEG equipment there is the movement of the cables, broken wire contacts, low battery, etc [3].

1.1.6. Project Framework

Summing up, both wet and dry electrodes have advantages and drawbacks. Yet, dry electrodes have a set of unique advantages that makes this electrode the most appropriate for some exams. For instance, the fact that it dispenses the use of gel makes them particularly suitable for long-term exams and for patients use at home.

However, one of the main problems of dry electrodes is the strong artefact susceptibility, much stronger than in the wet electrodes. [1] In this study, a solution to this problem is proposed. As the artefact generation is related with the skin hydration state, an emulsion was developed to hydrate the scalp and also decrease the skin-electrode impedance, by creating ionic paths for electrical conduction. The produced emulsion should fulfil the following requirements: the application must be simple, be promptly absorbed by the skin, it has to take effect in less than 30 minutes, cannot be greasy and it also cannot dirty the hair and remain active at least 4-8 h. Therefore, there is not the need for the patients to wash the hair after the exam, as the emulsion is absorbed by the skin. Most of these properties are not fulfilled by commercial EEG gels nor by hair products, which are not electrically conducive.

1.2. Skin Hydration

1.2.1. Skin

Skin is a functionally exceptional organ of uttermost importance for the equilibrium and maintenance of the human body [36]. In terms of body mass, skin concerns more than 10%, making it the largest organ of the body [37], [38]. Regarding skin, one vital aspect is the intimate interaction with the external environment, while protecting and maintaining a communication between internal and external environment [36], [37]. Moreover, skin can adapt to the surrounding environment, being a fundamental feature to keep body homeostasis [36], [37]. It is composed by tissue that has the capacity to grow, differentiate and renew itself consistently [39].

1.2.1.1. Skin Structure

Skin is divided in three distinct layers: epidermis, dermis and subcutaneous tissue.

1.2.1.1.1. Epidermis

Epidermis is a stratified epithelium that is separated from dermis by a basal membrane, being the most superficial layer of the three layers that constitute the skin [39], [40]. Epidermis, has a thickness around 50 to 100 µm [41]. This layer is constituted by four different types of cells, the keratinocytes, melanocytes, Langerhan's cells and Merkel cells [42], [39], [43].

Firstly, keratinocytes are the standard cell type in epidermis, being responsible for the production of a fibrous protein, denominated keratin [42]. Keratin is the component responsible for the protective function of the epidermis, being the main structural protein of this layer [42], [40]. On the other hand, there are melanocytes, which produce a pigment called melanin [42], [39]. Furthermore, there are the Langerhan's cells, which are macrophages and finally, epidermis has Merkel cells that are correlated with sensory functions [37], [42], [39].

Epidermis is itself divided in five different layers - Figure 1.3 [37]. The deepest layer is the *stratum basale* that is adjacent to the dermis. In its constitution there are all the types of cells described above, plus epidermal stem cells, which have the ability to divide and produce new cells, particularly new keratinocytes [37], [43], [40].

The following layer is the *stratum spinosum*. This layer is characterized by a pattern of adjacent keratinocytes [36], [44]. It also contains scattered Langerhan's cells and lamellar bodies, which are membrane-bound secretory organelles [42], [40], [44]. The next layer is the *stratum granulosum* [42]. All the cells above this layer are dead, since dermal capillaries don't reach this area, therefore, there are not enough nutrients to its survival [42]. Following, it is found *stratum*

lucidum, which is composed by three to four layers of flattened dead cells [42]. This specific layer is only present in the hands and soles of the feet [42].

Last, there is the outermost layer, the *stratum corneum* (SC) [42]. This layer has between 10 to 30 layers of flat dead cells, which correspond approximately to 5 to 20 µm [40], [42]. The number of cell layers doesn't change much with the sex, age and individual [45]. However, it is influenced by the location, meaning that, for example, the scalp SC has around 12 ± 2 layers, being thinner than the SC from the trunk, which has 13 ± 4 layers, and thinner than the SC of the extremities, with 15 ± 4 layers [45]. The described dead cells are called corneocytes and are non-viable cells that continue to be completely functional, predominantly regarding the barrier functions [42], [44].

Corneocytes are filled with keratin and are terminally differentiated cells that have their origin in the keratinocytes [42], [44]. In fact during the transition from the *stratum granulosum* to the SC, the keratinocytes loose the nuclei and organelles, turning into corneocytes [40]. Each corneocyte is constituted by 70% of keratin and 20% of lipids that are present in a cell envelope of cross-linked proteins [37], [41]. The cell envelope plays the essential role of keeping osmotically active material inside the corneocytes [41]. Moreover, extracellular non-polar lipids are around the corneocytes, leading to a hydrophobic matrix [41]. The main lipids present in this intercellular space are ceramides, representing 50% of the total lipid mass, plus 25% of cholesterol and 10% fatty acids [41].

The SC is one of the most important layers regarding skin function, being the one that offers the barrier function, protection against biological, chemical and physical assaults [42]. This is also the most important layer for this study, since it is the one that provides most of the resistance to bio-electric signals transfer, thus, main obstacle to overcome [42].

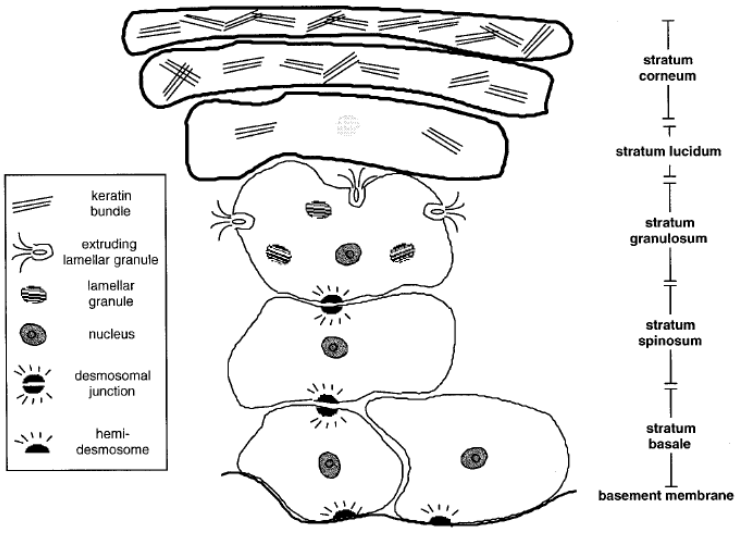


Figure 1.3 - Representation of the five principle layers of epidermis and correspondent components [37].

1.2.1.1.2. Dermis

Dermis is the middle layer of the skin, existing underneath the epidermis - Figure 1.4 [42]. This layer provides nutrients, immune protection and supports the epidermis through the basal layer [37], [42]. Moreover, it has a role in regulation of temperature, pressure and pain [37]. Dermis is thicker than epidermis, being around one to two millimetres [41]. It consists of collagen fibres, about 70% of the total mass [37]. Additionally, it has in its composition elastic connective tissue, which offers elasticity [37]. Contrary to epidermis, dermis has a vast vascular network, responsible for providing skin nutrition, repair, immune response and thermoregulation [37].

Furthermore, dermis also contains appendages such as hair follicles, oil/sebaceous glands and sweat glands [37], [42]. The hair follicles are dispersed across the entire skin [37].

Regarding sweat glands, these are simple structures, shaped as a tube that is coiled in a ball [37]. It secretes a dilute salt solution with a pH around five [37]. The secretion of this fluid is made by temperature-controlling determinants, as exercise, high external temperature and emotional stress [37].

Finally, there are oil glands that are associated with hair follicles [37]. This glands secrete sebum, which is composed by triglycerides, free fatty acids and waxes [37]. This particular fluid has the functions of protection and lubrication of the skin, while at the same time maintains the skin pH [37], [42].

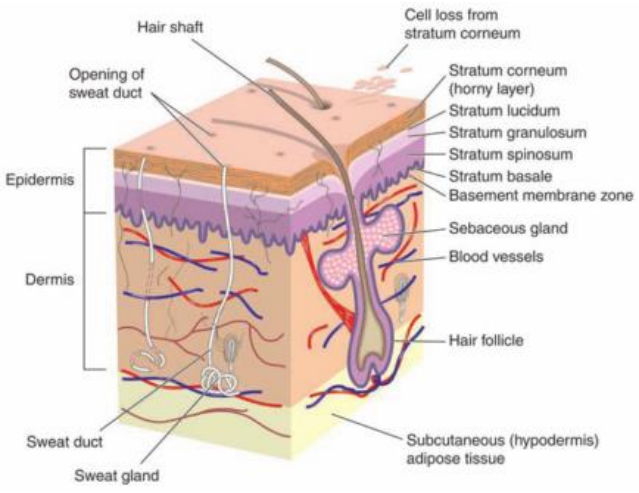


Figure 1.4 - Representation of the different layers of skin [42].

1.2.1.1.3. Subcutaneous tissue

With regard to the subcutaneous tissue it is not technically part of skin, however it contains some of the same protective functions as skin [42]. This tissue is the most in-depth skin layer and is mainly constituted by adipose tissue/fat cells, arranged in lobules, and some areolar connective tissue, that links this layer to the dermis [37], [42]. Adipose tissue is where excess energy is

stored, being also an endocrine organ that synthesizes active compounds that regulate metabolic homeostasis [42].

1.2.1.2. Skin Functions

One of the most crucial functions of the skin, is the barrier function. It is a barrier against dehydration, thus water evaporation is controlled and the equilibrium is always maintained [42].

In addition, this organ is the first layer of protection against mechanical injuries, such as bumps, abrasion and cuts, as well as chemical damages, not letting chemicals enter inside the body [37], [42]. It also works has a shield against bacterial invasions and it filtrates ultraviolet (UV) radiation and environmental pollutants [42], [46], [41].

Furthermore, skin has the ability to self-renew [38]. Skin renewal takes place at a constant rate, in a cyclic way [38]. This cycle is characterized by the desquamation of the SC approximately every 14 days, meaning that the skin has a new surface every 2 weeks [38]. Therefore, products that are designed to affect skin surface have to be applied in a continuous manner, due to the constant state of flux of the skin [38].

Skin, is likewise vital for homeostasis, since it maintains a relatively constant environment within the body [42]. This includes, thermoregulation, that is possible due to the blood supply and sweat glands, controlled by the nervous system [42], [46].

Lastly, but certainly not least, skin has components of the nervous system that can detect temperature, touch, pressure and pain [42]. Therefore, skin gives a lot of input about the surrounding environment to our body [42].

1.2.1.3. Skin barrier

The focal layer that performs this barrier function is the SC. It is a barrier in multiple ways, such as against loss of internal body components, predominantly water to the external environment [37]. Skin is a very efficient barrier, since the water loss from “insensible perspiration” is limited to 0.5 $\mu L/cm^2/h^{-1}$or 250 mL of water per day [37]. The small amount of water that is lost, serves to hydrate the outer layer of the SC [47]. This allows the maintenance of the flexibility and it also facilitates enzymatic reactions that lead to SC maturation [47].

The barrier function is possible due to a two compartment system, denominated “brick and mortar model” [44]. In this case, corneocytes are the bricks, which are surrounded by a continuous extracellular lipid matrix, the mortar [44]. The shape of the corneocytes, flat and hexagonal, plus their specific features, are ideal for the SC integrity and desquamation [44]. The physical packing of the corneocytes, produces a tricky path for the molecules to cross and the hydrophobic lipids are arranged in lamellar structures that also offer a water-tight barrier property

[43], [47]. Therefore, the diffusion path length is increased, which in return improves the SC barrier function [47].

Furthermore, cells of deeper SC layers have the ability to bind water [44]. This is related to the natural moisturizing factor (NMF) [47], [48]. NMF is found in the corneocytes and when it absorbs water it turns into a humectant [47], [48]. A humectant is a component that can attract water [38]. Hence, NMF maintains normal levels of water in the SC, even in low humidity conditions, which plasticizes this layer and, therefore SC keeps its resilience and prevents cracking and flacking [48].

The maintenance of the SC hydration is influenced by external humidity [47]. Thus, in dry environments, water loss decreases and the number of layers in the SC increases [47]. On the other hand, barrier repair occurs when the pH is acidic [47]. As a result pH is important for antibacterial protection, since some organisms can't survive in an acidic environment [41]. The pH of the skin is usually between 5,4 and 5,9 [49], [50].

1.2.2. Importance of hydration

Regulation of water loss is mainly of the responsibility on the SC. Hydration and SC water content are essential parameters for skin plasticization and barrier function [51]. Therefore, this tissue is as impermeable as possible, allowing a small amount of water loss to maintain the outer layers of the SC hydrated, as described above [41]. Consequently, flexibility is maintained and there are enough water to permit enzyme reactions that simplify SC maturation and desquamation [41]. Moreover, maintaining the skin barrier intact is vital since hydration affects skin appearance, mechanical properties of this organ and cell signal processes [52].

The ability of the SC to retain water depends of various aspects, such as SC thickness, the corneocytes phenotype and organization, and lipids barrier composition [41]. The skin reserves water through occlusion, due to the water permeability barrier, and through cellular humectancy, which is correlated with the NMF [48]. Both of these mechanisms are susceptible to disturbances and perturbations [48]. If the skin is not hydrated or dry, it is predisposed to cracks, when subjected to mechanical stress [41]. Dry skin is characterized by changes in the structure of the corneocytes namely in the corneocytes envelopes [41]. Thus, hydration influences SC deformability [41]. Dry skin is reliant on extrinsic factors such as climate, environment and exposure of skin to soaps, detergents, chemicals or medications. [53] Additionally, there are also intrinsic factors that contribute to dry skin as genetics, diseases, hormone imbalances and ageing [53].

From the electrical point of view, the SC can be characterised as a parallel combination of resistor and a capacitor - Figure 1.5 - , in which the capacitance characteristic is thought to come from the double-layer capacitance of the penetration routes of skin [54], [55]. On the other hand, the resistance is believed to be connected to the ohmic resistance of skin [55]. Therefore,

electrical impedance measurements demonstrate that resistance and/or capacitance can be affected by hydration, ionic strength of the skin-bathing medium, pH and chemical treatment [54].

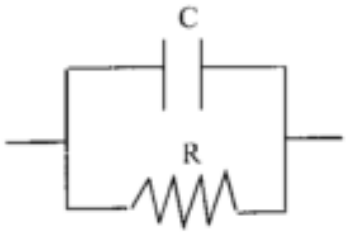


Figure 1.5 - Model of the skin electrical circuit, a resistor and a capacitor in parallel [56].

Thus, one strategy to increase drug delivery and permeability across skin is to increase skin hydration [57]. It was also demonstrated by Rosendal *et al*, that electrical properties of the skin are directly correlated with the water content of the SC [53]. Thus, changes in epidermal hydration result in changes in the electrical properties of the SC [53]. This was further reported by Clar *et al*, McAdams *et al*, Huang *et al*, etc [58], [59], [60]. Thus, due to the problem presented in Chapter 1, there is a need for an increase in skin hydration, to lead to a decrease in skin impedance.

1.2.2.1. Mechanisms of Skin Hydration

Moisturizers are used to re-store the skin moisture level or keeping it [38], [61]. Furthermore, they are also used to restore the barrier function, to cover small skin fissures and give a soothing protective film to the skin [62]. There are three physiological mechanisms for hydrating the SC: by occlusion, using humectants or with emollients [38], [61].

Regarding the first mechanism, occlusion, the skin is directly or indirectly covered by a layer that affects water evaporation [38], [63]. This can be achieved by applying oily substances on the skin, through which water cannot pass [38]. In other words, these substances reduce transepidermal water loss (TEWL) by creating a hydrophobic barrier, while contributing to the lipid phase between corneocytes [61]. There are some limitations associated with occlusive substances as odour, potential allergenicity and greasy sensation [61]. Occlusion, is a simple mechanism, extensively used to enhance the penetration of drugs [63]. There are multiple classes of substances that can play this role, such as hydrocarbon oils, waxes, silicone oils, fatty acids, etc [38].

With regard to emollients, they are generally lipids and oils that moisturize and enhance skin appearance by promoting skin softness, flexibility and smoothness [61]. Moreover, these substances fill the cracks between clusters of corneocytes, without being occlusive [61]. Usually, fatty acids and fatty alcohols are used as emollients, affecting the skin barrier by improving, repairing and influencing skin permeability [61].

As was already described a humectant has the capacity to attract water, using two mechanisms [38]. One consists in water extraction from the dermis into the epidermis; a second mechanisms consists in helping the SC to absorb water from external environment [38], [61]. Some humectants also contain emollient properties [61]. Substances with this property are glycerine, urea, propylene glycol, etc [38], [39]. These compounds are rather pleasurable to use, bringing a smoother skin feel by filling holes in the SC through swelling [38]. In contrast, certain humectants can lead instead to an increase in TEWL, since the enhancement of water absorption from dermis to epidermis can be lost to the environment [61]. Therefore, these components should be used in combination with occlusive substances [61].

Using substances that hydrate by different mechanisms creates a synergy, which alleviates the dry skin [61]. It has been demonstrated that some compounds interact synergistically if they are in a mixture, inducing higher values of skin permeation than that induced by each one individually [64]. Therefore, chemical mixtures can offer a way to overcome limitations sometimes imposed by individual chemicals [64].

1.2.3. Moisturizing substances

The following are examples of some substances with moisturizing properties.

1.2.3.1. Cetyl Alcohol

Cetyl Alcohol is a chemical that can be used as a coating, an emulsifying and stiffening agent [65]. It is used in cosmetics and pharmaceutical formulations, such as emulsions and ointments [65]. This component can be classified as an emollient, because it is absorbed and retained in the epidermis, leading to softening and lubrification of the skin [65]. It is considered safe, being used in topical and oral formulations [65]. It is included in the FDA Inactive Ingredients Database [65].

1.2.3.2. Dimethicone

Dimethicone belongs to the family of the silicones and can be used as a moisturizer [66]. Silicones are resistant to decomposition by ultraviolet radiation, acids and electrical charges [66]. Moreover, these components are odourless, colourless, non-toxic and nonirritant [66], [65]. Silicones are immiscible with water, therefore can be used to form emulsions. This substance is an emollient, making skin smooth [66]. It is included in the FDA Inactive Ingredients Database [65].

1.2.3.3. Glycerine

Glycerol or glycerine is a widely used humectant, which prevents the crystallization of the SC lipids, leading to a decrease in TEWL and an increase in the water content of the skin [52]. It is a colourless, odourless, hygroscopic liquid, meaning it can attract and hold water molecules from the surrounding environment [52]. This characteristic is due to the three hydrophilic hydroxyl groups [52]. This compound is miscible with water, also having some antimicrobial properties [52]. Moreover, it accelerates corneocytes maturation and enables the digestion of desmosomes, which in return boosts desquamation [61]. It is also a generally recognized as safe (GRAS) component by U.S Food & Drug Administration (FDA) [67]. It is included in the FDA Inactive Ingredients Database [65].

1.2.3.4. Sweet Almond Oil

Sweet almond oil is an emollient that can be used as a solvent or as an oleaginous vehicle [65]. This compound is colourless or with a pale yellow colour [65]. This oil is also used in food and therapeutically, therefore it is regarded as nontoxic and nonirritant [65].

1.2.3.5. Urea

This chemical is a humectant and hydrating agent, used in various skin conditions, that also improves the barrier function of the skin [68]. Urea has the advantage of being a physiological substance present in blood, urine and sweat [69], [70]. This substance is also an effective penetration enhancer of different substances [52]. Furthermore, urea leads to a decrease in TEWL [52]. Urea can also cause skin stinging and burning in concentrations between 4% and 10%, if used in very dry skin [52]. It is also a generally recognized as safe (GRAS) component by U.S Food & Drug Administration (FDA) [67].

1.2.3.6. White Wax

White wax is widely used in creams and ointments to increase consistency and to stabilize water-in-oil emulsions [65]. This compound also forms a film coating [65]. It is incompatible with oxidizing agents and it is nontoxic and nonirritant [65]. Moreover, it is widely used for oral and topical formulations, however there are some cases of people being hypersensitive [65]. It is included in the FDA Inactive Ingredients Database [65]. It is also a generally recognized as safe (GRAS) component by U.S Food & Drug Administration (FDA) [67].

1.2.4. Topical Formulations/ Moisturizers

To hydrate the skin it is common to use topical formulations, or commonly called moisturizers [62]. Therefore, moisturizers are one of the main products used in dermatology, since its application leads to skin integrity and well-being, providing also a healthy appearance [62].

Moisturizers can be of different kinds such as ointments, creams, lotions (emulsions and solutions), oils and gels [62]. Accordingly, lotions are normally thinner while creams are heavier, therefore, lotions are preferred for daytime application.

An emulsion is an opaque, heterogeneous system of two immiscible liquid phases that are mechanically agitated to form droplets of one liquid dispersed in another liquid [37], [71]. It has the advantage of being thermodynamically stable in a specific range of temperature [52].

Emulsions can be oil-in-water (O/W) - Figure 1.6 - or water-in-oil (W/O) [52], [71]. The surfactant, acts as an emulsifier making it easier to form the emulsion [71]. Furthermore, this compound helps in the stabilization of the emulsion by diminishing the surface energy of the droplets [71]. Therefore, the surfactants decrease the interfacial tension, preventing the occurrence of coalescence droplets [71].

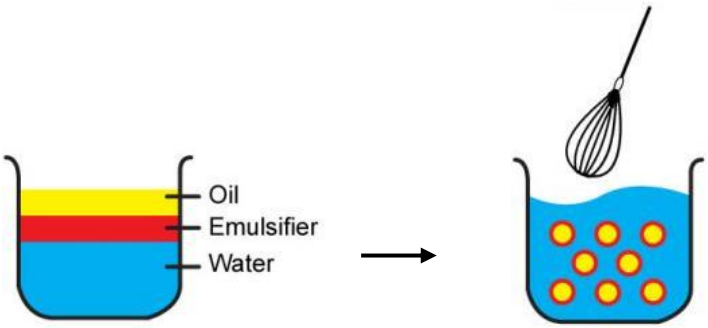


Figure 1.6 - Representation of an O/W emulsion, with the 3 essential components of an emulsion [72].

1.2.5. Moisturizing formulations

There are in the market multiple moisturizing products that can be used in the scalp However, the emulsion developed in this work have specific characteristics that are not present in most of the formulations marketed and are based on those described in literature. The following formulations serve as base for the development of the emulsion.

Friedman in 1957, patented electrically conductive emulsions to be used with electrodes to produce electrocardiograms [73]. The main objective of these formulations is to enhance the transfer of current between the skin and the electrocardiogram electrodes [73]. These emulsions are stable and had a pH between 4.2 and 6 [73]. However, contain components that can be prone to skin irritation, as the Sodium Lauryl Sulphate [74]. These formulations were non-ionic, cationic and anionic - Table 1.1:

Table 1.1 - Three formulations present in the patent [73].

Non-ionic	Percentage (%)	Cationic	Percentage (%)	Anionic	Percentage (%)
Sodium Nitrite	0,1	Methylene Bis-Stearmide	10,0	Sodium Lauryl Sulphate	1,0
Non-ionic Blend of Ethylene Oxide Derivatives of Lanolin	6,0	Stearyl Polyoxyethylamine	1,7	Glyceryl Monostearate	11,0
Cetyl Alcohol	2,0	Glacial Acetic Acid	0,3	Cetyl Alcohol	1,0
Sodium Chloride	5,0	Sodium Nitrite	0,1	Sodium Nitrite	0,1
Glycerine	5,0	Sodium Chloride	5,0	Sodium Chloride	5,0
pH 5 Buffer Solution	81,9	Glycerine	5,0	Glycerine	5,0
-	-	pH 5 Buffer Solution	77,9	Water	76,9

Moreover, Lichtenstein in 1971, also invented a formulation to be used with electrocardiographic diagnostic equipment [75]. The main objective of this preparation was to improve the transmission of current from the body to the equipment [75]. Additionally, it is not drying, irritating and harmful to the person and it also serves as a conditioning agent in the skin [75]. In this invention, salts were not used, due to the additives that were necessary to add, such as corrosion inhibitor, buffering agents and bacterial inhibitors [75]. Therefore, to substitute the salts, organic acids were used, such as benzoic acid, salicylic acid, lactic acid, etc [75]. The chemical composition of these formulations is presented in Table 1.2.

Table 1.2 - Compositions of Lichtenstein [75].

Emollient Lotion	Percentage (%)	Aqueous Solutions	Percentage (%)
Sodium Tartrate or Sodium Bitartrate	3,5	Sodium Citrate	4,0
Fatty Acid Groups	6,5	Glycerine	4,5

Natural or Synthetic Oils	4,0	Sodium Lauryl Sulfate	0,5
Triethanolamine	3,0	Alcohol	2,0
Glycerine	4,0	Water	89,0
Water	79,0	-	-

In 1988 was patented, by Bell, a stable pharmaceutical W/O emulsion that comprises in its composition an electrolyte [76]. The objectives of the invention are to offer a stable emulsion that may or may not contain topical drugs and an electrolyte [76]. In this emulsion can also be added preservatives, antioxidants, humectants, thickeners, etc [76]. The general formulation is presented in Table 1.3.

Table 1.3 - General Formulation invented by Bell [76].

A. Oil Phase:	
Mineral Oil	1-15%
Organopolysiloxane Emulsifier	1-4%
Volatile Silicone Fluid	5-30%
PPG-20 Methyl Glucose Ether Distearate	1-10%
B. Water Phase:	
Electrolyte Salt	0-5%
Water, q.s. 100%	
C. Drugs - add to phase A or B depending on the drug	0-20%

Additionally, Raab, in 1991, studied the effect of urea in solutions and problems that occur when using this substance in topical formulations [77]. However, a formulation using urea and NaCl is presented [77]. This preparation is used to moisturize the skin, to protect and treat scalp lesions. For this purpose, an O/W emulsion was developed - Table 1.4.

Table 1.4 - Formulation developed by Raab's article [77].

Urea	5,0%
Sodium Chloride	5,0%
Tween 80	20,0%
Distilled Water	40,0%
Vas. Alb. ad	100%

Hakim patented, in 2017, a lotion to be used in dry scalp, which help in hydrating and restoring the pH of the scalp [78]. This lotion is mainly composed by natural products, including salt from the Dead Sea, to guarantee the safety of the formulation [78]. The Dead Sea salt is used in this preparation since it can bring multiple advantages to the lotion, namely to treat certain

diseases such as psoriasis and canker sores [78]. The salt from this sea also contains more minerals than normal salt [78]. The proposed formulation is presented in Table 1.5.

Table 1.5 - Composition of formulation by Hakim [78].

Organic Aloe Vera Juice	60%
Organic Coconut oil	8%
Castor oil	7%
Dead Sea salt	6%
Olive oil	5%
Sweet Almond oil	5%
NF Emulsified Wax	4%
Potato Starch	2%
Leucidal Liquid	1,4%
Apple Cider vinegar	1%
Pure essential oils and extracts	1%
Phenoxyethanol	0,6%

2. Experimental Part

As was mentioned, dry electrodes have two main problems, the strong artefact susceptibility and the high skin-electrode impedances, much stronger than in the wet electrodes [1]. So, a solution is proposed in the form of an emulsion. As the artefact generation is related with the skin hydration state, the emulsion must hydrate the scalp which translates in a decrease in the skin-electrode impedance. The produced emulsion must also be easy to apply, take effect in less than 30 minutes, cannot be greasy and must remain active at least for four to eight hours.

The emulsion was formulated in the Department of Pharmaceutical Technology of the Faculty of Pharmacy of the University of Porto.

2.1. Materials and Methods

Throughout the development of the emulsion multiple components were used, which are reported in Table 2.1, with the respective origin and batch. Furthermore, a list of equipment used for preparation and analysis is provided in Table 2.2.

Table 2.1 - Raw materials used in this work.

Raw Material	Origin	Batch
NaCl	PROLABO	12H220019
Kathon® CG	Acofarma	62897
Urea	Fagron/ Acofarma	13G10-B08-284684/ 131688
Glycerine	Acofarma	151817/170601
Tween® 80	Acofarma	130287-P-1
Sodium Alginate	IMCD	S21721
Oleic Acid	Acofarma	120015-E-3
Sweet Almond Oil	Acofarma	161952
Dimethicone	Acofarma	131177-P-1
Miglyol	Acofarma	132249-P-2/ 170186-P-1/ 171252-P-1
HPMC	Colorcon	DT317684
Xantan Gum	Acofarma	141167
Aristoflex	Clariant	GBG0003931
Cetly Alcohol	Acofarma	130151-P-2
Glyceryl Stearate	Guinama	9415600006
Ceteareth-12/Eumulgin B1	Guinama	9405100010
Corn Starch	Acofarma	170222-P-2
White Wax	Acofarma	130761-P-1
Cutina HR	Cognis	CG00730001
Sodium Metabisulfite	José M. Vaz Pereira, Lda.	961773

Isopropyl Myristate	Acofarma	130239-P-1
Lanette N	José M. Vaz Pereira, Lda.	338149
Butylated Hydroxytoluene (BHT)	Fluka	1390957
Water	b.Tec.Farm.	
Phosphate buffered saline	Sigma	SLBW3999

Table 2.2 - Equipment used in this work.

Equipment	Origin
Centrifuge 5804	Eppendorf AG, Germany
Corneometer® CM 825	Courage-Khazaka, Germany
FT/IR-4000	Jasco,USA
Interface 1000E Potentiostat	Gamry Instruments, USA
Mastersizer 3000	Malvern Instruments Ltd, UK
pH Meter	Basic pH meter 20, Crison Instruments, Spain
Vibra Cell ™ VCX130	Sonics & Materials, INC, USA
Viscometer	HAAKE Viscotester, ThermoScientific, Germany
Visioscan® VC 98	Courage-Khazaka, Germany
Tewameter® TM 210	Courage-Khazaka, Germany
T25 Digital Ultra Turrax®	IKA, Germany

2.1.1. Preparation of moisturizing emulsions

The emulsion must have some essential components such as an electrolyte, a preservative, an oily phase, an O/W surfactant (since it will be an O/W emulsion), moisturizing agents, a thickener and water.

In a first study, two moisturizing agents, urea and glycerine, were analysed to assess whether these two substances had synergistic action. For this purpose, four formulations were prepared - Table 2.3.

Table 2.3 - Composition of formulations H9C, H10C, H11C and H12C.

Raw Material	H9C	H10C	H11C	H12C
Dimethicone	10%	10%	10%	10%
Glycerine	10%	-	10%	-
Kathon® CG	0.10%	0.10%	0.10%	0.10%
NaCl	2%	2%	2%	2%
Sodium Alginate	2.50%	2.50%	2.50%	2.50%
Tween® 80	5%	5%	5%	5%
Urea	10%	10%	-	-
Water	qsp 100%	qsp 100%	qsp 100%	qsp 100%

As can be seen, H9C is the formulation that has both urea and glycerine, H10C only contains urea, H11C has glycerine and H12C contains neither urea nor glycerine.

The protocol for the preparation of the above formulations is:

- In a beaker with water, NaCl, Kathon® CG, urea and glycerine were added in the appropriate quantities and stirred with a magnetic stirrer;
- This aqueous solution was heated at 50 ºC and Tween® 80 was added;
- Then, using a T25 Digital UltraTurrax® homogenizer, at 8000 rpm for 5 minutes, dimethicone was added to the aqueous solution. Following, the emulsion was submitted to ultrasonication using the Vibra Cell VCX130 ultrasonic homogenizer, with an amplitude of 70% for 5 minutes.
- Finally, the sodium alginate was dispersed in the emulsion with a magnetic stirrer. All formulations are kept in an amber coloured glass bottle, tightly closed, at room temperature.

To prepare the emulsion, a homogenizer and an ultrasonic probe were used. A homogenizer is an equipment that reduces the size of droplets in a liquid-liquid dispersion to form an emulsion [79]. In other words, it uses mechanical shear to mix the immiscible liquids into smaller droplets and these droplets are dispersed in the other liquid - Figure 2.1A [80]. It is necessary to choose the appropriate shear rate for the production of a homogeneous emulsion with a maximum reduction in particle size [81].

Regarding the ultrasonic probe it operates by generating sonic pressure waves in the liquid, which in return creates micro-bubbles - Figure 2.1B [80]. Such micro-bubbles will vibrate at a certain amplitude and implode, which creates enough energy to break droplets into even smaller droplets [80].

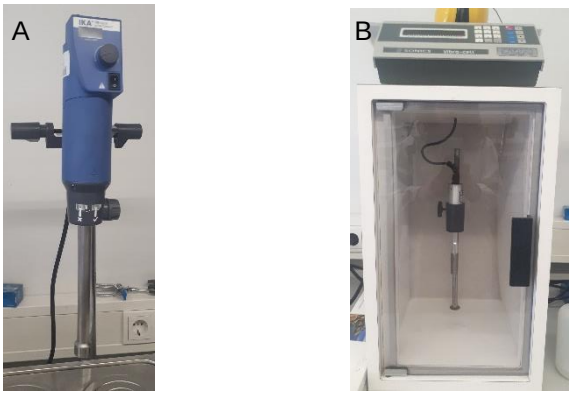


Figure 2.1 - Equipment; A - Mechanical homogenizer T25 Digital UltraTurrax®; B - Ultrasonic probe Vibra Cell VCX130.

2.1.2. Improvement of formulations

After the initial study, it was necessary to improve the moisturizing formulation. Various formulations were developed in the search for the one that fulfilled all the requirements (Annex Table 2.12, 2.13, 2.14). Thus, the one that presented the best results and fulfilled most of the requirements was the formulation H14 - Table 2.4.

Table 2.4 - Final formulation - H14.

Raw material	H14
NaCl	2%
Kathon® CG	0.10%
Urea	10%
Glycerine	10%
Miglyol	10%
Glyceryl Stearate	3.50%
Ceteareth-12	1.50%
Sodium Metabisulfite	0.10%
Water	qsp 100%

The protocol for the preparation was very similar to the previous one, with some slight differences:

- In a beaker with water, glycerine, Kathon® CG, NaCl and sodium metabisulfite were added (aqueous phase), and the mixture was heated at 60-70ºC and stirred with a magnetic stirrer;

- In another beaker, glyceryl stearate, Ceteareth-12 and Miglyol (oil phase) were added and also heated at 60-70ºC;

- When both phases were at the same temperature, the oil phase was added to the aqueous phase and stirred with the magnetic stirrer;

- The emulsion was stirred until room temperature, 20-25ºC, and finally urea and the remaining water were added.

2.1.3. Characterization of the emulsions

2.1.3.1. pH

An essential parameter of a topical formulation is pH. Therefore, there is the need to measure the pH, which can be done using a pH meter. This equipment measures the hydrogen-ion activity, acidity or alkalinity in a solution [82].

Therefore, preparations were then characterized regarding pH, using the Basic pH meter 20 - Figure 2.2 - after preparation, one month and two months after storage at room temperature. The measurements were performed three times and an average was calculated.

Figure 2.2 - Basic pH meter 20.

2.1.3.2. Organoleptic characteristics

Organoleptic characteristics can also be assessed, which concerns the colour, odour and appearance of a formulation [83]. This evaluation is made using the human senses, being important to know how the consumer will perceive the product [84]. This was performed after emulsion preparation, one month and two months after storage at room temperature.

2.1.3.3. Stability Assessment

Another essential aspect is the physical stability of a formulation, which influences the expiration date [85]. This can be evaluated through the test of Accelerated Stability Assessment Test by Centrifugation. This induces an acceleration on the aging process, reducing the time of evaluation [86]. It is assumed that stability is directly proportional to the gravitational force, so if separation of phases exists, it means that the formulation is not chemically stable [86].

This was achieved using the Centrifuge 5804 at 3000 rpm for 30 minutes - Figure 2.3. Emulsion was placed in a 15 mL Eppendorf tube until the 5 mL mark.

Figure 2.3 - Centrifuge.

2.1.3.4. Droplet size

The mean size and the size distribution of the emulsion droplets was measured using the Mastersizer 3000, Malvern (U.K.). This equipment measures light scattering, being a laser diffraction technique [87]. More precisely, it measures drops within a volume defined by the intersection between the laser beam and the fluid [87].

As an emulsion was prepared, it was possible to measure the size of internal phase droplets. Accordingly, Mastersizer 3000 was used, which makes five measurements for each sample and the results were expressed as D_v10, D_v50, D_v90, and the respective variation coefficients - Figure 2.4.

Figure 2.4 - Mastersizer.

2.1.3.5. Viscosity

Another important parameter is the viscosity, which can be measured using a viscometer [88]. When a force is applied to a certain fluid, a displacement of the fluid molecules occurs [88]. Consequently, a velocity gradient is established and since the molecules are located at different points on the liquid, the developed velocities will be different [88]. This leads to the deformation of the fluid, thus viscosity is the property that defines the extent to which the material resists deformation [88]. Another way to express viscosity is by applying a force to a plate while another parallel plate is stationary - Figure 2.5 [88]. The plate moves with a certain velocity, which in return also causes a deformation in the fluid [88]. The force per unit area is denominated shear stress, and the gradient of velocity field is the shear rate [88]. The shear rate is understood as the rate of deformation of the fluid [88]. So, viscosity is mathematically defined as the ratio of shear stress to shear rate, having the units of Pascal-seconds (Pa·s) [88].

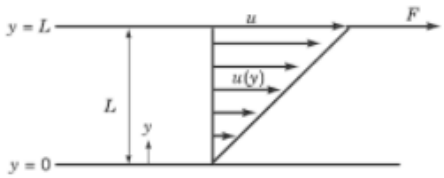


Figure 2.5 - Representation of a fluid between two walls, being one of the walls stationary and the other moveable. A force F is applied to one of the walls, which causes deformation of the fluid, creating a velocity field [88].

The viscosity depends on the temperature, pressure and chemical composition of the fluid in question [88].

In terms of viscosity a fluid can be Newtonian or non-Newtonian - Figure 2.6 [89]. Therefore, if the viscosity of a certain fluid is independent of the shear rate that fluid is Newtonian [89]. On the other hand, if the viscosity depends on the shear rate the fluid is called non-Newtonian [89]. In this class, some fluids can present elastic shear properties, meaning that part of the induced deformation is recovered when the stress stops being applied [88]. These fluids are denominates viscoelastic fluids [88]. Moreover, if the viscosity decreases with increasing shear rate the liquid is instead pseudoplastic, and if it increases it is dilatant [88].

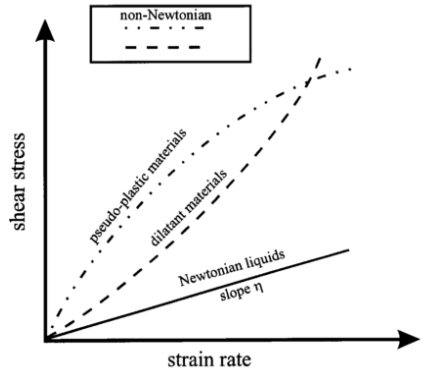


Figure 2.6 - Flow curves that demonstrate Newtonian and non-Newtonian fluid behaviour [89].

Viscosity was evaluated using the rotational viscometer HAAKE Viscotester - Figure 2.7. This equipment uses the principle of shearing the fluids between two walls, which creates a velocity profile on the liquids and also imposes a force on the stationary wall [88]. For this study, the attached thermostatic bath was kept at 32 ºC, since this is reported to be the temperature of skin surface at an ambient temperature of 20-22 ºC [90]. The measurement consisted in a stabilization period of 1800 seconds and a test period, in which the shear rate started at 1 s^{-1} until it reached its maximum of 500 s^{-1}, returning again to 1 s^{-1}. The viscosity was measured one week after preparation, and one month and two months after storage at room temperature.

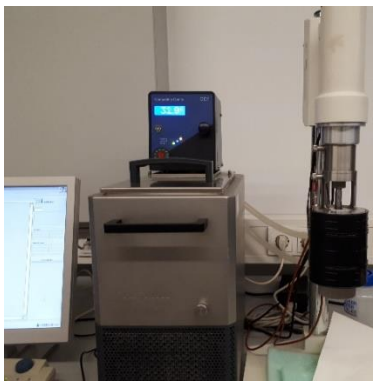


Figure 2.7 - Rotational viscometer with a thermostatic water bath attached.

2.1.4. *In-vitro* impedance studies

To further test the emulsion, an *in-vitro* study was carried out using a diffusion cell to perform impedance spectroscopy. Electrochemical Impedance Spectroscopy (EIS), is a non-destructive, non-invasive and real-time technique used to assess the electrical properties of materials and their interfaces with surface-modified electrodes [91], [92]. This technique can be used for bio-electrical impedance investigation, electrical impedance tomography, study of transdermal drug delivery, etc [93]. Furthermore, it is extensively used to evaluate the condition of animal tissues *in-vivo*, *in-vitro* and *ex-vivo* [94].

EIS is based on the concept of electrical impedance, which is the aptitude of a circuit to resist the electrical current flow and is measured in Ohm [91], [95]. Therefore, if an AC potential is applied to an electrochemical cell and the current that goes through the cells is measured, it is possible to know the electrochemical impedance [95]. For instance, if a sinusoidal potential excitation is applied the response will be an AC current signal. [95] Thus, it is possible to analyse the signal as a sum of sinusoidal functions [95]. Accordingly, impedance is assessed using a small excitation signal, such that the response from the cell is linear [95]. Therefore, EIS can be applied to monitor specific information within a certain region of interest with patterned electrodes and it translates the acquired data into a spectra of electrical impedance [92].

Electrochemical impedance spectroscopy can be performed in two modes, the potentiostatic and the galvanostatic [96]. Here was used the potentiostatic mode, in which a fixed DC potential is used and a perturbation in the form of sinusoidal potential is applied to the cell [96]. The result of the study is the system impedance [96].

Data can be represented as a Bode plot, being impedance vs frequency, or as a Nyquist plot which is imaginary component of the impedance vs real part [56]. Accordingly, the Bode plot has the perk of showing the frequency, since the impedance is plotted with log frequency on the x-axis and the absolute values of the impedance ($|Z| = Z_0$) [95]. On the other hand, the Nyquist plot is obtained by representing Z with an imaginary part.

Therefore, for this study there is the need to choose a membrane that replicates the biological conditions. Human skin should ideally be used to perform these kinds of tests, however it is expensive and difficult to obtain, so models need to be used [97]. One of the most widely used models for human tissue permeability studies, is mouse skin [98]. This is due to the fact that, mouse skin is cheap to purchase and maintain [98]. Moreover, there are some advantages of using animal skin instead of using human volunteers and whole animals, such as it is cheaper, quicker to obtain, better reproducibility is normally achieved and the parameters used are less restricted [99]. However, human and mouse skin have some differences that should be noted. For example, it is reported that mouse skin is normally more permeable than human skin for certain components [97].

So, the hydration ability of the emulsion was evaluated by testing the impedance change of mouse skin after being in contact with the emulsion for a certain time. To that end, a portion of skin is fixed between two chambers of a home-made diffusion cell - Figure 2.8A. Accordingly, mouse skin was acquired at FFUP on 14 of December 2017, on 9 of March and on 4 of April 2018. The dermis side of the skin was cleaned with isopropyl alcohol to remove the adherent fat and the skin hair was trimmed with a scissor [100]. Afterwards, the skin was washed with distilled water and wrap in aluminium paper and stored at -20ºC [100]. When used, the skin was once again washed with distilled water and the skin is cut such that it can cover the ring of the compartment (area of hole is 0,79 cm^2 and area to cover is 2,0 cm^2) - Figure 2.8B and 2.8C.

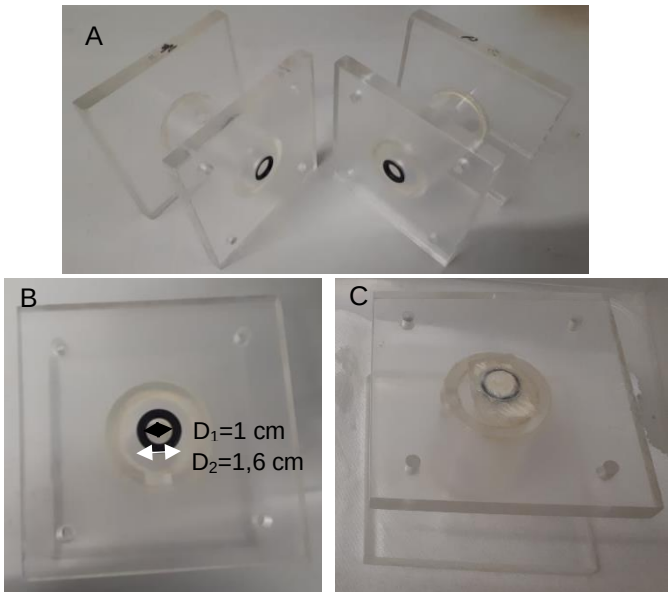


Figure 2.8 - Four electrode cell used; A - Two compartments of the cell; B - View of the hole and diameter values, in which the hole has 1 cm of diameter, but the membrane must cover the 1,6 cm diameter; C - Sample of mouse skin applied on the hole.

A four electrode cell is used to study processes that occur within the electrolyte, meaning between two measuring electrodes, separated by a membrane, which in this case is the mouse skin [96]. In this set up, the working and the counter electrodes (the outer electrodes, A and D in Figure 2.9) provide current flow [96]. The electrodes used for this purpose are of titanium with a platinum coating - Figure 2.9A and D. Accordingly, this type of cells enables for instance the study of ion transport through a membrane or it can also be used to analyse the conductivity of an electron or ion [96]. The inner electrodes measure the potential difference (inner electrodes, B and C in Figure 2.9) and from the measured current and potential, the software calculates the impedance, which corresponds essentially to the resistance of the skin [96]. These electrodes are reference saturated calomel electrodes (SCE) - Figure 2.9B and 2.9C. For this study, the Interface 1000E Potentiostat was used.

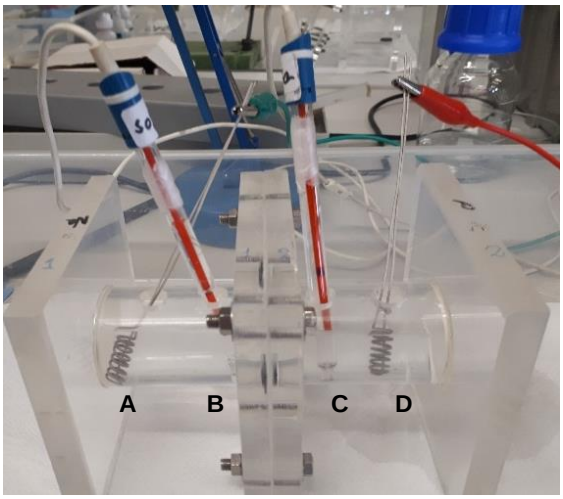


Figure 2.9 - Cell and layout of the four electrodes used in the impedance spectroscopy, in which A is the counter electrode and D the working electrode. Through these two electrodes there is current flow. Furthermore, B corresponds to the reference electrode and C to single electrode that measures the impedance between the two points [96].

The software used was the "Gamry Framework", being chosen the test "Potentiostatic EIS" with the parameters shown in Figure 2.10.

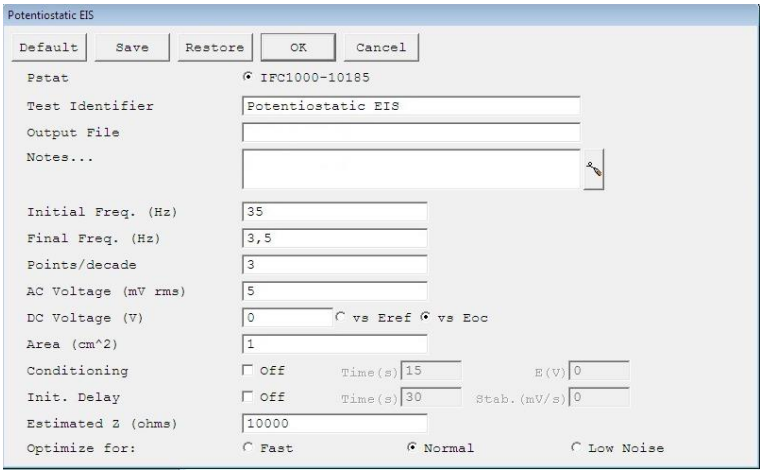


Figure 2.10 - Parameters of the measurement using the program "Gamry Framework" and test "Potentiostatic EIS".

The receptor media is allocated in the receptor chamber and depends on the permeant and membrane that are going to be studied, as well as, on the cell type going to be used. [98] Furthermore, the receptor fluid must have the capacity to solubilize the compounds being study and it cannot induce changes in the skin, since the fluid must be kept in contact with the underside of the skin until the end of the experiment [99].

Thus, an ideal receptor media for an *in-vitro* permeability study should mimic the *in-vivo* situation [98]. The chosen receptor media was phosphate buffer saline (PBS), since it is widely used for ionisable permeants and in impedance studies [98].

So, the two chambers were filled with PBS - Figure 2.11D-, which was prepared by adding a tablet of PBS to a volume of 200 mL of water. The skin was kept in PBS for approximately 40 minutes. This time was chosen by measuring the impedance of the skin for 5 hours and assess in which time the impedance turned constant - Annex Figure 2.32. Then, the PBS is replaced in the left chamber by the emulsion - Figure 2.11. Impedance measurements are performed for eight hours with the following periodicity: every two minutes until the 20 minutes mark, then every five minutes until the 60 minutes mark, afterwards every 15 minutes until the three hours mark and finally every 30 minutes until the eight hours are completed.

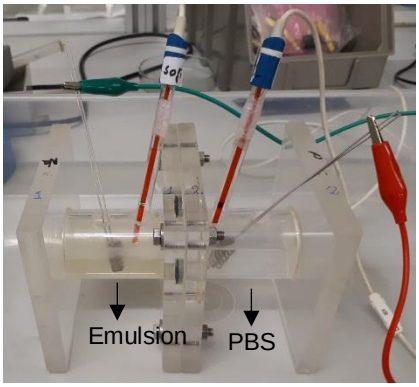


Figure 2.11 - Cell with emulsion in the left compartment and PBS in the right compartment.

2.1.5. Fourier Transform-Infrared Spectroscopy (FTIR)

To evaluate the *ex-situ* effects of the emulsion on the mouse skin, FTIR was used. Therefore, skin morphological changes, disruption of the lipid layer arrangement, etc., which were provoked by the emulsion can be analysed.

FTIR is a vibrational spectroscopic technique that uses electromagnetic radiation, more precisely infrared radiation (IR) [101]. Thus, IR radiation passes through the sample and some of the radiation is going to be absorbed while the rest is going to be transmitted [102]. It is a simple, reproducible, non-destructive, quick and sensitive technique [103], [104].

The information that is obtained is on the molecular-level, allowing the investigation of functional groups, bindings and molecular conformations of the materials at analysis [103]. FTIR evaluates the wavelengths of infrared radiation that are absorbed by the sample, and since each molecule has a specific vibrational spectral band, direct information about the biochemical composition of the sample is provided [103]. In other words, a molecular “fingerprint” is produced and obtained for each compound present in the sample [101]. Therefore, it can be used to know the composition of a certain sample, for quality control and quantification of components [104].

Therefore, FT/IR-4000 was used and measurements were performed on mouse skin - Figure 2.12A. The hairs in the mouse skin had to be cut, to not cause noise in the spectral acquisition, and this was achieved with a shaving machine, to ensure the integrity of the SC. The samples were analysed after being immersed in PBS for 40 minutes (reference spectrum), and

at the following time points: after one hour, 4 hours, 8 hours and 12 hours, in contact with the emulsion. The diffusion cell was once again used. The FTIR was used with the ATR PRO410-M, Multi-Reflection ATR accessory, which is a ATR prism of ZnSe, Ge - Figure 2.12B. The skin was placed directly on ZnSe ATR crystal with the SC facing down on the crystal surface. The spectrums were collected with a spectral resolution of 4 cm^{-1} and with 64 scans per minute.

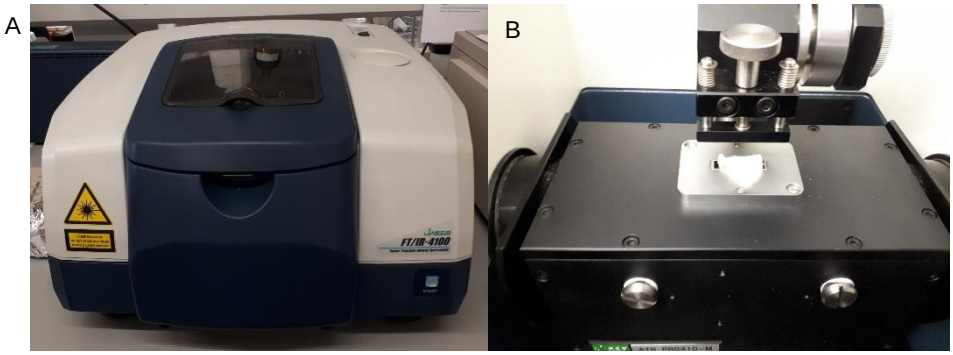


Figure 2.12 - A - FTIR; B - Multi-Reflection ATR accessory with mouse skin.

2.1.6. Biometry studies

Biometry is the study of the physical and anatomical characteristics of an individual [105]. Therefore, the biometry of the skin is the study of its biological and functional characteristics. Some examples of biometric parameters are skin hydration, transepidermal water loss, pH, lipid content, skin relief, etc.

The biometry studies were performed on the inner side of the forearm of human volunteers, in a circular area of 4 cm^2 - Figure 2.13. The area to be tested was not cleaned with any product before the measurements and the volunteers should not have applied any cosmetic product before the test. These studies were performed in FFUP in the Laboratory of Pharmaceutical Technology.

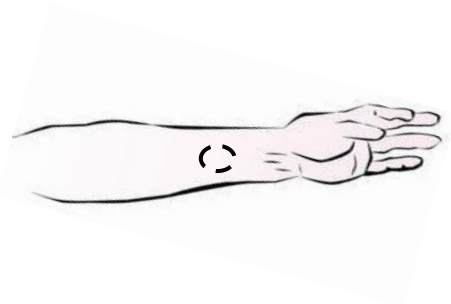


Figure 2.13 - Scheme of the area where the emulsion was applied and where the measurements were performed.

Three types of equipment were used. The first was the Corneometer® CM 825 - Figure 2.14A - that measures the SC hydration and is a capacitance measurement [106]. This equipment has a probe formed by two finger-type metal plates that are close to each other; the measurement depth is around 30 µm [106]. Additionally, it has high reproducibility, it is easy to operate and has

a short measuring time [53]. It uses the high dielectric constant of water to examine the water-related variations in the skin electrical capacitance [107]. Meaning that the dielectric constant is obtained, since it is proportional to the quantity of water present in the tissue [108]. The units of this equipment are system-specific arbitrary units [107]. For this equipment, three measurements were made in each site using the Corneometer, and the average and standard deviation were registered.

Another equipment used was the Tewamenter® TM 300 - Figure 2.14B and C - that measures the transepidermal water loss (TEWL). TEWL is the rate at which water vapour is lost from the body through the skin [109]. This is an essential parameter to assess skin hydration by monitoring the changes in the barrier function of the SC, which is correlated with the skin's ability to retain moisture [53], [110], [111]. Therefore, if the TEWL is low the skin water content will be high, however, if the there is an increase in the TEWL it suggests a damage function [111], [112]. The Tewameter® is an evaporimeter that measures through an open-chamber system [110]. This equipment uses two probes to assess the density gradient of the water evaporation from the skin, being one of the probes for temperature and the other for humidity [110]. Moreover, "the flux density of water is expressed in grams per square meter per hour ($g/m^2/h$)" [110]. TEWL was evaluated for two minutes and the average and standard deviation was registered.

The final equipment used was the Visiocan VC 98 - Figure 2.14D. This is a method that uses image analysis to assess various parameters of the skin such as hydration [113]. This equipment has the advantage of being a rapid measurement probe [114]. It is based on graphic representation of the skin under special illumination and the image is evaluated according to four parameters: roughness, smoothness, scaliness and wrinkles [114]. It measures the skin surface directly having also high resolution [115]. The obtained image is in different grey levels and the distribution of these shades is used to calculate clinical parameters to quantitatively and qualitatively describe skin surface [115]. Therefore, skin smoothness is evaluated by the size of the wavy skin surface, in which high smoothness corresponds to low surface values [115]. For the Visioscan® a photo of the skin relief was taken with a high resolution camera.

Figure 2.14 - Biometry equipment. A - Probe of the Corneometer®; B - Probe of the Tewameter®; C - Tewameter®; D - Camera of the Visioscan.

Firstly, skin hydration, TEWL and skin relief were evaluated in each site before sample application. Following, approximately, 20 milligrams of product were applied with 20 circular movements using a glove. After application of products, hydration and TEWL were measured at 15, 30 and 60 minutes and skin relief measurements were performed only at 30 and 60 minutes.

These tests were performed in 6 human volunteers (all women) with age between 21 and 23. The laboratory where the measurements took place had a controlled temperature around 20 ºC and a relative humidity of approximately 60%. All the volunteers were informed about the experience and signed an informed consent - Annex Figure 2.39 and 2.40. Moreover, longer tests were performed, with a duration of four hours, in which the measurements were done with an interval of 30 minutes.

2.1.7. *In-vivo* Impedance studies

A second *in-vivo* evaluation was performed at FEUP in the Department of Metallurgical and Materials Engineering, consisting in impedance measurements. The temperature and humidity of the laboratory were respectively, in average, 22.8ºC and 48.4%. Once again, the measurements were done in the forearm and a circular area of 4 cm^2 was marked to apply the emulsion. Afterwards, a sintered Ag/AgCl commercial electrodes (BIO, EasyCap GmbH, Germany) was applied on the skin. A second, commercial ECG electrode (Kendall Arbo, Tyco Healthcare Deutchland, Germany) was also applied on the forearm, about 10 cm away, after rubbing strongly with ethanol and applying a commercial gel (Electro-gel, Electro-Cap International, Inc.), to decrease electrode impedance - Figure 2.15. A measurement was performed before emulsion application; thereafter, the emulsion was applied, and impedance measurements were performed after 15, 30 and 60 minutes. These measurements were performed with 10 volunteers (5 women and 5 men), with ages between 22 and 27 years old. The same was done for a duration of 4h after emulsion application, in which the protocol was the same but after the 30 minutes mark, the measurements were done with an interval of 30 minutes. These measurements were performed in 3 volunteers (all women) with 22 years old.

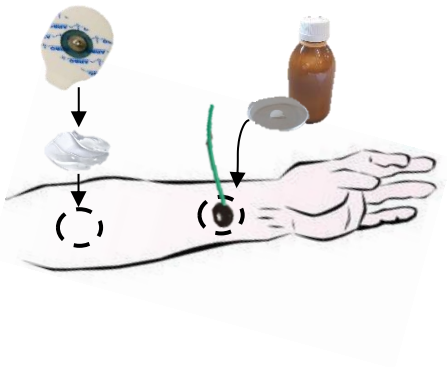


Figure 2.15 - Scheme the in-vivo study, where in the first area an ECG electrode was used with commercial gel and in the second area a Ag electrode was used with the emulsion [116], [117], [118], [119].

The measurements were made by using the software “Gamry Framework”, and an electronic system. In the software it was chosen the test “Potentiostatic EIS” with the parameters shown in Figure 2.10.

2.2. Results and Discussion

An emulsion was prepared to be used in junction with dry EEG electrodes. This emulsion is to be applied in the scalp and has to quickly hydrate the skin. This is mainly because, dry electrodes have a strong artefact susceptibility that is related with the skin hydration state. The emulsion must also create an ionic path to ease the electrical conduction. Additionally, to these requirements the formulation should be easy to apply, be readily absorbed, cannot be greasy, should not have an unpleasant smell, preferably, should not dirty the hair and remain active at least for four to eight hours. This way, the patients do not need to wash the hair after the exam, as the emulsion is naturally absorbed by the skin.

For this end, there are some essential components that the formulation must present. To hydrate the skin, moisturizers are used, so two humectants were chosen. These compounds were glycerine and urea [52], [120]. The way they hydrate the skin is by increasing the water content [52], [120]. It was decided that an emulsion would be prepared, as emulsion droplets can cause an occlusive effect at skin surface, important in hydration. Therefore, a surfactant and an oily component were needed, being the last one the dispersed phase. Additionally, most oily components are emollients that enhance skin appearance by promoting skin softness, flexibility and smoothness, being in the end translated in skin hydration [61]. A preservative was also needed to maintain the product fresh and safe [39]. Furthermore, as the emulsion is to be applied in the scalp a slightly viscous preparation is advised, to not drip to the eyes, as well as, to adhere promptly to the skin. Therefore, a thickening agent may or not be added. Lastly, the formulation contained an electrolyte, to increase electrical conductivity, one of the requirements. The electrolyte chosen was sodium chloride (NaCl), as it is the most abundant salt in our body [121]. This component can be used as a thickener and it presents effects in hydration, being extensively used in commercial cosmetics and pharmaceutical products [121].

2.2.1. Initial formulations and selection of moisturizing agents

The first four formulations, H9C, H10C, H11C and H12C are represented in Table 2.3. In relation to the components chosen, Kathon® CG was selected as the preservative since it is generally used in topical and hair formulations [122]. The concentration chosen was 0.10%, being in the range of concentration regarded as safe (0.05% to 0.1%) [122]. Regarding the surfactant, Tween® 80 was chosen. This compound is a non-ionic surfactant, therefore it is not as irritant as other surfactants [65]. Additionally, it is widely used in topical and pharmaceutical formulations [65]. Dimethicone was selected as the oil phase, being non-greasy and odourless [65]. In addition, it also works as an emollient, meaning it makes skin smooth and it fills the spaces between corneocytes [66]. Regarding glycerine and urea, both are components extensively studied and generally regarded as safe. A percentage of urea between 10% and 30% increases water uptake in the SC [120]. Furthermore, higher concentrations of urea are used for extreme cases of dry and rough skin [123]. Therefore, in this study, 10% of urea and 10% of glycerine were used.

Sodium alginate was used to increase the viscosity and it also works as a stabilizing agent in O/W emulsions [65]. It also has the advantage of being normally used in topical formulations [65]. The concentration of sodium alginate can be adjusted until the desired viscosity is achieved. In this case, a concentration of 2.50% was employed. These were the first formulations tested since they presented a good feel in the skin.

2.2.1.1 Accelerated stability

Accelerated stability by centrifugation of the four formulations was performed. The results are in Figure 2.16.

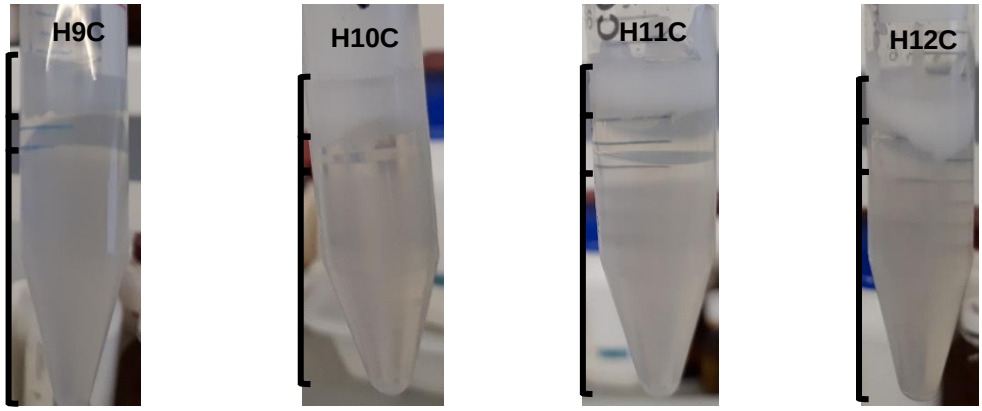


Figure 2.16 - Aspect of the emulsions after centrifugation, 30 minutes at 3000 rpm.

After centrifugation, all the formulations showed phase separation. This means that the formulations are not stable after a long period of storage. However, when performing accelerated stability studies, strong forces are applied in the formulation, inducing an aging process that might take, in reality, some time. So, this test is relevant as a primary evaluation of the long term stability of emulsions.

2.2.1.2 Organoleptic characteristics

Organoleptic characteristics were assessed at the moment of preparation and one and two months after storage, at room temperature. The results are reported in Table 2.5.

Table 2.5 - Organoleptic characteristics of each formulation.

Formulation	Time	Odour	Aspect
H9C	0 month	Almost Odourless	Homogeneous
	1 month	Almost Odourless	Phase Separation
	2 months	Almost Odourless	Phase Separation
H10C	0 month	Almost Odourless	Homogeneous
	1 month	Almost Odourless	Phase Separation
	2 months	Almost Odourless	Phase Separation
H11C	0 month	Almost Odourless	Homogeneous
	1 month	Almost Odourless	Phase Separation
	2 months	Almost Odourless	Phase Separation
H12C	0 month	Almost Odourless	Homogeneous
	1 month	Almost Odourless	Phase Separation
	2 months	Almost Odourless	Phase Separation

The formulated emulsions presented a light smell of raw materials that was not sensed when applied in the skin. Overall, there were no significant changes in the odour of the formulations. However, after 1 month all of them presented phase separation. Therefore, as was expected by the accelerated stability test, separation of phases occurred. The formulation that was the last to display phase separation was H9C and the one showing the quickest separation was H12C. This leads to the conclusion that H9C is more stable than H12C. The colour after preparation was off-white, not totally opaque colour, which is characteristic of emulsions. After phase separation there was one phase that remained white while the other was slightly transparent, as can be seen in Figure 2.7.

Figure 2.17 - Emulsions aspect after two months of preparation.

The separation of phases is an indicator of the instability of the formulations. Therefore, all formulations showed physical instability, however oxidation seems not to occur as the phases maintained a whitish colour and there were no changes in odour. These results suggest that some components of the formulation might be incompatible. So, it was necessary to optimize the formulation.

2.2.1.3 pH

A pH ranging between 5.4 and 5.9 would be most appropriate since skin pH is around these values [49]. However, the physiological pH range that is recognised for dermal formulations, range between 4.0 and 7.0 [124]. The values of pH after 1 month and 2 months of formulations preparation are represented in Table 2.6. As separation of phases occurred, it was necessary to shake the emulsions before measuring the pH.

Table 2.6 - pH of the 4 formulations.

pH	0 month	1 month	2 months
H9C	6.95	6.84	7.79
H10C	6.68	6.43	7.24
H11C	6.07	5.14	5.52
H12C	7.68	4.62	5.08

The preparation with the highest pH, at the beginning, was H12C and with the lowest was H11C (glycerine). After two months, H11C and H12C pH decreased. However, the pH of H9C and H10C slightly increased. Accordingly, the pH was not constant over the 2 months. Nevertheless, all the values were within the physiological pH range (4.0 – 7.0). Therefore, all the formulations were suitable for skin application regarding pH [124].

Stability of a pharmaceutical formulation is defined as the capacity of a product to maintain, to a certain extent, its physical, chemical, microbiological and toxicological properties, which were present at the time of its packaging [125]. This is for a period of storage and use [125]. So, pH can also be an indicator of stability. Urea is unstable in many pharmaceutical and cosmetic preparations since it hydrolyses into ammonium cyanate [120]. The degree of degradation is normally small, yet it translates into multiple effects, such as a shift in pH into alkaline and a strong ammoniacal odour [120]. Acidic preparations are disposed to accelerate the process of decomposition of urea [120]. This can be observed in H10C formulation, in which the pH increased by almost one unit after two months, suggesting degradation of urea. On the other hand, it is reported that glycerine can affect the pH by slightly decreasing it [126]. This aspect can be confirmed in the formulation H11C, which has only glycerine, and presented the lower initial pH. The formulation without any humectant (urea or glycerine) had a higher pH after preparation, which decreased significantly after two months, suggesting that the other components of the formulation may cause a decrease in initial pH.

2.2.1.4 Size of internal phase droplet

The results obtained from the Mastersizer for the internal phase droplets size is reported in Table 2.7.

Table 2.7 - Droplets average size (µm).

AV (µm)	D_v10	D_v50	D_v90
H9C	0.887	3.22	9.11
H10C	0.950	2.74	9.10
H11C	0.830	3.38	16.30
H12C	0.720	2.55	12.10

Mastersizer gives the values of droplets size in percentiles D_v10, D_v50 and D_v90. Thus, all the formulations had similar values for the D_v10 percentile, as well as for the D_v50. However, for the D_v90 is where there were large differences between the formulations, when comparing H9C and H10C with H11C and H12C.

To be considered a microemulsion the particle size has to be below 100-200 nm [127]. So, the formulations prepared are emulsions since the droplets size was higher than 200 nm. Thus, to achieve a lower droplets size, the velocity used in the mechanical homogenizer should be increased. Furthermore, more surfactant should be used, for instance 10% of Tween® 80, instead of 5%. It could also be added another surfactant. Mastersizer measurements were only performed right after preparation of the emulsions.

2.2.1.5 Viscosity

Viscosity was measured after one week of formulations preparation, one and two months. The results are presented in Figure 2.18. As all the formulation separated after approximately one month, thus, before measuring the viscosity, they were shaken manually.

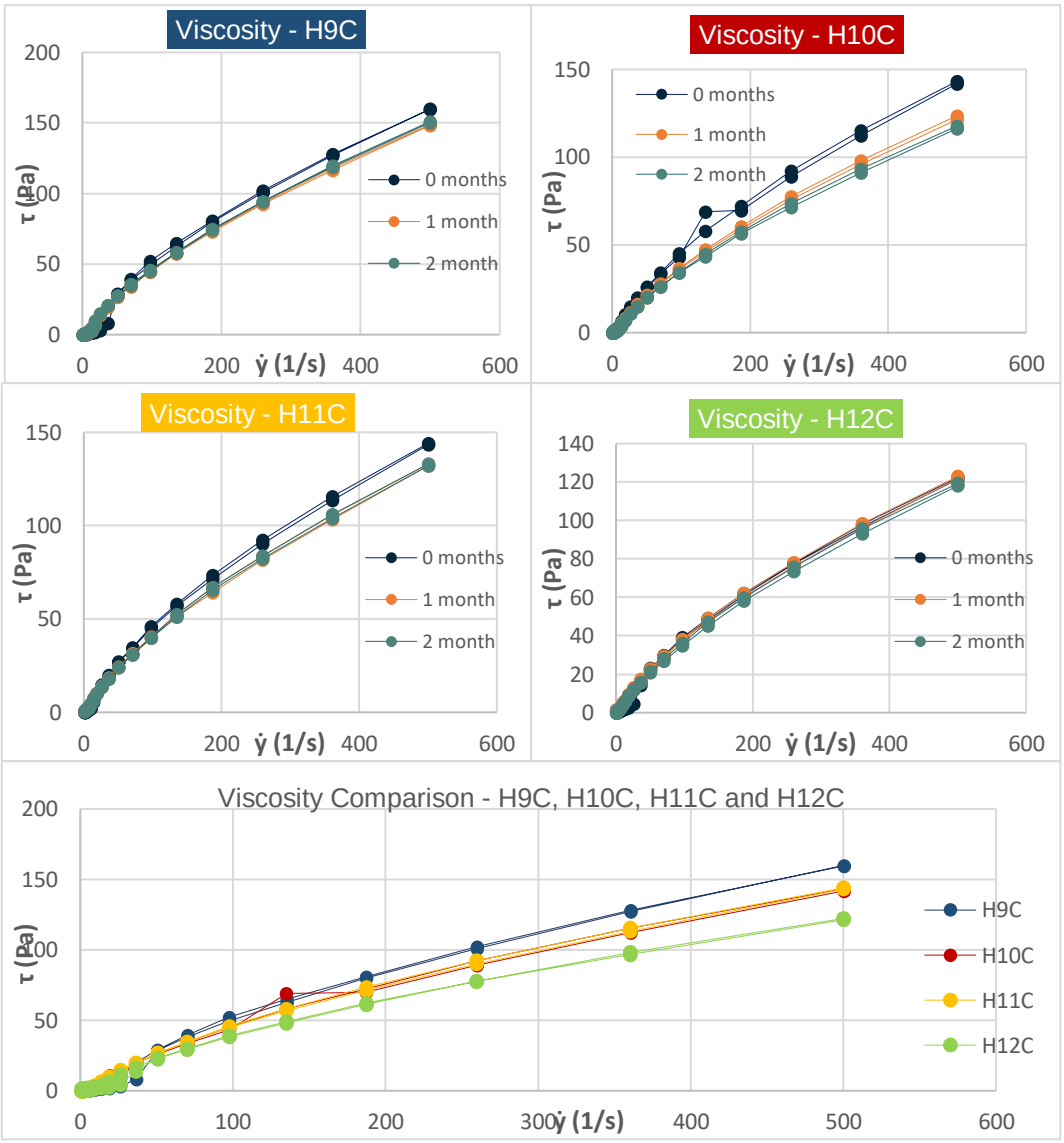


Figure 2.18 - Viscosity of the four formulations after preparation and two months after storage.

In general, all the formulations showed no appreciable changes in viscosity and rheological behaviour after 2 months of storage.

The four formulations are non-Newtonian liquids with a shear-thinning behaviour, since the shear stress increases with an increasing shear rate [88]. This is in agreement with the normal rheological behaviour of solutions containing sodium alginate since they exhibit a shear thinning or pseudo plastic behaviour.

Comparing the rheological profiles of the four formulations, it is possible to verify that H12C is the one with the lowest viscosity. H10C and H11C have similar values and H9C exhibits slightly higher viscosity values. This might be explained by the fact that H9C is the one with the least amount of water, and H12C was the one with the highest amount of water. The other two formulations, had the same amount of water, but H11C presents a slightly higher viscosity, that can be explained by the glycerine that has also viscosity-increasing properties [65].

2.2.1.6 Evaluation of skin Hydration and TEWL

As previously described, hydration and TEWL were analysed in human volunteers before any product application and 15, 30 and 60 minutes after the formulations application. The results can be observed in Figures 2.19 and 2.20.

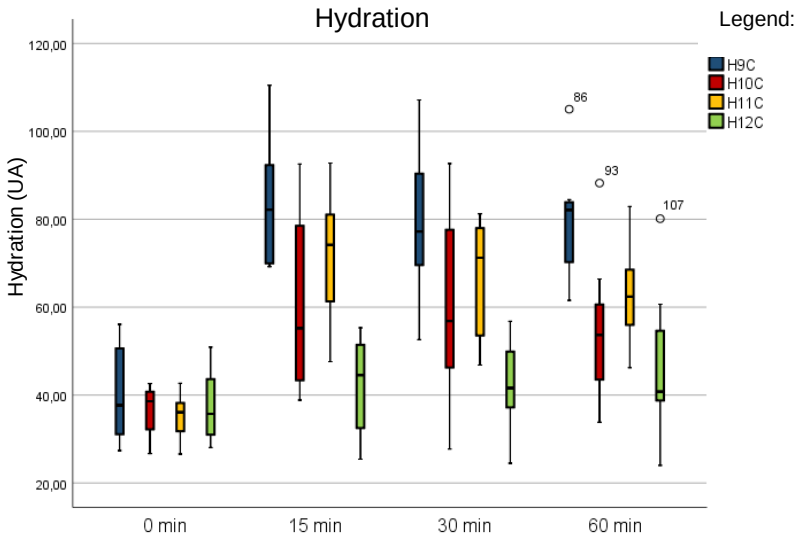


Figure 2.19 - Skin hydration, before emulsions application, and 15, 30 and 60 minutes after application.

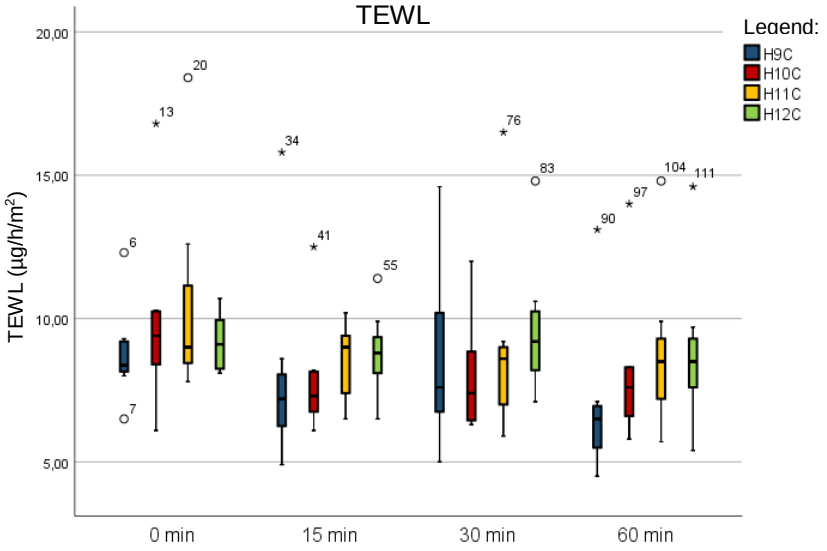


Figure 2.20 - Skin TEWL before emulsions application, and 15, 30 and 60 minutes after application.

A formulation has the ability to hydrate the skin if the values obtained using the Corneometer® are higher than they were before the product application. Therefore, regarding hydration, it is possible to conclude that H9C was the one that caused the highest values of skin hydration. Followed, by H11C, H10C and finally H12C. Globally, hydration values were higher 15 minutes after the formulation application, decreasing at 30 and 60 minutes. These results

demonstrate that H9C, containing both glycerine and urea, achieved a higher hydration than formulations only containing one of the components (H10C andH11).

Regarding transepidermal water loss (TEWL), it is expected to decrease after the products application,, meaning that less water is being lost and the skin is retaining its moisture [109]. Therefore, H9C once again was the formulation that presented the best results, causing the lowest values of TEWL. Followed by the urea based emulsion (H10C). The formulation H11C presented a result similar to H12C after one hour.

It is reported that glycerine solutions only reduce the TEWL for some hours, and if it is reapplied, the TEWL of the skin does not present significant changes [128]. Lóren studied the influence of emulsions with different hydrating compounds by measuring the TEWL and skin capacitance in volunteers [129]. One of the emulsions did not contain low weight humectants, another one included glycerine and the last one included urea [129]. The emulsions without urea (with glycerine) showed an increase in capacitance, however, the TEWL didn't increase significantly, leading to the conclusion that the hydration was not significant [129]. On the other hand, the urea formulations reduced greatly the TEWL, meaning it improves barrier function without any type of irritation [129]. This supports the results of the hydration and TEWL for these four formulations.

Accordingly, H9C presents the best results for hydration and TEWL - Annex Figure 2.33. It can also be expected since it contains 10% more of a humectant than the rest of the formulations. However, it also proves that urea and glycerine work jointly to improve the hydration and water content in the skin. Moreover, urea and glycerine, both present good results in relation with hydration, however, glycerine does not really have an effect in the TEWL values. On the other hand, urea greatly reduces the TEWL values. Therefore, urea and glycerine seem to work in synergy, being the best combination. These results are consistent with the findings of Gloor *et al*, in which the combination of the two components provided better results than a monotherapy [130].

However, it is not only the urea and glycerine that have moisturizing properties, NaCl can also help in this effect as was studied by Hagströmer *et al* [131]. In this study, a cream with urea was compared with a cream with urea and NaCl, to verify if the moisturizing effect increases when the NaCl is combined with urea [131]. Thus, it was found that the cream containing both urea and NaCl is slightly more effective than the one with just urea [131]. This is due to the fact that urea increases the water binding and NaCl absorbs and retains moisture [131].

Furthermore, surfactants can also have some skin penetration enhancer properties. Shokri *et al* evaluated the efficiency of different surfactants to promote diffusion of products through the skin [132]. The surfactants tested were sodium lauryl sulfate (SLS), cetyltrimethylammonium bromide (CTAB), benzalkonium choride and Tween® 80 [132]. SLS showed the best results, but all surfactants increased the rate of diffusion of the test compound

through skin [132]. However, SLS, as an anionic surfactant can cause skin irritation. Tween® 80, on the other hand, does not present this problem, being the one used in these four formulations.

It should also be noted, that the tests were performed with just a few volunteers, thus a higher population sample is needed to confirm the results. This is due to the fact that the properties of skin are influenced by race, gender, age, body site and genes [133], [134], [135], [136]. Therefore, a more heterogeneous population is advised. However, with these preliminary tests it is already possible to see a tendency.

Furthermore, the conditions, for instance, temperature and humidity of the room where the tests are performed can also influence the results. For example, absorption of the emulsion is higher at higher temperatures and at elevated humidity [137], [138].

Any volunteer presented skin irritation or discomfort after emulsions application.

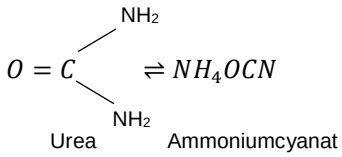
2.2.2 Optimization of moisturizing formulations

So, due to the good results obtained, it was decided to use both humectants in the emulsion. Anyway, the formulation presented stability problems (phase separation and changes in pH), thus, to try to overcome these issues various formulations were prepared. The emulsions formulations can be assessed at Annex in Tables 2.12,2.13 and 2.14. None of these prepared formulations was adequate, since most of them presented phase separation after centrifugation and after storage, as happened in the previous formulations. Additionally, most of the gelling agents are very sensible to electrolytes, which caused instabilities. Moreover, some of the formulations after one month started to present a change of colour to light brown, being an indication of oxidation. Furthermore, in some formulations high temperatures were needed to prepare the emulsions, which could cause instabilities, since many compounds are unstable at high temperatures. Lastly, due to the smell some formulations were discarded, since as was mentioned, odour is an important requirement.

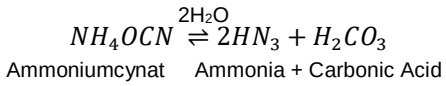
2.2.3. Final formulation

After several attempts, the chosen formulation to proceed with the tests was H14 -Table 2.4. This formulation, similarly to the others, contains NaCl as the electrolyte, Kathon® CG as the preservative, urea and glycerine as the humectants and moisturizing components. Miglyol® was employed as the oily phase. This component is a medium-chain triglyceride that has emulsifying properties [65]. It has some advantages, as the fact that it is easy to spread in the skin, has good penetration properties and it is also an emollient, leading to a feel of smooth skin after application [65], [139]. An antioxidant was also added since it was concluded that formulations containing oils have a tendency to oxidize after two weeks of preparation. The antioxidant chosen was sodium metabisulfite, which is commonly used in oral and topical formulations. Miglyol® is reported in the literature to be resistant to oxidation, therefore the cause of oxidation in other formulations might have been other component, namely urea [65]. As was mentioned previously, urea can be

unstable in many pharmaceutical and cosmetic preparations since urea hydrolyses into ammonium cyanate [120]. When urea is in solution, it decomposes into ammonium cyanate (2.1) and then it can be split into two molecules of ammonia and one molecule of carbonic acid (2.2) [77].



(2.1)



(2.2)

The degree of degradation is usually small, yet it translates into multiple effects, such as a shift in pH towards alkaline values and a strong ammoniacal odour [120]. Acidic preparations are disposed to accelerate the process of decomposition of urea [77],[120]. This leads to an increase in the pH, which in return leads to decomposition [77]. Other factors that promote the decomposition of formulations with urea are high temperatures and contact with the air [77]. Therefore, preparations comprising urea should be stored in normal room temperature or below, and in an airtight container [77]. This is the main reason why in this formulation urea is added in the end, since the solution is heated until 70ºC. The antioxidant added will also help in keeping the pH more stable and inhibit the oxidation of the urea. Additionally, Eumulgin® B1/ ceteareth-12 was employed in the formulation since it is an emulsion stabilizer, forms a thin film and increases the viscosity [65]. This component is used in a low concentration, 1.5% so the viscosity will not be very high, only providing a certain resistance to dripping in the skin. The film that forms, will help in reducing TEWL and is quickly absorbed by the skin. On the other hand, glyceryl stearate was added due to the stabilizing, emulsifying and emollient properties [65]. It is a non-ionic surfactant, used in pharmaceutical and cosmetic products [65]. This component also forms a film that is soluble in water and is absorbed promptly by the skin, helping in the occlusive effect [65]. All the components used in the emulsion preparation are regarded as safe, being in the FDA Inactive Ingredients Database or in the GRAS list [65].

2.2.3.1 Accelerated stability - Final formulation (H14)

The result of centrifugation of H14 is shown in Figure 2.21.

Figure 2.21 - Aspect of the emulsion after centrifugation, 30 minutes at 3000 rpm.

The formulation presented a slight phase separation, meaning that the formulation is still not stable.

2.2.3.2 Organoleptic characteristics - Final formulation (H14)

Organoleptic characteristics were assessed for two months, in the same conditions as the last four formulations. The results are shown in Table 2.8.

Table 2.8 - Organoleptic characteristics of H14.

Time	Colour	Odour	Aspect
0 month	Off-white (not totally opaque)	Odourless	Homogeneous
1 month	Off-white (not totally opaque)	Odourless	Homogeneous
2 months	Off-white (not totally opaque)	Odourless	Homogeneous

Accordingly, the emulsion preserved its colour for the two months, showing no sign of colour change. The odour also did not change during the two months. The formulation maintained a homogeneous aspect for two months. Therefore, the emulsion maintains its integrity at least for 2 months, even though it separated in the accelerated stability test.

2.2.3.3 pH - Final formulation (H14)

Another property tested was the pH variation during two months. The results are in Table 2.9.

Table 2.9 - pH of formulationH14.

	0 month	1 month	2 months
H14	5.62	6.17	6.80

The pH of the emulsion after preparation was slightly acidic, being in the range that can be used in skin (4.0-7.0). After one month it increased and the same happened after 2 months. This results also lead to the conclusion that the formulation is not completely stable, as there was an increase in the pH. These results are in agreement with the results of accelerated stability test.

As most of the components used in this formulation were also present in H9C the reasons for this instability can be the same.

2.2.3.4 Size of internal phase droplets - Final formulation (H14)

The size of droplets was measured right after preparation of the emulsion. Results are shown in Table 2.10.

Table 2.10 - Average and relative standard deviation of droplets for D_v10, D_v50 and D_v90 of H14.

	D_v10		D_v50		D_v90	
	AV (µm)	RSD (%)	AV (µm)	RSD (%)	AV (µm)	RSD (%)
H14	0.655	0.65	2.89	2.30	10.5	1.28

The average size of the droplets was 2.89 µm with a relative standard deviation of 2.30 %. Additionally, 10% of the droplets display a smaller size than 0.655 µm and 90% were smaller than 10.5 µm. The droplets size was smaller than those of the previous formulations, although in this case only a magnetic stirrer was used to emulsify the hydrophilic and the hydrophobic phases. Therefore, a possible solution to achieve even smaller droplets is to use the high speed homogeniser (Ultra Turrax®) and the ultrasound (Vibra Cell ™). This may also contribute to the stabilization of the emulsion for a longer time. However, the procedure was not used to make this formulation because it caused the formation of a lot of foam.

2.2.3.5 Viscosity - Final formulation (H14)

Viscosity and rheological behaviour were evaluated at 32ºC, one week, one and two months after emulsion preparation and also at 20ºC, one week after preparation. The results are reported in Figure 2.22.

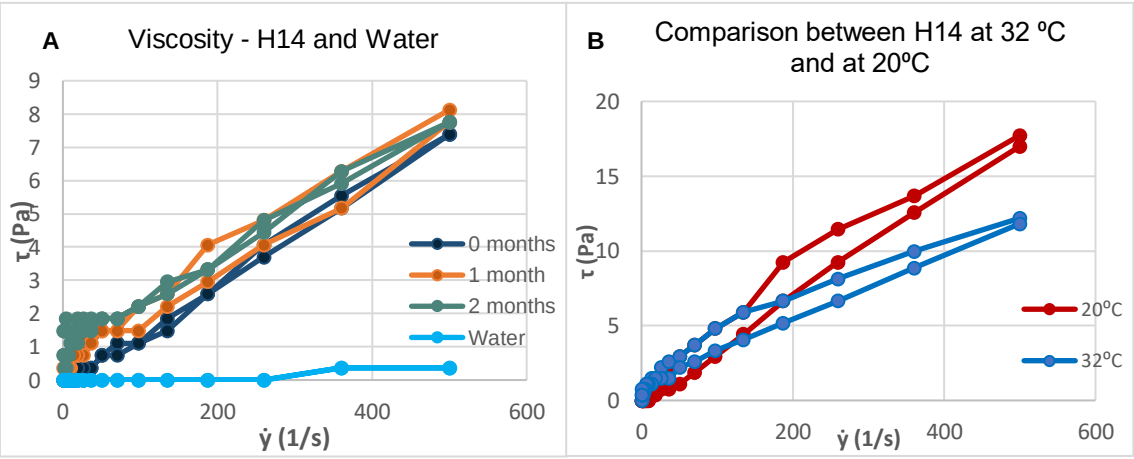


Figure 2.22 - Viscosity of H14. A - Measured at 32ºC for 2 months; B - Viscosity at 20ºC and at 32ºC 2 days after preparation.

The viscosity of H14 showed a slight increase with time. Again, changes in droplets dispersion, the degree of flocculation and changes in the structure influence the rheological properties [140]. The formulation presents a pseudo-plastic or shear-thinning behaviour. This is the suitable behaviour for a topical formulation since the apparent viscosity decreases with increasing tension, meaning that it turns more liquid, which in return makes it easier to spread the product evenly [165]. It also presents a higher viscosity than water, one of the characteristics that the emulsion was intended to have.

In Figure 2.22B, it can also be seen that, as expected, the viscosity was higher at lower temperatures. This also happens for example with water, in which its viscosity decreases exponentially with decreasing temperature [142]. As the formulation is constituted with more than 60% of water this behaviour is normal, and there were other components in the formulation, such as glyceryl stearate and ceteareth-12 that are solid at room temperature and melt at higher temperature [65].

Accordingly, in the market most of the products used in the scalp have a similar viscosity to water, being very fluid. However, if it is possible for the emulsion to exhibit some resistance to dripping and spreading, this may be advantageous as it will not flow, for example, to the eyes after application to the scalp. Furthermore, the measurement of viscosity for a certain time can be a measurement of the stability of a formulation. Therefore, if there are many changes in viscosity it is a signal of instabilities.

2.2.3.6 Evaluation of skin - Hydration and TEWL - Final formulation (H14)

Results of skin hydration and TEWL measurements are shown in Figure 2.23.

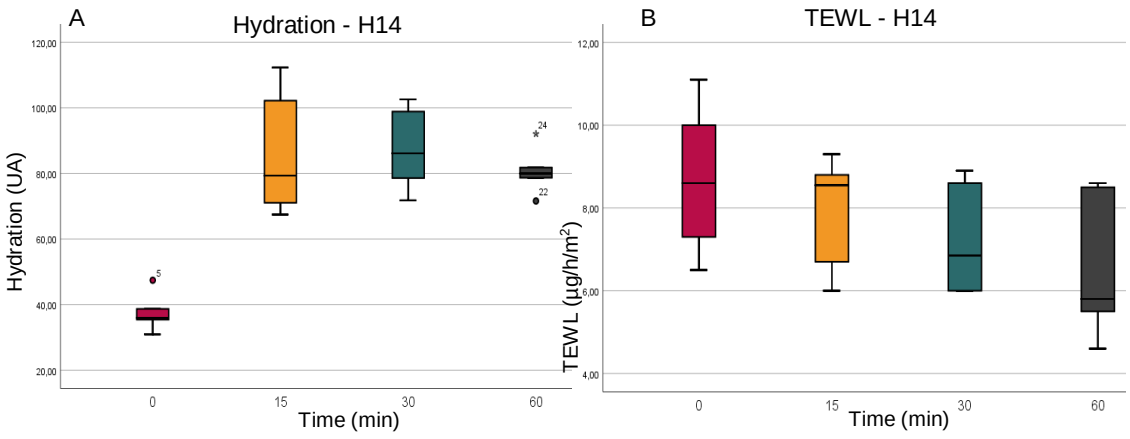


Figure 2.23 - Biometry studies for formulation H14; A - Skin hydration; B - TEWL results.

As can be seen in Figure 2.23, the emulsion increased significantly the skin hydration, maintaining the values even after one hour of application. The initial mean value for hydration was 37.40 UA and after 15 minutes it increased to 85.27 UA. For the TEWL, there was a decrease after emulsion application, however the values did not change significantly during the 60 minutes. Effectively, the emulsion seems to hydrate the skin with a slight decrease in transepidermal water

loss. So, the emulsion did hydrate the skin in a quickly, since after 15 minutes the hydration value was double of the initial. No volunteer presented skin irritation or discomfort, as well as there was not a need skin cleaning at the end of the test since the emulsion was absorbed, leaving a smooth feeling to the skin.

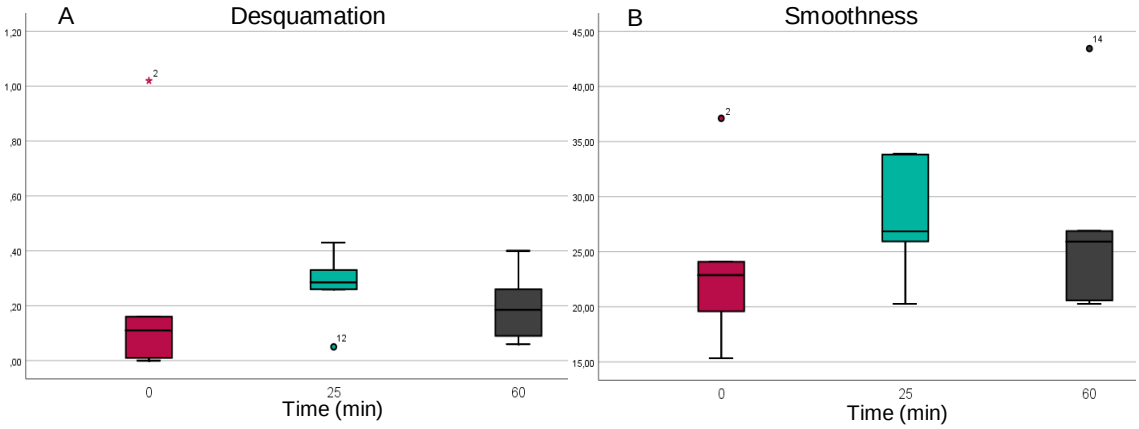


Figure 2.24 - Values of Visioscan for: A - Desquamation; B - Smoothness.

The results regarding the measurements for the relief parameters desquamation and smoothness, are in Figure 2.24. The desquamation increased after 25 minutes but returned to basal values after 60 minutes. Therefore, the emulsion did not have a big impact in desquamation, at least in the first hour of application, meaning that it does not contain exfoliating characteristics. Regarding the smoothness, the values, obtained 60 minutes after emulsion application were higher than the initial values, however, differences were not significant.

To further test the emulsion, biometric measurements were made to confirm that the skin hydration, TEWL, desquamation and smoothness were maintained for 4 hours. The results are presented in Figure 2.25, 2.26 and 2.27.

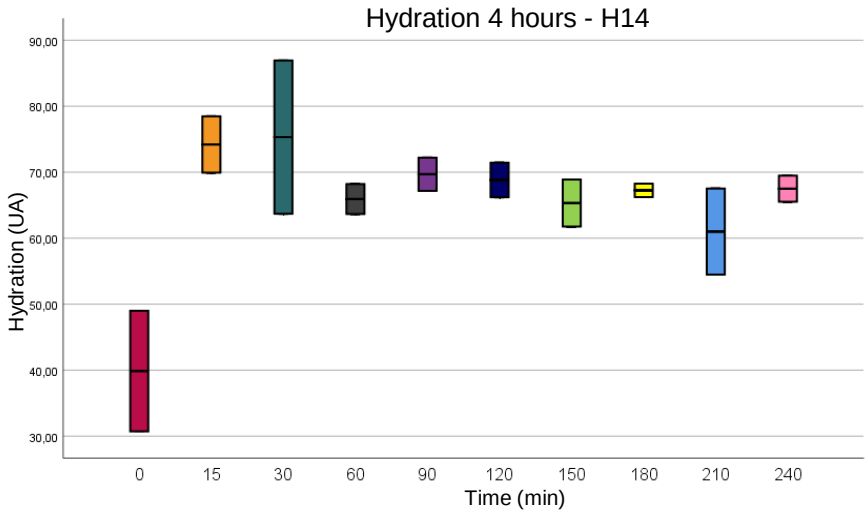


Figure 2.25 - Hydration of skin for 4 hours after application of H14.

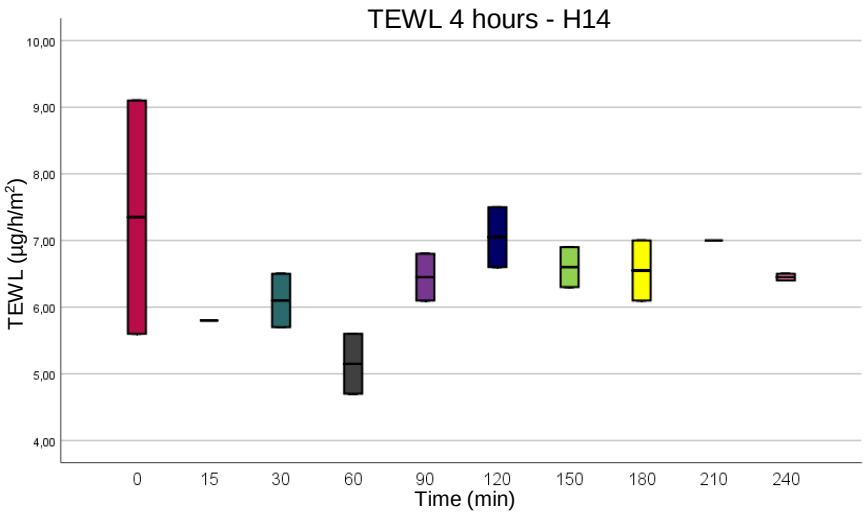


Figure 2.26 - TEWL of skin for 4 hours after H14 application.

The results showed that the hydration had its highest values at 15 and 30 minutes and then it remained constant. The effect of the emulsion was maintained during at least four hours. Confirming, once again, that the emulsion has hydrating properties.

Regarding TEWL, it can be seen that the lowest value was achieved at one hour mark and then the value increased and remained constant until the end of the four hours. Once again, the emulsion did not seem to have a significant impact in the reduction of TEWL. Therefore, the emulsion seems to hydrate using two physiological mechanisms: using humectants and with emollients, not presenting a significant occlusive effect.

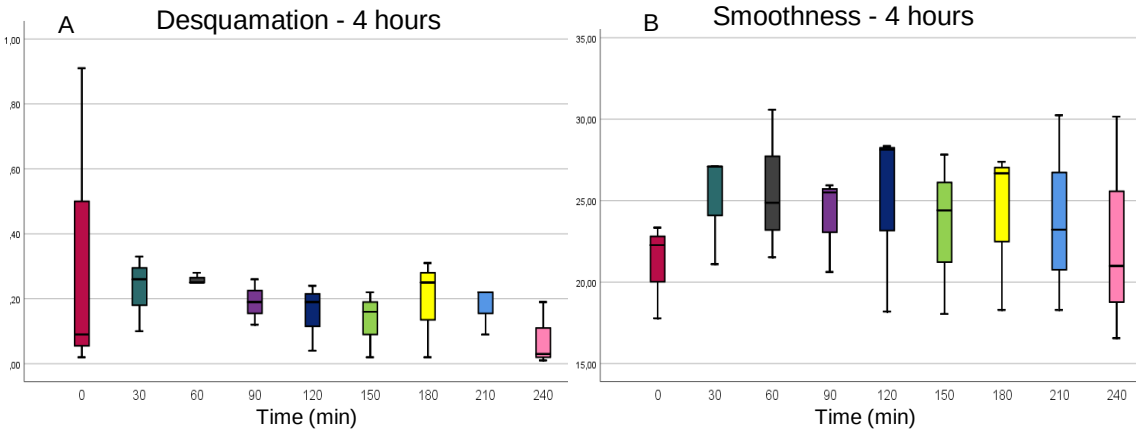


Figure 2.27 - Visioscan values for: A - Desquamation; B - Smoothness.

The values for desquamation and smoothness, measured for four hours, are in Figure 2.27. The desquamation values showed that the emulsion did not have a big influence in this parameter, since the baseline value is almost the same as the value obtained after four hours. The smoothness, has increased after 30 minutes, but this value did not remain constant since after four hours was the same as the baseline. Therefore, smoothness, seemed to improve for one or two hours, and after that the effect seemed to disappear gradually.

After four hours, no irritation was, once again, presented nor discomfort. One of the requisites for the emulsion was to present a hydration effect for at least four hours, which was achieved.

2.2.4 Impedance spectroscopy in mouse skin - Final formulation (H14)

Impedance spectroscopy was used to evaluate effects of the emulsion on the permeability of mouse skin to small ions. The results of impedance spectroscopy measurements performed for eight hours are in Figure 2.28. The impedance of just PBS and the emulsion, were recorded and subtracted to the *in-vitro* impedance values. The values of impedance for PBS and the emulsion are in Annex at Figure 2.34. These measurements were made in low-frequency range from 35.78 Hz to 1.628 Hz.

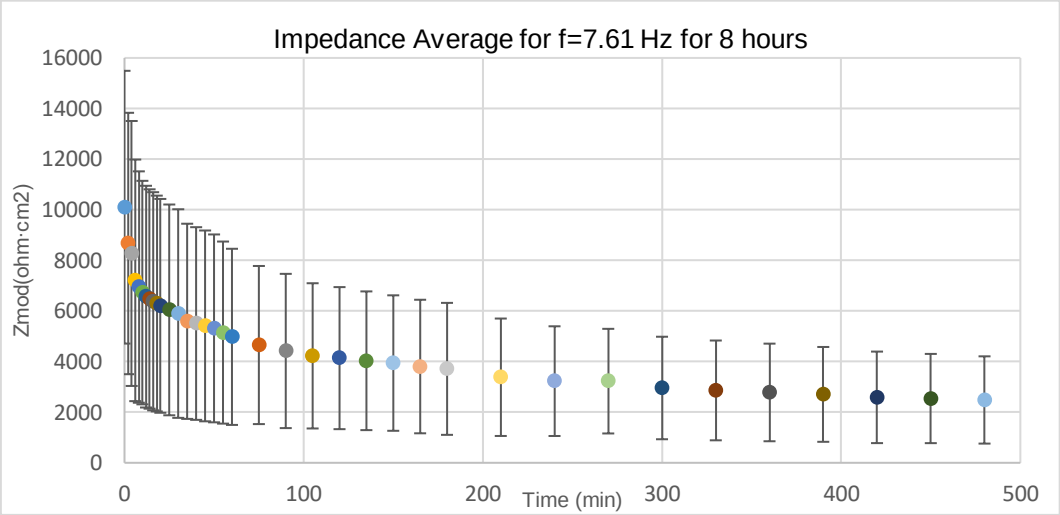


Figure 2.28 - Values of Impedance for a frequency of 7,61 at different time for eight hours.

Looking at Figure 2.28, it is possible to assess that the initial impedance was of 1.27E4 $\Omega \cdot cm^2$, and immediately after two minutes it decreased to 1.11E4 $\Omega \cdot cm^2$. Thus, there was an extreme decrease in the first 20-30 minutes. The final impedance, at eight hours was 2.48E3 $\Omega \cdot cm^2$. The same trend was verified for other frequencies, see Annex Figure 2.35. Therefore, with this *in-vitro* study it is concluded that the emulsion helps in decreasing the impedance values, by hydrating the skin and by creating and an ionic path, due to the NaCl in the emulsion.

These studies do not completely reflect how the emulsion will behave *in-vivo* since as the mouse skin is dead it cannot metabolite the emulsion and it is also in constant contact with the product, which does not happen in the real situation. However, this method is often used in the literature as it usually provides a reliable description of what happens in real conditions [56], [143], [144].

2.2.5 Impedance spectroscopy *in-vivo* - Final formulation (H14)

The impedance spectroscopy experiments performed *in-vivo* represents another step towards the assessment of the effect of the emulsion in what concerns the decrease of the scalp impedance. Impedance spectroscopy was performed in the forearm of volunteers for one hour and for four hours. The results relative to one hour are and four hours are reported in Figure 2.29 and 2.30, respectively.

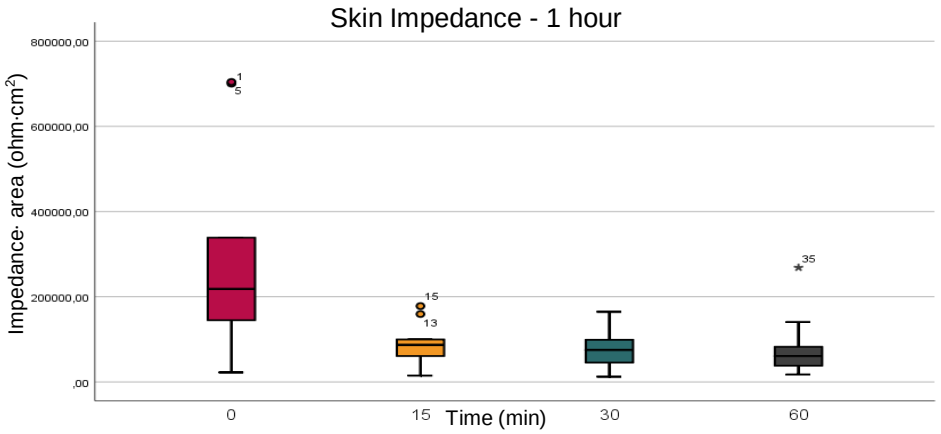


Figure 2.29 - Impedance in function of time. Average of the values for a certain frequency, being in this case f=7.575 Hz.

From Figure 2.29, it is possible to conclude that skin impedance before emulsion application, was 2.90E5 Ω·cm^2. However, after 15 minutes the impedance decreased about 25% of the initial value, 8.74E4 Ω·cm^2. The values of impedance were maintained constant for one hour. In the Annex Figure 2.36 it is possible to see that this trend is maintained for the frequency range 35.72 Hz to 1.624 Hz. With these results, it is concluded that the emulsion indeed had an effect in decreasing skin impedance and its effect is maintained at least for one hour. Moreover, this effect is promptly seen after 15 minutes, meaning tests can be performed after 15 minutes of emulsion application.

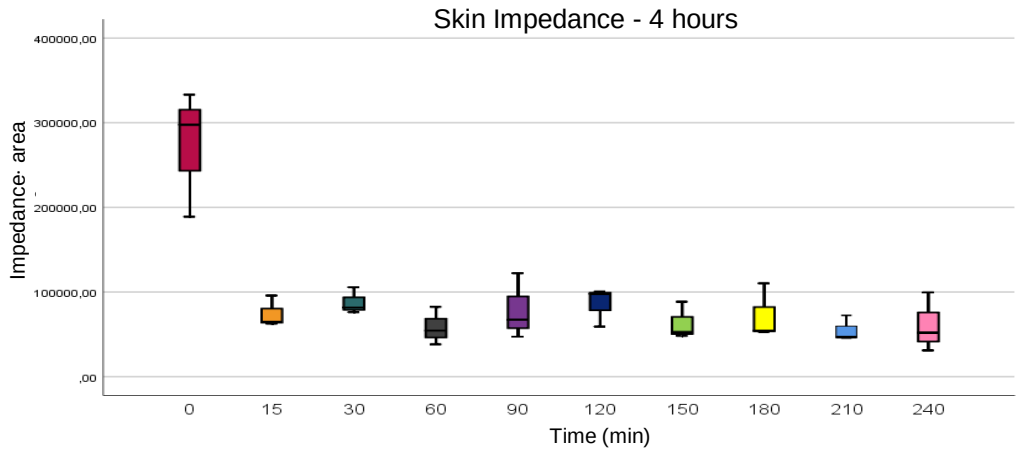


Figure 2.30 - Impedance in function of time. Average of the values for a certain frequency, being in this case f=7.575 Hz.

To further analyse the emulsion a four hours test was also performed. In Figure 2.30, it is possible to see that once again skin impedance decreased to 25% of the initial value, with the application of the emulsion and its effect after four hours is still strong. Overall, skin impedance maintained constant after emulsion application in the skin. Figure 2.37 on the Annex, contains the values of impedance for different frequencies. The values follow the same trend to decrease with time.

A relation can be seen with the results of the hydration, in Figure 2.23 and 2.25, and with the skin impedance results in Figure 2.29 and 2.30. Therefore, as the skin hydration is maintained almost constant for four hours this behaviour can also be seen in skin impedance for four hours, meaning that indeed skin hydration influences greatly the value of skin impedance.

In general, the results of the emulsion are positive, since the main objective of reducing skin impedance by hydration and creation of an ionic path are accomplished. In addition, the effect of the emulsion is still strong after four hours, which covers most of the test times for EEG, another achieved requirement. Furthermore, no volunteer presented any sign of skin irritation or discomfort caused by the emulsion. The emulsion did not seem to dirty the skin, being readily absorbed.

2.2.6 FTIR - Final formulation (H14)

To check the influence of the emulsion at the chemical level, FTIR analysis was performed in mouse skin. The FTIR spectra of the skin were acquired after 40 minutes in PBS, the period of equilibrium chosen for the skin, and after 40 minutes in PBS and 1h, 4h,8h and 12h in constant contact with the emulsion. The results are shown at Figure 2.31.

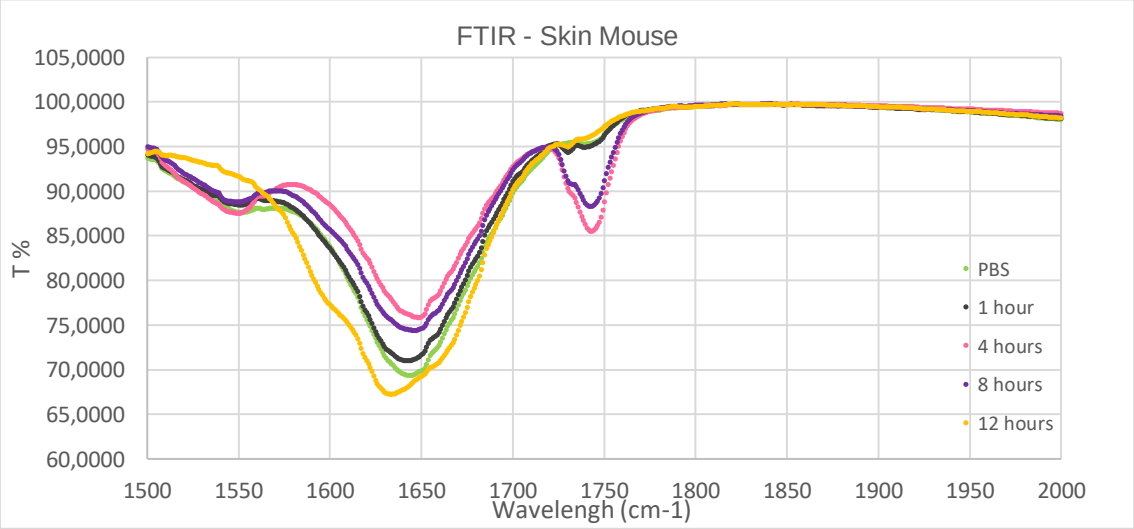


Figure 2.31 - FTIR spectra for different time points.

Table 2.11 - Ratios of Amide I/ Amide II for each measuring time.

Sample	Ratio (Amide I/AmideII)
PBS	2.02
1 hour	2.15
4 hours	1.94
8 hours	2.03
12 hours	8.64

The SC hydration is characterized by the absorption bands 1645 cm^{-1} and 1545 cm^{-1} corresponding respectively to the Amide I and Amide II bands [145], [146], [147]. Water does not influence the Amide II band, but it influences the Amide I band, that increases in the presence of water. This is due to the water absorption at 1640 cm^{-1} [147]. A FTIR spectra was acquired for the emulsion and it did present infrared absorption at frequency of 1645 cm^{-1}, that can be caused by the water, but not at 1545 cm^{-1}. Additionally, in the literature it is described that as the extent of hydration increases the ration between heights of peak for Amide I and Amide II also increases - Table2.11 [148]. The results present a slight increase in the ratio after 1 hour, but no changes after four and eight hours. However, after 12 hours the ratio is indeed higher than ratio of skin treated with PBS.

Therefore, no significant changes can be seen in one, four and eight hours, but after 12 hours it is indeed possible to see a bigger hydration. Thus, FTIR seems to be a poor analyser of hydration, at least in the first hours, on the other hand it can detect hydration if measured after longer hours of emulsion application. Yet, more samples need to be done to reach accurate conclusions.

Furthermore, the intensity of the peaks can be different due to the pressure that is impose when securing the skin to FTIR, meaning that if more pressure is applied the peaks turn out more intense [149]. Additionally normally the depth of analysis of an infrared beam is 1 to 5 µm and the SC has a thickness of 5 to 20 µm [40], [150]. Therefore, this analysis is only referent to the SC, not reaching the more in-depth layers of the skin.

3. Conclusion

Electroencephalography(EEG) is a widely used technique for brain imaging. This technique can be achieved using gel-based electrodes, the current gold standard, and also dry electrodes. Dry electrodes provide a cleaner and quicker measurement, having the advantage of not needing a gel. However, these electrodes still present some problems regarding the strong movement artefact susceptibility, which is higher than in the wet electrodes.

Thus, to overcome this problem an emulsion was developed in order to moisturize the skin, because artefact generation (skin impedance) is related to the dry state of the skin, and to create an ionic path between the electrode and the skin, for a better electrical conduction. Additionally, the emulsion should hydrate quickly, without dirtying the hair, and provide a skin hydration for at least four hours.

The formulation developed contains sodium chloride, urea and glycerine, as well as other components. The emulsion was stable for at least two months, presenting a pH in the physiological pH range recognized as safe for dermal formulations, and a slight viscosity, for an easier and safer application. Furthermore, regarding the organoleptic characteristics, it had an off-white colour and did not present any odour, an advantage given that the emulsion is to be applied to the scalp without the need to wash the hair after the exam. In relation to hydration, the *in-vivo* studies demonstrated that the emulsion had high hydration properties, lasting at least four hours. These conclusions were in agreement with the *in-vivo* impedance studies, in which skin impedance was measured after emulsion application. Thus, the skin impedance decreased about 25% of the initial value, maintaining the value constant for at least four hours.

Therefore, the developed emulsion accomplishes the main requirements of reducing skin impedance, by hydrating the skin, and the presence of sodium chloride allows to create an ionic path. In addition, the emulsion hydrates the skin rapidly, as high hydration values and low skin impedance were achieved after just 15 minutes. These values were also maintained for at least four hours. Moreover, no volunteer presented any irritation or discomfort during the use of the emulsion, which was readily absorbed without the need for skin cleaning at the end of the tests.

4. Future Work

Even though the formulation presented good results and accomplished the main objectives imposed, it still has stability problems. To overcome this problem, most of the components should be added after the aqueous phases and the oil phases are mixed together since they need to be heated until 60-70ºC. This is the case for urea, sodium metabisulfite and Kathon® CG. The last component should be added later because it is reported that at temperatures higher than 50ºC degradation can occur [122]. As for urea it was already described the oxidation that occurs at certain temperatures. Trying to make urea more stable it was patented in 1987 a solution, by Sakai & Quick, that was to add lactone to the formulation [120]. The addition of this component stabilizes urea regarding various aspects [120]. There are other components that present the same effect and can be used in topical formulations. As these components are thermo-sensible they should be added when the formulation is at room temperature. If this does not resolve the problem, mechanical and ultrasonic homogenizers could be used. These equipment will lead to a smaller droplet size which leads to a more stable emulsion. Additionally, the formulation could be optimized by increasing the concentration of surfactant. If no progress is shown a new formulation needs to be formulated.

Another aspect that could be improved is the odour of the emulsion. This formulation is to be applied to the scalp, therefore if the formulated emulsion contained, a not to overpowering, pleasant smell, it could be beneficial. This because fragrances can have calming effects in patients that might be nervous in the exam.

Furthermore, the emulsion should be further tested by increasing the volunteers of the *in-vivo* tests. Another test that could be performed is the *in-vivo* study using FTIR. This equipment is sensible to the SC hydration and it is quick.

In addition, the emulsion should be, in a first trial, used in junction with dry electrodes to see how it works. If the results are positive, the tests can be made in the head of volunteers after emulsion application.

5. Annex

Table 2.12 - Prepared Formulations.

Components	H9A	H9B	H9D	H9E	H9W	H9F	H9G	H9H
NaCl	2%	2%	2%	2%	2%	2%	2%	2%
Kathon CG	0,10%	0,10%	0,10%	0,10%	0,10%	0,10%	0,10%	0,10%
Urea	10%	10%	10%	10%	10%	10%	10%	10%
Glycerine	10%	10%	10%	10%	10%	10%	10%	10%
Tween 80	5%	5%	7,50%	7,5%	5%	5%	5%	5%
Sodium Aginate	2,50%	2,00%	2,50%	-	-	-	-	-
Oleic Acid	10%	-	-	-	-	-	-	-
Sweet Almond Oil	-	10%	-	-	-	-	-	-
Dimethicone	-	-	10%	10%	10%	-	-	-
Miglyol	-	-	-	-	-	10%	10%	10%
HPMC	-	-	-	2%	-	2%	-	-
Xantan Gum	-	-	-	-	-	-	1,11%	0,50%
Aristoflex	-	-	-	-	-	-	-	-
Water	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%

Table 2.13 - Prepared Formulations.

Components	H9I	H9J	H9L	H9M	H9N	H9O	H9P2	H9S
NaCl	2%	2%	2%	2%	2%	2%	2%	2%
Kathon CG	0,10%	0,10%	0,10%	0,10%	0,10%	0,10%	0,10%	0,10%
Urea	10%	10%	10%	10%	10%	10%	10%	10%
Glycerine	10%	10%	10%	10%	10%	10%	10%	10%
Tween 80	5%	5%	5%	5%	5%	5%	-	5%
Sodium Aginate	-	-	-	-	2%	1,50%	-	-
Miglyol	10%	10%	10%	-	10%	10%	10%	10%
HPMC	-	-	-	-	-	-	-	-
Xantan Gum	0,75%	-	-	-	-	-	-	-
Aristoflex	-	0,50%		-	-	-	-	-
Alcohol Cetilico	-	-	-	10%	-	-	-	-
Glyceryl Stearate				-	-	-	3,50%	-
Ceteareth-12/Eumulgin B1	-	-	-	-	-	-	1,50%	-
Corn Starch	-	-	-	-	-	-	-	8,73%
Water	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%

Table 2.14 - Prepared Formulations.

Components	H9T	H9U	H9V	H9X2	H9Y	H13
NaCl	2%	2%	2%	2%	2%	2%
Kathon CG	0,10%	0,10%	0,10%	0,10%	0,10%	0,10%
Urea	10%	10%	10%	10%	10%	10%
Glycerine	10%	10%	10%	10%	10%	10%
Tween 80	5%	5%	-	-	-	10%
Dimethicone	-	-	-	-	10%	10%
Glyceryl Stearate	-	-	-	-	3,50%	-
Ceteareth-12/Eumulgin B1	-	-	-	-	1,50%	-
White Wax	7,50%	-	-	-	-	-
Cutina HR	-	10%	-	-	-	-
Sodium Metabisulfite	-	-	-	-	0,10%	0,10%
Isopropyl Myristate	-	-	8%	8%	-	-
Lanette N	-	-	12%	6%	-	-
Butylated Hydroxytoluene (BHT)	-	-	0,05%	0,05%	-	-
Water	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%	qbp 100%

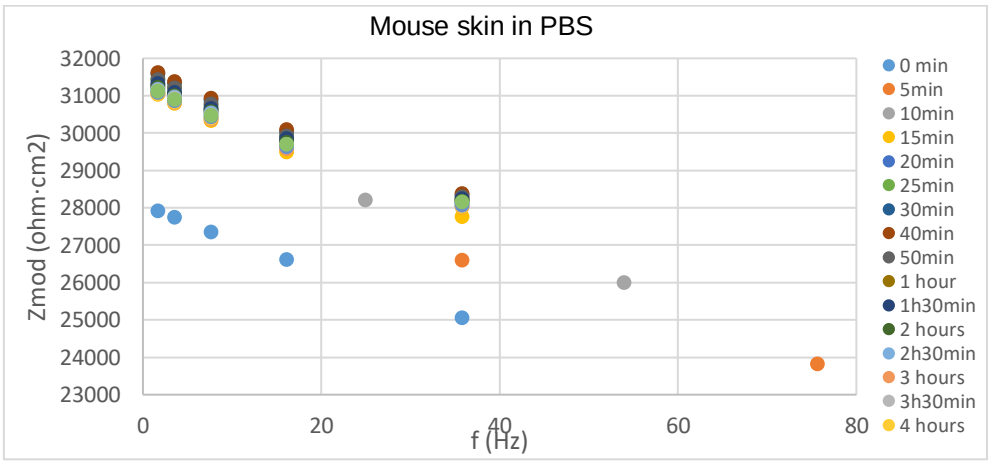


Figure 2.32 - Impedance of mouse skin in PBS for 5 hours.

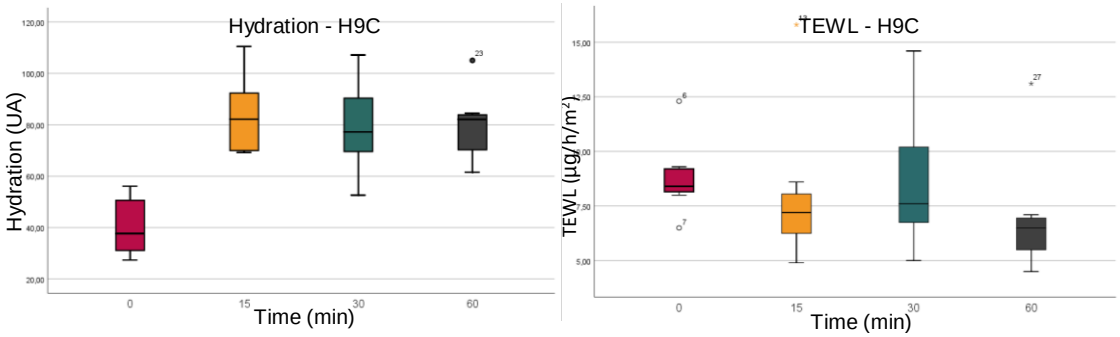


Figure 2.33 - Hydration and TEWL values for H9C.

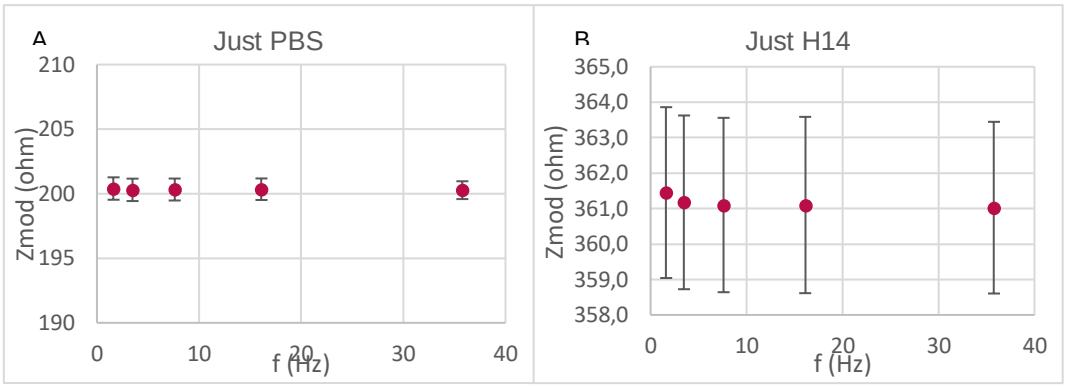


Figure 2.34 - Impedance values. A - Average of 4 measurements of PBS impedance; B - Average of 4 measurements of H14 emulsion impedance.

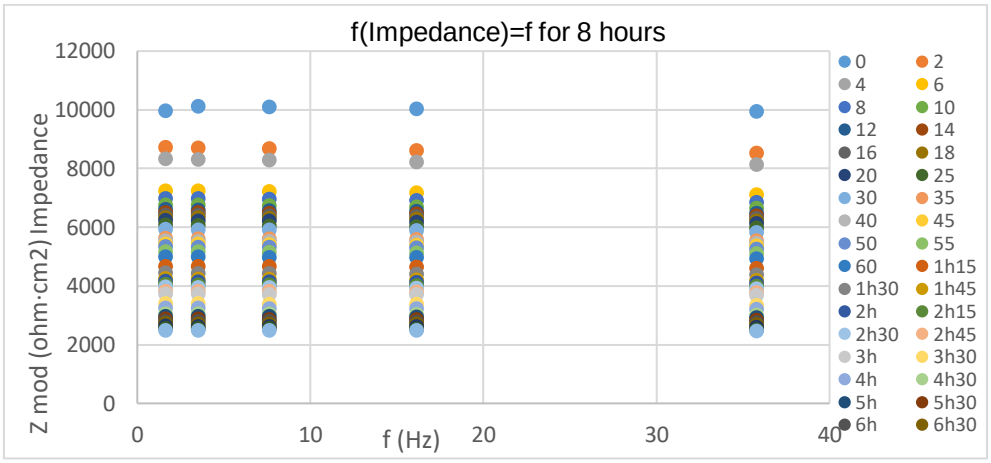


Figure 2.35 - Values of Impedance for different frequencies and times for mouse skin.

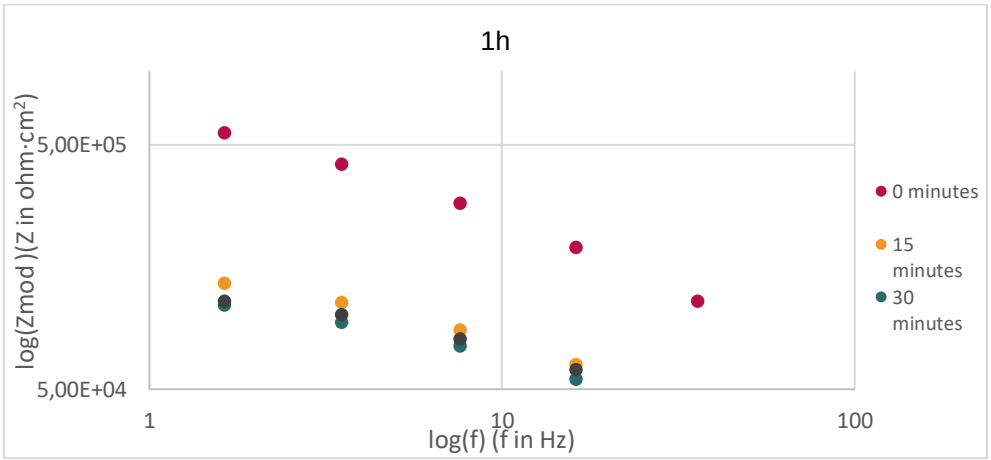


Figure 2.36 - Impedance spectroscopy values average for one hour before and after H14 application. Impedance in function of frequency.

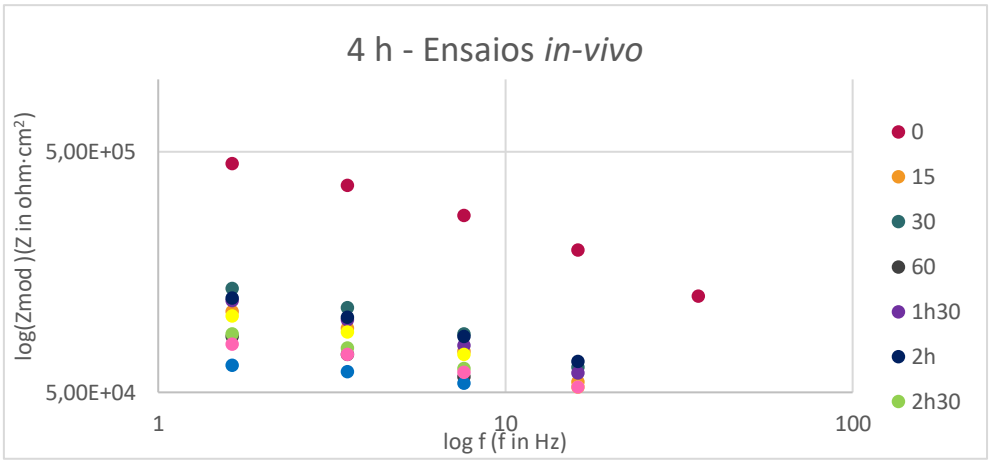


Figure 2.37 - Impedance spectroscopy values average for four hours before and after H14 application. Impedance in function of frequency.

Annex

DECLARAÇÃO DE CONSENTIMENTO INFORMADO

Designação do estudo: Estudo da influência de formulações hidratantes na função de barreira cutânea

Investigador responsável: Maria Helena dos Anjos Rodrigues Amaral
Data do parecer favorável pela respetiva Comissão de Ética: ..... / ..... / .....

Eu, abaixo-assinado [nome legível e completo do(a) voluntário(a) saudável],
_____, com ..... anos de idade, declaro que compreendi a explicação que me foi fornecida acerca da investigação que se tenciona realizar e concordo participar no estudo em que serei incluído(a). Foi-me dada a oportunidade de fazer as perguntas que julguei necessárias e, de todas, obtive resposta satisfatória.

Tomei conhecimento de que, de acordo com as recomendações da Declaração de Helsínquia, a informação e a explicação que me foram prestadas versou os objetivos, as condições, os procedimentos e a duração do ensaio, bem como a possibilidade de ocorrência de reações de intolerância/alergia ou irritação cutânea. Além disso, fui informado que não será atribuída qualquer compensação monetária pela participação no estudo.

Fui ainda informado que tenho o direito de recusar a todo o tempo a minha participação no estudo, sem que isso possa ter como efeito qualquer prejuízo para mim e que os registos dos resultados poderão ser consultados pelos responsáveis científicos e ser objeto de publicação, mas os elementos da identidade pessoal serão sempre tratados de modo estritamente confidencial.

Eu li e assinei este consentimento informado, com total conhecimento dos factos.

Data: dia ..... (mês por extenso) ..... ano .....

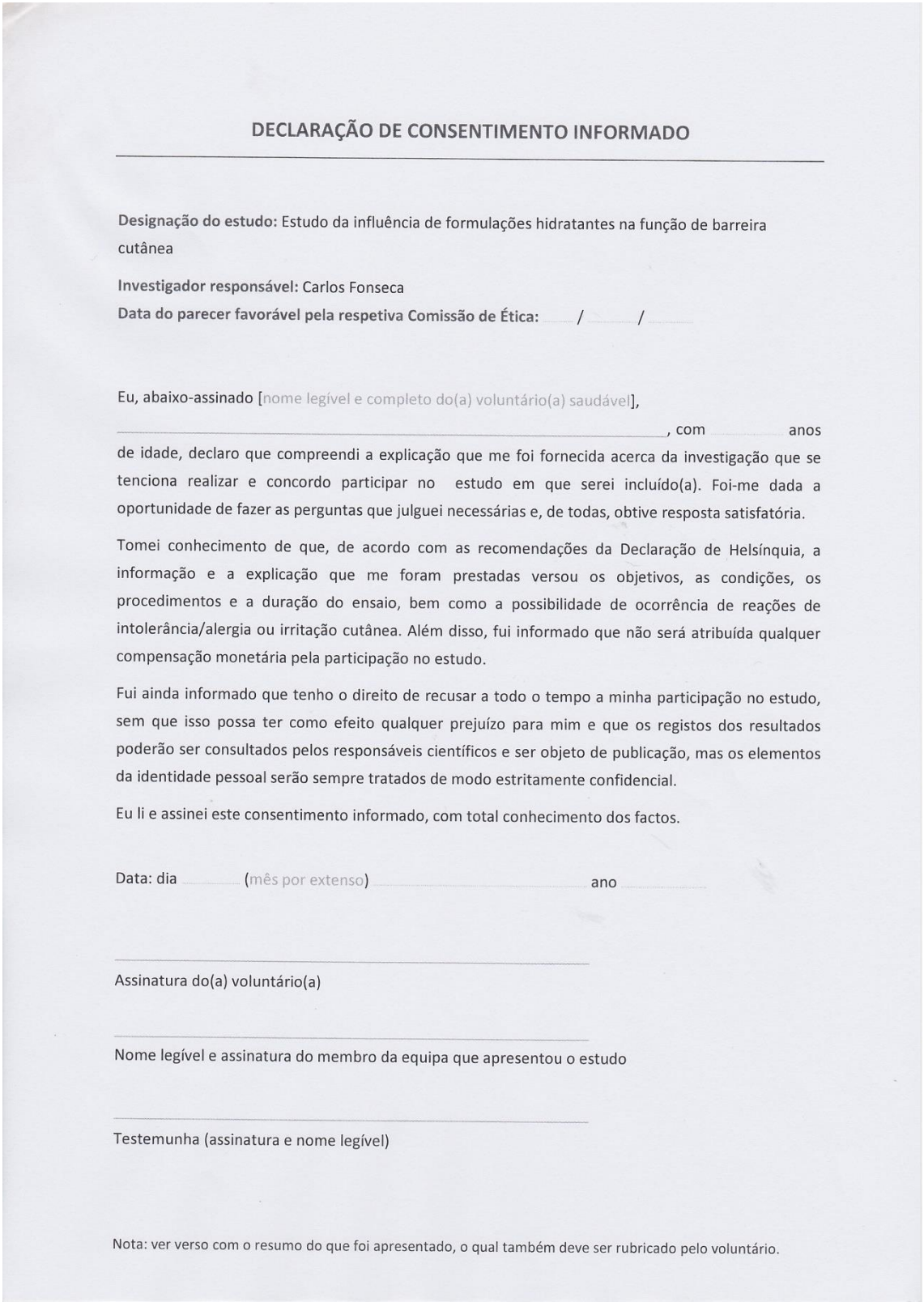
_____
Assinatura do(a) voluntário(a)

_____
Nome legível e assinatura do membro da equipa que apresentou o estudo

_____
Testemunha (assinatura e nome legível)

Nota: ver verso com o resumo do que foi apresentado, o qual também deve ser rubricado pelo voluntário.

Annex



DECLARAÇÃO DE CONSENTIMENTO INFORMADO

Designação do estudo: Estudo da influência de formulações hidratantes na função de barreira cutânea

Investigador responsável: Carlos Fonseca

Data do parecer favorável pela respetiva Comissão de Ética: ..... / ..... / .....

Eu, abaixo-assinado [nome legível e completo do(a) voluntário(a) saudável],

_____, com ..... anos de idade, declaro que compreendi a explicação que me foi fornecida acerca da investigação que se tenciona realizar e concordo participar no estudo em que serei incluído(a). Foi-me dada a oportunidade de fazer as perguntas que julguei necessárias e, de todas, obtive resposta satisfatória.

Tomei conhecimento de que, de acordo com as recomendações da Declaração de Helsínquia, a informação e a explicação que me foram prestadas versou os objetivos, as condições, os procedimentos e a duração do ensaio, bem como a possibilidade de ocorrência de reações de intolerância/alergia ou irritação cutânea. Além disso, fui informado que não será atribuída qualquer compensação monetária pela participação no estudo.

Fui ainda informado que tenho o direito de recusar a todo o tempo a minha participação no estudo, sem que isso possa ter como efeito qualquer prejuízo para mim e que os registos dos resultados poderão ser consultados pelos responsáveis científicos e ser objeto de publicação, mas os elementos da identidade pessoal serão sempre tratados de modo estritamente confidencial.

Eu li e assinei este consentimento informado, com total conhecimento dos factos.

Data: dia ..... (mês por extenso) ..... ano .....

_____

Assinatura do(a) voluntário(a)

_____

Nome legível e assinatura do membro da equipa que apresentou o estudo

_____

Testemunha (assinatura e nome legível)

Nota: ver verso com o resumo do que foi apresentado, o qual também deve ser rubricado pelo voluntário.

Figure 2.39 and 2.40 - Informed consent for the both *in-vivo* studied in FFUP and FEUP, respectively.

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