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FINAL DEGREE PROJECT

DEVELOPMENT AND OPTIMIZATION PROCESS OF SOLUBLE GLOVES

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1. ABSTRACT

This project aimed to develop a recyclable polymer-based liquid formulation capable of forming a protective barrier on the hands, simulating a glove. Tests included abrasion and wash-off resistance assays, elasticity analysis, and interlayer adhesion evaluation. An initial gel formulation failed under finger movement, prompting the development of new formulations using a purified monomer (MOP). The most promising formulation combined MOP with plasticizers to achieve flexibility, minimal tackiness, and no interlayer adhesion. Results support continuing this line of research, focusing on optimizing composition and performance under real conditions, and developing suitable application and removal systems for clinical use.

2. INTRODUCTION

2.1. Collaborating entity and its monomer

This whole project is a collaboration with Synkotech Biocompatible Materials (SBM), a biotechnology and chemical-pharmaceutical company founded in 2017, specialized in the synthesis and development of organic molecules, with a particular emphasis on monomers and organic biomaterials. It is located at Reus (Tarragona), where it carries out its main activity through the Research, Development and Innovation (R&D&I) department and, simultaneously, the production department.

The company has developed a new monomer derived from malonate, completely biocompatible and non-cytotoxic. Based on this monomer, several products have been developed with characteristics that vary depending on how it is treated before utilization. SBM uses two main approaches for handling this monomer: purifying it or leaving it unpurified. The purified monomer is more reactive and it is designed to cure upon contact with an activator located in the devices used for skin aplication, while the unpurified version is used in preliminary polymerizations in specific solvents to adjust mechanical properties of the final product, such as elasticity. We used both forms of the monomer at different stages of the project, depending on the specific objective at each stage. Throughout this report, the unpurified monomer will be referred to as MOU, and the purified monomer as MOP.

2.2. Background

Single-use medical protective equipment plays a critical role in safeguarding healthcare professionals and patients against infection and cross-contamination. This category includes items such as gloves, masks, and some other clothing items, all of which are intended for single use to maintain hygienic integrity. This medical equipment is widely used in many sectors and is especially important in healthcare, so using large amounts of these items every day is completely normal. In addition, its use inevitably increased during the Covid-19 pandemic. It was estimated that approximately 65 billion gloves were being used each month worldwide in 2020.[1] Following their use, these materials are converted into waste. The environmental impact of such waste is also significantly influenced by the raw materials used in the manufacturing process. Latex, a natural material that is extracted from the rubber tree. This production process involves extracting the raw material, transporting the latex, and processing it into gloves. There

are synthetic alternatives, such as nitrile and polyvinyl chloride (PVC), but they are derived from petroleum and present even higher footprints. The total carbon footprint of 200 latex gloves is approximately 42.07 kg of CO_2, equivalent to 210 grams of CO_2 per glove. Considering that a latex glove weighs about 5 grams, this implies an emission of 42 kg of CO_2 per kilogram of gloves.[2]

Their disposal is typically managed through incineration processes, which effectively eliminate potential pathogens and mitigate the risk of biological contamination in the environment. However, incineration of medical waste is not recommended since large amounts of greenhouse gases and harmful substances such as heavy metals, dioxins, polychlorinated biphenyls, and furans are emitted during this process.[3] In addition, the incineration of this waste further amplifies its environmental impact.

These data highlight the significant environmental impact caused by single-use medical products. From the moment they are produced until they are disposed of, these items generate considerable carbon emissions and require a large amount of energy. Therefore, it is important to find and apply more sustainable solutions in the healthcare sector to reduce their negative effect on the environment.

With this premise, SBM proposed using their adhesive technology to develop a soluble glove. Their product derived from malonate has mechanical properties similar to cyanoacrylates but without their toxicological limitations, since its decomposition generates biocompatible byproducts. This skin protector applied over the skin in liquid form, since it is dissolved in an organic solvent, which once evaporated creates a layer on the skin, creating a protective barrier against external agents.

The main advantage over current gloves is that this one would be reusable, as the material, once extracted from the skin, can be re-dissolved in solvent, sterilized and reformulated to obtain the original raw material. Therefore, we could replace a non-recyclable material with an infinitely recyclable sterile material. This innovation could represent a turning point in sustainable healthcare, allowing a significant reduction of the carbon footprint in this area. Furthermore, with an optimistic view, it could even be extrapolated to other industries in the future. For the time being, the target area of this project would be surgical, since the product dissolves in organic solvent, and introducing this glove into the chemical industry is currently a major challenge.

2.3. Theoretical foundation

2.3.1. Polymers: The structural basis of gloves

A polymer is a macromolecule composed of repeating monomer units, which may be identical, forming a homopolymer, or different, resulting in a heteropolymer or also called copolymer. These polymers can be of natural or synthetic origin, such as cellulose, proteins, or DNA, or synthetic, manufactured from, for example, petroleum-derived compounds through controlled polymerization processes. In these structures, monomers are linked together by covalent bonds, composing the structural entities called repeat units. This repetition can reach hundreds or thousands of units, generating materials with high molecular weight and specific physical and chemical properties.

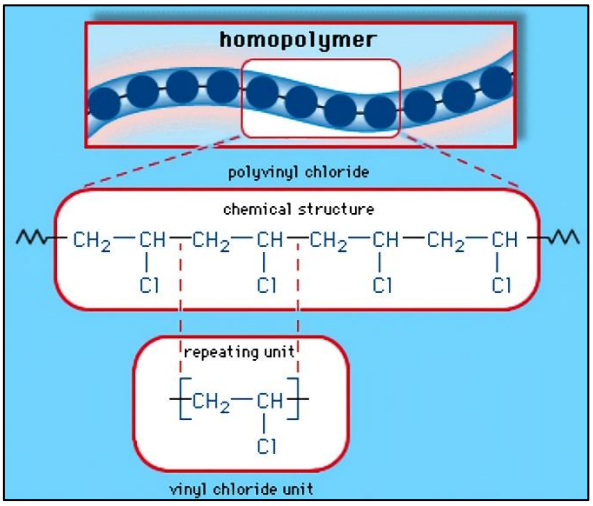

Figure 1. *PVC, an example of synthetic homopolymer. Source: The Editors of Encyclopaedia Britannica, 2024.*[4]

Polymer structures

Monomers also have a specific functionality depending on the number of bonds that they can form. On one hand, a given monomer can be bifunctional when it has an active bond that may react to form a two-dimensional chainlike molecular structure. On the other hand, if a monomer has three active bonds it is called trifunctional and it forms three-dimensional molecular network structures. These different functionalities lead to the possibility of linear, branched, cross-linked and network polymer structures, and this directly influences their thermal, mechanical, and chemical properties.[5] Branched structures are usually less rigid, dense, brittle and crystalline than linear polymers, mainly because linear chains can be placed more neatly, held together by Van der Waals forces and hydrogen bonding, leaving less empty space than branched chains.

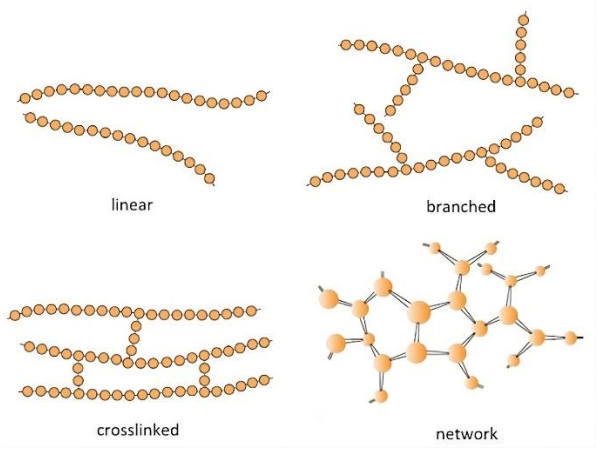

Figure 2. *Molecular structures found in polymers. Source: Callister WD, et al., 2020.*

Structural variations lead to a new classification of polymers based on their elasticity, tensile strength, and rigidity. This classification divides polymers into fibers, plastics, and elastomers. Fibers generally have low elasticity, high tensile strength, and moderate stiffness. Plastics are more rigid, with lower elasticity and tensile strength than fibers. Finally, elastomers are the most suitable polymer class for making gloves, as they exhibit high elasticity and very low rigidity.[6]

Glass transition temperature and plasticizers

This information suggests that the ideal polymer for a glove is a nonlinear polymer capable of behaving like an elastomer. However, the elasticity of a polymer depends significantly on its glass transition temperature (Tg), the specific temperature at which a polymer transitions from a rigid and brittle state to a more flexible and elastic form.

With this in mind, we can also consider the use of plasticizers, widely used additives in the polymer industry that modify the glass transition temperature. Plasticizers are typically non-volatile, low molecular weight compounds that reduce brittleness while increasing flexibility and resistance to temperature fluctuations.[7] They act through a physical, not chemical, mechanism: they do not form covalent bonds with the polymer. Instead, plasticizers insert themselves between polymer chains, weakening van der Waals forces and hydrogen bonds that hold the chains tightly together. As a result, the polymer's glass transition temperature is lowered, allowing the material to remain flexible at room temperature.[8]

Understanding the nature and behaviour of polymers is essential to evaluate glove properties such as tensile strength, elasticity, permeability, and durability. Therefore, the relationship between polymer science and its practical application in products like gloves

highlights how material selection and additive use directly influence the functionality, safety, and performance of the final product.

2.3.2. The skin

The skin is the largest organ of the human body (comprises approximately 16% of the human organism) and it is one of the most important, since it's a self-renewing organ that covers the entire external surface of the body, serving as a dynamic interface between the internal and the external environment. In this way, it plays a fundamental protective role against a wide range of external agents, including mechanical trauma, chemical substances, temperature changes, infectious organisms, ultraviolet (UV) radiation, and water loss.

Skin anatomy

The skin is composed by three main layers: the epidermis, dermis, and hypodermis, each with complex architecture that allows the fulfillment of specific functions, from the immune barrier to thermoregulation.

The **epidermis** is the layer of greatest interest for this project, because it is the outermost layer and serves as the primary barrier against the external environment. It lacks blood vessels, and it is composed mainly of keratinocytes. Nevertheless, it also contains melanocytes that produce melanin with obvious protective value against ultraviolet light, dendritic Langerhans cells that act as antigen-processing cells, and Merkel cells that are abundant in areas involved with sensory perception and have many neuroendocrine functions.[9,10] Epidermis consists of several strata, from the deepest to the most superficial:

- Basal or germinative layer (*stratum basale)*: Contains proliferating keratinocytes attached to the basement membrane by hemidesmosomes. Melanocytes, responsible for melanin synthesis, also reside here.
- Spinous layer (*stratum spinosum)*: Keratinocytes synthesize keratin, that mainly composes the lipid matrix of *Stratum corneum* and is responsible for skin impermeability. Keratinocytes are interconnected by desmosomes, forming a resistant framework.
- Granular layer (*stratum granulosum)*: In this layer is where keratinocytes lose their nucleus and become dead cells (corneocytes), the main cells of *Stratum corneum*.

- Lucidum or clear layer (*stratum lucidum)*: This layer is only present in thick skin (palms and soles).
- *Stratum corneum*: The outermost layer, formed by protein-enriched dead keratinocytes (corneocytes) surrounded by a lipid matrix that constitutes the body's main physical-chemical barrier. [11,12]

In addition, epidermal lipids (ceramides, cholesterol and free fatty acids) that surround cells form a hydrophobic extracellular matrix that maintains water homeostasis and protects against external antigens.[12]

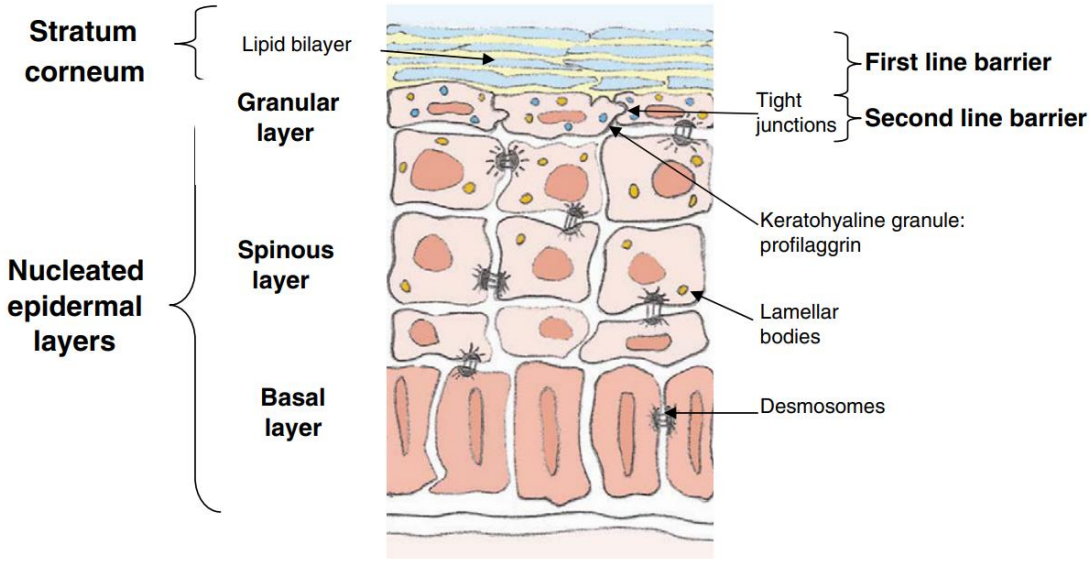

Figure 3. Layers of the epidermis. Source: Baroni A, et al., 2012.

A slightly inner layer is the **dermis**, that is made up of two layers of connective tissue: the papillary and reticular layers, that blend into one another. It is the thickest layer of the skin, and it is mainly composed of fibroblasts, a network of collagen type I and III fibers, elastin and a matrix rich in glycosaminoglycans (such as hyaluronic acid), which provide elasticity and mechanical resistance. Additionally, this layer contains specialized structures such as sweat glands, sebaceous glands, hair follicles, nerve endings (Meissner, Pacinian, Merkel, and Ruffini receptors), and blood and lymphatic vessels that regulate temperature and allow metabolic exchange with the epidermis.[13]

Finally, the **hypodermis**, or subcutaneous fascia, is the deepest layer of the skin. It is primarily composed of adipose tissue organized into lobules, and it also contains larger blood vessels and deeper nerves. The main functions of this layer are to serve as an energy reserve, enhance thermal insulation, and act as a shock absorber against trauma.

Additionally, it is populated by local and blood-derived immunocytes that contribute to the innate immune system in the skin. [14]

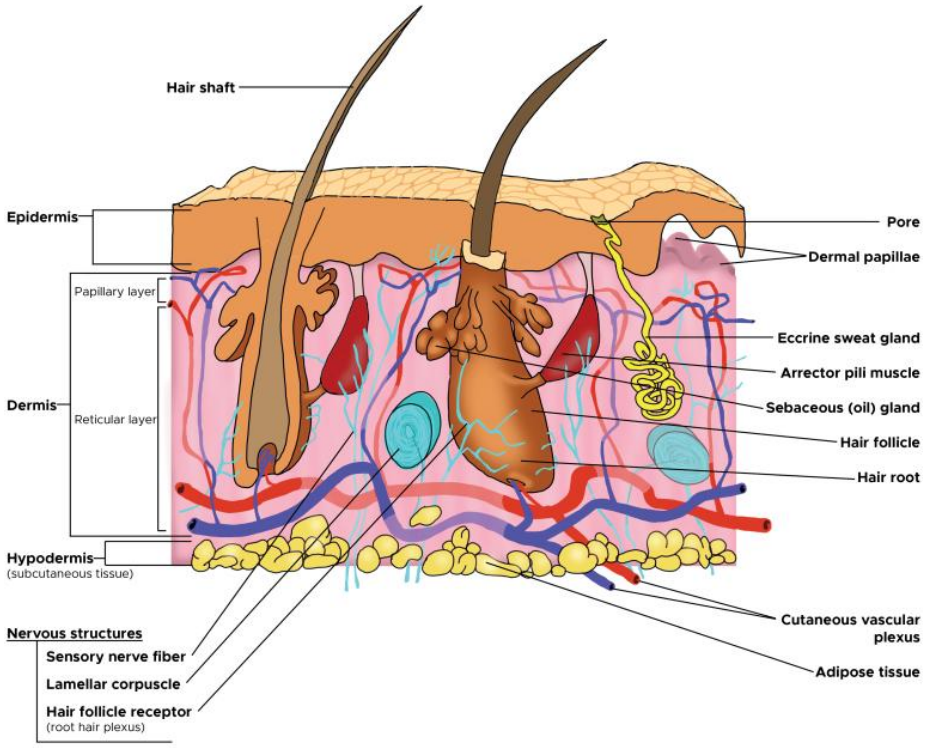

Figure 4. *The skin anatomy, layers and components of the skin. Source: Yousef H, et al., 2024.*

Skin of the hands

The skin of the hands has anatomical and functional characteristics that distinguish it from other regions of the human body. These differences are directly related to the essential role the hands play in interacting with the environment, manipulating objects, and performing both delicate and intense tasks, making this area one of the most exposed and functionally active in the body.

The epidermis of the palms of the hands is particularly thick due to the presence of a highly developed *Stratum corneum*, which provides effective mechanical protection against the constant friction hands suffer. While the epidermis on the rest of the body measures approximately 0.1 mm and is called thin skin, on the palms of the hands this layer can reach a thickness of up to more than 1 mm. Additionally, as mentioned before, hands contain a layer called *Stratum lucidum*, that is only found in thick skin types such as the palms and soles. It is distinguished as a highly eosinophilic zone between the stratum granulosum and the stratum corneum and is composed of a few layers of densely packed, flattened cells.[15]

Moreover, the dermis of the palms is rich in collagen and elastic fibers, as well as nerve endings and vascular structures, which contribute to its fine tactile sensitivity. Its high density of sensory receptors enables fine tactile perception, crucial for texture discrimination, pressure, and vibration.[16,17] In this context, the separation between the dermis and hypodermis is poorly defined compared to thin skin.

The palmar skin also lacks hair follicles and sebaceous glands, making it more susceptible to dehydration and irritation. Instead, it is rich in eccrine sweat glands, responsible for thermal regulation and keeping the skin moist, facilitating friction and grip. [18] Furthermore, the constant activity of the hands leads to a high rate of epidermal cell turnover, which contributes to maintaining the structural integrity of the skin, although it also implies a greater need for repair and regeneration mechanisms.[15]

It is important to keep in mind the variations between the palmar skin and the rest of the body's skin for this project, primarily because changes in the outermost layers can significantly affect glove adhesion, greatly influencing how easily it can be removed from the skin, especially from the palms.

Influence of pH and consequences of its deregulation

Skin pH is a fundamental physiological parameter that reflects the acidity of its surface. Under normal conditions, the pH of human skin ranges between 4.1 and 5.8,[19] creating a slightly acidic environment known as the acid mantle. This physiological acidity plays a crucial role in protecting the skin from external agents and is involved in regulating the skin barrier, the process of skin renewal, enzymatic activity, immune function, and the balance of the resident microbial ecosystem.[20,21]

The origin of the skin's acidic pH is mainly due to three physiological mechanisms that act together to maintain the acid balance on the skin's surface. The first is the degradation of the filaggrin protein, present in the upper layers of the epidermis. It breaks down into amino acids such as histidine and glutamine, which are then transformed into acidic compounds such as urocanic acid and pyrrolidone carboxylic acid. The second mechanism involves the secretion of lipids by the skin cells. These lipids are then transformed by enzymes into ceramides, free fatty acids, and other compounds. The free fatty acids generated in this process lower the pH of the skin's surface, making this mechanism one of the main sources of acidification. Finally, the third mechanism is the action of the SLC9A1/NHE1 ion exchanger, which expels protons (H^+) into the

extracellular space in the epidermis. This activity creates a more acidic environment in specific areas where it is needed and strengthens the skin barrier.[22]

This pH level is influenced by various endogenous factors (such as skin hydration, sweat, sebum production, anatomical location, and age) and external factors (as detergents, cosmetics, and topical antibiotics).[20] Disruptions in pH balance have been associated with the development of several skin disorders, including irritant contact dermatitis, atopic dermatitis, ichthyosis and acne. Additionally, sustained pH elevation promotes dysbiosis, facilitating the proliferation of opportunistic pathogens as *Candida albicans* or *Staphylococcus aureus* and decreasing microbial diversity, which can predispose to infections or inflammatory skin diseases.[22,23]

Given this information, skin pH is not just a passive biochemical characteristic but plays a dynamic role in skin physiology. Maintaining it within the physiological acidic range is critical for preserving the skin's structural, functional, and immunological integrity, as well as for ensuring a healthy and stable microbial ecosystem. Therefore, it’s important to avoid using chemical reagents with a significantly different pH from the skin's natural acidic range, as this may trigger damaging reactions. If the use of such contraindicated reagents is necessary, they must be used in very low concentrations and always in accordance with the regulations of the countries where the final product is intended to be distributed.

The importance of skin microbiome

Human skin also constitutes a dynamic and complex ecosystem, inhabited by a big community of microorganisms that play essential roles in skin health. This microbiota includes bacteria, fungi, viruses, and mites, whose composition varies according to intrinsic factors as age or sex, and extrinsic factors as environmental conditions.[24] For example, the skin microbiota shifts notably during puberty, with increased predominance of *Corynebacterium* and *Cutibacterium* and decreased abundance of Firmicutes (including *Staphylococcus* and *Streptococcus* species). In adulthood, despite the skin’s continuous exposure to the environment, the microbial composition remains surprisingly stable over time.[25] The main bacterial phyla present in the adult skin are Actinobacteria, Firmicutes, Proteobacteria, and Bacteroidetes. These microorganisms colonize different skin regions, adapting to the specific characteristics of each area. The hands are a unique anatomical site with high exposure to environmental factors, making them a hotspot for microbial diversity. Studies have shown that the hand microbiome is characterized by a high abundance of gram-positive bacteria, with species such

as *Staphylococcus spp.*, *Corynebacterium spp*, *Cutibacterium spp., Streptococcus spp., and Propionibacterium spp.* being predominant.[26,27] These bacteria are well-adapted to the dry and often perturbed environment of the hands, which is subjected to frequent washing, environmental exposure, and physical abrasion.

As regards the function of the skin microbiota, it does not only act as a physical barrier against pathogens by producing antimicrobial compounds and competing for resources but also modulates the host's immune response. For example, *Staphylococcus epidermidis* can induce the production of antimicrobial peptides in keratinocytes by recognizing pathogen-associated molecular patterns, strengthening the skin's innate defenses. Furthermore, the interaction between resident microorganisms and the immune system contributes to maintaining skin homeostasis.

Furthermore, it has been observed that excessive use of disinfectant products and frequent washing can cause skin irritation, which in turn alters the microbiota of the hands and can increase the risk of infections. In certain cases, disturbances in the microbiome may lead to the development of human diseases. Many common skin conditions, such as acne, eczema, impaired wound healing, and others, have been linked to alterations in the skin microbiota, a phenomenon known as “dysbiosis”.[28] Therefore, it is essential to maintain a balanced hand microbiota by avoiding the use of irritating products, which can not only damage the skin barrier but also disrupt the microbial ecosystem, potentially leading to additional complications.

3. AIMS AND HYPOTHESIS

To start this project, the following hypothesis was raised: Is it possible to develop a polymer-based liquid formulation that, when applied to the hands, forms a flexible and elastic protective barrier, resistant to external fluids and that can be redissolved later to allow its sterilization and reuse in a hospital environment?

Based on this hypothesis, the general objective of the work was to advance the development of a polymeric formulation that allows the creation of a liquid product that can be applied to the skin of the hands, forming a film that acts as a protective "second skin". To answer the hypothesis, several specific objectives were established: first, to formulate a combination of polymers that, when dried on the skin, generates a continuous and stable barrier. Next, evaluate the resistance of said barrier against common fluids in the hospital environment, such as urine.

In addition, special attention was paid to the mechanical properties of the resulting film, such as its elasticity, flexibility and resistance to tearing or abrasion, fundamental aspects for the product to be functional during the natural movement of the hands. Another key aspect was to verify that this barrier could redissolve in a controlled manner after use, which would allow its removal, cleaning and eventual reuse. Finally, in case of obtaining a formulation with adequate properties, it was proposed to design a method of application on the hands that was easy, comfortable and fast, thinking of a practical use within the hospital environment.

Although it was not expected to achieve a glove with ideal characteristics throughout my participation in the project, we sought to advance towards the objective proposing polymer-based formulations that would bring us one step closer to the desired final product, while also discarding those clearly inadequate.

4. MATERIALS AND METHODS

4.1. Starting formulation

By the time I started working on this project, it had already been in development for some time. Previous studies had led to the approach of creating the soluble glove from a gel formulation that was still in the process of being optimized. The main polymer in this formulation was made from MOU. More information about the composition of this gel formulation, referred to as BPAG, can be found in *Table 1*.

Table 1. *Standard composition of BPAG.*

Reagent	Concentration	Function	Precautions	PPE
Organic solvent	60,08%	Dissolves reagents, facilitates application and subsequent film formation upon evaporation.	 Flammable, irritant (eyes and respiratory tract).	Lab coat, Glasses, Gloves.
MOU Polymer	13,28%	Film former.	-	
BPAG Plasticizer 1	5,69%	Film former. Improves the adhesion of the film to the skin.	 May cause mild irritation.	
BPAG Plasticizer 2	12%	Provides flexibility and elasticity.	-	
BPAG Plasticizer 3	0,95%	Provides flexibility and elasticity. Adhesion promoter.	-	
Texture modifier 1	3%	Modifies viscosity, provides gel texture.	 Irritating on inhalation (only at high exposure).	
Texture modifier 2	5%	Provides greater mechanical resistance.	-	

The plasticizers listed in Table 1 include "BPAG" in their names to distinguish them from other plasticizers mentioned in later phases of the project.

4.2. Abrasion test

The aim of this test was to optimize the formulation so that it would be more resistant to abrasion and chafing. One method to evaluate the film resistance to abrasion is measuring its protective effectiveness through continuous abrasion cycles. This efficacy is quantified by evaluating transepidermal water loss (TEWL). This parameter can be measured with a vapor meter, which measures the values in g/m^2h.[29]

Figure 5. *VapoMeter, a TEWL meter from Delfin Technologies.*

To begin with this method, delineate and label the areas on the forearms of 10 volunteers, where the samples would later be applied. These areas should be square-shaped, measure approximately 3 centimetres on each side and be spaced slightly apart to prevent overlap of the samples.

Next, take an initial measurement of TEWL using the vapor meter, before applying any samples (all measurements, including this one, must be performed in triplicate, and then calculate the average).

Subsequently, apply 0.2 millilitres of each sample to their respective delimited areas, spreading them evenly. After 10 minutes, take measurements with the vapor meter on the areas where the samples had been applied, which are now completely dry.

Then, the abrasion cycles begin. Using a scouring pad, make ten passes over each area with a sample. Fifteen minutes later, take the measurements again with the vapor meter. After this, perform three more abrasion cycles, with the expectation of seeing increasing TEWL values after each cycle. The protective effectiveness percentage of the samples after every cycle can be calculated with the following equation:

$$\text{Protective effectiveness } (\%) = 100 - \frac{\text{sample measurement}}{\text{blank measurement}} * 100$$

Equation 1. *Calculation of protective effectiveness.*

4.3. “Wash off” test

To evaluate which additives provides the formulation a better water and urine resistance, we performed a “Wash off” test. This is a colorimetry test, made with a colorimeter that detects extremely small colour changes in the skin.

Figure 6. *Colorimeter from Cortex Technology.*[30]

To perform this method, mark square-shaped areas on the forearms of 10 volunteers, where the samples would later be applied, as in the abrasion test. These areas, again, should measure approximately 3 centimetres on each side.

Next, measure the baseline (skin without any sample applied) at each position using the colorimeter. Simply place the tip of the colorimeter against the skin, applying slight pressure. Record the results given by the colorimeter (baseline measurement) and, subsequently, apply 10 µL of a 1000 ppm dye solution in isopropanol to each marked position. Wait 10 minutes and apply each of the samples to be evaluated to their assigned positions, over the dried dye. After 5 minutes, measure the new baseline values through the formed film (post app measurement).

To start with the washing cycles, place a piece of sterile gauze soaked with synthetic urine on each position. Cover the arm with plastic film to prevent the gauze from moving and wait 20 minutes. After this time, remove the film and gauze, and clean each point using the aloe cleaning spray, wiping twice upwards and twice downwards. Wait 10 minutes and take a new measurement with the colorimeter (post washing measurement). This will be the measurement after the first cycle. Repeat these steps two more times to complete a total of three cycles.

The measurements are reported in the CIE L*a*b* colorimetric notation system, where L* represents the lightness (from black to white), a* indicates the green-red component, and b* indicates the blue-yellow component. Thus, 1/L* can be interpreted as a relative measure of greyness, a* is a measure of redness, and b* is a measure of yellowness in

relative terms. In this context, the variable “x” quantifies the total colour intensity, and it is calculated as the Euclidean distance in the CIE L*a*b* colour space using the formula:

$$x = \sqrt{L^2 + a^2 + b^2}$$

Equation 2. *Calculation of the total colour intensity.*

To obtain the final result, the baseline measurement is taken as representing 0% dye, while the measurement taken just before washing begins is considered 100%. Based on this, the amount of dye lost after each cycle can be calculated, and the final outcome can be expressed as the percentage of dye remaining after three washes.

$$\text{Remaining dye } (\%) = \frac{(\text{baseline measurement} - \text{post washing measurement})}{(\text{baseline measurement} - \text{post app measurement})} * 100$$

Equation 3. *Calculation of the remaining dye percentage.*

Since the samples are applied over the dye, any damage to their integrity caused by the synthetic urine will result in the progressive removal of the dye during the washing cycles. Therefore, the sample that retains the highest percentage of dye after three washes can be considered the most resistant to this fluid.

4.4. Additives evaluated by Abrasion and “Wash off” tests

Using the BPAG formulation as a base, it was decided to evaluate various additives that could improve the qualities of abrasion and urine resistance. These additives and concentrations were selected based on previous studies conducted by the collaborating company, in which they yielded good results in similar tests. Nevertheless, it was necessary to evaluate whether they would produce comparable effects in this formulation.

Table 2. *Additives evaluated in BPAG formulation.*

Additive	Concentration	Precautions	PPE
Reagent 1	0,75%	 May cause eye and respiratory irritation.	Lab coat, Glasses, Gloves.
	2%		
Reagent 2	1%	 May cause eye irritation.	
	3%		

	5%		
Reagent 3	0,75%	 May cause eye irritation.	
	1,5%		
	3%		
Reagent 4	0,5%	 May cause irritation in sensitive skin or in high concentrations.	
	2,5%		
	5%		
Reagent 5	0,25%	 May cause mild irritation.	
	1%		
	3%		

4.5. Application on fingers

It is necessary to perform finger tests progressively throughout the project, as the final product is intended to be practical for hand application. In this way, weak points of the formulation under development can be identified, such as bad resistance to repeated finger bending or to friction between application areas.

To begin this method, wash your hands and ensure they are completely dry (wait 5 to 10 minutes if necessary). Then, apply the desired amount of formulation along a finger using a syringe. It is advisable to repeat the test using different sample volumes to determine which amount yields the best results. A standard quantity cannot be established, as it depends on the size of the individual's fingers.

Using your other hand, wrap the treated finger and rotate your hands 10 times (5 times to the left and 5 to the right, alternating) to ensure the finger is fully coated with the sample. Since the sample dries quickly, this step must be completed immediately after application.

If multiple fingers are being tested (for example, to observe how they interfere with each other during application) make sure to wash the other hand thoroughly before using it

again, as any leftover formulation may affect the distribution. Assistance may be required for this step.

After application, wait 10 minutes to allow the samples to fully dry, keeping finger movement to a minimum and avoiding contact between fingers or with other surfaces. Finally, evaluate the characteristics of the films formed on the fingers. These include bending resistance, interlayer adhesion, skin sensation, tackiness, and ease of application.

In this project, the method was performed twice: once with the BPAG formulation, and once with new formulations based on polymer made from MOP, which were selected for testing after the project's direction was adjusted in response to the results obtained throughout its development.

4.6. Film formation and interlayer adhesion test

Interlayer adhesion test was carried out following the initial finger application test, due to the need to identify additives that could prevent interlayer adhesion. This test also served to evaluate the characteristics of the films formed with diverse formulations.

To evaluate interlayer adhesion, prepare films on Teflon molds using the formulations containing the additives of interest. The intention is to produce square-shaped films, measuring 5 cm per side, with a target final weight of 2 grams. To calculate the amount of formulation to pour into the mold, it is important to consider that the solvent will fully evaporate in the oven. For example, if the formulation contains 80% solvent, 10 grams should be poured into the Teflon mold to obtain a 2-gram film after drying.

Once the molds are filled with the calculated amount, place them in an oven at 80 °C overnight. The next day, remove the molds and let them cool slightly for 5 minutes. Then, carefully remove the films from the molds using a spatula.

Next, cut each film into four equal parts, labelled fragments 1, 2, 3, and 4. Place the fragments in overlapping pairs (e.g., fragment 1 on top of 2, and 3 on top of 4). Keep one pair at room temperature, while place the other in an oven at 40 °C overnight to see if heat influences adhesion.

Finally, examine the samples to determine whether any of the film pairs fused or adhered over time, and record the results.

At this stage of the project, the BPAG formulation had been discarded for this project (as explained in the Results section 5.3.). Therefore, this test was conducted with alternative formulations, with a focus on finding formulations that showed no interlayer adhesion. At this point, polymer formation with MOP was also introduced.

First, the test was performed without additives, testing the interlayer adhesion using MOU polymer and MOP polymer. Samples are detailed in *Table 3*.

Table 3. *Formulations without additives prepared for interlayer adhesion test.*

Formulations for polymer films without additives		
Reference	**Components**	**Concentrations**
1	MOU polymer Organic solvent	20% 80%
2	MOP polymer Organic solvent	20% 80%
3	MOU polymer MOP polymer Organic solvent	10% 10% 80%
4	MOU polymer MOP polymer Organic solvent	10% 15% 75%
5	MOU polymer MOP polymer Organic solvent	5% 15% 80%

After analysing the results from the previous films, it was decided to test different concentrations of certain plasticizers in MOP polymer films. All plasticizers and concentrations were selected based on data from previous projects carried out by the collaborating entity. Details about these new formulations are found in *Table 4*, where formulations 1 to 3 contain different concentrations of Plasticizer 1, and formulations 4 to 6 contain different concentrations of Plasticizer 2.

Table 4. *First batch of formulations with additives prepared for interlayer adhesion test.*

First batch of formulations with additives		
Reference	**Components**	**Concentrations**
1	MOP polymer Plasticizer 1 Organic solvent	12,5% 4% 83,5%
2	MOP polymer Plasticizer 1 Organic solvent	12,5% 8% 79,5%
3	MOP polymer Plasticizer 1 Organic solvent	12,5% 12% 75,5%
4	MOP polymer Plasticizer 2 Organic solvent	12,5% 12% 75,5%
5	MOP polymer Plasticizer 2 Organic solvent	12,5% 18% 66,5%
6	MOP polymer Plasticizer 2 Organic solvent	12,5% 25% 62,5%

A second batch of formulations with additional additives is detailed in *Table 5*, where formulations 7 to 10 and 11 to 14 contain 4% and 8% of Plasticizer 1, respectively.

Table 5. *Second batch of formulations with additives prepared for interlayer adhesion test.*

Second batch of formulations with additives		
Reference	**Components**	**Concentrations**
7	MOP polymer 4% Plasticizer 1 Plasticizer 2 Organic solvent	12,5% 4% 2% 81,5%
8	MOP polymer	12,5%

	4% Plasticizer 1 Plasticizer 3 Organic solvent	4% 2% 81,5%
9	MOP polymer Plasticizer 1 Plasticizer 4 Organic solvent	12,5% 4% 2% 81,5%
10	MOP polymer Plasticizer 1 Plasticizer 5 Organic solvent	12,5% 4% 2% 81,5%
11	MOP polymer Plasticizer 1 Plasticizer 6 Organic solvent	12,5% 4% 2% 81,5%
12	MOP polymer Plasticizer 1 Plasticizer 2 Organic solvent	12,5% 8% 2% 77,5%
13	MOP polymer Plasticizer 1 Plasticizer 3 Organic solvent	12,5% 8% 2% 77,5%
14	MOP polymer Plasticizer 1 Plasticizer 4 Organic solvent	12,5% 8% 2% 77,5%
15	MOP polymer Plasticizer 1 Plasticizer 5 Organic solvent	12,5% 8% 2% 77,5%
16	MOP polymer Plasticizer 1	12,5% 8%

	Plasticizer 6 Organic solvent	2% 77,5%

4.7. Elasticity test

To test the elasticity of the films formed by each formulation depending on its additives, a test was carried out with a universal testing machine (UTM), specifically the MTE-1L model from Techlab Systems and using METROTEST software. With this test, we evaluated samples 7 to 16 from *Table 5*, excluding references 8 and 13 because they did not give good results in the interlayer adhesion test.

To perform this test, prepare films in the same way as in the interlayer adhesion test, in square-shaped molds that measure 5 centimetres per side, ensuring that each film have a weight of 2 grams. Once the films are prepared, divide each film obtained into three strips, 1,5 centimetres wide. Each strip from the same film is a replicate, measuring 5 centimetres in height and 1.5 centimetres in width. Then, weigh the strips, and record their weight to ensure consistency across samples.

Then, perform elongation tests using the UTM. The UTM has grips specifically designed to prevent slippage or tearing of delicate materials like plastic or polymer films. To begin, open the upper grip and place the top end of the film squarely between the jaws, ensuring the film is centred laterally and hangs straight down. Carefully tighten the grip to hold the specimen firmly to prevent it from loosening during the test. Repeat the process with the lower grip, gently tightening the sample to remove any slack or wrinkles. Ensure that the alignment is vertical so that the load is applied evenly during the test.

Once the strip is secured, use the METROTEST software. Select the tensile test mode and input the sample dimensions. Start the test using the software interface and the UTM will begin applying a controlled tensile force by moving the crosshead, gradually stretching the film. The test continues until the film breaks, at which point the machine automatically stops, and the software provides tensile strength (N) and elongation at break (mm).

Figure 7. *Tensile test of a film strip using the UTM.*

After the test, calculate the elongation at break as a percentage, as this allows for easy comparison of elasticity between samples. To do this, keep in mind that the strips measure 5 centimetres in height and 1.5 centimetres in width. Each grip covers 1.5 centimetres of the strip's height, so the actual stretch section is interpreted as measuring 2 centimetres in height.

$$\text{Elongation at break (\%)} = \frac{\text{Sample elongation at break (mm)}}{20} * 100$$

Equation 4. *Calculation of the elongation at break percentage.*

5. RESULTS AND DISCUSSION

This section presents and discusses the results obtained throughout the project, following the chronological order in which the various tests were conducted. At the end of each subsection, where applicable, the way each step of the project led to the next is explained. Furthermore, some examples of data processing can be found at *Annexes* section.

5.1. Abrasion test results

After the performance of this method with 10 volunteers, the obtained data was used to calculate the average protective effectiveness after every abrasion cycle for each sample. Results obtained from the fourteen evaluated formulations are shown in *Figure 8*.

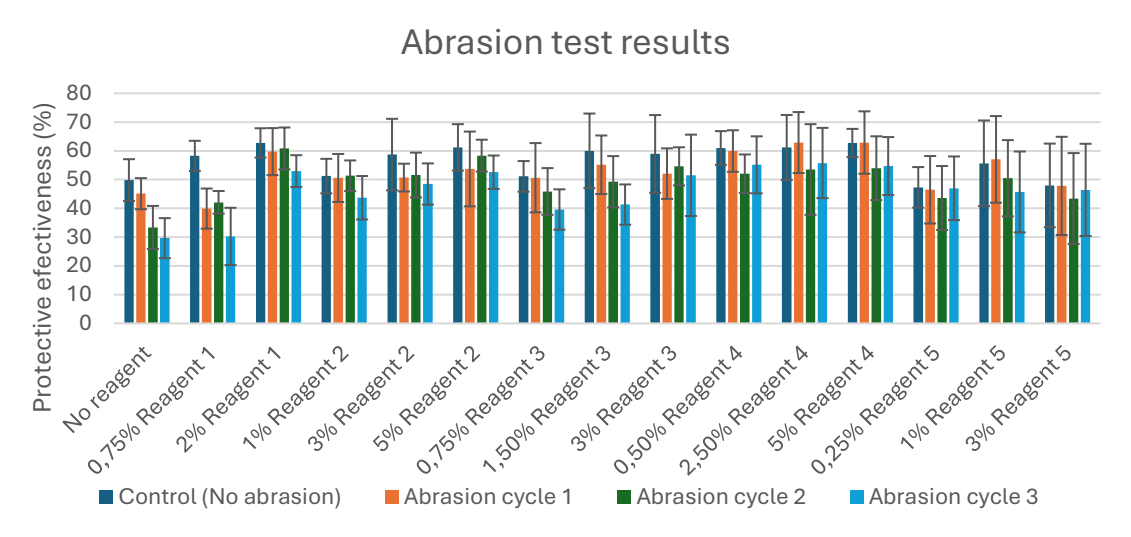

Figure 8. *Abrasion test results for each reagent, represented on a graphic as the evolution of the protective effectiveness percentage throughout consecutive abrasion cycles.*

In addition to protective effectiveness, the difference between the first and the last measure for each reagent was calculated to estimate the stability of the formulations against deterioration.

The BPAG formulation without a reagent showed the lowest effectiveness from the start, with a value of 49.8% that progressively decreased to 29.6% after the third abrasion cycle. This percentage drop of 20.2% confirms that in the absence of a reagent, there is no effective or stable protection against abrasion. This group serves as the baseline for comparing the improvement offered by the various formulations with reagents.

In the case of Reagent 1, the 0.75% concentration showed an acceptable initial effectiveness (58.2%) but suffered a significant loss after the third cycle, dropping to 30.2%. It showed high variability with a reduction of a 28% between first and last measure, indicating low abrasion resistance, even less than the original BPAG formulation. However, the formulation with 2% of Reagent 1 was noticeably more stable: it started with an effectiveness of 62.7% and maintained 52.9% by the end of the test, decreasing by only 9.8%, suggesting reliable performance even after abrasion. These data position 2% of Reagent 1 as a very solid option for applications requiring good protection and durability against abrasion. This concentration represents the most promising formulation within this group.

The formulations with Reagent 2 were characterized by a good combination of efficacy and stability. The 1% concentration showed consistent performance, only reducing from 51.2% to 43.7%, offering a small difference of 7.5% between the measurement before abrasion and the last cycle. Nevertheless, protective effectiveness can be considered low from the start. The gel with 3% of this reagent maintained good resistance, ending with 48.5% and a difference of 10,2%. The highest concentration (5%) achieved one of the best efficacies and abrasion resistances in the group, starting at 61.2% and maintaining 52.6% after three abrasion cycles (reduction of 8.6%). This concentration represents the most promising formulation within this group.

The 0.75% concentration of Reagent 3 started with an effectiveness of 51.1% but fell to 39.6%, providing a significant decrease of 11.5%. The 1.5% formulation started with a high value (59.9%) but decreased to 41.3%, suggesting greater sensitivity to wear. The 3% concentration showed more balanced behavior, finishing at 51.5% with moderate decrease of 7.4%. Overall, this reagent at higher concentrations offers medium protection with reasonable stability, although it does not match the performance of Reagents 2 or 4.

The formulations with Reagent 4 were the most effective and stable overall. At the 0.5% concentration, the formulation managed to maintain effectiveness above 55% throughout the test, with a difference of 5.9% between first and last measure. The formulation with 2.5% of Reagent 4 concluded the test with a value of 55.8% and a difference of only 5.4%. Meanwhile, the formulation with 5% of Reagent 4 was the most protective of all at the start of the test (62.7%) and finished at 54.7%, with a slightly greater difference of 8%. These results clearly position Reagent 4, as one of the best options for abrasion resistance, in any of the tested concentrations.

Finally, Reagent 5 was the least promising. Despite having the lowest differences between first and last measures (particularly the 0.25% formulation, with only 0.4% of difference), its protective effectiveness levels were consistently low. The 1% concentration slightly improved the global results of this Reagent but still fell short compared to other more effective reagents. Even the highest concentration (3%) failed to reach notable protective levels, finishing at only 46.4%.

In conclusion, the results confirm that Reagent 4 is a good choice to improve abrasion resistance at any concentration from 0,5% to 5% and also has one of the best protective effectiveness, closely followed by Reagents 1 and 2, but only at their higher concentrations (2% and 5%, respectively).

5.2. “Wash off” test results

After three wash cycles with synthetic urine were performed to 10 volunteers, results observed in *Figure 9* were obtained. As mentioned in materials and methods section, the percentage of remaining dye after each wash serves as an indicator of the resistance of the applications on the skin, assuming that greater dye loss implies greater degradation or washout of the application due to the action of urine.

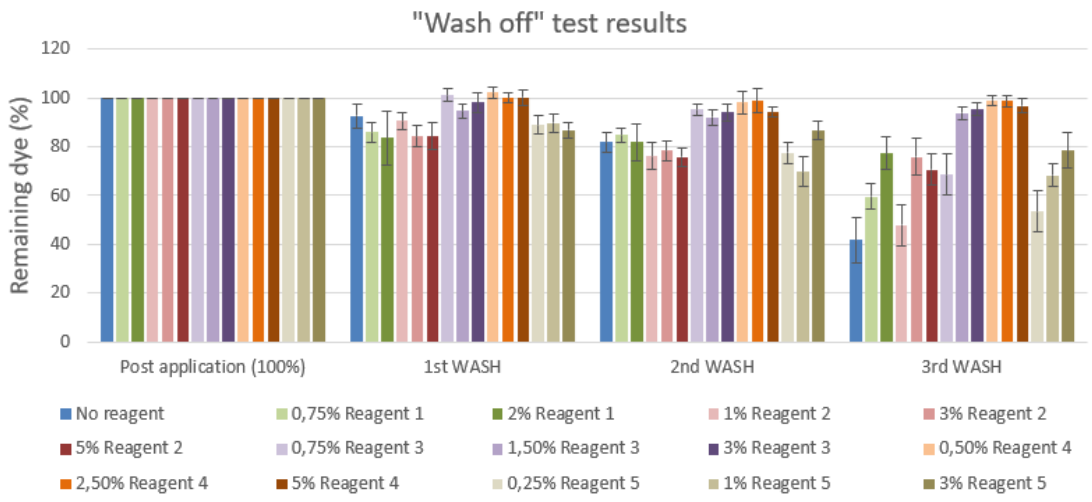

Figure 9. *“Wash off” test results for each reagent, represented on a graphic as the remaining dye percentage throughout consecutive washing cycles.*

The sample without additional reagents showed progressive and significant loss after washing. In the first cycle, retention was 92.65%, dropping to 81.81% after the second wash and drastically decreasing to 41.65% after the third cycle. These data reflect that the application is highly vulnerable to urine. This result establishes a clear baseline

against which the efficacy of the different reagents used in the experiment can be compared.

In the case of Reagent 1, both tested concentrations showed significant loss of application from the first wash, reaching low values by the third cycle, especially at a concentration of 0.75%, where only 59.56% of the dye remained. Although the 2% formulation showed a slight improvement (77.31% after the third wash), the data reflect limited efficacy.

Reagent 2, on the other hand, showed concentration-dependent behaviour. At a 1% concentration, the application loss was very pronounced (47.91% after the third wash). However, increasing the concentration to 3% and 5% showed a considerable improvement in resistance (75.88% and 70.66% after the third wash, respectively). Although these values do not exceed those of the control in the first wash, they do indicate that, over time, the application resists continued exposure to urine better. Additionally, at higher concentrations, the reagent offers some protection against washouts, although not particularly effectively.

The formulations with Reagent 3, evaluated at concentrations of 0.75%, 1.5%, and 3%, showed clearly positive behaviour with respect to concentration. While significant loss was recorded at the lowest concentration (68.65% after the third wash), the 3% formulation managed to preserve 95.36% of the original dye, followed by a remaining 93.72% by the 1.5% concentration of this reagent. This result suggests that this reagent, at appropriate concentrations, offers effective protection against the action of synthetic urine.

Results of Reagent 4 were excellent at all concentrations tested. Treatment loss was practically zero even after three washes, with values between 98.68% and 100%. This sustained efficacy indicates that this reagent provides great and reliable protection, even at minimal concentrations. Therefore, Reagent 4 is positioned as the most effective and stable option among the formulations studied.

Finally, Reagent 5 showed intermediate efficacy. At very low concentrations (0.25%), performance was poor, with considerable loss (53.52% by the third wash). Increasing the concentration to 1% and 3% improved the results (80.22% and 78.53%, respectively), but without reaching the values obtained by Reagent 3 or 4. Although variability was moderate, the data suggests that this reagent requires higher concentrations to achieve acceptable efficacy, without matching the best results of the study.

In summary, the results show a clear influence of both the reagent type and its concentration on the resistance of applications to washing with synthetic urine. Reagent 4 stands out as the most effective, providing almost complete and stable protection, while Reagent 3 also offers very high performance at adequate concentrations. In contrast, Reagents 1, 2 and 5 showed limited protection, especially in their more diluted formulations.

If we compare these results with those obtained in the abrasion test, we can clearly observe that Reagent 4 is a good option to be added to the BPAG formulation, at any of the tested concentrations. Since all tested concentrations of this reagent produced very similar results with negligible differences, it was decided to use the lowest concentration of Reagent 4 (0.5%) to minimize the risk of adverse reactions, given that this reagent has a slightly more acidic pH (3.4) than that of the skin (4.1-5.8). As mentioned in the Introduction (Subsection 2.3.2. *The Skin*), a reagent with a pH that differs from that of the skin, especially at high concentrations, is more likely to cause skin disruption, potentially leading to irritation or dysbiosis and its associated consequences. Keeping this in mind, it was finally decided to add a 0.5% concentration of reagent 4 to the BPAG formulation.

5.3. First application on fingers

This section of the project marked a turning point after observing the results of applying the optimized BPAG formulation to the fingers.

Diverse quantities of the new BPAG formulation were applied to the fingers following the process explained in subsection 4.5. *Application on fingers* from Materials and methods. The tested quantities were 0.5, 1, and 2 mL per finger, in order to assess whether variations in the thickness of the layer formed on the skin would affect the results. All quantities yielded very similar outcomes, except for the 2 mL application, which was too large to be applied comfortably and did not result in a uniform layer being susceptible to being removed by rubbing against other surfaces. The other gel quantities led to an easy and uniform application, completely covering the fingers and not providing any uncomfortable sensation in this regard.

A notable advantage observed with this formulation was that, once dried, it did not exhibit stickiness upon contact with other surfaces, including the skin, while remaining well-adhered to the hands. However, two negative factors were noted, both related to finger bending.

Firstly, when the fingers were bent, the coating adhered to itself, compromising its stability, especially during prolonged contact or under pressure. The film adhered more strongly to itself than to the skin, tending to detach from the skin and stick to itself. This behaviour could be attributed to the molecular forces between the layers of the film, which exceeded any interactions with external surfaces, including the skin. This represented a significant drawback in the progress of glove optimization that had not been previously considered.

Additionally, fissures formed on the upper side of the fingers when they were bent, regardless of the amount applied. This suggests that the film obtained with this formulation lacked enough elasticity to withstand the elongation generated by finger flexion.

These results led to a reconsideration of the project, as it became unclear whether the BPAG formulation (originally adapted from another project) was truly suitable for the glove application, or whether it would be better to adjust the overall approach.

Finally, it was proposed that the simplest way to continue would be to start from a new, original formulation, focusing on one that did not show interlayer adhesion but was still elastic enough to withstand finger bending effectively. It was considered that, in any case, the knowledge acquired from the abrasion and “wash off” tests could later be applied, mainly with the intention of confirming whether reagent 4 is as effective in the new formulation as it was in the BPAG formulation.

5.4. Interlayer adhesion test and film formation results

Until now, the project had been carried out only with unpurified polymer, the MOU polymer, but it was suggested that it would be interesting to start the new approach by testing whether the MOP polymer (purified) had different interlayer adhesion properties.

First, two films were prepared as indicated in subsection 4.6 from Materials and methods. One of these films contained only MOP polymer and the other contained only MOU polymer (References 1 and 2 from *Table 3*). Regarding interlayer adhesion, the MOU polymer film showed very high interlayer adhesion, fusing with itself at both room temperature and 40°C. In contrast, the MOP polymer film showed no interlayer adhesion at all, at either temperature. This suggested that the impurities present in the MOU polymer were what caused this greater number of intermolecular forces that led to the adhesion of the polymer to itself.

Nevertheless, the MOP polymer film presented high rigidity and fragility, was not elastic at all and in its final form contained many bubbles, while the MOU polymer film was elastic, flexible and did not have any bubbles. For that very reason, it was decided to combine MOP and MOU polymers in different concentrations, trying to find a combination with the desired characteristics: that there was no interlayer adhesion but that at the same time the film was elastic, malleable and bubble-free.

The tested combinations were prepared with MOP:MOU polymer ratios of 1:1, 3:2 and 3:1 (References 3, 4 and 5 in *Table 3*, respectively). Once the interlayer adhesion test was carried out, Reference 3 was the only one that exhibited adhesion at both room temperature and 40 °C. Additionally, this film was not very elastic and had bubbles. The other two combinations yielded good results regarding interlayer adhesion: they did not show adhesion at either temperature. Nevertheless, these two films were fragile, lacked elasticity, and also presented bubbles.

Table 6. *Summary of physical characteristics of the polymer films without additives.*

Characteristics of polymer films without additives.				
Reference	**Polymer used**	**Film characteristics**		
		Bubbles	**Fragile**	**Elastic**
1	MOU	No	No	Yes
2	MOP	Yes	Yes	No
3	MOP:MOU (1:1)	Yes	Yes	No
4	MOP:MOU (3:2)	Yes	Yes	No
5	MOP:MOU (3:1)	Yes	Yes	No

Table 7. *Summary of adhesion test results of the polymer films without additives.*

Adhesion test results of polymer films without additives.			
Reference	**Polymer used**	**Interlayer adhesion**	
		Room temperature	**40ºC**
1	MOU	Yes	Yes
2	MOP	No	No
3	MOP:MOU (1:1)	Yes	Yes

4	MOP:MOU (3:2)	No	No
5	MOP:MOU (3:1)	No	No

Due to these results, it was decided to experiment with various plasticizers combined with MOP polymer, taking advantage of its potential to not present interlayer adhesion, but seeking to obtain better film characteristics with regard to elasticity, fragility and presence of bubbles.

First of all, to find a film composition that did not present bubbles, MOP polymer was combined with Plasticizer 1 and Plasticizer 2 at diverse concentrations as shown in *Table 4*. The films with Plasticizer 1 showed practically no bubbles, especially those with lower concentrations (4% and 8%). However, these films remained fragile and exhibited low elasticity. In contrast, all films containing Plasticizer 2 presented numerous small bubbles.

Table 8. *Summary of physical characteristics of the first batch films with additives.*

Characteristics of first batch of formulations.				
Reference	**Additives and concentration**	**Film characteristics**		
		Bubbles	**Fragile**	**Elastic**
1	4% Plasticizer 1	No	Yes	No
2	8% Plasticizer 1	No	Yes	No
3	12% Plasticizer 1	Yes	Yes	No
4	12% Plasticizer 2	Yes	No	Yes*
5	18% Plasticizer 2	Yes	No	Yes*
6	24% Plasticizer 2	Yes	No	Yes*

*Although these films demonstrated greater elasticity, they broke easily when stretched, likely due to the high number of bubbles.

On the other hand, regarding the interlayer adhesion test, both plasticizers in all their tested concentrations gave good results, preventing the film layers from fusing both at room temperature and at 40°C.

Table 9. *Summary of adhesion test results of the first batch films with additives.*

Adhesion test results of first batch of formulations.			
Reference	**Additives and concentration**	**Interlayer adhesion**	
		Room temperature	**40ºC**
1	4% Plasticizer 1	No	No
2	8% Plasticizer 1	No	No
3	12% Plasticizer 1	No	No
4	12% Plasticizer 2	No	No
5	18% Plasticizer 2	No	No
6	24% Plasticizer 2	No	No

Given these results, and since Plasticizer 1 at concentrations of 4% and 8% was the only one that prevented bubble formation, it was proposed to prepare a second batch of formulations. These would combine those concentrations of Plasticizer 1 with other plasticizers, with the aim of improving elasticity and reducing fragility while maintaining the absence of interlayer adhesion. These combinations are specified in *Table 5* from the subsection 4.6.

Almost all formulations in this second batch provided exceptionally good results, both in terms of film characteristics and in the interlayer adhesion test itself. The only films that did not present optimal outcomes were those corresponding to references 8 and 13 in *Table 5*, both with Plasticizer 3 and different concentrations of Plasticizer 1 (4% and 8%, respectively). These films passed the interlayer adhesion test, but they were rigid, not elastic and they also presented bubbles.

Table 10. *Summary of physical characteristics of the second batch films with additives.*

Characteristics of second batch of formulations.				
Reference	**Additives and concentration**	**Film characteristics**		
		Bubbles	**Fragile**	**Elastic**
7	4% Plasticizer 1 2% Plasticizer 2	No	No	Yes
8	4% Plasticizer 1 2% Plasticizer 3	Yes	Yes	No

9	4% Plasticizer 1 2% Plasticizer 4	No	No	Yes
10	4% Plasticizer 1 2% Plasticizer 5	No	No	Yes
11	4% Plasticizer 1 2% Plasticizer 6	No	No	Yes
12	8% Plasticizer 1 2% Plasticizer 2	No	No	Yes
13	8% Plasticizer 1 2% Plasticizer 3	Yes	Yes	No
14	8% Plasticizer 1 2% Plasticizer 4	No	No	Yes
15	8% Plasticizer 1 2% Plasticizer 5	No	No	Yes
16	8% Plasticizer 1 2% Plasticizer 6	No	No	Yes

Table 11. *Summary of adhesion test results of the second batch films with additives.*

Adhesion test results of second batch of formulations.			
Reference	**Additives and concentration**	**Interlayer adhesion**	
		Room temperature	**40ºC**
7	4% Plasticizer 1 2% Plasticizer 2	No	No
8	4% Plasticizer 1 2% Plasticizer 3	No	No
9	4% Plasticizer 1 2% Plasticizer 4	No	No
10	4% Plasticizer 1 2% Plasticizer 5	No	No
11	4% Plasticizer 1 2% Plasticizer 6	No	No

12	8% Plasticizer 1 2% Plasticizer 2	No	No
13	8% Plasticizer 1 2% Plasticizer 3	No	No
14	8% Plasticizer 1 2% Plasticizer 4	No	No
15	8% Plasticizer 1 2% Plasticizer 5	No	No
16	8% Plasticizer 1 2% Plasticizer 6	No	No

Based on these results, the formulations presented in Table 5, except for samples 8 and 13 (those that involve Plasticizer 3), emerged as strong candidates for serving as the base formulation of the soluble glove. It was then proposed to compare the elasticity provided by the different formulations in order to identify which combination stood out in this regard, potentially making it the most resistant to hand movements and finger bending.

5.5. Elasticity test results

The main parameter evaluated in this test was the average elongation at break (%), which proportionally corresponds to the elasticity of the film. However, the average elongation force (N) was also measured because it was considered relevant to search for formulations that offered moderate or low tensile resistance, assuming that this would contribute to greater comfort when wearing the formulation on the hands. Once the test was performed, the results clearly showed that the concentration of Plasticizer 1 plays a decisive role in modulating the properties of the films, but this effect was significantly influenced by the nature of the second plasticizer employed.

Table 12. *Elasticity test results: Average elongation at break and tensile strength.*

Elasticity test results			
Reference	**Additives and concentration**	**Average elongation at break (%)**	**Average tensile strength (N)**
7	4% Plasticizer 1	558,27	17,22

	2% Plasticizer 2		
9	4% Plasticizer 1 2% Plasticizer 4	241,93	28,34
10	4% Plasticizer 1 2% Plasticizer 5	410,95	24,46
11	4% Plasticizer 1 2% Plasticizer 6	444,60	32,91
12	8% Plasticizer 1 2% Plasticizer 2	512,08	20,46
14	8% Plasticizer 1 2% Plasticizer 4	195,97	39,00
15	8% Plasticizer 1 2% Plasticizer 5	352,58	29,10
16	8% Plasticizer 1 2% Plasticizer 6	291,47	31,53

As shown in *Table 12*, among all tested formulations, the combination of 4% Plasticizer 1 with Plasticizer 2 yielded the highest average elongation at break, reaching 558.27%. This indicates excellent flexibility. Interestingly, when the concentration of Plasticizer 1 was increased to 8%, the elongation at break slightly decreased to 512.08%. This moderate decline suggested that higher Plasticizer 1 content increases the rigidity of the network, partially counteracting the flexibility imparted by Plasticizer 2. In addition, it is worth mentioning that the samples with Plasticizer 2 provided the lowest tensile strength results, both with 4% of Plasticizer 1 and with 8% (17.22N and 20.46N, respectively).

The behaviour of Plasticizer 4 was markedly different. For both Plasticizer 1 concentrations, this formulation produced the lowest elongation values. The film containing 4% Plasticizer 1 and Plasticizer 4 exhibited an average elongation at break of 241.93%, while the 8% Plasticizer 1 film dropped further to 195.97%. These results indicate that Plasticizer 4, unlike Plasticizer 2, does not perform as effectively as a plasticizer with this polymer. The reduction in elongation with increasing Plasticizer 1 suggests that this additive reinforces the matrix in this case, restricting deformation. This is further supported by the fact that these films required the highest average elongation forces, with the reference 14 formulation reaching a remarkable force of 38.99 N. While

this implies excellent tensile resistance, it also denotes poor elasticity compared to the other samples.

Films with Plasticizer 5 presented intermediate mechanical profiles. At 4% Plasticizer 1, the average elongation at break was 410.95%, whereas at 8%, this value declined to 352.58%. These films showed improved flexibility compared to those with Plasticizer 4, but less than those with Plasticizer 2. The corresponding elongation forces were also moderate, averaging 24.46 N for the 4% formulation and 29.10 N for the 8% formulation. This balance between ductility and strength suggests that Plasticizer 5 provides a dual effect: it allows for acceptable flexibility while enhancing mechanical integrity.

The film with 4% Plasticizer 1 and Plasticizer 6 showed an elongation at break of 444.60%, demonstrating considerable flexibility. The 8% Plasticizer 1 version, however, experienced a noticeable drop to 291.47%, indicating that the increase in Plasticizer 1 content in this case once again introduces rigidity. Nonetheless, both Plasticizer 6 films required relatively high forces to break, with average elongation forces of 32.91 N and 31.53 N for the 4% and 8% formulations, respectively. These values highlight the potential of Plasticizer 6 as an additive capable of enhancing tensile resistance without excessively compromising elasticity, particularly at lower Plasticizer 1 concentrations.

Overall, as Plasticizer 1 content increased from 4% to 8%, the elongation at break tended to decrease across all additives, which aligns with the expectation that higher Plasticizer 1 concentration promote crosslinking density or structural stiffness. However, the extent of this reduction and its mechanical consequences vary significantly depending on the additive.

In conclusion, the combination of 4% Plasticizer 1 and Plasticizer 2 (reference 7 in *Table 5*) proved to be the most effective formulation for maximizing film elasticity. Nevertheless, the other tested formulations also exhibited good elasticity compared to the MOP polymer film without additives. However, those containing Plasticizer 4 showed the most limited performance in this regard.

Although the formulation with the lowest elasticity still nearly doubled its original length, it was decided not to exclude any samples from further testing at this stage. All formulations demonstrated acceptable elastic properties. Based on these results, all samples were selected for direct application on fingers to assess whether the properties observed in film form were consistent during practical use.

5.6. Last application on fingers

Based on the previous tests, it was considered appropriate to perform a finger test on the formulations to determine whether their outcomes could be extrapolated to the real application intended: use as a glove. The objective of this section was to check whether, even if completely optimal results were not obtained, the formulations currently being worked with provided better results than the original BPAG formulation. Thus, it was decided to test on the fingers the formulations corresponding to references 7 to 16 of Table 5, excluding references 8 and 13 that had already been previously discarded.

As expected, in this regard, all the new formulations far exceeded the characteristics presented by the formulation originally chosen for development, the BPAG formulation. In this case, 0.4 mL was applied to each finger, a smaller amount than with the gel, as these samples were more liquid and applying larger volumes was more difficult and less comfortable. All the new formulations produced very similar results in terms of comfort, tackiness, interlayer adhesion, and resistance to cracking or breaking at the upper part of the fingers when bent. No notable difference was observed between samples.

These new formulations performed very well on the upper part of the fingers when bent, no cracks were observed after closing hands repeatedly. Nevertheless, although briefly bending the fingers without applying force no longer resulted in interlayer adhesion, when the fist was kept closed for a longer period and applying pressure, the layers adhered slightly, once again compromising the stability of the protective barrier. This behaviour could be due to alterations in the film due to the pressure exerted and the increase in temperature inside the hand when it remains closed. However, there was a noticeable improvement in this characteristic compared to the BPAG formulation as shown in *Figures 10, 11* and *12*, where Reference 11 was used as an example of the new formulations behaviour. To take these images, 50 ppm of blue dye was added to each formulation in order to facilitate the visualization of differences.

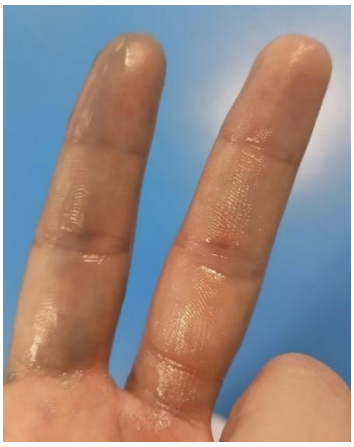

Figure 10. *Comparison of BPAG (left finger) and Reference 11 (right finger) applications.*

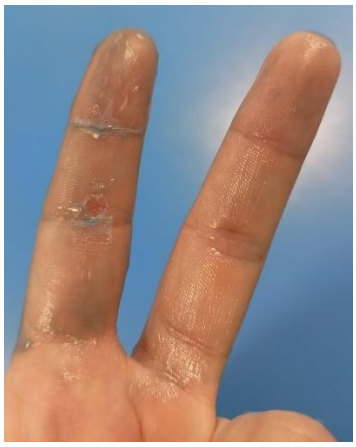

Figure 11. *Comparison of brief bending resistance between BPAG (left finger) and Reference 11 (right finger) applications.*

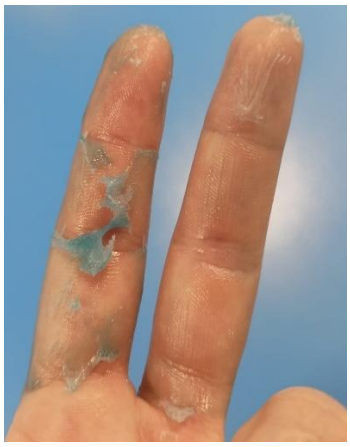

Figure 12. *Comparison of sustained bending resistance between BPAG (left finger) and Reference 11 (right finger) applications.*

As shown in *Figure 11*, brief contact does not appear to compromise the integrity of the new formulation, unlike the BPAG formulation. However, prolonged contact leads to greater destabilization of the new film, particularly at the fingertips, while the BPAG film is already completely degraded at this point, as shown in *Figure 12*.

Unfortunately, as a setback, it was observed that these new formulations exhibited a slight tackiness in contact with skin or other surfaces, which was not present in the BPAG formulation. Nonetheless, the progress was so significant in terms of barrier stability and reduced interlayer adhesion during brief and sustained contact that the new formulations were considered optimal for further investigation.

In summary, results suggest that substantial progress was made in understanding the chemical, physical, and mechanical properties that such a formulation must possess to be viable in a clinical context, particularly in surgical environments.

Initial efforts focused on optimizing the BPAG formulation by incorporating various reagents aimed at improving resistance to mechanical abrasion and degradation by synthetic urine. Notably, Reagent 4 demonstrated superior performance in both the abrasion and “wash off” tests, consistently preserving the integrity of the protective film. This positioned it as a key additive candidate in any future formulation.

However, despite promising results in these tests, the BPAG formulation showed critical limitations when applied to fingers, it showed significant interlayer adhesion upon finger bending and the formation of fissures in the upper areas of the fingers. These findings marked a turning point in the project, prompting a reconsideration of the overall strategy and leading to the exploration of new formulations of MOP based polymer.

The second phase of the project started by characterizing the behaviour of MOP polymer based films, both alone and in combination with MOU polymer, and evaluating their performance through interlayer adhesion tests. It was discovered that MOP polymer, in contrast to MOU polymer, prevents interlayer adhesion due to its lack of impurities, but is inherently fragile, lacks elasticity and forms bubbles when it dries. To overcome these drawbacks, several plasticizers were introduced in a controlled and systematic manner. Two rounds of additive screening yielded a selection of formulations that were able to maintain desirable film characteristics (including elasticity, flexibility, and absence of bubbles) while also preventing interlayer adhesion under test conditions.

The elasticity tests conducted using the UTM showed that the combination of 4% Plasticizer 1 with 2% Plasticizer 2 resulted in the best mechanical performance, with an elongation at break exceeding 550%, and low tensile strength, which suggests high comfort in practical use. Several other formulations also showed adequate elasticity, indicating a broad range of viable options for continued development.

Subsequent finger application tests confirmed a substantial improvement over the BPAG formulation. The new formulations resisted brief and moderate flexion without cracking or significant interlayer adhesion. However, they still presented partial adhesion when the hand was closed under sustained pressure and warmth. Additionally, a slight tackiness to touch was noted in some formulations and that was a not present issue in the gel formulation BPAG. Despite this, the improvements in film integrity, elasticity, and comfort firmly establish the new formulations containing MOP polymer as a superior starting point for future optimization.

5.7. Future challenges based on the results obtained

Although the latest formulations tested in this project represented a substantial improvement over the original BPAG formulation, especially in terms of elasticity, interlayer adhesion, and film stability, some challenges remain that must be solved in future stages.

To continue with this project, it is considered essential to conduct an additive screening before proceeding to the next phase of testing. Since a 2% concentration was used as a guideline for the plasticizers based on other projects results of the collaborating entity, it would be advisable to evaluate a range of concentrations for each additive to determine whether the desired properties can be achieved. If the target characteristics are not obtained, further tests should be carried out using the concentrations that appear most promising.

One of the main observed issues is the persistence of interlayer adhesion under prolonged pressure and warmth, such as when the hand remains closed in a fist. While brief contact no longer compromises the integrity of the film, sustained pressure still leads to partial adhesion and destabilization, especially at the fingertips. This indicates that, despite the progress made, behaviour of the film under real usage conditions (such as surgical procedures) is not yet optimal. To solve this problem, tests similar to those used for interlayer adhesion could be adapted to include the application of pressure to the layers when they are slightly hot. This could be achieved through compression testing using the UTM.

Another point for future investigation is the slight tackiness exhibited by the new formulations when in contact with skin or other surfaces. This property is uncomfortable for prolonged use and could limit dexterity in professional settings, so strategies such as surface modifying agents that could mitigate this effect without compromising flexibility should be explored.

Additionally, it would be beneficial to complement these results with long-term wear simulations, including repeated hand movements, sweating, and interaction with common hospital substances. These studies would help better approximate real-life wear and degradation of the glove.

Once an optimal formulation is consolidated, it will also be necessary to reintroduce resistance tests (such as the abrasion and “wash off” tests) to confirm whether the promising additives from the BPAG phase, especially Reagent 4, remain effective in the new polymeric formulation based on MOP.

Finally, future development could explore new application methods to improve uniformity, ease of use, and drying speed, which are critical factors in a hospital environment. The integration of custom dispensers or spray systems could significantly enhance practical application, providing an optimal thickness that provides not only comfort but also confidence to the user.

6. CONCLUSIONS

1. It has been demonstrated that it is possible to formulate a polymer-based liquid product that forms a continuous film on the skin when applied, confirming the viability of the base concept.
2. The abrasion resistance test showed that specific additives, particularly Reagent 4, can significantly improve the film's stability under repeated friction, which is essential for practical use in healthcare settings.
3. The protective barrier showed high resistance to external fluids, particularly synthetic urine, when optimized additives (such as Reagent 4) were included, supporting its suitability for clinical environments.
4. The final selected formulations generated flexible and elastic films capable of adapting to hand movement, thus fulfilling the mechanical requirements raised in the hypothesis.
5. The new formulations did not present interlayer adhesion under brief contact, although prolonged pressure still caused partial adhesion, which needs to be improved.
6. All films tested could be redissolved in organic solvent, validating the potential for removal, sterilization, and reuse of the material, as initially hypothesized.
7. The hypothesis is partially confirmed: while the project achieved a recyclable, biocompatible barrier with promising mechanical and chemical properties, further optimization is needed to ensure full functionality under prolonged clinical use.

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8. ABBREVIATIONS AND ACRONYMS

SBM	Synkotech Biocompatible Materials
R&D&I	Research, Development and Innovation
MOP	Purified monomer from SBM
MOU	Unpurified monomer from SBM
BPAG	Starting gel formulation
TEWL	Transepidermal water loss
UTM	Universal Testing Machine

9. ANNEXES

9.1. Example of abrasion test data processing

The data shown in *Table 13* correspond to the TEWL values measured with the vapor meter for one of the volunteers. The yellow bands correspond to the average value of the three replicas above.

***Table 13**. TEWL results obtained from a volunteer.*

Sample	Transepidermal water loss (g/m²h)				
	Blank	**Post application**	**1st cycle**	**2nd cycle**	**3rd cycle**
No reagent	8,6	3,6	4	4,4	6,4
	8,3	3,2	3,5	3,8	7,5
	7,9	2,1	2,9	4,3	4,8
	8,3	3,0	3,5	4,2	6,2
0,75% Reagent 1	3,2	2,2	2,5	1,8	3,1
	4,2	1,9	3	2,3	3,7
	3,6	1,6	2,8	2,3	3,4
	3,7	1,9	2,8	2,1	3,4
2% Reagent 1	7,8	3	4,2	1,9	3,4
	7,8	2,3	1,8	2,2	3,9
	6,9	2,8	2,2	3,7	4,3
	7,5	2,7	2,7	2,6	3,9
1% Reagent 2	5,9	3,1	2,6	2,1	3,3
	6,1	3,2	2,7	2,1	4
	5,4	3	3,1	2,4	3,6
	5,8	3,1	2,8	2,2	3,6
3% Reagent 2	6,3	3,7	3,5	3	4,7
	5,8	3,2	3,4	2,4	2
	6,6	2,6	3,4	3	3,3
	6,2	3,2	3,4	2,8	3,3
5% Reagent 2	6,4	2,9	3,4	2,3	2,3
	6,7	2,8	3	2,9	4,1
	6,0	3	3,9	2,7	3,7
	6,4	2,9	3,4	2,6	3,4
0,75% Reagent 3	6,1	1,7	2,5	2,8	3,1
	6,2	2,6	3,1	2,8	3,3
	5,3	3,6	3,6	2,5	3,3
	5,9	2,6	3,1	2,7	3,2
1,50% Reagent 3	6,9	3,3	2,4	2,1	3,8
	5,8	3	2,8	3,9	3,6
	6,7	2	2,6	3	3,3
	6,5	2,8	2,6	3,0	3,6

3% Reagent 3	6,7	3,9	3,3	3,6	4,5
	7,0	2,5	3,7	3,6	4
	7,1	3,7	3,4	3,3	4,4
	6,9	3,4	3,5	3,5	4,3
0,5% Reagent 4	8,6	3,1	3,8	3,4	3,4
	9	3,6	1,5	3,8	2,8
	8,6	3,3	2,2	3,5	2,7
	8,7	3,3	2,5	3,6	3,0
2,50% Reagent 4	8,3	3,2	1,4	2,2	2,8
	8,5	3	2,4	2,4	3
	7,7	2,7	2	3,6	2,5
	8,2	3,0	1,9	2,7	2,8
5% Reagent 4	8,7	1,6	2,1	3,8	2
	8	2,6	2,1	2,3	2,2
	8,1	2,7	2	2,4	2,2
	8,3	2,3	2,1	2,8	2,1
0,25% Reagent 5	6,6	3,2	3,3	2,7	3,4
	5,9	2,8	3,2	3,4	3,9
	6,3	3,8	3	3,7	3,6
	6,3	3,3	3,2	3,3	3,6
1% Reagent 5	8,1	1,6	1,7	2,5	1,8
	7,8	1,9	2,1	2,7	2
	7,3	3,2	2,4	2,9	2,1
	7,7	2,2	2,1	2,7	2,0
3% Reagent 5	8	2,9	2	3,1	2,2
	7,8	1,1	1,4	3,1	2,7
	7	2,2	2,5	3,4	2,2
	7,6	2,1	2,0	3,2	2,4

Using *Equation 1* on page 18 (Subsection *4.2. Abrasion test*) we carry out the data processing and obtain the protective efficacy presented for this volunteer, shown in *Table 14*.

Table 14. *Protective effectiveness obtained from a volunteer.*

Sample	**Protective effectiveness (%)**			
	Post application	**1st cycle**	**2nd cycle**	**3rd cycle**
No reagent	63,86	57,83	49,40	25,30
0,75% Reagent 1	48,65	24,32	43,24	8,11
2% Reagent 1	64,00	64,00	65,33	48,00
1% Reagent 2	46,55	51,72	62,07	37,93
3% Reagent 2	48,39	45,16	54,84	46,77
5% Reagent 2	54,69	46,88	59,38	46,88
0,75% Reagent 3	55,93	47,46	54,24	45,76

1,50% Reagent 3	56,92	60,00	53,85	44,62
3% Reagent 3	50,72	49,28	49,28	37,68
0,5% Reagent 4	62,07	71,26	58,62	65,52
2,50% Reagent 4	63,41	76,83	67,07	65,85
5% Reagent 4	72,29	74,70	66,27	74,70
0,25% Reagent 5	47,62	49,21	47,62	42,86
1% Reagent 5	71,43	72,73	64,94	74,03
3% Reagent 5	72,37	73,68	57,89	68,42

After obtaining these results, the average is made with the rest of the volunteers.

9.2. Example of “Wash off” test data processing

The data shown in *Tables 15* and 16 correspond to the CIE L*a*b* colorimetric notation system values measured with the colorimeter for one of the volunteers. The yellow bands correspond to the average value of the three replicas above.

Using *Equation 2* on page 20 (Subsection *4.3. “Wash off” test*) we carry out the data processing and obtain the total colour intensity for all the samples tested on this volunteer. Results are shown in *Tables 17 and 18.*

Then, with *Equation 3* on page 20 (Subsection *4.3. “Wash off” test*) the percentage of dye is calculated. Results are shown in *Tables 19 and 20*.

Finally, when results are obtained, the average is made with the rest of the volunteers.

Table 15. *Colorimeter results obtained from a volunteer. Samples: without reagent, and concentrations of Reagent 1, Reagent 2 and Reagent 3.*

No reagent	0,75% Reagent 1	2% Reagent 1	1% Reagent 2	3% Reagent 2	5% Reagent 2	0,75% Reagent 3	1,50% Reagent 3	3% Reagent 3	
60,44	59,39	58,17	60,4	58,89	58,79	60,01	59,39	62,49	Base line
6,45	6,28	6,5	5,13	7,65	6,12	6,27	6,36	4,07	
17,56	18,12	18,13	16,93	17,81	16,78	18,28	17,93	16,4	
42,84	45,85	42,39	43,89	45,43	46,93	46,16	42,75	41	Dye application
10,33	11,51	13,11	13,16	11,5	11,51	11,48	13,86	14,52	
-4,55	-2,13	-6,79	-0,88	-2,65	-1,3	-0,59	-4,44	-6,82	
38,84	39,59	40,01	42,17	39,7	41,29	44,81	38,99	39,66	Post sample application
2,62	5,63	4,91	4,76	4,23	4,44	4	7,36	5	
-7,69	-14,65	-9,8	-12,06	-12,7	-13,07	-6,38	-16,88	-13,76	
40,96	46,83	47,51	45,67	41,13	43,96	42,48	39,97	41,19	1st wash
5,72	4,11	5,18	4,64	5,28	5,41	5,43	7,07	6,99	
-13,53	-5,74	-17,8	-6,33	-16,24	-13,53	-11,21	-14,03	-16,56	
45,29	46,26	45,8	51,27	41,62	46,09	45,72	40,39	39,57	2nd wash
5,1	4,39	5,91	3,52	5,23	5,47	4,07	7,64	7,58	
-7,39	-5,43	-17,36	1,89	-17	-14,06	-6,79	-13,61	-16,69	
49,09	47,64	43,58	50,78	41,39	47,1	53,93	42,46	40,38	3rd wash
4,45	5,39	6,22	4,42	5,11	5,14	4,4	6,76	7,26	
-7,33	0,78	-13,26	6,03	-12,47	-11,65	8,1	-10,79	-13,9	

Table 16. *Colorimeter results obtained from a volunteer. Samples: Reagent 4 and Reagent 5.*

0,50% Reagent 4	2,50% Reagent 4	5% Reagent 4	0,25% Reagent 5	1% Reagent 5	3% Reagent 5	
60,9	60,76	60,27	59,87	60,61	60,98	Base line
5,62	5,29	5,34	6,11	5,02	5,13	
18,07	15,25	16,86	16,37	17,67	18,19	
45,16	45,78	45,64	45,17	46,01	47,61	Dye application
12,19	11,96	12,57	12,59	11,69	11,81	
-5,52	-1,26	-3,51	-3,02	-1,11	-1,61	
41,94	41,74	43,56	41,38	42,05	40,1	Post sample application
4,61	3,85	4,33	5,97	4,45	3,17	
-13,24	-10,51	-8,92	-13,16	-11,48	-14,24	
42,11	41,01	42,64	43,97	42,05	38,63	1st wash
5,74	5,37	5,31	5,65	5,74	6,44	
-11,86	-12,56	-10,16	-8,1	-11,32	-23,77	
43,28	41,2	44,69	46,9	44,29	41,67	2nd wash
4,77	6,63	5,27	4,58	6,17	7,07	
-8,42	-12,73	-8,08	-4,4	-12,64	-18,7	
43,47	42,03	42,65	55,24	48,68	45,69	3rd wash
4,61	5,21	5,67	4,37	6,29	7,22	
-6,22	-9,81	-9,34	8,94	-11,86	-22,02	

Legend		
	L	Luminance
	a	Green to red
	b	Blue to yellow

Table 17. *Total colour intensity obtained from a volunteer. Samples: without reagent, and concentrations of Reagent 1, Reagent 2 and Reagent 3.*

No reagent	0,75% Reagent 1	2% Reagent 1	1% Reagent 2	3% Reagent 2	5% Reagent 2	0,75% Reagent 3	1,50% Reagent 3	3% Reagent 3	
63,27	62,41	61,28	62,94	62,00	61,44	63,04	62,36	64,73	Base line
44,30	47,32	44,89	45,83	46,94	48,34	47,57	45,16	44,03	Dye application
39,68	42,59	41,48	44,12	41,90	43,54	45,44	43,12	42,28	Post sample application
43,51	47,36	51,00	46,34	44,53	46,31	44,27	42,95	44,94	1st wash
46,17	46,78	49,33	51,43	45,26	48,50	46,40	43,30	43,61	2nd wash
49,83	47,95	45,98	51,33	43,53	48,79	54,71	44,33	43,32	3rd wash

Table 18. *Total colour intensity obtained from a volunteer. Samples: Reagent 4 and Reagent 5.*

0,50% Reagent 4	2,50% Reagent 4	5% Reagent 4	0,25% Reagent 5	1% Reagent 5	3% Reagent 5	
63,77	62,87	62,81	62,37	63,33	63,84	Base line
47,10	47,33	47,47	46,99	47,48	49,08	Dye application
44,22	43,21	44,67	43,83	43,82	42,67	Post sample application
44,12	43,23	44,15	45,07	43,92	45,81	1st wash
44,35	43,63	45,72	47,33	46,47	46,22	2nd wash
44,15	43,47	44,03	56,13	50,50	51,23	3rd wash

Table 19. Percentage of dye on the skin. *Samples: without reagent, and concentrations of Reagent 1, Reagent 2 and Reagent 3.*

No reagent	0,75% Reagent 1	2% Reagent 1	1% Reagent 2	3% Reagent 2	5% Reagent 2	0,75% Reagent 3	1,50% Reagent 3	3% Reagent 3	
0	0	0	0	0	0	0	0	0	Base line
100	100	100	100	100	100	100	100	100	Post sample application
83,75	75,93	51,93	88,20	86,88	84,50	100	100	88,13	1st wash
72,48	78,83	60,33	61,17	83,26	72,30	94,54	99,06	94,06	2nd wash
56,96	72,94	77,31	61,69	91,88	70,66	47,33	93,72	95,36	3rd wash

Table 20. Percentage of dye on the skin. *Samples: Reagent 4 and Reagent 5.*

0,50% Reagent 4	2,50% Reagent 4	5% Reagent 4	0,25% Reagent 5	1% Reagent 5	3% Reagent 5	
0	0	0	0	0	0	Base line
100	100	100	100	100	100	Post sample application
100	99,95	100	93,34	99,45	85,16	1st wash
99,35	97,89	94,24	81,13	86,40	83,25	2nd wash
100	98,69	103,57	33,65	65,76	59,57	3rd wash