# Using quality by design to optimize hydrogel semi-solid sheet masks for enhanced skin barrier: *in vitro* and *in vivo* studies

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## Abstract

**Background**: The COVID-19 pandemic led to the increased use of personal protective equipment (PPE) that exert continuous pressure, tension and friction forces on the skin, causing lesions. The current study aimed to develop and test the efficacy of a low-cost and easy to produce gelatin-based hydrogel sheet-mask to be placed between the PPE mask and the facial areas, to prevent skin injuries through redistribution and reduction of the pressure and to avoid the friction triggered by mask displacement.

**Methods**: Biodegradable and environmentally friendly excipients were added to enhance the physical and mechanical properties of the sheet-mask. The development of the hydrogel sheet-mask was achieved using Design of Experiment (DoE) with a Quality by Design approach (QbD). *In vitro* mechanical characterization was performed through elasticity and adhesivity evaluation using Kinexus Lab+ Rheometer (Malvern Instruments, Malvern, UK). The hydrogel sheet-mask performance was also tested using tribology tools and its efficacy was assessed by *in vivo* biometric studies of facial skin surface hydration and temperature.

**Results**: The addition of the excipients enhanced the elasticity and adhesiveness parameters of the sheet-mask, which maintained its integrity under all tested conditions. The *in vivo* study showed that the use of the sheet-mask slightly decreased the facial temperature due to pressure relief, and increased the skin hydration.

**Conclusion**: In conclusion, the resilient physical properties of the developed hydrogel sheet-mask and the attenuation of the physiological alterations in the facial area during its use are good indicators that this polymeric film-forming system can prevent skin lesions caused by the use of PPE.

**Keywords:** COVID-19; PPE; Hydrogel sheet-mask; Tribology; *In vivo* study.

## Introduction

The coronavirus SARS-CoV-2 which is responsible for the COVID-19 pandemic has led to the general use of personal protective equipment (PPE), a daily measure aiming to minimize human-to-human transmission. During the worst stages of the pandemic this measure was imposed not only on healthcare professionals but also the general public, with PPE being mandatory in every public facility and workplace [1].

The prolonged and continuous use of PPEs exerts sustained pressure, tension forces, friction, and also increases the local humidity and temperature, which may originate skin lesions [2,3]. A possible way to prevent such lesions is to incorporate a "barrier" between the skin and the PPE to serve as a protective shield in areas where the pressure is higher and where the skin suffers repeated rubbing.

A practical form to avoid PPE-skin damage related injuries is to place a reusable and low-cost hydrogel sheet-mask between the skin and the PPE mask. The use of natural polymers, such as gelatin, is an interesting approach to develop biocompatible hydrogel sheet-masks due to their excellent film-forming properties [4–9]. However, the physical properties of gelatin are sensitive to water and temperature, thus one way to improve the physical and mechanical attributes of this polymer is the addition of low-cost and ecofriendly excipients [9,10].

This work aimed to develop and test the efficacy of a low-cost and easy to produce hydrogel sheet-mask to be placed between the PPE mask and the facial area where pressure is more concentrated and suffers repeated rubbing, such as the upper edge of the nose bridge and cheekbones. This polymeric film-forming system might prevent skin injuries through redistribution and reduction of the pressure and also by avoiding the friction triggered by mask displacement, increasing HCPs skin health and personal wellbeing without compromising the protective function of PPEs.

## Materials and Methods.

### *Materials*

Gelatin type B was purchased form Acopharma (Spain). Glycerin was purchased form Lacrilar (Portugal). Polyvinyl alcohol (PVA) was purchased from JMGS (Portugal). Betaine anhydrous was purchased from T.C.I. Europe (UK). Silica (Aerosil 200 Pharma) was from Kremer and Martin GmbH (Germany). Sodium chloride was obtained from AppliChem (Spain), L-histidine was from Alfa Aesar (USA), sodium phosphate monobasic was from Panreac Applichem (Spain). Sodium methylparaben and sodium propylparaben were acquired from Fagron (Spain). Purified water was obtained by reverse osmosis and electrodeionization (Millipore®, Elix 3), followed by filtration (filter pore 0.22 µm).

## Methods

### *Preparation of gelatin-based hydrogel sheet-mask*

The hydrogel sheet-mask was prepared by mixing gelatin and each excipient with purified water under gentle stirring and heated at 75 °C in a thermostatic water bath for 45 minutes. Afterwards, the solution was poured into a Petri dish and cooled at room temperature overnight, resulting in the formation of a hydrogel sheet-mask.

### *Quality by Design (QbD) and Risk Assessment*

A quality target product profile (QTPP) was defined according to the sheet-mask chosen features, and the critical quality attributes (CQA) were selected. From the previous definition, failure modes associated with each excipient and process parameter were identified.

***Design Space and Formula Optimization***

A 2 Level Fractional Factorial Design Resolution V+ was used to optimize the formulation (MODDE® software, Umetrics, Umeå, Sweden, $p < 0.05$). According to the risk assessment, the percentages of betaine, PVA, silica, gelatin and glycerin were defined as the independent variables, while gelation temperature, adhesiveness, elasticity and lubricant properties were defined as the dependent variables.

***In vitro characterization of the gelatin-based hydrogel sheet-mask***

Temperature sweep tests were carried out with a plate–plate geometry and a gap of 0.5 mm (sheet-mask height simulation) using a controlled stress Malvern Kinexus Lab+ Rheometer (Malvern Instruments, Malvern, UK) with frequency of 1 Hz and shear strain of 1% at a 2.5 °C/min rate. The sample was cooled down from 75 °C to 25 °C while the G' and G'' moduli were determined.

The adhesive strength and elasticity were determined simultaneously using a plate- plate geometry in the following conditions: 0.1 mm/s velocity, 0.1 $mm/s^2$ acceleration rate, a target force of 1.5 N (simulate the pressure excreted by FFP2 mask), and 0.1 $mm/s^2$ deceleration rate. The elasticity value matches the distance through the sample until the probe detects 1.5 N of force and the adhesive value is the force required to detach the probe from the sample.

***Tribology assay***

The lubricant evaluation was performed with a controlled stress using a three-ball-on-plate tribometer geometry in rotational mode with a Malvern Kinexus Lab+ Rheometer (Malvern Instruments, Malvern, UK). Normal forces of 0.5 N or 1.5 N were used to simulate surgical and FFP2 mask forces, respectively. A gap of 1 mm with an initial and final velocity of $1x10^{-4}$ rad/s and 1 rad/s, respectively, were the conditions defined. This test was performed at 25 ºC and at 32 ºC and repeated with the addition of artificial sweat. The artificial sweat was prepared according to ISO105-E04-2008E. The friction, or torque (τ), resulting from the angular velocity under constant force at different speeds was measured for every condition tested.

### *In vivo study*

The studies were performed according to the rules of Good Clinical Practices and under the standardized procedures of PhD Trials, a company certified with ISO 9001 Quality System. The protocols and test conditions were reviewed and approved by the Ethical Commission of PhD Trials (Opinion nº 005/2012, 15th June 2012).

Volunteers used the hydrogel sheet-mask underneath the FFP2 mask for 4 hours to test its efficacy. As a control, the measurements were performed only with the FFP2 mask. The measurements of the skin surface hydration were performed using the Corneometer® (Dual-Cutometer MPA 580®), and the facial temperature was recorded with a 14-bit digital infrared camera (FLIR SC660 QWIP, Flir Systems, Danderyd, Sweden).

## Results

### *QbD Approach and Risk Assessment*

The development of the sheet-mask was designed based on the desired goal, which is to alleviate the pressure exerted by the PPE without compromising its protection efficacy. The risk assessment stressed that the amount of solvent and of the ingredients used to produce the sheet-mask are crucial to develop a high-quality product with the expected features. These variables may influence the gelation temperature, the elasticity, adhesivity and the lubricant properties of the final product since the CQAs of the sheet-mask may be compromised if the exact amount is not accessed properly. Table 1 summarizes the QTPP elements to produce an effective sheet-mask, as well as the CQAs classification and the risk analysis outcome.

**Table 1 - QTPP of the hydrogel sheet-mask and risk assessment result.**

| QTPP Element | Target | CQAs | Justification | Variables Which May Influence |
|---|---|---|---|---|
| Cosmetic form | Hydrogel Sheet-Mask | - | Formulation to place between the mask and facial area | - |
| Gelation temperature | 37 – 45 °C | Yes | Maintain integrity at physiological temperature | Ingredients and solvent amount |
| Lubricant properties | 0.005 – 0.05 N.m | Yes | Not too rough nor slippery | |
| Elasticity | Flexible and elastic | Yes | To avoid breakage | |
| Adhesiveness | 0.3 – 0.8 N | Yes | To be fixed on the skin and not cause damage | |

*Design of Experiment Application*

The risk analysis identified the amount of solvent, gelatin, glycerin, PVA, betaine and silica as crucial to produce an effective and efficient sheet-mask. Hence, the dependent variables chosen were the gelation temperature, adhesiveness, elasticity and lubricant properties based on the characteristics the sheet-mask should present to comply with the goal of its use. To optimize the formulation, the effect of each ingredient amount and their interactions on the dependent variables were considered, using MODDE® software. The software output led to the construction of a design space plot with a range of each studied parameter that will not affect the previously defined dependent variables. Figure 1 shows the design space created by the software.

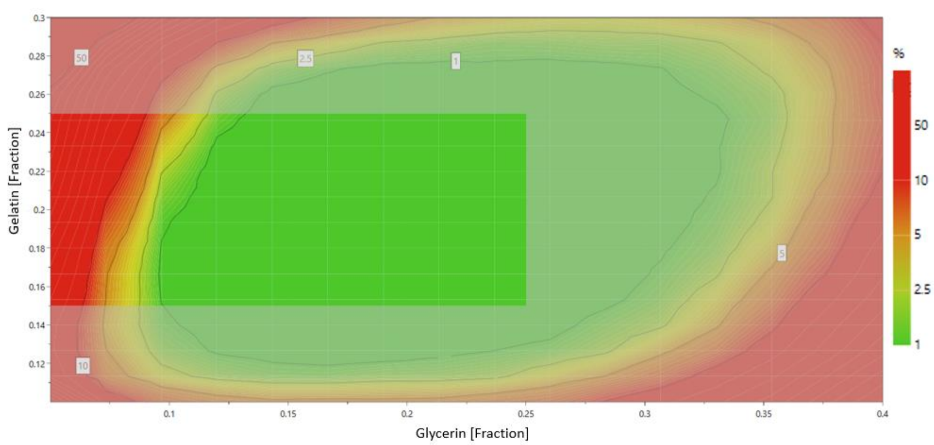


**Figure 1 - Design Space of the hydrogel sheet-mask.**

*In vitro characterization of the hydrogel sheet-mask*

From the proposed values given by the DoE, a final formulation of the hydrogel sheet-mask was prepared. A gelatin film was also prepared and used as a control to evaluate the mechanical properties of the improved hydrogel sheet-mask.

Rheological measurements of the hydrogel sheet-mask were performed, and the adhesiveness and elasticity were determined. The results in Table 2 show that the sheet-mask is approximately 34% less hard and also 50% more adhesive than the gelatin film.

**Table 2- Compression test and respective values of elasticity and adhesiveness measurements (n=3).**

| | Elasticity (mm) | Adhesiveness (N) |
|---|---|---|
| **Hydrogel Sheet-Mask** | 0.81 ± 0.08 | 0.24 ± 0.03 |
| **Gelatin Film** | 0.54 ± 0.06 | 0.11 ± 0.03 |

The use of a PPE mask increases the temperature, pressure and sweat in the facial area. To understand the adaptability of the hydrogel sheet-mask when subjected to these conditions, tribology evaluation was performed. Two temperatures, room temperature and skin temperature (25 ºC and 32 ºC, respectively), and two forces, 0.5 N to simulate surgical masks use and 1.5 N to simulate FFP2 use, were tested. Furthermore, the same test was performed but with the addition of artificial sweat. The tribology results are presented in Figure 2.

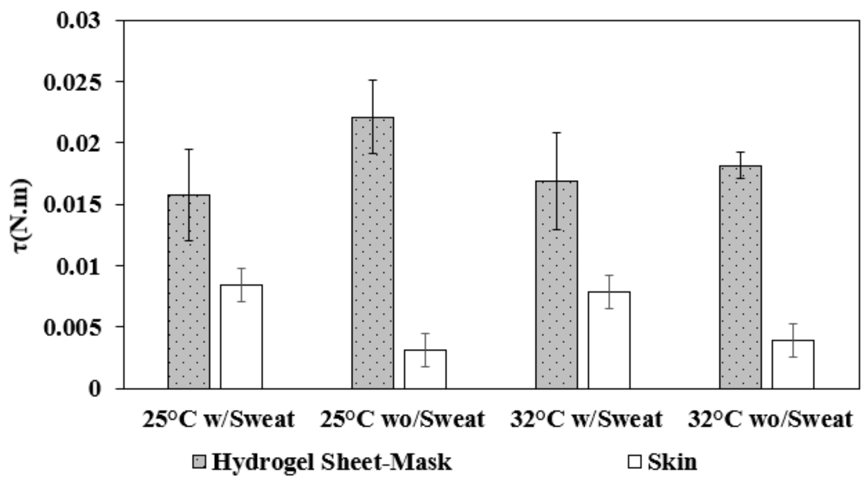


**Figure 2- Friction of hydrogel sheet-mask and skin (n=3).**

The results of the tribological evaluation show that the hydrogel sheet-mask presented similar friction values for both tested temperatures, which were slightly decreased in the presence of sweat. The friction values obtained in the no-sweat conditions were $1.1x10^{-2}±4.3x10^{-4}$ N.m at 0.5N and $2.0x10^{-2}±2.8x10^{-3}$ N.m at 1.5N, which decreased to $1.0x10^{-2}±9.9x10^{-5}$ N.m at 0.5N and $1.6x10^{-2}±8.0x10^{-4}$ N.m at 1.5N, when sweat was added.

### *In vivo Efficiency Evaluation of the Hydrogel Sheet-Mask*

To evaluate the efficacy of the hydrogel sheet-mask, infrared images of the facial area were registered, capturing the temperature distribution without the mask application, after 4 hours of FFP2 mask use, and after 4 hours of FFP2 mask used over the hydrogel sheet-mask. The infrared thermography images (Figure 3) showed an increase of 0.49±0.93 °C and 0.96±0.65 °C after the mask application, in the nose bridge and cheekbone area, respectively. However, when the hydrogel sheet-mask was placed underneath the PPE mask, the temperature increased only 0.22 ± 0.21 °C and 0.56 ± 0.45 °C, respectively (Table 3).

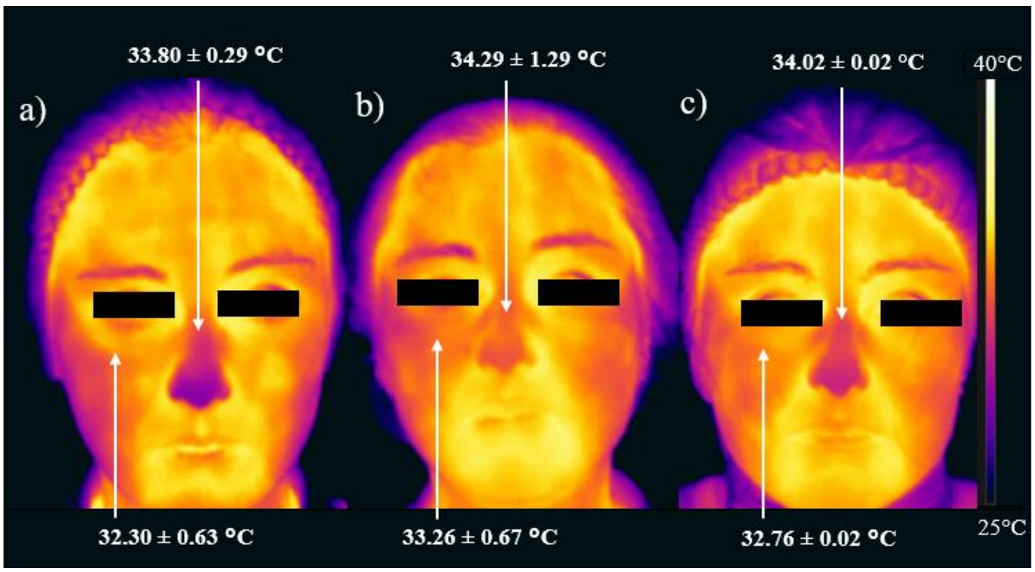


**Figure 3- Infrared images of facial skin temperature distributions associated with the use of a FFP2 mask at baseline (a), after 4 hours PPE mask use (b), and after 4 hours of PPE mask use over the hydrogel sheet-mask (c) (n=2).**

**Table 3- Temperature differences before and after mask application in the nose bridge and cheekbone area, with or without hydrogel sheet-mask use (n=2).**

| | Temperature Differences (°C) | |
|---|---|---|
| **Area** | **After 4 hours of PPE mask use** | **After 4 hours of PPE mask use over the hydrogel sheet-mask** |
| **Nose bridge** | 0.49±0.93 | 0.22±0.21 |
| **Cheekbone** | 0.96±0.65 | 0.56±0.45 |

The hydration of the *stratum corneum* and how it is affected by the hydrogel sheet-mask was also evaluated in the previously described conditions. The results in Figure 4 show that the skin hydration decreased when the PPE mask was used alone, from 43±8 AU to 38±28 AU but increased to 76±11 AU when the hydrogel sheet-mask was used underneath the PPE mask for 4 hours.

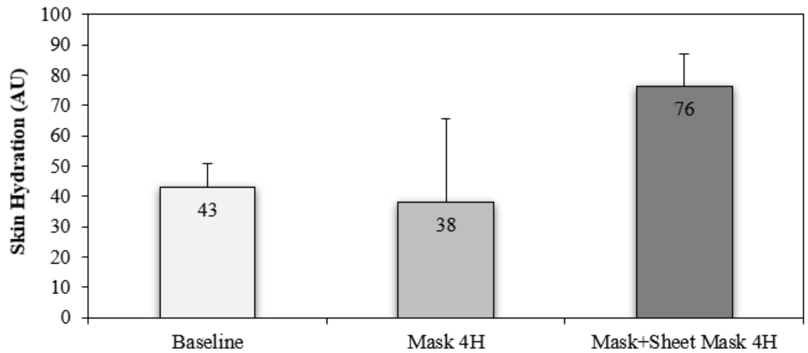


**Figure 4- Skin hydration measurements at baseline, after 4 hours of PPE mask use, and after 4 hours of PPE mask use over the hydrogel sheet-mask (n=2).**

## Discussion

The exact amounts of each ingredient to use in the development of the hydrogel sheet-mask were defined using QbD tools, which emphasized the most important variables that might compromise the final quality of the sheet-mask, and the construction of a design space using DoE. This methodology is a simple and timesaving strategy to guarantee the reproducible production of a hydrogel sheet-mask with the desired characteristics of an efficient cosmetic product.

Since the goal of the hydrogel sheet-mask is to alleviate the shear forces provided by the PPE mask, it is important to test if the product maintains its characteristics throughout the use, i.e., if the sheet-mask resists the pressure forces and keeps its placement, effectively providing a protective barrier. The compression and adhesivity assay results showed an increase of elasticity and also adhesiveness when glycerin, betaine, silica and PVA were added to the gelatin hydrogel, suggesting interactions between the gelatin and the added ingredients. The addition of these ingredients favored the development of a stable, resistant and adherent sheet-mask.

The tribology evaluation evidenced that the developed sheet-mask had high friction values in all tested conditions. At first glance, this result might not seem satisfactory. However, if the goal of the sheet-mask is to diminish the pressure exerted on the skin by the PPE mask, then the sheet-mask needs to absorb the shear loading and distribute the shear stress over an extended area to properly function as a protective sheet [11]. To do so, the mask must be kept fixed on the area where it is placed, and friction plays an important role in providing such placement.

To understand the hydrogel performance, facial temperature and skin hydration were determined *in vivo* when the PPE mask was used by itself or over the sheet-mask. The images captured with the infrared camera showed a slight decrease of facial temperature when the sheet-mask was used underneath the PPE mask, compared to when the PPE mask was used alone. These results suggest that the use of the hydrogel mask causes a pressure relief through pressure redistribution, since pressure and temperature are linearly related according to Gay-Lussac's law. Regarding skin hydration results, the increase in hydration on the *stratum corneum* suggests that the skin barrier integrity is maintained resulting in an improvement of the epidermal barrier function [12]. Taken together, these *in vivo* results showed that the incorporation of the hydrogel sheet-mask significantly improved skin health by redistributing and reducing the pressure exerted by the PPE mask, while also maintaining the integrity of the *stratum corneum*.

## Conclusion

The DoE and QbD tools used in this study were useful to optimize the formula and masks manufacturing process, making it easier, more economical, and more reproducible. The characteristics of the developed and optimized semi-solid hydrogel sheet-mask contributes for its efficiency as a skincare mask that reenforces the skin barrier, preventing lesions and promoting a healthier skin during pandemic times.


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**Conflict of Interest Statement**

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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