

Article

Nanoemulsion Gel Formulation Optimization for Burn Wounds: Analysis of Rheological and Sensory Properties

Małgorzata Miastkowska , Agnieszka Kulawik-Pióro  and Mariola Szczurek

Institute of Organic Chemistry and Technology, Department of Chemical Engineering and Technology, Cracow University of Technology, Warszawska 24, 31-155 Cracow, Poland; agnieszka.kulawik-pioro@pk.edu.pl (A.K.-P.); mariolakot94@gmail.com (M.S.)

* Correspondence: malgorzata.miastkowska@pk.edu.pl; Tel.: +48-126-283-072

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Abstract: Background: Despite the variety of treatment methods for wounds and scars after burns, there are still few effective preparations that can be used in a non-invasive therapy. Recent years have seen significant development of nanomedicine and nanotechnology in the treatment of infection in burn wounds. Proposal: The aim of this work was to develop a formula of a nanoemulsion gel for skin regeneration after burns, and to compare its rheological and sensory properties, as well as the effectiveness of post-burn skin regeneration with preparations available on the market. Methods: At the first stage of studies the composition and parameters of the preparation of sea buckthorn oil-based O/W (oil-in-water) nanoemulsion containing hyaluronic acid and aloe vera gel, as the active ingredients were optimized. Then, the nanoemulsion was added to the gel matrix composed of carbomer (1%) and water which resulted in receiving nanoemulgel. The physicochemical parameters of the obtained samples were characterized by means of dynamic light scattering method and scanning electron microscope. Rheological, sensory and influence on skin condition analysis was conducted for selected market products and developed nanoemulgel. Results: Nanoemulsion gel ($d = 211 \pm 1.4$ nm, polydispersity index (PDI) = 0.205 ± 0.01) was characterized by semi-solid, non-sticky consistency, porous structure, low viscosity, good “primary” and “secondary” skin feelings and pleasant sensorical properties. It improves the condition of burned skin by creating a protective layer on the skin and increasing the hydration level. Conclusion: Due to the fact that the obtained nanoemulsion gel combines the advantages of an emulsion and a gel formulation, it can be a promising alternative to medical cosmetics available on the market, as a form of formulation used in skin care after burns.

Keywords: nanoemulgel; burn wound healing; rheological properties; sensory properties

1. Introduction

Burn wound healing is a multi-stage biological process that involves a number of tissue interactions to restore the skin integrity and homeostasis after an injury. This process can be divided into four overlapping stages: homeostasis, inflammation, proliferation, and reconstitution of tissues [1–3]. If the basement membrane is not damaged, the epidermis regenerates slowly and normally without leaving a scar. One of the factors contributing to scar formation is the depth of the wound. If the basement membrane of the epidermis is damaged, together with partial damage of the dermis, non-hypertrophic scars can be formed during healing. If the dermis is deeply damaged, it is necessary to remove dead tissues and use surgical methods, cell therapies and/or tissue engineering procedures. In such cases, healing is a long-term process, which leaves hypertrophic scars. The treatment of a burn wound is usually carried out in three ways: treatment without a dressing, treatment with a dressing, early surgical removal of dead tissues and reconstruction [4].

Despite the variety of treatment methods of wounds and scars after burns, there are still few effective preparations that can be used in a non-invasive therapy. These restrictions are related to cytotoxicity and adverse reactions caused by medicinal substances that are present in preparations. As a result, more recent studies are devoted to alternative propositions from plant origin, which could potentially represent safer therapeutic agents with lower minimal side effects. These plant-derived preparations could be used in support of skin hydration and overall protection as medical cosmetics, the so-called cosmeceuticals, which support skin regeneration [5].

Out of many preparations that are applied topically to the skin after burns, the most common forms available on the cosmetics and pharmaceutical market are classic emulsions (including lotions and balsams), gels, ointments and aerosols.

1.1. Emulsions

Emulsions are widely used as cosmetic and pharmaceutical formulations because of their excellent solubilizing capacities to lipophilic and hydrophilic active ingredients and application acceptability. Emulsions are heterogeneous systems comprising at least two immiscible liquid phases, where one liquid is dispersed as globules (dispersed phase) in the other liquid (continuous phase) [6]. The emulsions available on the market are preparations that both support burn treatment and provide skin care after the burn. The market offers a wide variety of emulsions (o/w and w/o types) at relatively low prices and in various consistencies—liquid (lotions, which include less than 20–30% fatty substances), semi-liquid (balsams, which include more than 20–30% of the oil phase), or semi-solid (creams, in which the share of the internal phase is between 30–70%) [7]. Emulsions are easy to apply and spread well on the skin. They are also characterized by a high rate of absorption and they do not leave an as oily film on the skin as ointments. What makes them a favorable option is the phase system, whose structure resembles a water-lipid coat of the epidermis, effectively transporting active components into the skin. The oil phase components that are included in an emulsion form a protective layer, which shields the epidermis against external factors [7,8].

1.2. Gels

Gels are semisolid formulations, usually made up of two components, a solvent and gelator phase. The three dimensional network gelator molecules physically entraps the solvent phase, resulting in a viscoelastic gel. They have a broad range of applications in food, cosmetics, biotechnology, pharmatechnology, etc. Typically, gels can be distinguished according to the nature of the liquid phase. For example, organogels (oleogels) contain an organic solvent and hydrogels contain water. Recent studies have reported other types of gels for dermal drug application, such as proniosomal gels, emulgels, bigels and aerogels [9].

The gels are characterized by by the semi-solid consistency which allows longer contact time of the preparation with a burn. Due to the content of moisturizing and cooling (e.g., menthol) substances which soothe pain and irritation, they may act as first-line formulations for the treatment of skin burns [10,11].

Hydrogels are a type of gel in which the dispersed phase is water and the gelling agents are polymers. Unlike gels, hydrogels are not absorbed from the surface of the burn, but they leave a semi-transparent layer on the skin to protect it against external factors and microbes. They also have absorption properties and, as a result, they can absorb exudate from wounds and accelerate healing by regenerating the damaged tissues [9].

1.3. Ointments

Ointments are semi-solid preparations and they have the highest percentage of fatty substances in their composition out of all formulations. On the one hand, they inhibit the multiplication and penetration of microbes to the wound by creating an occlusive barrier on the skin. On the other, they have heavy consistency and a long absorption time. If the substrate in which the active substances

are dispersed has been properly selected, ointments have good spreadability and adhesion. Once they are applied, they leave an oily film that creates a barrier to protect and moisturize the skin after injury [12,13].

1.4. Aerosols

Aerosols exist in the form of liquid or solid particles with the size of 0.01–100 μm in diameter, and they are stably suspended in air. The aerosol form of the preparation allows easy and quick application to a large area of the damaged skin and eliminates pain and unpleasant burning sensation, which is particularly important in the case of sunburns. The structure of the dosing packaging prevents the cosmetic mass from being infected with microbes. The foam that is created after the application is not sticky. Aerosols also do not leave a greasy deposit on the skin, as is the case with ointment preparations. Although aerosols allow quick healing of burns, their prolonged use on the damaged skin is inadvisable because of the propellants present in the recipe, as they can cause irritation and sensitization [14,15].

1.5. Nanostructured Systems

In recent years, there has been significant development of nanomedicine and nanotechnology in the treatment of infection in burn wounds. Nanostructured systems are effective media for the active and medicinal substances used to treat bacterial infections and to stimulate wound healing. The nanoformulations used in the treatment of skin burns can be divided into two classes: organic nanostructures (polymer nanoparticles, nanoemulsions, nanogels, liposomes and lipid nanoparticles) and inorganic nanostructures (nanoparticles of gold, copper or silver) [16].

Nanoemulsions are colloidal systems composed of an aqueous phase and an oil phase, stabilized with surfactants and sometimes with the addition of auxiliary surfactants. They are characterized by the very small size of the dispersed phase droplets (20–500 nm), large interphase surface and low interphase and surface tension [17]. Due to these properties, nanoemulsion increases the absorption rate and eliminates variability in the absorption, helps in solubilizing lipophilic drugs, and hence increases their bioavailability. However, the low viscosity of nanoemulsions makes their direct topical application inconvenient and modifies skin permeation profile. These problems can be solved by incorporating a nanodispersion into the polymer solution to form an in situ nanoemulgel. In a nanoemulgel, hydrophobic drugs are loaded in the oil cores and the droplets of emulsion are entrapped in the hydrogel cross-linked network [18,19]. The incorporation of nanoemulsions into hydrogel systems, commonly referred to as nanoemulgels (NEGs), has improved the topical efficacy of various, otherwise poorly permeable, therapeutics [20]. It also solves the problem of hydrophilicity of hydrogels, which limits the applications for the delivery of hydrophobic drugs [18].

The combination of a nanoemulsion and hydrogel provides sustained/controlled drug delivery and easy administration, thus, enhancing patient compliance. A nanoemulgel, as a prolonged release system, allows to extend the duration of the substance concentration in the therapeutic range, while it also has a reduced dose of the active component. The internal phase (nanoemulsion) contains an active substance that is released into the skin by the dispersing phase. This phase is further cross-linked and, as a result, the particles of the penetrating substance are captured and slowly released by the cross-linked structure. This prolongs the skin exposure to the medicine [19]. Moreover, the combination of both of these forms provides an improvement to the rheological and sensory properties of the nanoemulsion, and facilitates its application to the skin. The addition of rheological modifiers makes it possible to prolong the contact time of the preparation with the skin, and to increase the skin hydration level by forming a hydrophilic film on the skin surface and reducing the transepidermal water loss. This is a very important aspect of skin regeneration. A great advantage of a nanoemulgel is the increased content of hydrophilic ingredients, which distinguishes it from creams and ointments. Usually, this physicochemical form of a drug delivery system also does not show stability problems such

as phase inversion (emulsions) or rancidity (ointments). In addition, a nanoemulgel formula protects active ingredients from degradation, due to external factors such as light or temperature [16,21–23].

The widespread use of a given physicochemical form of a preparation is closely related to its effectiveness, market availability, price, as well as a number of characteristics, which result from the rheological and sensory properties of a product. The most important ones include simple application, consistency, spreadability, absorption rate and sensation on the skin after the application.

The aim of this work was to develop the formula of a nanoemulgel for skin regeneration after burn and to compare its rheological and sensory properties, as well as the effectiveness of post-burn skin regeneration with other physicochemical forms of preparations (emulsions, ointments, gels) available on the market.

2. Results

2.1. Characterization of the Obtained Nanoemulsions

In the first stage of the study, a number of parameters were investigated with regard to their impact on the stability of the basic emulsions. These parameters were: emulsifier concentration (4% w/w or 6% w/w), oil phase concentration (3% w/w, 5% w/w, or 10% w/w), stirrer speed (300 rpm or 500 rpm), and emulsification time (10 min or 15 min) during pre-emulsification and during ultrasonication (60 s or 120 s). The oil phase of the obtained oil-in water type (O/W) nanoemulsions was sea buckthorn oil, while decyl glucoside acted as an emulsifier. The quantitative and qualitative composition of the basic nanoemulsions and the process parameters are shown in Table 1.

Table 1. The composition and the process parameters of the basic nanoemulsions preparation.

Phase	Raw Material Name	Composition (%mass)													
		N1	N2	N3	N4	N5	N6	N7	N8	N9	N10	N11	N12	N13	N14
A	Decyl glucoside	4.0	6.0	4.0	4.0	4.0	4.0	4.0	4.0	6.0	6.0	6.0	6.0	4.0	6.0
	Water	86.0	84.0	91.0	86.0	91.0	91.0	91.0	91.0	89.0	89.0	89.0	89.0	93.0	91.0
B	Sea-buckthorn berry oil	10.0	10.0	5.0	10.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	3.0	3.0
Process parameters															
Mixing speed (rpm)		300	300	300	300	300	300	500	500	300	300	500	500	500	500
Pre-emulsification time (min)		10	10	10	15	15	10	10	10	10	10	10	10	10	10
Ultrasonic homogenization time (s)		60	60	60	60	60	120	60	120	60	120	60	120	120	120
Stability															
- creaming or phase separation															
+/- stable after 7 days		-	-	-	-	-	-	-	+/-	-	-	-	-	+/-	+
+ stable after 14 days															

As can be seen from the data in Table 1, a stable basic nanoemulsion was obtained by increasing the concentration of the emulsifier to 6%, reducing the concentration of the oil phase to 3% and extending ultrasonication time to 120 s. According to the literature [24–26], in most cases, the extended ultrasonication time, increased concentration of the emulsifier and reduced concentration of the oil phase have a positive effect on obtaining stable formulations. However, it should be noted that each formulation has to have its own optimization of parameters obtained, and should be selected according to a given qualitative and quantitative composition of the formulation. It should be also noted that if the concentration of a surfactant is too high, it may result in a lower diffusion rate of surfactants and may cause the coalescence of emulsion droplets [27,28]. Additionally, if sonication time is increased, it may result in monomodal size distribution, but the input of energy should be at

the optimum level. The researchers explain this phenomenon by the fact that an increase in the energy density causes greater droplet deformation and the disruption of the interfacial layer of the stabilized emulsifier [25,29–31].

Moisturizers are one of the most important classes of cosmetic products, because they prevent xerosis, delay premature ageing and help in dermatological therapies of a wide variety of skin disorders [32]. According to the state of the art, the next stage was an attempt to add the following moisturizing substances to the basic nanoemulsion: hyaluronic acid, allantoin, aloe vera gel and D-panthenol. The recipe of the nanoemulsion was also enriched with a preservative (sodium benzoate) and antioxidant (vitamin E). In the final stage of the recipe optimization, the nanoemulsion was thickened with an aqueous carbomer solution to obtain the desired consistency. The acid-base reaction was brought to pH = 6 with a citric acid solution.

In the case of each formulation, the emulsification parameters were used as established in the previous stage: pre-emulsification time was 10 min, pre-emulsion stirring speed was 500 rpm, ultrasonic homogenization was 120 s. The qualitative and quantitative composition of the emulsions enriched with other ingredients is shown in Table 2.

Table 2. The qualitative and quantitative composition of the emulsions enriched with other ingredients.

Phase	Raw Material Name	Sample Name								
		N14-A	N14-B	N14-C	N14-D	N14-E	N14-F	N14-G	NG-Carb-0.5	NG-Carb-1.0
A	Decyl glucoside	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0	6.0
	Water	79.0	83.8	90.0	90.0	90.0	90.0	88.8	86.8	86.3
	Hyaluronic acid 1%	5.0	-	1.0	-	-	-	1.0	1.0	1.0
	Allantoin	1.0	1.0	-	1.0	-	-	-	-	-
	Aloe vera gel	1.0	1.0	-	-	1.0	-	1.0	1.0	1.0
	D-panthenol 75%	4.0	4.0	-	-	-	1.0	-	-	-
B	Sodium benzoate	0.1	0.1	-	-	-	-	0.1	0.1	0.1
	Vitamin E	0.1	0.1	-	-	-	-	0.1	0.1	0.1
	Sea-buckthorn berry oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
C	Carbomer	-	-	-	-	-	-	-	0.5	1.0
	5% r-r citric acid	-	-	-	-	-	-	-	0.5	0.5
	Stability	-	-	+	-	+	-	+	+	+

The data in Table 2 show that stable nanoemulsions were obtained by adding hyaluronic acid (N14-C), aloe vera gel (N14-E) to the aqueous phase, and by combining these moisturizing ingredients (N14-G). The addition of moisturizing agents, antioxidant and a preservative did not influence the emulsified particle size, polydispersity index (PDI) and stability in time (Table 3).

Table 3. Particle size and polydispersity index (PDI) of stable formulations.

Nanoemulsion Name	Storage Time (Days)	Particle Diameter (nm)	PDI
N14	1	214.0 ± 0.7	0.214 ± 0.01
	14	217.2 ± 1.1	0.197 ± 0.20
N14-C	1	213.5 ± 1.6	0.208 ± 0.02
	14	236.5 ± 2.3	0.173 ± 0.01
N14-E	1	202.2 ± 2.6	0.212 ± 0.02
	14	228.9 ± 1.5	0.185 ± 0.02
N14-G	1	211.5 ± 1.4	0.205 ± 0.01
	14	227.4 ± 1.8	0.288 ± 0.01

2.2. Nanoemulgel Characteristics

The desired nanoemulgel consistency was obtained by adding a carbomer to its recipe at a concentration of 1% (Table 2). As the result of the study, a stable nanoemulgel containing sea buckthorn berry oil, hyaluronic acid, and aloe vera gel as the active ingredients was obtained. It was characterized by pH = 6.0. The slightly acidic environment of the formulation applied to skin burn is advisable, because it reduces the skin susceptibility to microbes and, thus, accelerates the regeneration of the damaged tissue [33].

The addition of a carbomer to the N14-G formulation caused the change of the nanoemulsion from liquid to semi-solid consistency (NG-Carb-1.0). The scanning electron microscope (SEM) images of the nanoemulgel (Figure 1) show interconnected pores with random size distribution. This porous structure may provide sufficient space for high drug loading, movement of drug throughout and enhance the drug release rate [18].

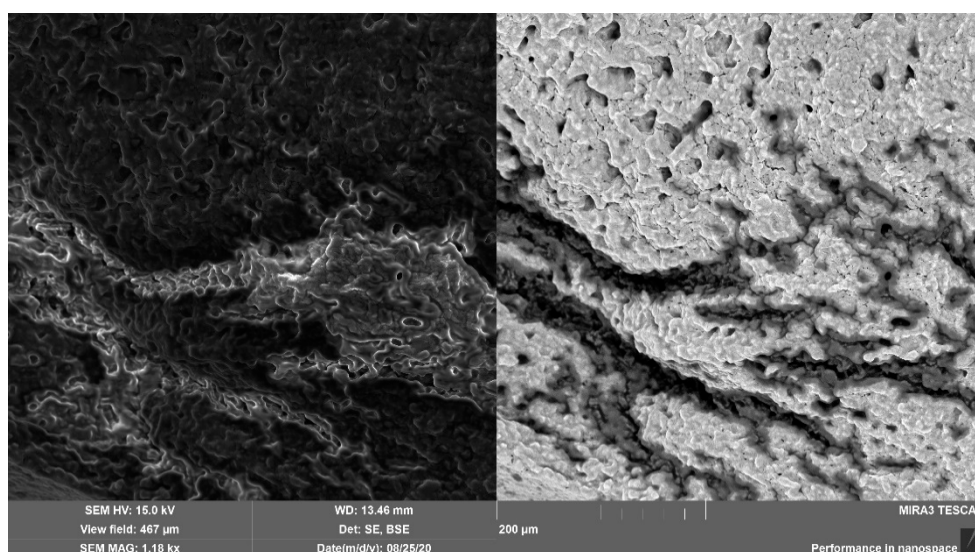


Figure 1. Scanning electron microscope (SEM) images of the obtained nanoemulgel NG-Carb-1.0.

2.3. Rheological Properties of the Preparations Supporting Burn Healing

Skin care after burns is often a long and complicated treatment process, which depends on the condition of the epidermis basement membrane, and which is affected by several aspects. One of them is the application of multi-component preparations with a wide spectrum of activity.

In order to be effective, the skin-applied preparations which support the healing process after burns have a semi-solid consistency and plastic properties at human body temperature to allow easy spreading and adequate skin adhesion. The longer the preparation remains on the injured skin, the more the burned area is protected against external factors and the preparation itself can form a protective layer. These characteristics can be established by determining the apparent viscosity and the yield stress of the sample.

The products with an exceedingly high yield stress show heavy consistency and difficult spreadability on the skin. These may cause irritation and lower the regularity and frequency of the product use, which is necessary for burn wound healing, and, as a result, decrease the therapeutic and/or care effect. Such preparations are also less efficient, which is also important for consumers in the case of long treatment [34–36].

In practice, the inadequate apparent viscosity of the preparation means shorter contact time between the preparation and the skin, more difficult spreading, and, in certain cases, an occlusive layer will not be created as a result [34,35,37,38].

By taking the physicochemical form as a criterion, the studied market preparations which support the regeneration of burned skin (Table 4) were divided into three groups: emulsions, ointments and gels.

Table 4. Characteristics of the tested market preparations.

Item	Preparation Name	Physicochemical Form	Scope of Application	Composition (acc. to the Information Provided by the Manufacturer)
1	A	balsam	1st degree burns (sunburns)	Aqua, Panthenol, Cyclopentasiloxane, Cetearyl Ethylhexanoate, Glycerin, Aloe Barbadensis Leaf Juice Powder, Macadamia Ternifolia Seed Oil, Aleurites Moluccana Seed Oil, Sodium Hyaluronate, Tocopherol, Tocopheryl Acetate, Sodium Polyacrylate, Dimethicone, PEG-40 Hydrogenated Castor Oil, Polyacrylamide, C13-14 Isoparaffin/Laureth-7, Acrylates/C10-30 Alkyl Acrylate Crosspolymer, Triethanolamine, Phenoxyethanol, Ethylhexylglycerin, DMDM Hydantoin, Parfum, Butylphenyl Methylpropional, Citronellol, Hexyl Cinnamal, Limonene, Linalool
2	B	balsam	1st degree burns (sunburns)	Aqua, Ethylhexyl Stearate, Glycerin, Caprylic/Capric Triglyceride, Polyglyceryl-3 Methylglucose Distearate, Prunus Amygdalus Dulcis Oil, Gossypium Herbaceum Seed Oil, Cetearyl Alcohol, Dimethicone, Poliacrylamide, C13-14 Isoparaffin, Laureth-7, Panthenol, Parfum, Allantoin, Tocopheryl Acetate, Soluble Collagen, Glycogen, Hydrolysed Elastin, Sorbic Acid, Sodium Succinate, Bisabolol, Disodium EDTA, Citric Acid, Phenoxyethanol, DMDM Hydantoin, Methylparaben, Propylparaben, Coumarin, Eugenol
3	C	hydrogel	1st and 2nd degree burns	Carbomer, Octylene Glycol, Glycerin, Polyethylene Glycol, Sodium Hydroxide, Aqua
4	D	hydrogel	1st and 2nd degree burns (sunburns and thermal burns)	Aqua, Glycerin, Propylene Glycol, Ethanol, Triethanolamine, Carbomer, 1-Methylnicotinamide Chloride, Methylparaben, Eucalyptus Globulus Leaf Oil
5	E	cream	Scars after burns and surgery	Active substances: Allium Cepa Extract, Chamomilla Recutita Extract, Sodium Heparin, Allantoin Excipients: Cetearyl Alcohol (and) Sodium Lauryl Sulfate, Paraffinum Liquidum, Petrolatum, Mono- and Diglycerides (and) Potassium Stearate, Glycerin, Methyl Parahydroxybenzoate, Propyl Parahydroxybenzoate, Aqua
6	F	cream	1st and 2nd degree burns (sunburns, thermal burns, after radiation therapy)	Aqua, Olea Europaea Fruit Oil, Sorbitan Olivatate, Cetearyl Olivatate, Glycerin, Panthenol 75%, Isostearyl Neopentanoate, Myreth-3 Myristate, Scutellaria Baicalensis Root Extract, Sorbeth-30, Squalane, Silybum Marianum Extract, Carbomer, Sodium Hydroxymethylglycinate 50%, Menthol, Sodium Hyaluronate

Table 4. Cont.

Item	Preparation Name	Physicochemical Form	Scope of Application	Composition (acc. to the Information Provided by the Manufacturer)
7	G	cream	Skin irritated after sunbathing, acceleration of wound healing, stimulation of regeneration and restoration of the epidermis	Aqua, Glycine Soja (Soybean) Oil, Simmondsia Chinensis (Jojoba) Seed Oil, Cera Alba, Vitis Vinifera (Grape) Seed Oil, Hippophae Rhamnoides (Seabuckthorn) Berry Oil, Betulin, Sodium Stearate, Citric Acid
8	H	ointment	1st degree burns (sunburns, after radiation and photo therapy)	Active substances: Allantoin, D-Panthenol Excipients: Lanolin, Paraffinum Liquidum, Petrolatum, Ethyl Parahydroxybenzoate, Aqua
9	I	ointment	1st degree burns (thermal)	Petrolatum, Lanolin, Linum Usitatissimum Oil, Propolis, Parfum, Linalool, Citronellol, Limonene, Coumarin, Geraniol
10	J	ointment	1st degree burns Skin sanitizing after burn	Zinci Oxydum, Tormetillae Extractum Fluidum, Ammonii Bituminosulfonas, Natrii Tetraboras, Vaselinum Flavum, Lanolinum, Aqua, Vanillinum
11	K	lotion	1st degree burns (sunburns)	Aqua, Hexyl Laurate, PPG-15 Stearyl Ether, Butyrospermum Parkii (Shea Butter), Glycerin, Steareth-2, Steareth-21, Dimethicone, Panthenol, Menthyl Lactate, Tocopheryl Acetate, Calcium Gluconate, Sodium Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Isohexadecane, Polysorbate 80, Cetearyl Alcohol, Carbomer, Phenoxyethanol, Methylparaben, Ethylparaben, Propylene Glycol, Diazolidinyl Urea, Parfum (Fragrance), Benzyl Salicylate, Butylphenyl Methylpropional, Citronellol, Limonene, Linalool, Benzyl Benzoate, Geraniol, Benzyl Alcohol, Sodium Hydroxide
12	L	lotion	Skin irritated after sunbathing, epidermis regeneration	Water, Paraffinum Liquidum, Dimethicone, Propylene Glycol, Cetyl Alcohol, Cetearyl Alcohol, Cetearyl Ethylhexanoate, Isopropyl Myristate, PEG-8 Distearate, Glyceryl Stearate, PEG-100 Stearate, Parfum, Caprylyl Glycol, Hydrolyzed Collagen, Allantoin, Imidazolidinyl Urea, Triethanolamine, Carbomer, Glycerin, Tetrasodium EDTA, Niacinamide, Carrageenan (Chondrus Crispus), Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer, Phenoxyethanol, Xanthan Gum, Polyisobutene, Helix Aspersa, Ethylhexylglycerin, Avena Sativa Kernel Extract, PEG-7 Trimethylolpropane Coconut Ether, Glucose
13	M	gel	1st degree burns (sunburns, thermal burns)	Aqua (modified solution of medicinal water: bicarbonate-chloride-sodium-bromide-iodide-boron from Uzdrowisko Rabka S.A.), Aloe Vera (Aloe Barbadiensis) Extract, Propylene Glycol, Glycerin, Symphytum Officinale Extract, Panthenol, Triethanolamine, Allantoin, Carbomer, DMDM Hydantoin
14	N	gel	1st degree burns (sunburns)	Aqua, Panthenol, PEG-40 Hydrogenated Castor Oil, Phenoxyethanol, Methylparaben, Ethylparaben, Propylene Glycol, Allantoin, Retinyl Palmitate, Calcium Gluconate, Carbomer, 2-Bromo-2-Nitropropane-1,3-Diol, Parfum (Fragrance), Sodium Hydroxide

Due to the fact that the obtained nanoemulgel combines the characteristics of an emulsion and a gel, the flow curves for NG-Carb-1.0 are included for both these groups for comparative purposes.

The results of the flow curve analyses (Figures 2–4) show that the tested preparations were characterized by a complex rheological behavior. They belonged to the group of non-Newtonian liquids with a clear yield stress.

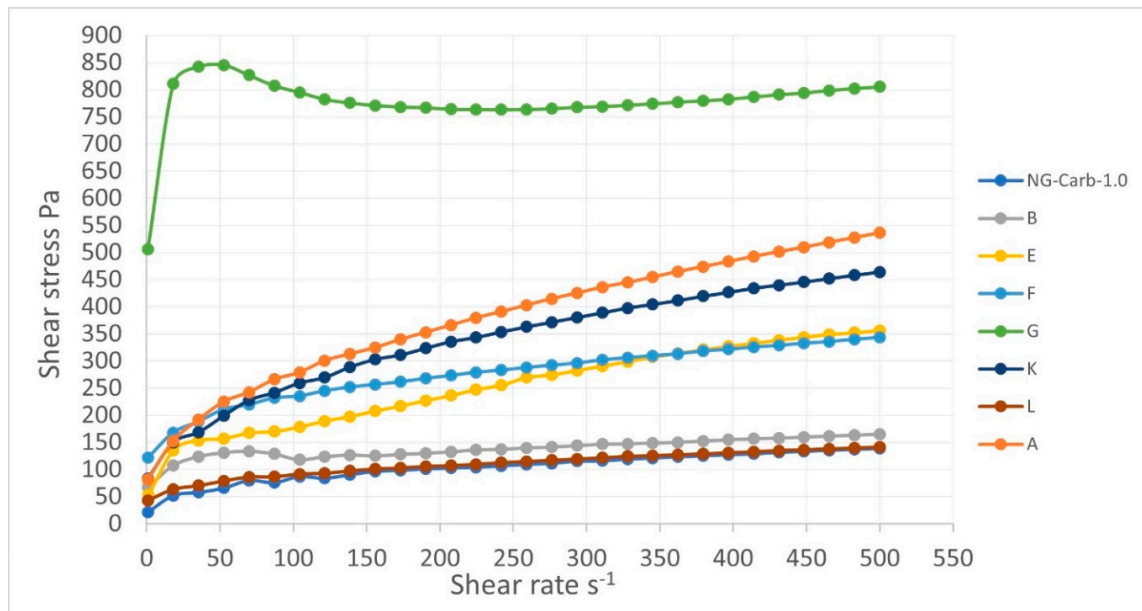


Figure 2. Flow curves of samples belonging to a physicochemical form group—emulsions.

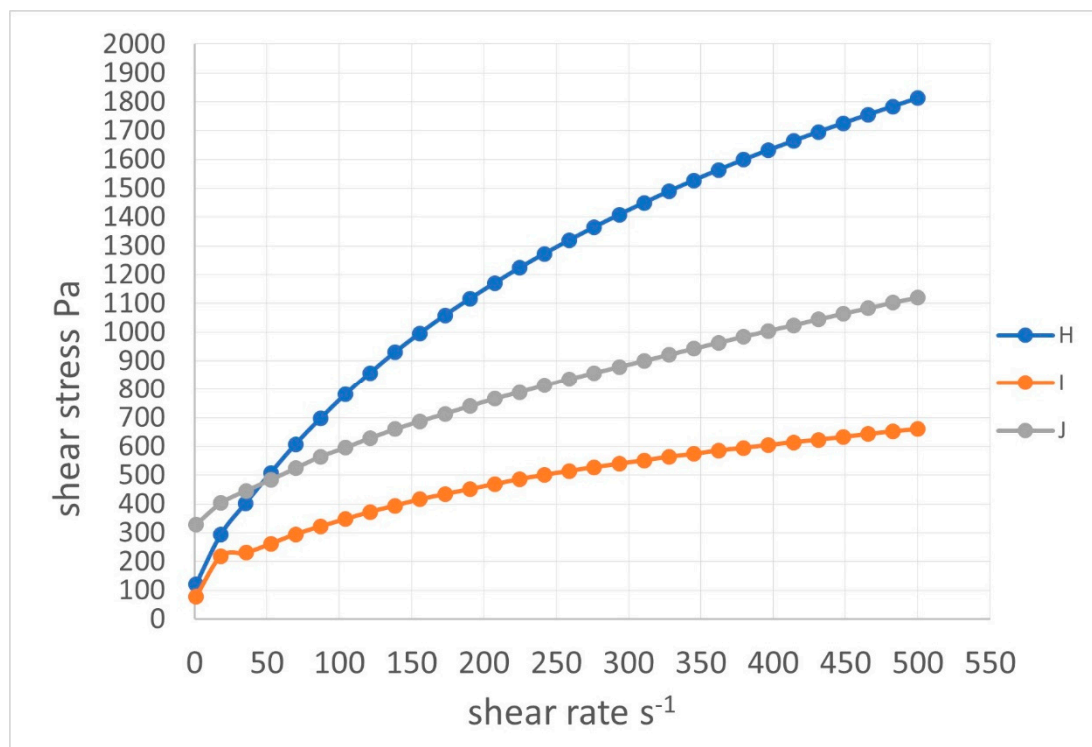


Figure 3. Flow curves of samples belonging to a physicochemical form group—ointments.

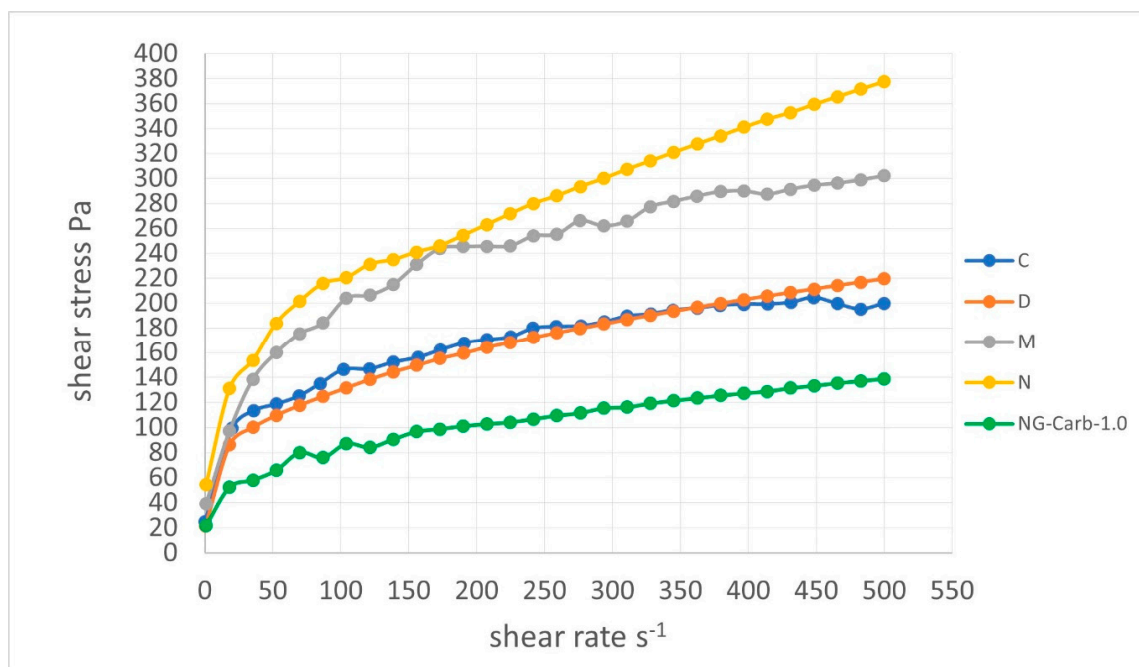


Figure 4. Flow curves of samples belonging to a physicochemical form group—gels.

The application of cosmetic and medicinal preparations on the skin is accompanied by physical operations that are closely related to a specific shear rate: draining under gravity for lotions on hands (shear rate ranges between $0.01\text{--}10\text{ s}^{-1}$), cream spooning and pouring (shear rate ranges between $10\text{--}100\text{ s}^{-1}$), and cream rubbing (shear rate around 1000 s^{-1}) [35,39–41]. The numerical values of apparent viscosity of the tested preparations at $1, 10, 100, 500\text{ s}^{-1}$ shear rates, at temperatures of $25\text{ }^{\circ}\text{C}$ and $32\text{ }^{\circ}\text{C}$, and yield stress and dissipation energy are shown in Table 5.

Table 5. Rheological characteristics of the tested preparations.

Sample	Physicochemical Form	Apparent Viscosity, Pas at $\dot{\gamma} = 1\text{--}500\text{ s}^{-1}$				Yield Stress τ_0, Pa
		1.0 s^{-1}	10.0 s^{-1}	100.0 s^{-1}	500 s^{-1}	
A	balsam	81.86	8.385	2.670	1.079	81.80
B	balsam	68.45	5.910	1.330	0.335	67.47
C	hydrogel	30.45	4.810	1.445	0.390	24.94
D	hydrogel	55.58	4.740	1.263	0.439	21.42
E	cream	52.89	7.425	1.710	0.712	52.77
F	cream	123.4	9.215	2.255	0.686	121.6
G	cream	530.5	44.40	7.670	1.610	505.8
H	ointment	123.6	16.10	7.491	3.623	119.7
I	ointment	78.97	11.94	3.330	1.319	77.14
J	ointment	229.7	14.72	3.805	1.494	327.8
K	lotion	84.39	8.230	2.490	0.928	83.20
L	lotion	43.71	3.495	0.880	0.282	43.18
M	gel	40.16	5.330	2.102	0.604	39.28
N	gel	55.93	7.197	2.110	0.757	54.32
NG-Carb-1.0	nanoemulgel	22.43	2.863	0.836	0.277	21.75

In the course of the study, Herschel–Bulkley (1) model parameters were determined ($R^2 > 0.990$). This equation is used to describe the flow curves of non-Newton liquids that are shear-thinned with a yield stress. The test results are presented in Table 6.

$$\tau = \tau_y + k\dot{\gamma}^n \quad (1)$$

where:

τ —shear stress, Pa

τ_y —yield stress, Pa

k —consistency parameter, Pa·sⁿ

$\dot{\gamma}$ —shear rate, s^{−1}

n —flow behavior index, -.

Table 6. Rheological parameters of a Herschel–Bulkley model for test samples.

Name of the Sample	Physicochemical Form	τ_y (Pa)	k (Pa·s ⁿ)	n (-)	R^2
A	balsam	61.860	19.796	0.506	0.999
B	balsam	4.936	35.240	0.236	0.991
C	hydrogel	12.650	16.456	0.391	0.999
D	hydrogel	4.942	23.328	0.323	0.997
E	cream	53.839	8.723	0.5573	0.993
F	cream	61.931	40.001	0.306	0.994
G	cream	513.4	35.12	0.34	0.990
H	ointment	20.258	51.872	0.570	0.998
I	ointment	19.973	62.201	0.380	0.998
J	ointment	304.048	17.493	0.629	0.999
K	lotion	60.798	18.658	0.491	0.999
L	lotion	40.510	9.232	0.389	0.996
M	gel	85.435	4.211	0.605	0.993
N	gel	41.108	20.170	0.445	0.998
NG-Carb-1.0	nanoemulgel	14.938	7.371	0.436	0.997

2.4. Sensory Analysis of the Preparations Supporting Burned Skin Regeneration

2.4.1. The Correlation of Feelings on the Skin after the Application of Preparations and Rheological Measurements

A decrease in apparent viscosity together with an increase in shear rate was observed for all tested preparations. Different apparent viscosity for certain shear rates of the tested preparations determines their different skin application, sensory properties, and, as a result, healing effect.

In the literature [34–36], it was shown that the product flow properties, established by rheological measurements, may be correlated with the empirically subjective assessment of skin feeling.

“Primary” and “secondary” skin feelings are distinguished for the products applied topically to the skin. The primary skin feeling refers to the sensation that occurs when the preparation is taken out of its container, applied and spread on the skin. This feeling is closely related to the yield stress and apparent viscosity in the lower ranges of the shear rate. The secondary feeling refers to the moment when the applied layer of the care/medicinal product covers the skin evenly. Secondary feeling is the absorption capacity perceptible on the skin and how the product feels on the skin after the application. Absorption capacity perceptible on the skin increases as viscosity decreases. This is related to the apparent viscosity determined, in this case, for the upper range of shear rate ($\dot{\gamma} = 500 \text{ s}^{-1}$) and allows an assessment of the final degree of spreading the test sample on the skin.

The study method proposed by Brummer [34] was used to determine the primary skin feeling caused by the studied preparations supporting skin healing after burns.

The correlation of the assessments by sensory testing panels with the values measured for the flow onset and maximum viscosity gave the initial “window of measured values”, shown as rectangles in Figure 5.

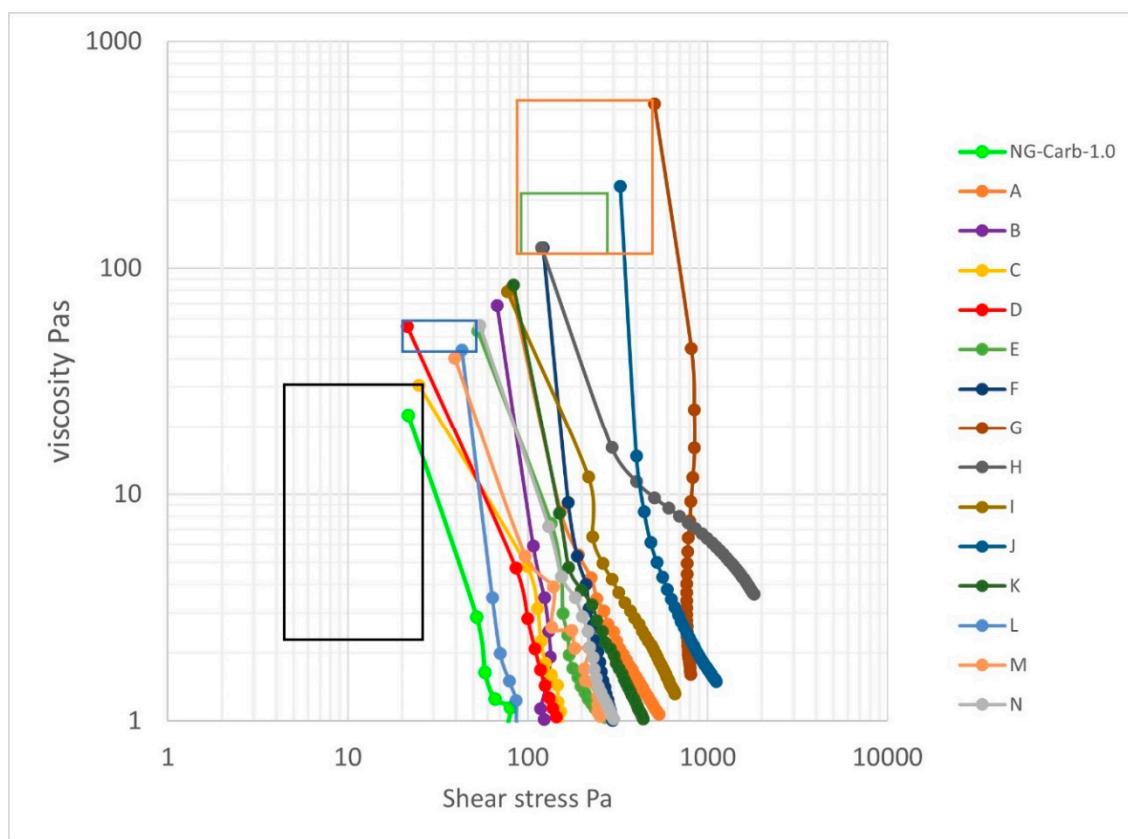


Figure 5. Viscosity curves of samples A–N and NG-Carb-1.0 plotted on the Brummer et al. window for the evaluation of good primary skin feeling. Ointments—green rectangle; emulsions—orange rectangle; gels—blue rectangle; nanoemulsion—black rectangle.

The window (rectangle) boundaries are determined on the basis of the preparation with a given physicochemical form with the lowest sensory assessment. The values measured with this method for the shear stress and viscosity at flow onset provide the upper and lower limits for the respective measured variable. The limits include the values measured for the preparation assessed as good. Products falling within these rheology boundaries were assessed as good when they were first spread on the skin, whereas products with less acceptable skin feeling fell outside of these parameters [34–36].

In the literature, the window boundaries were determined only for the o/w emulsion [34,36] and for petroleum jelly samples with Fischer–Tropsch wax [36]. In our study, we defined them for the following type systems: ointment, gels and gel nanoemulsions. In the case of the nanoemulsion, the results of our previous studies [42] were used. These boundaries are, respectively: ointments 120–320 Pa, 120–230 Pas; emulsions: 120–505 Pa, 120–530 Pas; gels: 20–55 Pa, 40–55 Pas; nanoemulsions 3.5–23 Pa, 2.3–26 Pas. The window boundaries for the ointment were within the window boundaries for the emulsion. Our study showed that the boundaries of good sensory assessments depended on the physicochemical form and consistency of the tested preparations. This is in line with the results published by Beeker et al. [36]. The highest are for emulsions and ointments, and the lowest for nanoemulsions. Viscosity values are 4.6 times lower than for emulsions and ointments, while for gels, it is a factor of 1.5 times.

The data in Figure 5 show that, in the case of emulsions, the products G, F are within the boundary window for good primary skin feeling; for ointment: H, for gels: D, M. The obtained nanoemulsion was also within the boundary window for nanoemulsion products. The viscosity value, which characterizes the secondary skin feeling, occurs only at a very high shear rate, at which the apparent viscosity of the preparation does not change. The products, such as nanoemulsions, gel D, M, with lowest viscosity gave the best secondary feeling on the skin.

2.4.2. Sensory Assessment Profile of Selected Preparations

The obtained gel nanoemulsion combines the advantages of emulsions and gels. Therefore, the products of both these physicochemical forms, i.e., emulsions (preparation G) and gels (preparation M), were chosen for a detailed sensory analysis and assessment of the influence of the preparations on burned skin condition. The chosen preparations (G and M), as well as the nanoemulgel, also had the best primary feeling on the skin. In the case of the preparation M and nanoemulgel, they also had the best secondary skin feeling.

Eleven characteristics of the preparations were evaluated in the sensory assessment. The assessed parameters were color, fragrance, consistency, uniformity, spreadability on the skin, adhesion, smoothing, pillow effect, stickiness, oiliness and absorption. The sensory assessment profiles obtained are shown in Figure 6.

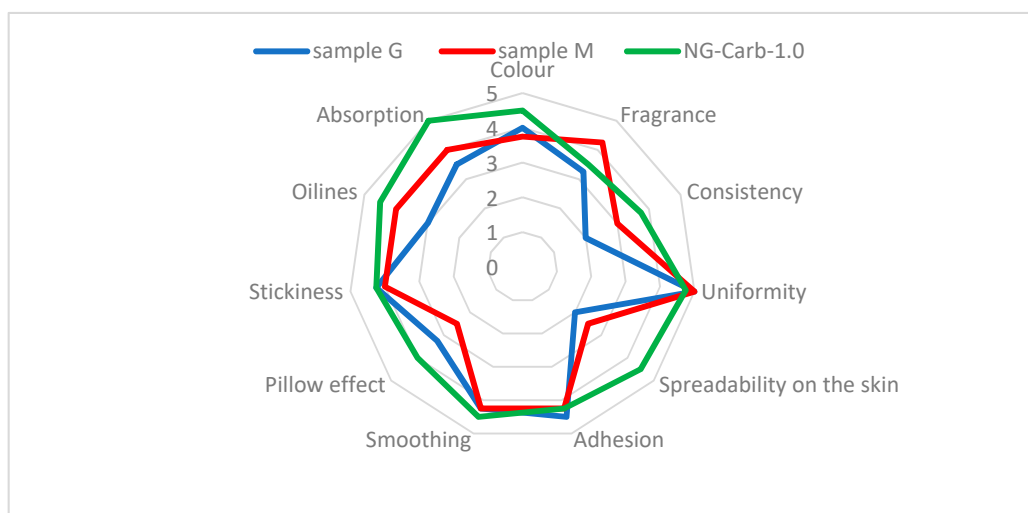


Figure 6. Results of the organoleptic evaluation of sample G, M and nanoemulgel (NG-Carb-1.0).

A large variation in the assessments of individual preparations was seen in the following assessed characteristics: consistency (difference of 1.75 points), spreadability (difference of 2.5 points), absorption (difference of 1.5 points) and oiliness (difference of 1.5 points). According to the assessors, the preparations differed least among themselves (difference of 0.25 points) in uniformity, adhesion, smoothing and stickiness.

The obtained results for the sensory analysis of the preparations are consistent with their rheological properties.

The obtained nanoemulgel has the lowest values for the yield stress and apparent viscosity, and, out of the assessed preparations, it received the best evaluation in: color (4.5), consistency (3.75), spreadability (4.5), smoothing (4.5), pillow effect (4.0), stickiness (4.25), oiliness (4.5), absorption (5). It received equally high values for uniformity (4.75) and adhesion (4.25). The lowest evaluated nanoemulgel property was fragrance (3.5), which probably was due to the absence of fragrance substances in the composition. According to the assessors, the nanoemulgel as a skin burn preparation was characterized with light consistency, which was quickly absorbed, and did not leave the feeling of oiliness and stickiness after application. The gel form allows good spreadability and skin adhesion of the product.

According to the assessors, the preparation G, which has the highest yield stress and viscosity, had the worst spreadability. It also had heavy consistency and medium oiliness. As a result of the combination of these properties, the preparation showed medium absorption, as it left a medium-oily film on the skin after the application. Nevertheless, the assessors noted its good adhesion, smoothing and low stickiness.

The preparation M obtained intermediate results between the cream G and the nanoemulgel. In comparison with the preparation G, it had better absorption as well as heavier consistency and oiliness. Even though the yield stress for the preparation M was lower than for the preparation G, the assessors noted that it was difficult to spread, which might be the result of its thin consistency.

For the validity level of $p < 0.1$, the statistically significant differences were related only to the spreadability property. The variance analysis results are shown in Table 7.

Table 7. Summary statistics from one-way analysis of variance (ANOVA).

Groups	N	Sum	Average	Variance	Post-Hoc
Preparation G	4	8	2	2	b
Preparation M	4	10	2,5	3	ab
NG-Carb-1.0	4	18	4,5	1	a

The cream G was the preparation with the lowest average, the gel nanoemulsion was the preparation with the highest average. There are significant differences between these preparations. However, such relationships are not found for the preparation M and nanoemulgel, as well as between the preparation M and preparation G.

2.5. Effect of the Gel Nanoemulsion and Market Preparations on the Skin after Burns

Skin wound healing is a systematic process, traditionally including four overlapping classic phases: hemostasis, inflammation, proliferation and maturation. Several factors influence skin healing after burn injuries, e.g., the causes, the degree and size of burn, the patient's general condition and the types of graft or materials for covering burn wounds [43]. In the case of our study, we first focused on the effect of the preparations on the skin after first-degree thermal burns and sunburns. Thermal burns result from tissue exposure to an external heat source [44]. On the other hand, sunburns differ significantly from thermal burns, as they result from infrared radiation. Although infrared radiation gives sunlight its warmth, it is not the heat of the sun that burns the skin.

Over the course of the conducted studies, the obtained nanoemulsion NG-Carb-1.0 and market preparations (cream G and gel M) were examined for their impact on skin burns of four assessors. Assessors 1 and 2 had first-degree burns caused by the contact of the skin with a hot iron and an electric heater, respectively. In the case of assessors 3 and 4, the effectiveness of the above-mentioned preparations was examined for first-degree sunburns. The examined preparations were applied to the burn area twice a day for 7 days. Tables 8 and 9 present the obtained results of the effect of the gel nanoemulsion and two market preparations on the skin affected by thermal burns and sunburns.

Table 8. Results of the examined preparations concerning their effect on the skin after thermal burn.

Results	Parameter Value (%)		
	Hydration	Elasticity	Smoothness
Preparation G (emulsion)			
Assessor 1	16 ± 1.24	22 ± 1.24	3 ± 0.94
Assessor 2	15 ± 1.41	26 ± 0.47	8 ± 0.47
Preparation M (gel)			
Assessor 1	25 ± 0.94	11 ± 0.47	2 ± 0.94
Assessor 2	28 ± 1.24	13 ± 1.69	8 ± 0.47
NG-Carb-1.0 (nanoemulgel)			
Assessor 1	22 ± 1.69	15 ± 0.47	3 ± 0.47
Assessor 2	18 ± 1.24	20 ± 1.24	7 ± 0.47
Control test (without the use of the preparation)			
Assessor 1	5 ± 0.94	5 ± 0.94	−4 ± 0.47
Assessor 2	3 ± 0.47	8 ± 0.94	−3 ± 0.47

Table 9. Results of the examined preparations concerning their effect on the skin after sunburn.

Results	Parameter Value (%)		
	Hydration	Elasticity	Smoothness
Preparation G (emulsion)			
Assessor 3	10 ± 0.81	19 ± 0.47	5 ± 0.47
Assessor 4	6 ± 0.47	10 ± 0.47	13 ± 0.47
Preparation M (gel)			
Assessor 3	11 ± 0.47	12 ± 0.47	9 ± 0.47
Assessor 4	8 ± 0.47	7 ± 0.47	17 ± 0.94
NG-Carb-1.0 (nanoemulgel)			
Assessor 3	16 ± 0.94	15 ± 0.94	2 ± 0.47
Assessor 4	15 ± 0.47	18 ± 0.47	16 ± 0.47
Control test			
Assessor 3	11 ± 0.47	−3 ± 0.47	2 ± 0.47
Assessor 4	8 ± 0.47	−1 ± 0.47	1 ± 0.94

Taking into account the examination results of the thermal burn skin presented in Table 8, it can be concluded that improved hydration and elasticity of the skin smoothness were observed for each of the examined preparations for assessors 1 and 2 in comparison with the control sample

Similar observations were made after the examination of the skin with first-degree sunburns. On the basis of the data in Table 9, it can be concluded that all the analyzed preparations had a positive impact on the condition of the skin after sunburn, as also shown in Figure 7.

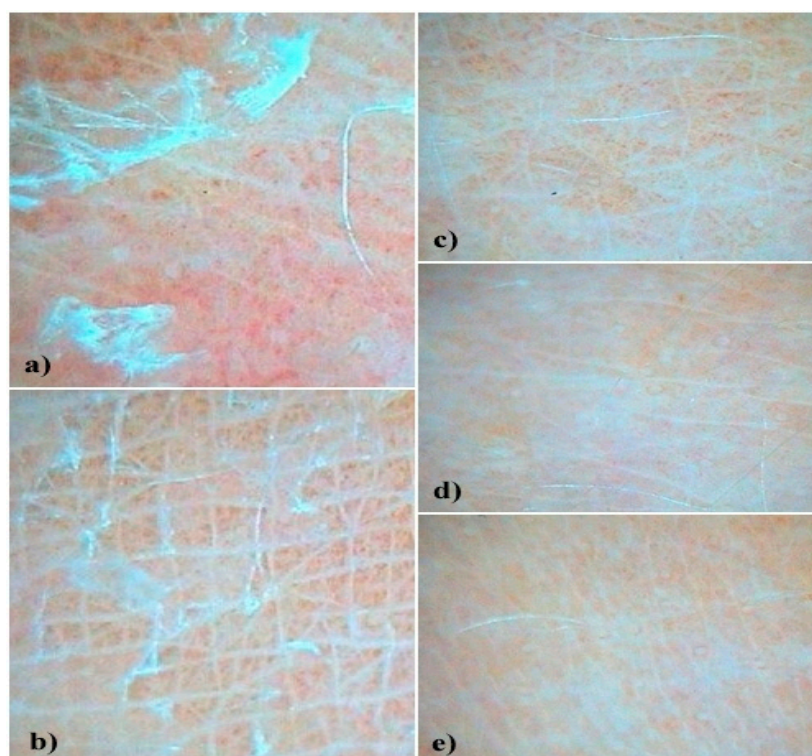


Figure 7. Photographs of the skin of assessor 3 with sunburns: (a) skin burn before the preparations were used. Skin burn after 7 days: (b) control group; (c) preparation G; (d) preparation M; (e) nanoemulsion NG-Carb-1.0.

3. Discussion

3.1. Discussion of the Rheological Results

The significant differences in the rheological properties (apparent viscosity, yield stress) of the analyzed samples depended on their physicochemical formulation, and on the concentration of bodying substances, emollients and emulsifiers in the formulation (Table 4).

These differences are also the result of the presence of various modifiers of rheological properties in their formulation, as they show different mechanisms of thickening or gelling. Solid lipids, such as waxes, fatty alcohols or butters, create crystal networks, which increase emulsion rigidity [45,46]. In the case of hydrocolloids (e.g., xanthan gum, carbomers), hydration reaction, as well as polymer chain swelling occur, and a network structure is formed [47–49].

All the analyzed ointment products had a semi-solid, heavy and sticky consistency, as their recipes included lanolin and petroleum jelly—non-polar emollients with a thick and very sticky form. Out of the examined physicochemical forms, ointments were characterized with some of the higher values of yield stress and apparent viscosity (Figure 3, Table 5). Moreover, high consistency of hydrophobic substances with high melting temperatures in their composition result in poorer spreadability [50,51].

The market of products for supporting burn healing includes emulsions with various consistencies and types (o/w and w/o). The tested lotions and balsams were semi-liquid, while the creams were semi-solid. Out of the examined creams and all the other preparations, the cream G (w/o-type emulsion) showed the highest yield stress and apparent viscosity, as it included beeswax and sodium stearate that regulate the consistency and viscosity of the product [52]. Beeswax also served as an emulsifier in this preparation. The preparation E included the mixture of cetearyl alcohol and sodium lauryl sulfate (emulsifying wax), which thickened the product to improve application properties, and, as a result, it had lower apparent viscosity (in the examined range of shear rate) and yield stress (52.7 Pa) out of all the analyzed creams. The lotion K had almost 2-times higher yield stress (83.2 Pa) than lotion L (43.18 Pa). This is probably due to the use of a thickening complex of sodium acrylate, sodium acryloyldimethyl taurate/copolymer/isohexadecane/polysorbate 80 and shea butter. The consistency of this lotion was heavier, and the preparation had lower spreadability on the skin. The lotion L contains dimethicone, which lowers the viscosity of preparations and lowers surface tension to improve the applicability of the product. The values of the yield stresses and viscosity of the balsams were similar to those of the analyzed lotions (K, L). The balsams A and B contained a rheological modifier (thickening complex): polyacrylamide, C13-14 isoparaffin, Laureth-7. Additionally, the balsam B contained a fatty substance (caprylic capric triglyceride) to facilitate glide during the application, thus, improving its utility capabilities and skin adhesion. This resulted in a slight reduction of its yield stress in relation to product A (Figure 2, Table 5).

The gel nanoemulsion obtained by us (NG-Carb-1.0) showed the lowest yield stress (21.75) and apparent viscosity out of all the analyzed physicochemical forms. It has a low content of fatty substances (sea buckthorn berry oil, 3.0% mass) and, therefore, it had a light and non-sticky consistency and very good spreadability on the injured skin.

The tested hydrogel preparation (C, D) showed similarly low yield stress value 17 Pa and apparent viscosity (as in the case of the nanoemulgel). The gels (N, M) had higher yield stress values (39–55 Pa) and viscosity in comparison with the analyzed hydrogels. A common feature of all these systems was a carbomer. In both the nanoemulgel and gel preparations, the carbomer allowed to obtain products with low values of the yield stress and apparent viscosity (Figure 4, Table 5). Taking into account the rheological characteristics of the studied products, it can be concluded that, just like gels, the nanoemulgel obtained by us can be a first-choice preparation in the treatment and/or the healing support of the skin after burns.

Correlation Relating Rheological and Sensory Measurements

With regard to application, consistency, spreadability, absorption, adhesion and stickiness were considered to be the most important properties of a preparation for skin treatment after burns.

In order to determine the relationship between the sensory assessment parameters and the specific rheological parameters, an attempt was made to develop a correlation between the results of the rheological and sensory studies. A linear relationship was determined between the sensory assessment parameters and the rheological parameters (k , n in Herschel–Bulkley model) [53,54]. According to the results obtained (Table 10), a strict relationship was obtained for the following pairs: spreadability, k ; spreadability, n .

Table 10. Correlation coefficients among rheological and sensory parameters. Statistically significant relations are in bold.

Property	k	n
consistency	0.546	0.424
spreadability	0.895	0.814
adhesion	0.140	0.070
stickiness	0.378	0.493
absorption	0.793	0.692

Both the analysis of variance (ANOVA) analysis and the correlation that combines the results of the rheological and sensory examinations confirm that the assessors with skin burns regard the skin spreadability as the most important property, which may determine their willingness to regularly use the preparation for post-burn skin regeneration, and also may result in its effectiveness. In our opinion, this is related to the fact that the sensations after the product application to the skin are different for burned skin in comparison with the healthy skin. It may also be important that, in order to ensure its effectiveness, it is necessary to touch the damaged skin several times when applying the preparation. As a result, the application process should not increase the discomfort or cause pain [55].

3.2. Effect of the Gel Nanoemulsion and Market Preparations on the Skin after Burns

3.2.1. The Active Components of the Nanoemulgel Supporting Burned Skin Regeneration

Our nanoemulgel recipe includes sea buckthorn (*Hippophae rhamnoides*) berry oil, which is one of few vegetable oils containing a rare palmitoleic acid (ω -7, omega 7)—a component of skin lipids. The palmitoleic acid stimulates regenerative processes in the epidermis and supports wound healing. In other words, this oil has the ability to activate physiological skin functions for skin regeneration and minimization of scarring. The regeneration of burned skin is positively affected by oil also containing unsaturated ω -3 and ω -6 fatty acids, carotenoids and tocopherols, which stimulate fibroblast proliferation, collagen biosynthesis and induce tissue reparation and angiogenesis [56–58]. Out of the analyzed market preparations, this ingredient was found in only one cream (preparation G, Table 4), which was intended for irritated skin after sunbathing, recommended for accelerated wound healing, the stimulation of regeneration and restoration of the epidermis.

The most important moisturizing substances found in the formula of the analyzed market preparations (Table 4), which are intended for skin regeneration after burns include: dexpanthenol (present in the composition of seven products), allantoin (present in the composition of six products), aloe vera gel (present in the composition of two products), and hyaluronic acid (present in the composition of two products).

Dexpanthenol is an alcohol derivative of pantothenic acid, a component of the B complex vitamins. It acts like a moisturizer, improving stratum corneum hydration, reducing transepidermal water loss and maintaining skin softness and elasticity. Activation of fibroblast proliferation, which is of relevance

in wound healing, has been observed both *in vitro* and *in vivo* with dexpanthenol. Dexpanthenol has been shown to have an anti-inflammatory effect on the experimental ultraviolet-induced erythema [59].

Allantoin, a derivative of urea, stimulates cell division and epithelization, thus, accelerating the regeneration process of damaged skin inflammation. It also has strong moisturizing properties, and it is highly safe during the therapy—it is not allergenic and it does not cause skin irritation. Thanks to allantoin, the proliferation of epithelial cells is accelerated, which leads to quicker recovery of the skin. Allantoin makes the skin easily retain additional water, and can therefore rebuild the protective hydro-lipid coat. Pharmaceutical and cosmetics products with allantoin are also recommended as an adjunct in the treatment of chronic skin diseases with impaired keratosis or skin damage, such as atopic dermatitis, contact dermatitis, psoriasis, ichthyosis, ulcers or burns [60].

Aloe vera is an herbaceous and perennial plant that belongs to the Liliaceae family, and it is used for many medicinal purposes. Scientific studies have shown that the gel can increase the flexibility and reduce the fragility of the skin, since 99% of the gel is water. Additionally, mucopolysaccharides along with amino acids and zinc present in aloe vera can have a positive effect on skin integrity, moisture retention, erythema reduction and they also help in preventing skin ulcers. Aloe vera is known for its anti-tumor, anti-inflammatory, skin protection, anti-diabetic, anti-bacterial, anti-viral, antiseptic and wound healing properties [61]. Gels obtained from aloe vera include, among others, polysaccharide, amino acids, plant sterols, enzymes, vitamins, minerals, sugars, as well as 75 other components. Due to its antibacterial, anti-inflammatory and moisturizing effects, aloe vera is successfully used to treat wounds and skin burns [62].

Hyaluronic acid (hyaluronan), a naturally occurring polymer is polysaccharide belonging to the glycosaminoglycan family. The hyaluronic acid molecule is readily soluble in water, producing a viscous liquid or gel that behaves like a lubricant. It enhances keratinocyte proliferation and migration, as well as the angiogenic response from the wound bed. Topical application of hyaluronic acid derivatives stimulates the healing of not only fresh wounds, but also ulcers and chronic wounds [63].

It was possible to add only the hyaluronic acid (N14-C), aloe vera gel (N14-E), and the combination of both ingredients (N14-G) to the nanoemulsion recipe (Table 2).

This is probably due to the fact that hyaluronic acid and aloe vera gel could additionally act as agents that thicken the aqueous phase and create a steric barrier [64]. As reported in the literature, water-soluble polysaccharides are frequently employed as texture modifiers, e.g., thickening agents or gelling agents. Thickening agents typically comprise of soluble polymers displaying extended structures and they can achieve higher solution viscosity since they can modify the fluid flow profile. Gelling agents are capable of generating chemical or physical cross-linking with its neighbors and impart solid-like properties to a nanoemulsion solution. The improved stability of the nanoemulsions by texture modifiers can be attributed to the inhibition of droplet movement and the resulting retardation of gravitational separation [65]. Therefore, apart from decreasing the droplet size, gravitational separation can also be reduced by adding thickeners to improve the viscosity of the aqueous phase, or by adding weighting agents to decrease the density differences. Droplet aggregation due to flocculation and coalescence can be limited by making sure that the steric and electrostatic interactions of droplets exceed their attractive interactions (e.g., hydrophobic, van der Waals). This is often accomplished by changing the aqueous phase composition [65,66].

The preparations intended for burned skin should contain non-irritating and non-allergic components. Therefore, a decyl glucoside was used as a nanoemulsion emulsifier.

Decyl glucoside is a non-ionic surfactant made from renewable, plant-derived feedstocks, an effective alternative to polyethoxylated group /sulfate-containing formulations. It belongs to the group of alkyl glucosides (APG), surfactants synthesized through the condensation of long-chain fatty alcohols and glucose, extracted from vegetal, renewable sources. They are used in various leave-on and rinse-off cosmetics and are considered to have low irritancy and allergenicity. Due to its invaluable mildness, this surfactant is also a perfect choice for sensitive skin and baby products [67,68].

3.2.2. Effectiveness of Skin Regeneration after Burns—Analysis of Functional Parameters of the Skin

Our criterion for the effectiveness of a nanoemulgel in skin regeneration after burns was the increase in skin functional parameters such as: hydration, elasticity, and smoothness compared to the control test during 7 days of use of the preparations on the skin area after thermal burns or sunburns. For comparative purposes, the products G (cream) and M (gel) were selected, due to their physicochemical form but also due to the results of rheological and sensory examinations.

The improvement in hydration, elasticity and smoothness of the skin after a thermal burn was observed in assessor 1 and assessor 2 for each of the tested preparations in comparison with the control group. This indicates that the preparations for the regeneration of burned skin require moisturizing ingredients and ingredients that affect skin hydration and elasticity.

The emulsion (the preparation G) and nanoemulgel had a greater impact on the improvement of skin elasticity in comparison with the gel preparation M. This is most likely related to the emollients contained in those preparations, sea buckthorn oil, among others. The results of the skin parameters after the use of the preparation G were also influenced by betulin present in its recipe. Betulin is known for its effective action in the treatment of burns. According to Frew et al. [69], betulin allows the wounds to heal faster as well as it reduces scar formation.

On the other hand, when the improvement of skin hydration is concerned, the gel M and nanoemulgel were more effective. Good hydration of burned skin is probably associated with the gel form of these preparations and the content of moisturizing ingredients, such as: brine water, aloe vera (aloe barbadensis extract), propylene glycol, glycerin, symphytum officinale extract, panthenol and allantoin (in the case of the preparation M) and hyaluronic acid and aloe vera gel (in the case of the nanoemulgel). Moreover, the gel matrix makes it possible to prolong the contact time of the preparation with the skin, and to increase the skin hydration level by forming a hydrophilic film on the skin surface and reducing the transepidermal water loss. The whole results in adequate nutrition, hydration and skin tension, as reflected by the results obtained by us.

After the tested products were applied, the increase in the hydration and elasticity levels in the skin after sunburns was observed, and this indicates good regeneration of the epidermis. Damaged epidermis after long exposure to sunlight creates erythema, which increases in proportion to the overall dose and if excessive exposure occurs, the skin has desquamation of the outer dead skin cells after some days. Out of the analyzed products, the highest hydration level was achieved by the application of the obtained gel nanoemulsion. The nanoemulgel in the analyzed cases prevented skin exfoliation and softened the reddened areas. The light formula and consistency, as well as the increased rate of absorption of the received preparation, can allow the application of the preparation to the skin several times a day and reduce discomfort during the application to the damaged skin. All the results of our analysis of the rheological and sensory properties of the products with a new physicochemical form for the regeneration of burned skin indicate that a nanoemulgel, like gel preparations, can be a first-line product to treat first-degree thermal burns and sunburns.

4. Materials and Methods

4.1. Materials

Based on our previous studies [42], in order to obtain the nanoemulsion gel, Plantacere 2000UP (International Nomenclature of Cosmetic Ingredients (INCI): Decyl Glucoside, HLB = 12.8) from BASF Company (Warsaw, Poland) was used as the emulsifier. The oil phase was composed of sea buckthorn oil (INCI: *Hippophae rhamnoides* Berry Oil) and tocopheryl acetate as the antioxidant (Ecospa Company, Warsaw, Poland). The aqueous phase included deionized water and moisturizing agents such as: *Aloe Barbadensis* leaf juice and 1 wt% sodium hyaluronate solution, allantoin, and D-panthenol. All of them were purchased from Ecospa Company, Warsaw, Poland. OPTASENSE™ G-40 (INCI: Carbomer) was used as a rheology modifier and purchased from Croda Company (Cracow, Poland). Sodium benzoate plays the role of a preservative (Brenntag Company, Kędzierzyn-Koźle, Poland).

The material for the comparative analysis included the obtained nanoemulgel and 14 market preparations (manufactured in the European Union (EU)) for the treatment of first and second-degree burns, scars after burns and surgical procedures, sanitizing wounds after burns, skin regeneration after burns. The detailed characteristics of the market preparations and their scope of use, in accordance with the manufacturers' declarations, are set out in Table 4.

4.2. Nanoemulsions Gel Preparation and Characterization

The preparation process of the nanoemulsion by ultrasound method was described in our previous study [42]. At the initial stage of the process, a basic emulsion was prepared, which consisted of an emulsifier, sea buckthorn oil and water. For this purpose, part of the aqueous phase with the emulsifier and oil phase was heated to 40–50 °C in a water bath. In turns, both phases were combined, adding the oil phase to part of the aqueous phase and they were mixed with PHOENIX Instrument RSM-10HS magnetic stirrer for 10 min or 15 min, 300 rpm or 500 rpm. Then, the prepared pre-emulsion was processed with an ultrasonic homogenizer UP200Ht (Hielscher), where the emulsification time was 60 s or 120 s, and ultrasound power was 40 W (40%), amplitude 89% (Table 2). Stability assessment of the prepared samples was carried out visually. In the next stage, the pre-emulsion was prepared and enriched with the following ingredients: aloe vera juice, 1% hyaluronic acid solution, allantoin, D-panthenol, as well as an anti-oxidant (vitamin E), and preservative (sodium benzoate). For all the enriched nanoemulsions, the pre-emulsification time was 10 min, pre-emulsion stirring speed was 500 rpm, ultrasonic homogenization was 120 s (Table 3). Stability assessment of the prepared samples was carried out visually and by means of the dynamic light scattering (DLS) method. To prepare the nanoemulgel, the procedure described by Mao et al. was applied. The first hydrogel matrix was prepared by soaking the carbomer in a water carbomer gel matrix. Then, the nanoemulsion was added to the gel matrix with slow continuous stirring. The final nanoemulgel was obtained by adjusting pH to 6 with the addition of a citric acid aqueous solution [18].

4.2.1. Determination of Nanoemulsion Droplet Size

The measurement of the nanoemulsion droplet size was made with Zetasizer Nano ZS analyzer by Malvern Instruments. Dynamic light scattering (DLS) technology was used in the analysis. The device is equipped with an analyzer with a laser, which emits light of a wavelength equal to $\lambda = 633$ nm. The measurements were carried out at 25 °C. The device measures diffused light at an angle of 173°. In order to avoid multiple scattering effects, the samples were diluted to 1 wt% with deionized water before the analysis. The measurements were carried out at a temperature of 25 °C and a relative humidity of 60%.

4.2.2. pH Measurements

The measurement of pH was made with a SevenMulti pH-meter (by Mettler Toledo Inc., Columbus, OH, USA) equipped with an InLab®Expert Pro electrode. The measurement was made by immersing the electrode in the resulting nanoemulsion. The results presented show the arithmetic mean from three measurements. The measurements were carried out at 25 °C.

4.2.3. SEM Analysis

The morphology of the obtained nanoemulgel was observed by a scanning electron microscope (Mira3-FEG-SEM, Tescan, Brno—Kohoutovice, Czech Republic) with a pole emission (Schottky emitter), equipped with an X-ray energy dispersive spectrometer EDX (Oxford Instruments) and a cooling table (Peltier), operating in a temperature range from as low as −30 °C. The microscope allows work in high, low and variable vacuum modes. For SEM investigations, the samples were prepared by rapid freezing in liquid nitrogen followed by freeze-drying for 48 h [18,70].

4.2.4. Rheological Studies

The flow curves and viscosity curves of the examined market products used in supporting burn healing and of the obtained nanoemulgel were determined with a rotational rheometer, model R/S Plus Brookfield. The cone-plate C-25-2 measurement system was used. Shear speed range was $1\text{--}500\text{ s}^{-1}$, measurement time 30 s, number of measurement points 30. The measurements were carried out at a constant temperature ($25\text{ }^{\circ}\text{C}$) with a Huber Ministat 125 thermostat and RheoWin 3000 rheometer firmware to automate and save measurements. The presented results of the study for each sample show the average from three measurements.

4.2.5. Sensory Analysis

The conducted sensory analysis focused on the assessment of the examined products, beginning with their appearance and accompanying sensations during and after the application of the preparation on burned skin. The analysis consisted of two stages.

The first stage was the empirical assessment of the primary and secondary feeling on the skin left by the analyzed preparations (the nanoemulgel and 14 market products) [34,35]. The assessors were asked to evaluate (on a 5-point scale) what sensations the tested product produced on the skin during and after the application. The obtained results of the assessor's evaluation (number-average) were then compared with the results of the rheological measurements. On this basis, the window boundaries of a good primary and secondary sensations on the skin were determined, as defined by Brummer et al. [34].

The second stage of the study was a detailed sensory assessment of three selected preparations and combined with the assessor's evaluation of the effect of the preparations on the skin affected by thermal burns and sunburns. The selected preparations were the cream G (which contained sea buckthorn oil), the gel M (with an aloe vera extract) and the nanoemulgel obtained by us, which was a combination of the form and active ingredients present in these preparations. The preparations selected for the second stage of the sensory assessment had the best sensations on the skin after the application according to the criteria defined by Brummer et al.

In first stage, the assessors were 10 women, aged 23–25, who had no previous experience in the evaluation of cosmetic preparations. In order to train the assessors and to obtain correct and repeatable results, the assessments were conducted under standardized conditions. The standardization of the conditions concerned the definition of the characteristics selected for the evaluation and the establishment of sensory assessment conditions.

In the first part, the assessors were informed about the general concept of the study, and then the test procedure was described in detail, as well as the assessment method for different characteristics of the tested preparations. Individual characteristics are defined in accordance with the ASTM D1490 Standard Guide for descriptive analysis of creams and lotions and our previous works [35,71]. The assessors gave their informed and written consent to participate in the study.

The assessment was conducted in a laboratory with controlled temperature and humidity and appropriate lighting conditions. The temperature of all the samples tested was the same and was $21 \pm 0.5\text{ }^{\circ}\text{C}$. The tested PCs were placed in tight, plastic containers.

The assessors were asked to evaluate the individual characteristics of the tested preparations on a 5-degree scale, where 1 was the lowest and 5 the highest score. The given numerical value of the evaluated characteristic for each sample is the average of the scores from each assessor. Eleven characteristics of the preparations were assessed in the test. The evaluated parameters were color, fragrance, consistency, uniformity, spreadability on the skin, adhesion, smoothing, pillow effect, stickiness, oiliness and absorption [35,72].

4.2.6. Test of the Effect of the Nanoemulsion and Market Preparations on the Skin Condition after Burns

The assessment of the effect of the gel nanoemulsion and two market preparations on the skin regeneration rate after burns was made with the ARAMO[®] TS device (by EURTEX, Warsaw, Poland) and synchronized software ARAMO Skin XP PRO Diagnostic System. The device allows the analysis of the skin condition with sensors which measure, among others, elasticity, hydration, hydration level, the amount and size of discoloration or wrinkle depth.

The study of the impact on the burn healing process and comparative evaluation of the prepared nanoemulgel and two market preparations was conducted on four assessors, aged 23–25, who had thermal burns and/or sunburns on the skin of upper limbs. The assessors gave their informed and written consent to participate in the study. The examined preparations were applied to burned skin twice a day for 7 days. At the beginning of the study, an analysis of the burn areas was carried out before the preparations were applied and after seven days of use. The measure of the effectiveness of the regenerating preparation was the effect on such factors as: elasticity, hydration, smoothness. The study also covered the area of burned skin that was not treated with any of the preparations (control test). The results given are the difference in the measurements of the above parameters of the skin condition before and after use of the tested preparations. Negative values mean deterioration and positive values mean improvement in a given skin parameter.

All subjects gave their informed consent for inclusion before they participated in the study. The scope of the study is in line with the Regulation (EC) No 1223/2009 of the European Parliament and of the Council, Cosmetics Europe–The Personal Care Association Guidelines “Product Test Guidelines for the Assessment of Human Skin Compatibility 1997”, Cosmetics Europe–The Personal Care Association “Guidelines for the Evaluation of the Efficacy of Cosmetic Products 2008”, World Medical Association (WMA) Declaration of Helsinki–Ethical Principles for Medical Research Involving Human Subjects.

4.2.7. Statistical Analysis

All data concerning the mean droplet size of nanoemulsions, PDI, and skin parameter were presented as a mean of three different experiments \pm SD. The analyses of variance (ANOVA) were conducted to check the differences between the sensory attributes of the samples. A value of $p < 0.1$ was considered statistically significant. In case of significant difference at 90%, Tukey’s HSD for post hoc comparisons were then carried out.

5. Conclusions

The nanoemulsion gel combines the advantages of an emulsion and a gel due to its light, non-oily, semi-solid consistency, transportability of hydrophobic substances, while, at the same time, it creates a protective layer on the skin and increases the hydration level. The obtained formulation showed the lowest yield stress and apparent viscosity out of all the analyzed physicochemical forms. According to the sensory analysis, the nanoemulgel as a skin burn preparation was characterized with light and non-sticky consistency, which was quickly absorbed and did not leave the feeling of oiliness and stickiness after application. The gel form allows good spreadability and skin adhesion of the product on the injured skin. Nanoemulsion gel is a promising alternative to medical cosmetics available on the market as a form of formulation used in skin care after burns.

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Abbreviations

NEGs	Nanoemulgels
PDI	Polydispersity index
SEM	Scanning electron microscope
DLS	Dynamic light scattering

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