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Reduction of Irritation Potential Caused by Anionic Surfactants in the Use of Various Forms of Collagen Derived from Marine Sources in Cosmetics for Children

Surfactants, which are the basic ingredients in washing cosmetics, can cause skin irritation. In the case of washing cosmetics intended for children, it is necessary to select the composition in such a way as to ensure maximum reduction or even elimination of the irritant effect of the final product. The paper attempts to improve the safety of bath cosmetics for children by introducing: collagen amino acids (150 Da), hydrolysed collagen (12 000 Da) and soluble collagen (300 000 Da). Zein value, bovine albumin, and human keratinocyte cell line studies have shown a significant decrease in the irritant potential of the compositions following the introduction of the proposed additives, which is more pronounced with the increase in the molecular weight of marine collagen. An important part of this work is a mechanism proposed by the authors, according to which the addition of the proposed additives can increase safety of the application of cosmetics for children.

Key words: Surfactants, marine collagen, cosmetics for children, safety

Verringerung des Irritationspotentials durch anionische Tenside bei der Verwendung verschiedener Kollagenformen, die aus marinen Quellen in der Kosmetik für Kinder stammen. Tenside, die Basisinhaltsstoffe in Waschkosmetika, können Hautreizungen verursachen. Bei Waschkosmetika, die für Kinder bestimmt sind, muss die Produktformulierungen so ausgewählt werden, dass die Reizwirkung des Endprodukts maximal verringert oder ganz beseitigt ist. In diesem Paper wird versucht, die Sicherheit von Badekosmetik für Kinder zu verbessern, in dem Kollagensäuren (150 Da), hydrolysiertes Kollagen (12 000 Da) und lösliches Kollagen (300 000 Da) den Produkten zugesetzt werden. Zein-Wert, Rinderalbumin und die Keratinozyten-Zelllinienstudien haben gezeigt, dass das Reizpotential der Produktformulierungen nach der Einführung der vorgeschlagenen Additive signifikant abnimmt. Die Abnahme ist mit zunehmendem Molekulargewicht von Meereskollagen ausgeprägter. Ein wichtiger Bestandteil dieser der Arbeit ist ein von den Autoren vorgeschlagener Mechanismus, nach dem die Zugabe der vorgeschlagenen Additive die Produktsicherheit bei der Verwendung von Kosmetika für Kinder erhöht werden kann.

Stichwörter: Tenside, marines Kollagen, Kosmetika für Kinder, Sicherheit

1 Introduction

Bath liquids intended for children are among the main cosmetic products used in this consumer group. Cosmetics of this type should, above all, be mild on the skin of children. Bath liquids are typically aqueous solutions of surface-active agents and various additives such as humectants, refatting agents, preservatives and pH regulators. The surface-active agents conventionally employed in formulations of this type for their good cleaning performance, satisfactory foaming properties and their responsiveness to thickening with salt are anionic surfactants [1–4].

However, beside the properties that determine the satisfactory quality parameters for product functionality, anionic surface-active agents also have certain disadvantages. The literature [5–9] contains a number of reports on interactions between anionic surfactants and the skin, particularly the stratum corneum. Possible consequences of such interactions include impairment of the skin barrier function, increase in transepidermal water loss and decrease in skin hydration. As a result, the skin may become irritated, dry or red. It is generally recognized that the skin irritation effect of anionic surfactants is due to the presence of monomers and micelles in the aqueous solution [1, 9]. Various methods for reducing the irritant effect that is induced by anionic surfactants have been proposed in literature. Studies have been focused on incorporating “mild” surface-active agents [6], protein hydrolysates [10–12], hydrophobic substances [13, 14] or polymers [15] into formulations. The mechanism of action in additives of this type is usually associated with an increase in the size of micelles or the formation of appropriate complexes in order to reduce the concentration of free monomers of surface-active agents (SAAs) in the solution.

In recent years, much attention has been paid to the application of active ingredients of marine origin in the cosmetic industry because of their great benefits for the health of human skin. Bioactive substances which are derived from marine resources can perform various functions as natural skin care ingredients, and their properties can be employed in the process of formulating new types of cosmetics. Therefore, oceans and seas should be considered as a huge reservoir of potential new cosmetic raw materials. An example is marine collagen, which may provide an alternative to the well-known bovine and porcine collagen types [16–23].

The described characteristics of collagen and its derivatives, however, do not take into consideration the impact of the material on the skin irritation effect of cosmetics. Consequently, the present study examined the possibilities for

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using various forms of marine collagen in wash cosmetics intended for children in order to minimize the irritant effect induced by anionic surfactants. The formulated product prototypes were analyzed empirically to assess the impact of additives of this type on the safety-in-use of bath liquids intended for children. The results were correlated with the molecular mass of added collagen forms.

2 Materials and Methods

2.1 Materials

The raw materials which were used to make the prototypes of bath liquids for children were the one commonly used in the cosmetics industry: sodium laureth sulfate (Texapon N70; BASF, Ludwigshafen, Germany, cocamidopropyl betaine (Dehyton K; BASF, Ludwigshafen, Germany), aqua (and) collagen amino acids (Collasurge, Croda Poland, $M_w = 150$ Da), hydrolysed collagen (Crotein M; Croda Poland, $M_w = 12000$ Da), aqua (and) soluble collagen (Collasol M; Croda Poland, $M_w = 300000$ Da), glycerin (Cremerglyc; Cremer Hamburg, Germany), citric acid (Citric Acid; HSH Chemie, Poland), sodium chloride (NaCl; POCH, Gliwice, Poland), sodium benzoate and potassium sorbate as preservatives (KEM BS; Pol Nil S.A., Poland), distilled water.

2.2 Methods

2.2.1 Determination of the irritant potential of bath cosmetics for children based on the zein value

The irritation effect of the prototypes of bath cosmetics for children was investigated by determining the zein value on the basis of own methodology, which is based on the descriptions presented in the literature [24, 25]. For this purpose, 2 g of zein was mixed with 40 g of 10% aqueous solution of the preparation (mixing time: 1 h, 35 °C) and the amount of solubilized zein was determined on the basis of Kjeldahl nitrogen method. The determination was carried out with the aid of the Digester 8 AR automatic digester and the Kjeltec 8400 automatic nitrogen analyzer. The study resulted in obtaining the zein value expressed in milligrams of the nitrogen mass determined in a 100 mL sample. The final result was an arithmetic mean of 3 independent determinations.

2.2.2 Determination of the irritant potential of bath liquids for children based on changes in the pH value of the bovine albumin solution

The study was carried out according to the methodology developed by Imokawa [26] and Tavss [27]. The degree of protein denaturation was determined by measuring the pH of the bovine albumin solution in the bath liquid solution.

The test was carried out as follows: 50 ml of 2% aqueous solution of bovine albumin and 50 ml of 10% solution of the detergent were prepared. In both solutions, the pH was adjusted to 5.5 (pH value of healthy human skin) by applying aqueous solution of 50% citric acid. The two solutions were then thoroughly mixed. The mixture was left to stand for 48 h, after which the pH value was measured. The higher the pH increase, the more irritant the product will be. The results were presented by calculating the percentage increase in pH value, in relation to the value assumed for healthy human skin (pH = 5.5), using the equation:

$$\Delta\text{pH} = \frac{\text{pH}_r - \text{pH}_0}{\text{pH}_0} \cdot 100\%$$

where: ΔpH : change of pH value, pH_0 : assumed pH value of healthy human skin, pH_r : mean pH value for the bovine albumin solution in the washing agent solution.

For each of the analyzed solutions 3 determinations were made, the value of which was averaged.

2.2.3 Particle size

The particle size distribution of the 1% aqueous solution of prototype of bath cosmetics for children was determined by Dynamic Light Separation (DLS) using the Zetasizer Nano (Malvern) instrument. Particle size was measured 24 h after preparation. The measurements were made in the range from 0.6 nm to 6000 nm, at a dispersion angle of 173° and at a temperature of 25 °C. The values shown are an average of the 3 series of at least 10 measurements.

2.2.4 Cell culture

The immortalized non-tumorigenic human epidermal cells (HaCaT, ATCC Cell Lines) were used in this study. HaCaT cells were cultured in DMEM (Dulbecco's minimum essential medium, Gibco), supplemented with 10% FBS and 1% penicillin/streptomycin (Sigma). The cells were incubated in a humidified atmosphere at 37 °C, 5% of CO₂.

For experiment, HaCaT cells were seeded on 96-well plates at a density 2×10^4 cells/well one day prior to drug exposure.

2.2.5 Resazurin assay

The resazurin sodium salt (Alamar Bule) (Sigma, R7017), was used to assess the cell viability. HaCaT cells were seeded in transparent 96-well plates and exposed to different bath liquids concentration ranging from 0.16% to 10% for a 30 min and 24 h. Unexposed cells were chosen as control group. After exposure, the resazurin solution was transferred into the plates for a final volume of 250 µL/well and a final concentration of 60 µM resazurin and incubated for 1 hour at 37 °C. The absorbance was measured at the wavelength $\lambda = 570$ nm using a microplate reader (Filter Max F5, Molecular Devices). The experiments were performed in triplicates for each bath liquid concentration. Results were expressed as percentage cell viability versus the control (100%).

2.2.6 Statistical analysis

Each analysis of bath liquids for children was performed in triplicates. Obtained values were presented as mean $\pm$ SD. Significant differences between obtained values were analyzed using Graph Pad Prism5.0 software using One-way ANOVA and Tukey's test. Assumed level of significance $\alpha = 0.05$.

3 Results and Discussion

3.1 Development of formulations and technologies for obtaining collagen-enriched bath liquids for children

Based on the literature data [1–3, 7–12, 16–28] and our own experience in the field [4–6, 13–15], the prototypes of bath liquids for children containing different types of marine collagen were formulated and prepared (Table 1). The raw materials were analyzed to determine how they affect the parameters associated with the safety-in-use of the product prototypes. Marine collagen with different molecular

masses (150 Da; 120 000 Da; 300 000 Da), commercially available as a cosmetic raw material, was added to the prototype bath liquids at a concentration of 0.1%. The reference bath liquid was formulated without any addition of marine collagen.

Marine collagen was obtained from specially selected warm-water fish (tilapia). This type of collagen is characterized by a higher level of the amino acid hydroxyproline than collagen which was obtained from cold-water fish [29, 30]. The study examined various forms of collagen of marine origin: Soluble collagen with a molecular mass of 300 000 Da, hydrolysed collagen with a molecular mass of 12 000 Da and collagen amino acids with a molecular mass of 150 Da. Soluble collagen has a characteristic triple helical structure and its total content of hydroxyl amino acids is the same as in bovine collagen. Collagen amino acids contain high levels of hydrophilic ammonium and carboxyl groups. The collagen hydrolysates are the most widely used form of collagen in the cosmetic industry. Marine collagen hydrolysate has the same amino acid composition as human tissues. Raw materials of this type have already been used in cosmetics as skin and hair conditioning substances however, the effect of such additives on the skin irritation effect of body wash cosmetics has not been studied to date.

The bath liquids for children were formulated in the technological process which is detailed below. An appropriate type of marine collagen, depending on the formulation, was added to a measured amount of water, while constantly stirring. The baseline formulation contained no collagen. Next, sodium salt of *ethoxylated lauryl alcohol sulphate* was introduced into the formulation. After mixing the contents, cocamidopropyl betaine was added. The next ingredient introduced into the mixture was glycerine. Sodium chloride was added to the homogeneous system. The entire contents were mixed. Finally, a preservative (sodium benzoate and potassium sorbate) was added and the pH was adjusted with citric acid to approximately 5.5. The following process parameters were employed: temperature approximately = 22 °C, rotational speed of the stirrer = 400 rpm.

Component [INCI name]	Formulations of bath liquids for children			
	P1	P2	P3	P4
	Concentration/wt%			
Sodium Laureth Sulfate	6			
Cocamidopropyl Betaine	2			
Glycerine	3			
Collagen Amino Acids		0.1		
Hydrolysed Collagen			0.1	
Soluble Collagen				0.1
Sodium Chloride	3			
Lactic Acid	to pH ≈ 5.5			
Sodium Benzoate and Potassium Sorbate	0.5			
Aqua	ad 100			

Table 1 Formulations of prototype bath liquids for children, % by weight

3.2 Skin irritation potential of bath liquids for use in children

The skin of children is very delicate and sensitive, so the irritant potential of bath liquids intended for use in this group of consumers should be as low as possible. The skin irritation potential of the formulated bath liquids was evaluated by determining the zein value (Fig. 1) and the change in pH of bovine serum albumin (BSA) solution (Fig. 2).

The surface active ingredients used in bath liquids may adversely affect the skin surface, causing it to become irritated. Research in the field indicates that surfactants, particularly of the anionic type, can interact via electrostatic forces with proteins in the stratum corneum. The consequences of such interactions include tissue swelling, elution of water-soluble small molecular components and enzyme inactivation. The main role in interactions of this type is attributed to the presence of surfactant monomers in the washing bath. The compounds cause denaturation of epidermal proteins, disrupt the orderly structure of the cellular cement and elute its constituent lipids. The above effects result in the development of skin irritation in the contact area, impairment of the epidermal barrier and, in extreme cases, skin inflammation [2, 9–15].

As the literature shows, determining the effect of surfactants on the denaturation of bovine serum albumin (BSA) belonging to the class of globular proteins, or their effectiveness in partial denaturation and solubilization of the water-insoluble protein zein makes it possible to evaluate the tendency of surfactants and surfactant-based products to induce irritation of human skin. Both proteins show structural similarity to keratin, a structural protein of the epidermis [31, 32].

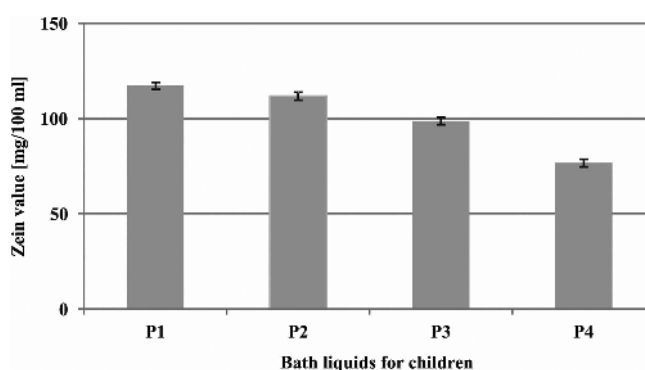


Figure 1 Zein value of 10% solution of bath liquids for children

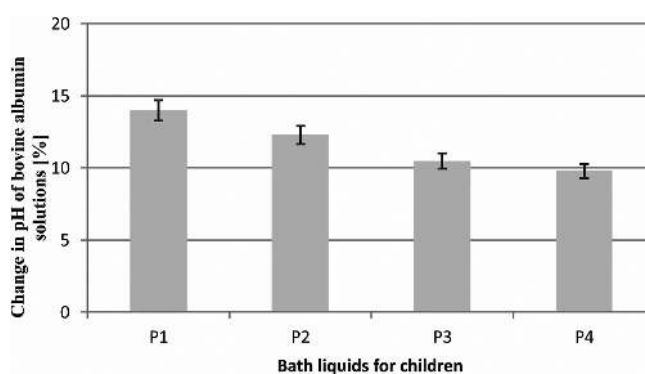


Figure 2 Change in pH of bovine albumin solutions of bath liquids for children

The zein value measurement involves determining the quantity of the water-insoluble protein zein solubilized by surfactants in the aqueous solution after previous partial denaturation by contact with an amphiphilic compound in the solution. The result is expressed as the mass of free nitrogen derived from protein in the solution, and it represents a measure of the irritant potential. The higher the test result, the higher the skin irritation potential of the test sample [5, 6, 14, 15, 24, 25]. In the BSA test, the structure of albumin changes as a result of denaturation of the protein caused by exposure to surfactants, ultimately leading to changes in the pH of the solution. The introduction of anionic surfactants into the albumin solution causes surfactant binding to the cationic groups of the albumin protein. In order to neutralize the negative protein charge resulting from the predominance of anionic groups in its molecule, the adsorption of protons from the solvent takes place, causing a rise in the pH of the solution. The greater the increase in the pH of the test solution, the stronger the skin irritation effect associated with that solution [5, 6, 14, 15, 24, 25]. Figure 1 presents the results of zein value evaluation in aqueous solutions of bath liquids for use in children.

The test found that the molecular mass of marine collagen had an effect on the zein value of the bath liquids. The highest value of the measured parameter was shown in the bath liquid not containing any marine collagen (117.32 mg/100 ml). The addition of marine collagen led to a decrease in the zein value of the body wash cosmetics compared to the reference product. Furthermore, higher molecular mass of marine collagen was shown to correlate with lower zein values. The lowest values, nearly 65 % lower than in the reference liquid, were recorded in the formulation P4 containing marine collagen with a molecular mass of 300 000 Da.

The results are consistent with the literature findings. For example, Bujak et al. [15] assessed the effect of the molecular mass of polyvinylpyrrolidone on the properties of facial cleansing foam. The addition of the polymer was found to improve the safety-in-use of the cosmetics under study. Among other effects, it was shown to decrease the irritant potential evaluated by measuring the zein value.

The findings of the zein test were verified by determining the change in pH of bovine serum albumin (BSA) solution. The measurement results are shown in Fig. 2.

The results of the zein test were confirmed. The highest increase in pH, by approximately 14 %, was observed in the reference liquid. The addition of marine collagen with differ-

ent molecular mass to the bath liquids for use in children led to a decrease in the value of the test parameter by about 12 %–30 % in comparison to the reference liquid. It was found that an increase in the molecular mass of marine collagen correlated with a smaller pH increase. The application of marine collagen with the highest molecular mass (300 000 Da; formulation P4) was linked to an increase in the pH level of just 9.8.

The denaturation of structural proteins in the stratum corneum and the disruption of the highly ordered liquid crystalline structure of intercellular lipids induced by surfactants used in bath liquids may damage the protective barrier of the skin. As a result of the processes, the compounds in the form of monomers are able to migrate more freely into deeper layers of the epidermis, where keratinocytes, i.e. living epidermal cells, are present. By combining with the cytoplasm of keratinocytes, surfactant monomers can cause a degradation of the cytoplasmic structure, which can also be accompanied by a damage to the building proteins of living cells. The processes may result in an irreparable damage of keratinocytes or even cell death. The capacity of surfactants to induce a permanent damage to living skin cells is referred to as skin toxicity. Also, the presence of surfactants and their interactions with proteins in living cells may destroy the structures of living epidermal cells, triggering topical inflammatory skin reactions. This type of reaction to an irritant agent is defined as irritant contact dermatitis (ICD) in the literature, and it is among the most prevalent cutaneous diseases [5]. In order to determine the potential impact of the analyzed product prototypes on living epidermal cells, the effect of the formulations on the metabolism of human skin keratinocytes (HaCaT) was evaluated. To this end, the resazurin (Alamar Blue) assay was performed. The dye used in the assay is a stable compound, non-toxic to cells, and contains an oxidation-reduction indicator using physiological cellular processes. The addition of resazurin to the cell culture causes a reduction in the mitochondria of living cells and a colour change of the dye from blue to red. The degree of reduction can be determined by spectrophotometry or fluorimetry, and the results obtained via these methods enable the assessment of the metabolic activity of cells and the degree of their proliferation [33, 34].

The cell proliferation was evaluated after their incubation for 30 min (Fig. 3) and 24 h (Fig. 4) in the bath liquids containing collagen in the concentration range from 0.16 % to 10 %.

In the formulations P1 and P2, the results obtained after 30 min of exposure to the test agent showed an increase in

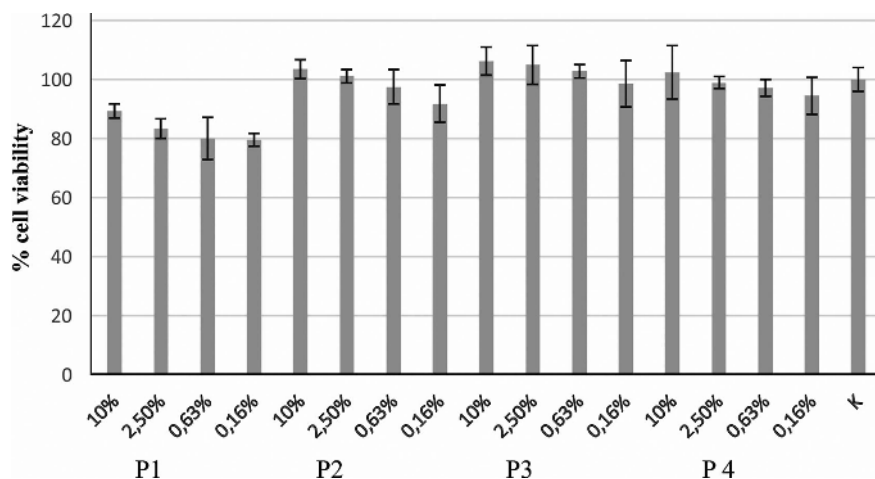


Figure 3 Cells viability after 30 min of incubation. Different letters indicate that means differ significantly, while same letters are used when there are no significant differences. Assumed level of significance $\alpha = 0.05$. K– control sample

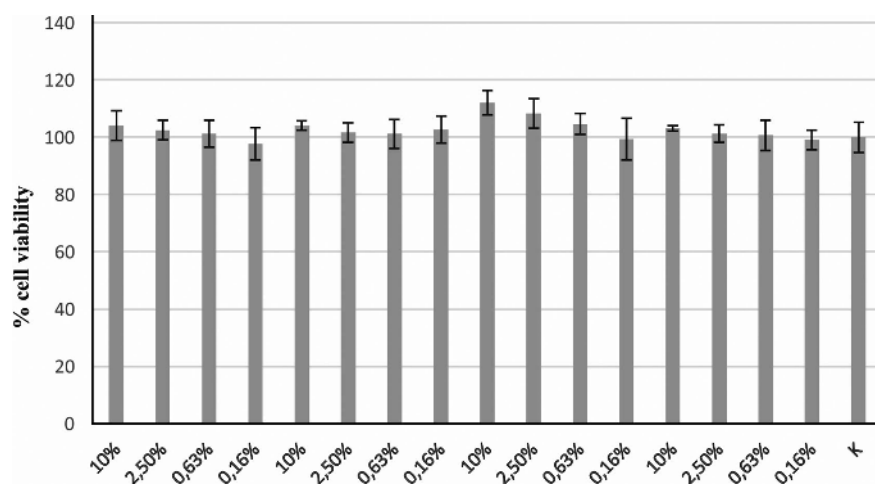


Figure 4 Cells viability after 24 h of incubation. letters indicate that means differ significantly, while same letters are used when there are no significant differences. Assumed level of significance $\alpha = 0.05$. K– control sample

cell viability in proportion to the collagen concentration used in the bath liquid. However, no statistically significant differences between collagen concentrations were noted in the formulations P3 and P4. Comparing different bath liquids with one another, for each of the tested concentrations it was shown that the cells subjected to the reference liquid without any marine collagen (formulation P1) were characterized by a significantly lower level of viability than that observed in the formulations P2, P3 and P4.

The results obtained after HaCaT cell incubation with the analyzed substance for 24 h indicate no significant differences in proliferation between the evaluated collagen concentrations in the formulations P1, P2 and P4. In the formulation P3, significant differences were found between the collagen concentrations of 0.16% and 10%. Furthermore, an increase in the concentration of collagen in the bath liquid was shown to be linked to an improved cell viability. Significant differences in the metabolic activity of keratinocytes between different bath liquids were observed only at the collagen concentration of 10% in the formulation P3 containing hydrolysed collagen. The viability of cells in the formulation P3 was shown to be higher than in the formulations P2 and P4. There were no significant differences at the other concentrations.

Summarizing, the tests showed that the addition of marine collagen in bath liquids intended for children blocks the migration of surfactant monomers into the deeper layers of the epidermis where living cells are present, resulting in higher cell viability compared to the collagen-free reference formulation.

The test results (Fig. 1–Fig. 4) demonstrate that the application of collagen in bath liquids significantly reduces the skin irritation potential of the formulations.

Research indicates that the skin irritation effect induced by body wash products is attributable primarily to free monomers of anionic surfactants present in the washing bath. Importantly, the severity of such interactions with the skin depends on the type and concentration of the used compounds [5–15]. Studies that investigate methods to reduce the irritant effect of body wash products, are focused on lowering the concentration of free monomers in the solution or stabilizing the arising surfactant micelles. This is achieved by employing auxiliary surfactants (nonionic, cationic or amphoteric) [5, 9, 35], adding hydrophobic substances to the formulation [5, 13, 14] or using anionic surfactants with a proven limited irritant effect as the main cleaning agents [5, 6]. The incorporation of other types of

compounds into the solution of anionic surfactants results in the formation of mixed micelles which are characterized by much greater stability and size compared to aggregates formed by a single type of surfactant molecules. More permanent binding of monomers in the aggregate reduces the access of individual molecules to the skin surface, which translates into a decrease in the irritant effect. The use of hydrophobic substances, which solubilize in the solution, also leads to the formation of aggregates displaying greater stability and size. In addition, free monomers present in the solution gain an additional interface, where their adsorption may take place. A reduction in the concentration of individual surfactant molecules in the solution and the production of more stable and larger aggregates in the solubilization process result in a decrease of the irritant effect associated with this type of system [5].

The bath liquids were formulated with a combination of surfactants with relatively low CMC values, which indicates that micelles are formed in the washing bath. The presence of such structures does not, however, eliminate surfactant monomers existing in equilibrium with micelles from the system. As a result, the skin irritation potential of the formulation is not sufficiently reduced.

The incorporation of a natural polymer-marine collagen into the prototypical bath liquids for children can also contribute to diminishing the irritant effect. The phenomenon can be explained by interactions (electrostatic, dipole-dipole and hydrophobic) taking place between the polymer and the surfactants found in the solution. Incorporating macromolecules of this type into the surfactant solution provides the monomers present in the solution with an additional interface at which they are adsorbed. Since collagen chains disentangle in response to a solvent, there is a possibility to increase the available sites to which surfactant molecules are able to attach. As a result, they are permanently bound to the polymer chain, forming a polymer-surfactant complex. Consequently, their concentration in the solution is decreased. Micelles arising in the solution from surfactant molecules are also capable of interacting with collagen chains, forming permanent bonds with them. In addition, polymer chains have the capacity to penetrate into micelles, which reduces the mobility of surfactant aggregates and increases their stability and size [5]. In the studied product prototypes formulated with various forms of collagen, the interactions between surfactants and this natural polymer described above bring about a decline in the amount of free monomers in the system, while increasing the size and stability

of the emerging aggregates, which ultimately reduces the skin irritation potential of the formulations. A drop in the concentration of monomers also lowers the intensity of migration of individual surfactant molecules to deeper layers of the skin, as demonstrated in the test performed with human keratinocyte cell lines (HaCaT) (Fig. 3 and Fig. 4). The observed effect becomes more prominent along with an increase in the molecular mass of the additive incorporated into the formulation. The hypothetical mechanism by which the addition of marine collagen contributes to a reduction in the skin irritation effect of the bath liquids is shown in Fig. 5.

Own elaboration based on [35] the mechanism according to which the irritant effect of bath liquids is reduced, as proposed by the authors, involves surfactant interactions with polymers. Interactions between compounds of this type have been widely discussed in the literature, and the observed decrease in the irritant effect is consistent with the results of studies conducted by Draelos et al. [36] who assessed the hydrophobically modified potassium acrylate copolymers in body wash cosmetics. The authors note that the addition of compounds of this type contributes to a significant reduction of the irritant effect of such formulations. Similar results were obtained by Fevol et al. [37] and Walters et al. [1] who, in their studies, analyzed the effect of hydrophobically modified polymers on the irritant effect of cosmetics intended for use in children. Also, Bujak et al. [15] observed a sharp decrease in zein value which was used as a measure of the skin irritation potential of shower gels containing polyvinylpyrrolidone and its complex with wheat protein hydrolysate. In another study the same authors described a potential method to reduce the skin irritation effect of facial cleansers through the application of polyvinylpyrrolidone with different molecular mass. As demonstrated in their study, the observed effect of reduced interaction between the washing formulations and the skin is more pronounced with increasing molecular mass of the used polymer [38]. The authors point out that the decrease in the irritating effect of the formulations which is observed after addition of the polymer is related to decreasing concentrations of free surfactant monomers in the solution due to their adsorption to polymer chains, and micelle stabilization resulting from the formation of complexes with the polymer.

Since the irritant effect may also be linked to the size of aggregates forming in the aqueous solution, the particle size of aqueous solutions of the bath liquids for children was assessed, too. In cosmetics of this type, which are usually aqueous solutions of surface-active agents (anionic, nonionic, amphoteric), mixed micelles are formed. Their shape is linked very closely to the type and concentration of surfactants and additives used in the formulation. Figure 6 shows the particle size distribution in 1% aqueous solutions of bath liquids for children.

The test solutions were shown to be polydisperse systems. One characteristic peak in the 4–10 nm range was observed in all bath liquids under study. Particles of this size are, most likely, surfactant monomers and spherical micelles arising in the process of aggregation of surface-active agents. The highest intensity in the particle size range of 4–10 nm was noted in the reference liquid P1, formulated without marine collagen. This finding might suggest that the solutions contained the largest amount of this type of particles and monomers. Peaks in the range from about a dozen to about 400 nm were probably indicative of the presence of mixed micelles (aggregates of surfactants, polymer and other components of the bath liquids). It was observed that an increase in the molecular mass of marine collagen

added to the bath liquids for children led to an increased size of aggregates forming in water, which translates into an improved level of safety-in-use of the studied product prototypes.

4 Conclusion

The study was an attempt to incorporate various types of commercially available marine collagen in the formulations of bath liquids intended for children to improve the safety-in-use of such products. By adding various forms of marine collagen to bath liquid formulations, products with reduced irritancy can be obtained. As the molecular mass of marine collagen increases, the zein value decreases proportionally. A 65% decrease in zein value was observed in the bath liquid that contains marine collagen with the highest molecular mass (aqua (and) soluble collagen), as compared to the collagen-free reference formulation. The incorporation of various forms of marine collagen into the bath liquids for children resulted in a lower pH increase of BSA tests by about 12%–30% compared to the reference liquid, which improves the safety-in-use of such formulations in relation to skin contact. Tests performed with human keratinocyte cell lines showed a significantly lesser cell viability after the addition of the reference liquid than after the incorporation of marine collagen into the liquids. Also, an increase in the molecular mass of marine collagen in the bath liquids led to an increase in the size of aggregates formed in water. The obtained results are attributed to the hypothetical mechanism proposed in the study based on surfactant-polymer interactions.

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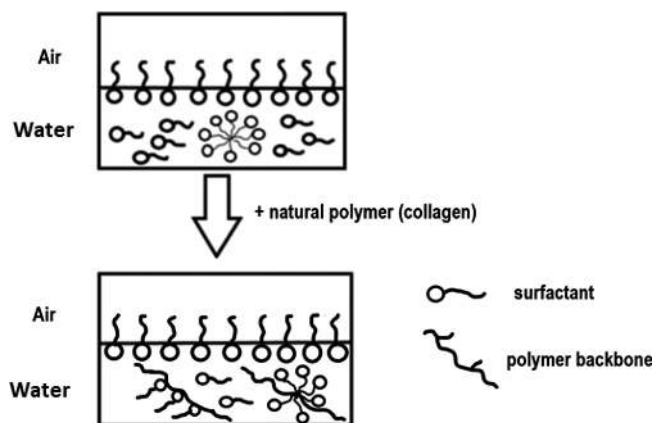


Figure 5 Mechanism of action of polymers with surfactants in aqueous solutions

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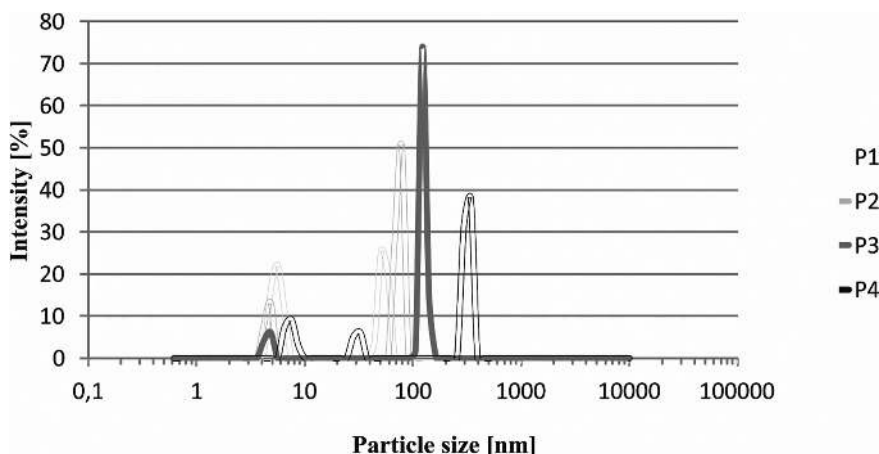


Figure 6 The size distribution for 1% aqueous solutions of bath liquids for children

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