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NEW MICRO AND NANO-STRUCTURED EMULSIONS BASED ON COLLAGEN AND KERATIN HYDROLYSATES

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Abstract: *The aim of the paper is to create new micro and nano-structured emulsions based on multifunctional surfactants (bolaform and gemini) with potential applications in designing foliar fertilizers based on collagen and keratin additives. Collagen and keratin additives were extracted from leather industry by-products using chemical and chemical-enzymatic hydrolyses. Bolaamphiphiles and gemini are new classes of amphiphilic surfactants with large potential of applications due to the high ability to deliver active substances. The preparation of nano and micro emulsions was based on optimisation of main parameters system composition, emulsifiers, shearing speed and temperature in a two-stage process.The used lipophilic non-ionic emulsifier is a long-chain fatty acid ester - isopropyl oleate, the hydrophilic emulsifier is a diester of sucrose and a vegetable oil in order to obtain a multiple water-oil-water emulsion due to the ability of surfactants to orient and make honeycomb formations, at nano and micro scale. The saturation of an aqueous solution of collagen and keratin hydrolysates with microelements was done up to 40% by using 2% of diester of sucrose. Due to properties such as biodegradability, nontoxicity, adherence to surfaces, surfactants based on sugar may be successfully used as fertilizers in agriculture. In our research we have elaborated a new method for including microelements and collagen or keratin hydrolysates in stable emulsions with the final purpose of application as a new class of foliar fertilizer.*

Key words: *surfactant, bolaform and gemini, smart emulsion, collagen, keratin hydrolysate*

1. INTRODUCTION

The aim is to create new micro and nano-structured emulsions based on surfactants (bolaform and gemini) with potential applications in biomaterial design based on leather and wool by-products. Industrial surfactants are divided into four categories based on the presence or absence of electrical charge in solutions: anionic surfactants have a negative ion in the polar group and the cationic ones have positive, nonionic and ampholytic ions. Surfactants having in the same molecule non-polar structural elements (or weakly polar, such as alkyl chains), and strongly polar structural elements (functional groups ionized or not) are adsorbed at interfaces in oriented monomolecular layers. Bolaamphiphiles and Gemini are new classes of amphiphilic surfactants with large potential of applications due to the high ability to deliver active substances [1]. Changes in interactions of surfactants at separation surfaces between phases result in changes in physicochemical properties of heterogenous systems such as: change in superficial tension, change of adhesion energy between phases, of shape and size of volume occupied by a certain phase, etc. These changes underlie a great number of phenomena with significant industrial applications: wetting, emulsification, foaming, detergent, etc.

2. GENERAL INFORMATION

2.1. Obtaining nano-structured emulsions

2.1.1. General characteristics

In this study the nonionic lipophilic emulsifier is a long-chain fatty acid ester - isopropyl oleate, the hydrophilic emulsifier - sucrose diester, and thyme oil. The aim is saturation of an aqueous solution of microelements or collagen/keratin hydrolysate (<40%) in an aqueous solution of sucrose diester (2%) in order to obtain a new foliar fertiziler.

The hydrophilic properties of non-ionic surfactants are provided by hydroxyl groups (-OH), ether linkages (-O-), amide groups (-CONH-), etc. Such groups are found in sugar, polyethers and similar combinations. Nonionic surfactants have a series of important characteristics different from anionic and cationic surfactants. This is due to the lack of electrostatic rejection found in ionic surfactants at the phase separation limit and in the micellar interior, which facilitates the adsorption of non-ionic surfactants on the interphase surfaces and their aggregation in the mycelia. Sugar esters and ethers with tenside properties are known as surfactants based on carbohydrates. Surfactants used in this study are sucrose esters - R alkyl radicals C_8-C_{18} as it is presented in Figure 1.

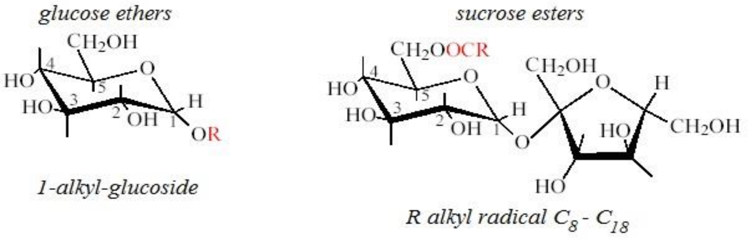

Fig. 1*: Representation of sucrose ethers and esters*

The most important features of these surfactants are: lack of toxicity, high biodegradability, low wetting potential and compatibility with other surfactants. The sucrose diester used in this study is obtained using an excess of methyl ester (sucrose: methyl ester = 1:2 molar ratio) and a small amount of dimethylformamide (12.5:1 relative to sucrose). The formation of diesters is also favored by the presence of small amounts of water. An excess of catalyst is undesirable because in the presence of K_2CO_3 saponification of the methyl ester occurs. Recovery of unreacted sucrose is carried out as follows: the reaction product, after removal of the dimethylformamide, is dissolved in an aqueous NaCl solution (5%) in a proportion of 3-4 parts solution to one part of raw ester, heated to 80-90°C, and maintained at this temperature until the sucrose ester has been decanted completely. After cooling, the ester is separated by filtration or centrifugation. From the aqueous solution, sucrose is recovered by evaporating the water.

2.1.2. Technological process

The two-step emulsification process (Figure 2) is used in this paper, where the result is a multiple nanostructured emulsion due to the properties of surfactants used to orient and form honeycomb formations at the nano and micro levels. Multiple emulsions are complex systems, also called ‘emulsions of emulsions’, in which the dispersed phase droplets contain a continuous phase with other dispersed droplets. The main types of multiple emulsions are water-oil-water and oil-water-oil. Two types of emulsifiers are used: a hydrophobic I one, isopropyl oleate (for W/O emulsion) and a hydrophilic II one - diester of sucrose based on sugar (for O/W emulsion). In the first step, water, vegetable oil (3%), lipophilic-isopropyl oleate surfactant (2%) are added and homogenized by stirring at 60°C to obtain a water-oil emulsion. In step II a solution of microelements saturated up to 40% (or collagen/keratin hydrolysate [2-4]) and a sugar-based surfactant - hydrophilic-diester (2%) are added to the water-oil emulsion, and homogenized by stirring at 60°C, obtaining a multiple water-oil-water emulsion (W/O/W).

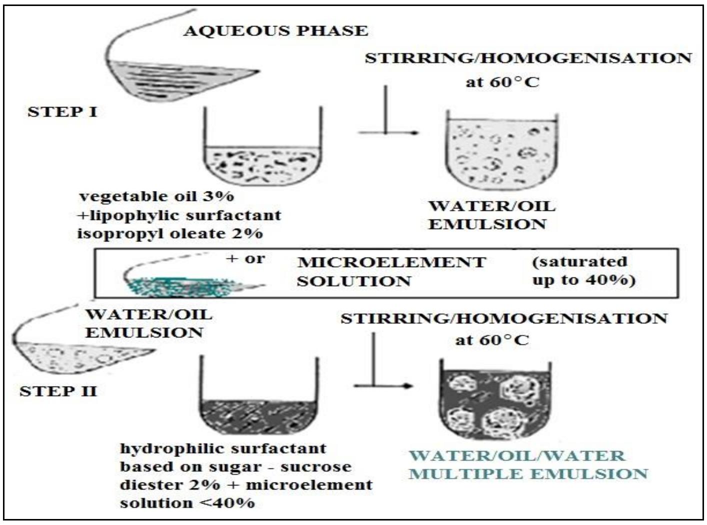

Fig. 2*: Two-step technological proces of obtaining nano- and micro- structured emulsions*

The thermal introduction of phase inversion for oil-water concentrated emulsions is preceded by obtaining a W/O/W emulsion. When an aqueous solution of a hydrophilic emulsifier is introduced into an oil containing a lipophilic surfactant, the W/O/W emulsion is obtained due to the inversion of the phases by modifying the HLB of the surfactant mixture. The phase inversion of multiple emulsions occurs when the dispersed droplets are tightly packed, nano and microstructured into the fluid in which they are suspended. The technological process is analogous to obtaining emulsions by replacing microelements with collagen hydrolysate or a 1:1 hydrolysed collagen-keratin blend. In order to obtain new structured nano and microemulsions an important part is to select the characteristics of the working parameters: system composition, emulsifiers, shear rate, tension, stirring speed, temperature. Long-chain fatty acid esters, including isopropyl oleate, vegetable oils, have been used to vary the physico-chemical properties of multiple emulsions as well as to attempt to control the transfer rate of the solute through the oil phase. Apart from the oil concentration used and the chemical nature, the behavior of the system is also influenced by the physico-chemical characteristics of the oil used, such as density and viscosity. Nonionic emulsifiers are preferred due to their low toxicity and because they hardly interact with other components. It has been demonstrated in literature [1] that it is preferred to use nonionic surfactants to obtain W/O/W emulsions with good yields. To obtain multiple W/O/W emulsions, at least two emulsifiers are introduced into the system, a lyophilic one, in the primary emulsion, and the hydrophilic one to form the secondary emulsion. Generally, multiple emulsions with a single emulsifier cannot be obtained. In a W/O/W emulsion, the optimal HLB value of the primary surfactant ranges from 2 to 7, while for the secondary surfactant it is 6 to 16. To stabilize a multiple system, the second emulsifier that disperses the primary emulsion in a continuous phase is generally less than 1/5 of the primary emulsifier and the HLB value of the emulsifier mixture is less than 10. If the value is high, there is a risk of reversing the phases and forming a single emulsion. If the second emulsifier is in high concentration, a portion of the primary surfactant may be incorporated into the micelle of the secondary one, thereby reducing the concentration of the primary surfactant that stabilized the W/O system. This would lead to the breakage of the oil layer with the loss of internal aqueous droplets. The temperature at which the phase inversion occurs depends on the concentration of the emulsifier mixture and on the HLB values. The higher the temperature, the more stable the O/W emulsion at ambient temperature. Temperature is one of the parameters that must be precisely controlled during the preparation of both the primary and the multiple emulsions. The minimum preparation temperatures are 60°C for the primary emulsion and 10°C for the multiple one. The stirring rate must be at least 800 rpm for the first emulsification and 200 rpm for the multiple emulsion. If the rates were lower, the system would show the tendency to coalesce and/or cream. Multiple emulsions are fragile systems, so the choice of emulsification methods is of particular importance in the success of obtaining the dispersed system with the desired properties. The two characteristic parameters for each multiple system are shear rate and tension. The yield of multiple drop formation decreases rapidly as the homogenization time increases. Structured and stable micro and nanoemulsions are formed, able to incorporate microelements or collagen/keratin hydrolysates with a 40% saturation, and the properties derive from the surfactants used, as well as the conditions and working parameters. In our research we have elaborated a new method for including micoelements and collagen or keratin hydrolysates in stable emulsions with the final purpose of application as a new class of foliar fertilizer.

2.2. Characteristics of nano-strucures emulsions

The three types of emulsions with: microelements (MC), collagen hydrolysates (HC) and collagen/keratin hydrolysate mixture (HK) were characterized by dynamic light scattering and optical microscopy. Dynamic light scattering test showed that all three types of emulsions are nano and microstructured. The size, percentage of the particles and Zeta potential were determined (indicating their stability). Figure 3 shows that MC emulsions have sizes ranging between 42 nm, 225 nm and 5269 nm without stirring (3a) and of 87.7 nm and 449 nm nm after 10 minutes mechanical stirring (3b). Nano size emulsion particle concentration increased from 6.5% to 22% after stirring. Zeta potential is -20.7 mV (3c) without stirring and -21.7 mV after stirring.

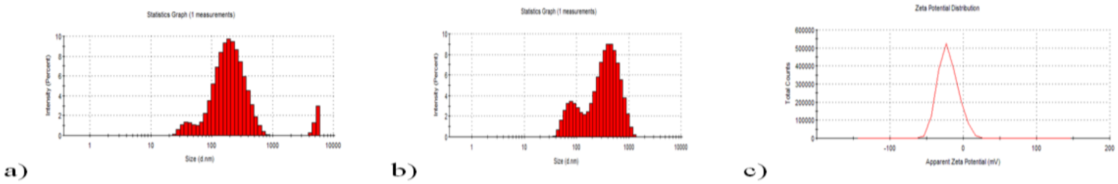

Fig. 3: *Particle size of MC emulsion: a) without stirring; b) after 10 min stirring; c) Zeta potential*

The analyses of HK sample with a collagen/keratin hydrolysate mixture in a 1:1 ratio, particle size and zeta potential, with stirring only, at different times, the experimental results are given in figure 4.

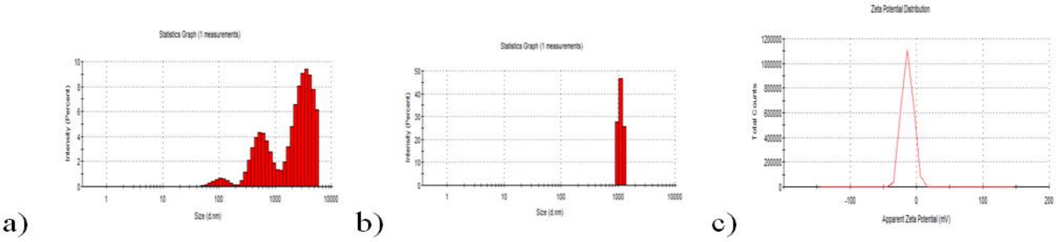

Fig. 4*: Particle size of emulsion HK : a) without stirring; b) after stirring; c) Zeta potential*

Figure 4 shows that HK emulsions have particle sizes ranging between 109.7 nm, 621.8 nm nm and 3371 nm without stirring (4a) and after 10 minutes stirring, the emulsion size is of 1109 nm (4b) with almost same values of -13.5 mV and respectively, -14.2 mV for Zeta potential (4c). For the third sample, HC, with collagen hydrolysate, experimental results are given in figure 5 and show that the emulsion particles are of 44.8 nm and 776.3 nm without stirring (5a) and of 1314 nm (5b) after 10 minutes stirring. Zeta potential is -2.74 mV (5c) without stirring and -4.52 mV, 5 minutes after stirring, showing a tendency to aglomeration. The influence of high molecular weights of collagen and keratin were revealed through higher particle size of emulsions after stirring and lower value for Zeta potential.

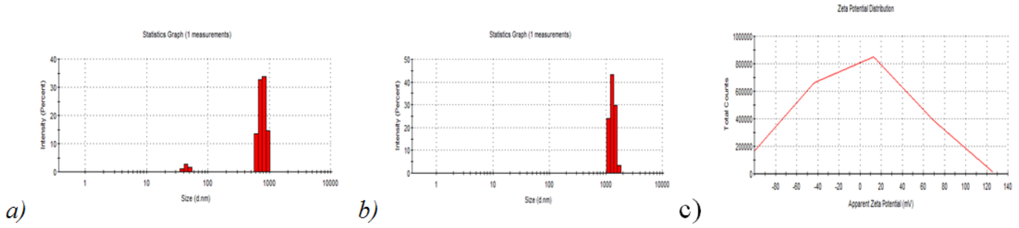

***Fig.** 5: Particle size of emulsion HC: a) without stirring; b) after stirring* ; c) *Zeta potential*

Optical microscopy images (Figure 6) show that emulsions with collagen/keratin hydrolysate (HK) and emulsions with microelements (MC) are structured in honeycomb formations and only the collagen hydrolysate (HC) emulsions are oriented and agglomerated in a chain. The results are in agreement with literature data [5] related to the formation of honeycomb and chain structures in multiple water-oil-water emulsions.

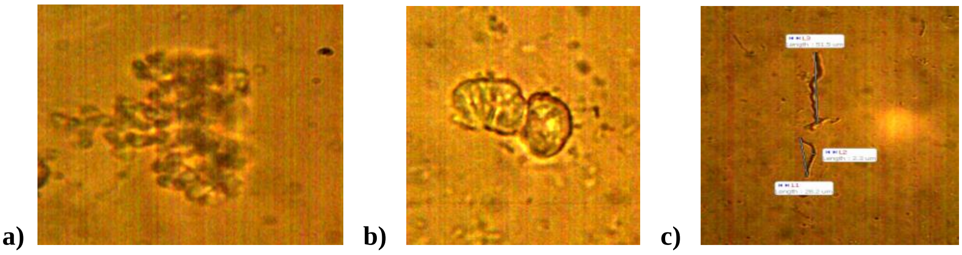

***Fig.** 6: Optical microscopy images (1000x) of emulsions structures:a) honeycomb of HK emulsion; b) honeycomb of MC of ;c) chain structure of HC emulsion*

The three samples were also microbiologically analysed, to determine behaviour to fungal attack of *Fusarium spp*, *Penicilium spp*, *Aspergillus flavus*, and carrying out analysis three days from inoculations, with images presented in figure 7.

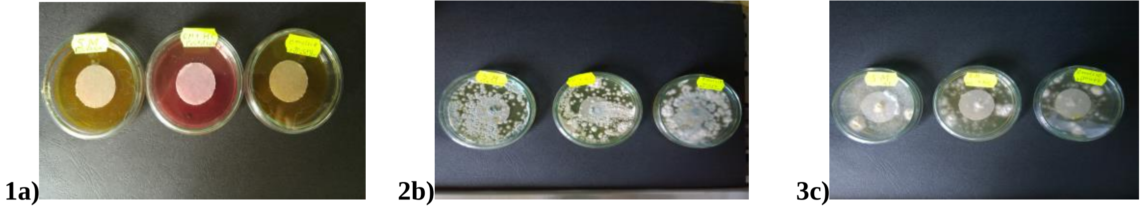

***Fig.** 7: Microscopy images of 1) HC; 2) HK;3) MC emulsion upon attack of: a) Fusarium spp; b) Penicilium spp; c) Aspergillus Flavus*

The best results were obtained for *Fusarium spp*, a specific saprotrophic and pathogenic fungus for its colonization of cereal grains and legumes. Due to properties such as biodegradability, nontoxicity, adherence to surfaces [6], surfactants based on sugar may be successfully used for designing new foliar fertilizers for agriculture. The new multiple emulsions are original due to the succesful inclusion of collagen and keratin hydrolysate with high potential for plant and seed biostimulation and nutrition. The research are in progress regarding the experimental of the new multiple emulsions in biostimulation and nutrition of cereal plants.

3. CONCLUSIONS

- The most effective method of obtaining multiple emulsions is the two-step emulsification process; inversion of multiple emulsion phases occurs when the dispersed droplets are packed tightly into the fluid in which they are suspended.
- The obtained multiple emulsions showed structures of honeycomb and chain with size particles of 42 nm to 5269 nm and Zeta potential from -2.74 mV to -21.7 mV and good resistance to *Fusarium spp* which recommend them for agriculture use.

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