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Olive oil and hyperthermal water bigels for cosmetic uses

F R. Lupi[1], L. Gentile[2], D. Gabriele[1], S. Mazzulla[3], N. Baldino[1], B. de Cindio[1]

[1] Department of Information, Modeling, Electronics and System Engineering, (D.I.M.E.S.) University of Calabria, Via P. Bucci, Cubo 39C, I-87036 Rende (CS), Italy

francesca.lupi@unical.it; domenico.gabriele@unical.it; noemi.baldino@unical.it; bruno.decindio@unical.it

[2] Department of Chemistry and Chemical Technologies, University of Calabria, Via P. Bucci, Cubo 14D, I-87036 Rende (CS,) Italy

luigi.gentile@unical.it

[3] Department of Biology, Ecology and Earth Science (Di.B.E.S.T.), University of Calabria, Via P.Bucci Cubo 6C, I-87036 Rende (CS,) Italy

sergio.mazzulla@unical.it

Corresponding author

Dr. Domenico Gabriele

Department of Information, Modeling, Electronics and System Engineering (D.I.M.E.S.)

Via P. Bucci – Cubo 39C,

I-87036 Arcavacata di Rende (CS), Italy

Email: domenico.gabriele@unical.it

Tel. +39 0984 496687; Fax +39 0984 494009

ABSTRACT

Bigels are biphasic systems produced with an organogel (or oleogel) and a hydrogel mixed together at high shear rates. These systems are promising for different uses, among them the formulation of new cosmetic matrices for cosmetic agents delivery is under investigation. In the present paper, a common cosmetic formulation for skin care was enriched with increasing fractions of monoglycerides of fatty acids/olive oil organogels, in order to understand the rheology and the microstructure of these systems. Small Amplitude Oscillation Tests, NMR-self diffusion analysis, contrast phase microscopy and electric conductivity confirmed that the addition of the organogel caused a microstructural change of the starting material, which turned from O/W to a more complex system where, probably, a matrix-in-matrix structure is present at the highest fractions of added organogel.

Keywords: *organogel, oleogel, hydrogel, bigel, cosmetic emulsion, olive oil, monoglycerides of fatty acids*

1. INTRODUCTION

Structured two-phase systems are used in a great variety of industrial fields (food, cosmetic, pharmaceutical) owing to their peculiar properties coming from the presence of two different phases (usually a lipophilic and a hydrophilic ones) and to the possibility of finely tuning the consistency of the final product by modifying both the consistency of the single phases and their relative amount. Different systems can be obtained, according to the structure level of the single phases. When only the continuous phase is structured these systems are, usually, named “emulgels” or “filled gels” to take into account both the emulsion-like behaviour of these materials and the relevant role played by the solid-like dispersing phase [1-5]. When only the internal phase is gelled they behave mainly like suspensions made of soft (and deformable) particles with a maximum packing fraction according to the particle rheological properties [6]. When both phases are structured a more complex behaviour, according to the properties of both phases, is observed and the systems can be classified as “bigels” or “biphasic gels” [5, 7, 8]. Emulgels and bigels are particularly interesting owing to their potential use in the cosmetic and pharmaceutical area because they can merge the advantages of emulsions and gels (both hydrogels and organogels) to control drug delivery for topical and transdermal use [5].

In the case of cosmetic formulations, in particular face and body/skin care creams, all the mechanical and textural properties requested by consumers should be guaranteed by manufacturers. In the light of this, these materials have to be humectant, emollient, skin softeners and, often, a carrier for active ingredients (antioxidants, vitamins, colouring agents or pigments) which should permeate through the skin [9]. In addition, in order to

meet consumers' preference they should ensure specific rheological properties i.e. smoothness, thickness but also spreadability, often influenced by the texture of the product [10]. Another very important characteristic of cosmetic preparations is high storage stability, which has to be maintained over several months or, in some cases, years [11]. In fact, cosmetic products such as lotions or creams are formed by two immiscible phases, one polar (the aqueous phase) and the other one nonpolar (the oil phase). According to their different physical and chemical properties, biphasic systems can incur phases instability such as creaming, coalescence, flocculation, sedimentation, Ostwald ripening, and phase inversion [12]. The modification of the mechanical properties of both the aqueous and the oil phase (by thickening and/or gelation) is, therefore, necessary for improving the spreadability and the smoothness of the cream and, on the other hand, for enhancing its storage stability. In fact, owing to the difficulty of droplets to move easily through the three-dimensional network of the gel, they cannot aggregate or flocculate, stabilizing the system.

Bigels are interesting innovative two-phase systems that have been recently proposed [5, 13] as structured systems able to properly control the delivery of active ingredients; they possess the advantages typical of both gels, such as: ease of preparation, absence of huge quantities of surfactants sometimes toxic, and possible controlled delivery of both lipophilic and hydrophilic active agents [5, 8]. Looking at these interesting properties, a growing number of authors is studying bigels as active agent carriers. Rhee et al, proposed bigels for ketoprofen transdermal administration [13]; Behera et al. investigated the physical properties of bigels made of synthetic polymers hydrogels (PVA and PVP), and sunflower oil and span 40 oleogels, for the delivery of metronidazole [14]; metronidazole was also added to carbopol hydrogel/Sorbitan

monostearate-sesam oil organogel studied by Singh et al., [15], and the controlled delivery of ciprofloxacin was studied, again by Singh et al. [8], with a guar gum hydrogel and sorbitan monostearate-sesame oil based organogel.

All the cited papers discuss the mechanical and microscopic characteristics of bigels. Singh et al. [8] studied the microscopic morphology of samples and the rheological properties in terms of viscosities and 'creep and recovery' tests; the same group carried out also small amplitude oscillation tests for similar systems [7] and fluorescent microphotographs were moreover taken in their work based on carbopol-based bigels [15]. Texture analysis (creep recovery tests) were also taken by Satapathy et al. [4] in their study comparing gelatin-based hydrogels, emulsion hydrogels, and bigels, while Rehman et al. [16] characterized the polarized optical microscopy, viscosity and texture properties of polymer-fish oil bigels. Finally, flourence microscopy and rheological tests (texture analysis, viscosity, stickiness and stress relaxation) was investigated by Behera et al. [14]. Apart from these techniques of investigation, no other works were published dealing with the microstructure of the systems and further investigations can be very useful to better understanding structure and behaviour of these materials.

In the present paper, structured two-phase systems (bigels) were prepared and investigated by using rheological methods and Nuclear Magnetic Resonance (NMR) techniques aiming at better understanding the relationship between rheological properties and microstructure and to describe the effects of the structured dispersed phase on final product behaviour. In fact, different fractions of olive oil organogels were added to a typical basis for oil-in-water cosmetic creams, and different techniques were

used to study the effect of this addition on the rheological and microstructural properties of the systems.

Colloidal suspensions characterisation by using Nuclear Magnetic Resonance (NMR) techniques is a vast and even expanding scientific endeavour [17, 18]. Owing to its non-invasive nature, and ability to probe structures over a broad range of length scales, NMR has established itself as a tool for the structural characterisation of anisotropic domain sizes in liquid crystals [19], fluctuations in ordered lamellar phases under weak shear [20] and the sizing of shear-induced multi-lamellar vesicles [21, 22]. It was also used for cosmetic emulsions [23] and food emulsions [24-27] characterisation. With respect to two-phase systems, NMR offers a variety of unique insights into both molecular orientation and transport kinetics within the various phases. One of the major contributions arguably is the possibility of easily deciding whether a given emulsion is of the discrete oil-in-water (O/W), discrete water-in-oil (W/O), or bicontinuous type from the oil and water self-diffusion coefficients [28]. Moreover, it is used to provide the droplet size distribution (DSD) of the emulsions [17, 27] and even long-range order of droplets in structured emulsions [24, 29, 30]. Even though a vast literature is available on emulsion studies, to the best of our knowledge, there is no available work on NMR characterisation of bigels. Starting from these considerations it has been considered interesting to use this non-invasive powerful technique to investigate bigel evolution with increasing oleogel content. Moreover, the characterisation was completed by using phase contrast microscopy and electrical conductivity determination to evaluate potential changes in relative distribution of the phases.

Bigels investigated in this paper are based on sulphurous hyperthermal water, and olive oil. Hyperthermal water is well-known because it exerts anti-inflammatory, anti-pruriginous, keratoplastic and keratolytic effects [31]; olive oil, used alone or as an ingredient in dermo-cosmetology and topical applications, shows therapeutic effects (e.g., anti-inflammatory, anti-neoplastic, and anti-aging), and prevents skin alterations physiologically caused by time and different external irritating factors [32].

The aqueous phase was structured by using a common hydrocolloid, acting as gelling agent and thickener, such as potato starch. Olive oil was organogelled with a mixture of monoglycerides of fatty acids. Previous studies were carried out to investigate the main characteristics of different kinds of organogels [33, 34], and in particular, monoglycerides/olive oil organogels alone [34, 35], and mixed with other semisolid matrices [36] were investigated. The organogel was added to the base cream at room temperature, and the bigel preparation resulted easy, giving very smooth and homogeneous materials.

2. MATERIALS AND METHODS

2.1 Materials

Bigels were produced by adding different amounts of an olive oil/monoglycerides of fatty acids organogel to a typical cosmetic oil-in-water structured emulsion basis for skin care, named E0. A schematic description of bigel production is sketched in Fig. 1.

2.1.1 Cosmetic base cream preparation

Sample E0 (Table 1) is a newly formulated O/W emulsion (base) which was found stable after evaluating for pH, electrical conductivity, centrifugation, phase separation, temperature stability tests (data not shown). It was prepared according to a commercial formulation kindly suggested by SA.TE.CA. S.r.l, a local company that produces skin care products with sulphurous hyperthermal water. All ingredients adopted for E0 preparation were supplied by A.C.E.F. (Italy) except for modified potato starch (from Maycos, Italy) and essential oils (from Muller & Koster, Italy).

For emulsion preparation, both the oil and the aqueous phases were separately heated up to 70°C, and the emulsions were prepared by the addition of oily phase to the aqueous phase in a standard turbo-emulsifier for 20 minutes at 900 rpm (CCT, Timatic, Italy). The mixing operation continued at different speeds at 35°C and finally essential oils were also added to the mixture.

This system can be considered as an "emulgel" or a "filled gel", according to the classification previously described, because the dispersing water phase is structured by potato starch gelatinisation that occurs when potato starch is heated in the presence of water at temperatures ranging, approximately, between 60°C and 70°C [37].

2.1.2 Organogel preparation

Organogel was prepared with an extra virgin olive oil (Gabro, Italy) as the solvent, and with a mixture monoglycerides of fatty acids (Myverol 18 04 K, Kerry Group, Ireland, referred to as Myverol throughout the paper) [35], antioxidant agents (α-tocopherol and ascorbyl palmitate, A.C.E.F., Italy) and a certain amount of *algae*, an extract of algal microflora, kindly supplied by SA.TE.CA. S.r.l. Algal cultures, mainly consisting of *cyanobacteria* and *sulfobacteria*, were grown into a "*sulphuretum*" made of shallow-

water hyperthermal tubs (46° C). The products of their metabolism constitute an organic matrix containing significant amounts of unsaponifiable lipids, carotenoids and retinoids that possess some antioxidant function and anti-inflammatory properties [38]. The exact composition of the organogel is listed in Table 2. The Myverol amount is intermediate between the values of samples previously investigated in the literature [39], and therefore, its rheological properties should be intermediate as well. It was produced, following a procedure already adopted, in the literature, for organogel preparation [1]: the oil, with α-tocopherol, *algae* and ascorbyl palmitate, was mixed using a laboratory stirrer (RW 20, IKA, Germany) and it was heated up to 70°C in a water bath thermostated by a plate heater (Jolly 2, Falc Instruments, Italy). Then the organogelator was added and the mixture was stirred for 15 minutes. Finally the system was gently stirred and cooled down with a fast cooling rate quenching it in a thermostatic cold bath (at 0 °C) down to the final temperature of 20 °C. In this case the oil phase was poured into a round aluminium vessel, where the final thickness of the cold hard fatty phase reached a maximum value of about 1 cm, in order to obtain a fast and, approximately, uniform cooling [1].

2.1.3 Bigel preparation

The base emulgel (sample E0) was modified by introducing the organogel and varying the aqueous phase/oil phase ratio, according to Table 3. In order to promote the organogel dispersion within the emulgel E0, vegetable glycerol (A.C.E.F., Italy) was also added, according to the company empirical knowledge and as suggested by the literature [40], in a total amount of 0.5 g of glycerol per 100 grams of final samples for *E5*, and 3.5 grams per 100 grams of final sample for the other emulsions. Samples E5-E25 were prepared by mixing sample E0 with the right amount of organogel and

glycerol at room temperature, by using a commercial blender (Minipimer MQ100, Braun, Germany) working at 700 rpm for 5 min. In this way, a bigel made by a starch hydrogel and a Myverol organogel was prepared. Anyway, it is worth noting that the investigated system is more complex than a "common" bigel because also oil droplets, coming from the starting emulgel, are still present in the final material.

2.2 Rheological characterisation

All samples were investigated with a rheological analysis aimed at characterising the mechanical properties and their possible relationship with the bigels microstructure.

Small Amplitude Oscillation Tests (SAOTs) were performed in the linear viscoelastic region. In particular, frequency sweep tests at 25°C and 36°C (with the aim of simulating the typical temperature of human body) were carried out in the range 0.1 - 10 Hz. Moreover, temperature ramp tests were performed at the fixed frequency of oscillation of 1 Hz, increasing temperature from 25°C up to 70°C with a heating rate of 1°C/min. Both tests were performed on a stress control rheometer DSR-500 (Rheometric Scientific, USA) equipped with a parallel plate geometry (ϕ=25 mm, gap 2.0±0.2 mm). Temperature was controlled with a Peltier system acting under the lower plate of the rheometer.

From a rheological point of view the investigated systems can be considered as weakly structured materials with rheological "units" that are connected by weak bonds and build a three-dimensional network [1, 41]. Being the complex modulus G^* a combination of the storage modulus G' and the dissipative one G'', as shown in eq. (1),

according to the model, data can be fitted using a two-parameter power law equation [41]:

$$G^*(\omega) = \sqrt{(G')^2 + (G'')^2} = A \cdot \omega^{\frac{1}{z}} \quad (1)$$

where the parameter *A* is the "strength of interactions" or the "strength of the gel" and is related to the strength of the interactions among the rheological units, whereas *z* is the "coordination number" or the "network extension" and it is related to the number of interacting rheological units within the 3-D network [41]. When *A* increases, the interaction forces within the network increase, whereas a high *z* value indicates a large number of interacting units cooperating and increasing the network connectivity making the system more "structured"; therefore *z* by extension, could be considered a "structuration degree". Data fitting is carried out through Table Curve 2D Software (Jandel Scientific, USA).

2.3 Electric conductivity

The electric conductivity (κ) was measured by means of an Orion model 120 Microprocessor Conductivity Meter fitted with an electrode having a cell constant of 1.02 cm^{-1}. The temperature was kept at 25.0 ± 0. 1°C and was maintained by a Lauda M-20 thermostat. The error limit of conductance measurements was ±0.02 μS cm^{-1}.

2.4 ^{1}H Self-Diffusion NMR

Self-diffusion coefficients were measured by a Diff30 Z probe with a maximum gradient strength of 1200 G·cm^{-1}. They were determined on a Bruker Avance 300 spectrometer operating at 300.0 MHz on ^{1}H nuclei. The diffusion experiments were performed using the longitudinal eddy current delay - pulse gradient stimulated-echo (LED-PGSTE) [42] sequence. LED is a method to avoid eddy current artefacts placing an eddy current delay after the application of a gradient pulse. In this case a delay of 6 ms was used for all experiments. In the LED-PGSTE sequence, as well as in the PGSTE a pair of trapezoidal narrow magnetic field gradient pulses with amplitude g and duration δ encode for spin displacement over a controlled observation time Δ. By applying the pulsed magnetic field gradients along the z-direction the corresponding diffusion coefficient can be determined. The experimental conditions to measure water self-diffusion coefficients were preliminary optimised for these sample and they are: Δ = 140 ms, δ = 2 ms and g were varied from 1 to 50 G·cm^{-1} in 16 gradient steps, while for oil self-diffusion Δ = 30 ms, δ = 2 ms and g were varied from 10 to 400 G·cm^{-1} in 16 gradient steps.

The spin−echo decays were analyzed according to Stejskal and Tanner [43], however some of the acquired data did not fit to a mono-exponential decay, consequently the following equation was used to fit the experimental data

$$I = I_0 \sum_{n=1}^{k} f^n e^{\left[-D_n(\gamma\delta g)^2\left(\Delta-\frac{\delta}{3}\right)\right]} \qquad (2)$$

Where I and I_0 represent the water resonance peak intensity in the presence and absence of field gradients and γ is the proton gyro-magnetic ratio, while f^n is the weight of the n-th diffusion coefficient D_n (i.e. the fraction of molecules with that coefficient). For

water self-diffusion experiments *k* was equal to 2, while for the oil self-diffusion a mono-exponential decay was fitting well the data. All measurements were performed at 25.0±0.5 °C., which was controlled using the standard variable temperature unit of Bruker spectrometers based on an air-flow system.

2.5 Contrast phase Microscopy Tests

The inner microstructure of the emulsions was investigated with optical microscopy. The microphotographs were taken using a contrast phase microscopy (MX5300H, MEIJI, Japan) equipped with phase contrast objective 40X. Samples were diluted to reduce both the potential presence of droplets aggregates (and therefore making photomicrographs more easily analysable) and the sample opacity [44]. All samples were placed onto a glass slide inside a cover-imaging chamber (Sigma Aldrich, Germany) on which a cover slide was put. The cover chamber forms a sealed volume for thick and free-floating specimens.

3. RESULTS AND DISCUSSION

3.1 Rheological characterisation

The rheological characteristics of the systems investigated in the present work are similar to those of solid-like weakly structured gel creams commonly used in cosmetic or pharmaceutical applications. In a frequency sweep test, at both the investigated temperatures of analysis, complex modulus is slightly dependent on the frequency of oscillation, and loss tangent is lower than unity in the whole range of investigated

frequency (Fig. 2). According to weak gel model, parameters *A* and *z* were evaluated (coefficient of determination R^2 for all fittings ranges between 0.92 and 0.99) for each emulsion and at both temperatures of analysis (i.e. 25 and 36°C, see Fig. 3). It is worth noticing that the network strength, described by parameter *A*, increases with the organogel content, or, by extension, with oil phase, up to sample E10. When the oil phase content is further increased, a first decrease of *A* is evidenced, for sample E15, and a following new increase is observed in samples E20 and E25.

On the contrary, the network extension follows an opposite trend: structuration seems to decrease starting from E0 up to E10 (see parameter *z*), then it increases in sample E15 and afterwards it decreases again up to sample E25. Similar behaviours are observed at both 25°C and 36°C, even though the trend of the structuration degree (parameter *z*) is less evident at 36°C with respect to lower temperature data.

The observed trend is quite unexpected because with increasing organogel content (and therefore with dispersed structured fraction) an increase in moduli (and therefore in A) was expected.

On the other hand it is known that in the case of O/W emulsions, the increase of the dispersed phase fraction is responsible for the increase of emulsions viscosity up to a maximum oil fraction [45]. Exceeding this value, a phase inversion can occur, and, at first, viscosity decreases, increasing again if further fractions of oil are added to the system. As an example, Abdurahman et al. [46] studied the case of crude oil in water emulsions, finding that a slope change in the curve of emulsions viscosity *versus* oil fraction identified a phase inversion of the emulsion. It is obvious that systems shown in the present paper are different and more complex than the cited crude oil emulsions,

also because the rheological properties of both the oil and aqueous phase are different. Anyway, the change in viscosity, as function of oil fraction, observed in the present work is very similar to that observed in the cited paper suggesting that a structural change in biphasic microstructure could occur between samples E10 and E20, which can be generally attributed to either the inversion of phases or the formation of bicontinuous bigels.

Nevertheless, the rheological characterisation, alone, is not able to give more detailed information about microstructure, therefore different techniques were used in order to better understand the potential changes.

The addition of an organogel within the starting emulsion matrix changes also the stability of the system. Recently, temperature ramp tests were proposed as a valid method to simulate the forced accelerated destabilisation of cosmetic emulsions, if compared with the common accelerated aging tests [2], and a constant trend of both moduli with temperature can be attributed to a more stable system. Fig. 4 shows the temperature ramp tests in terms of complex moduli and loss tangents *versus* temperature of sample E0 compared with E25. Both samples evidence an almost constant trend of complex modulus at low temperature, followed by a more pronounced reduction of G* starting at approximately 45°C. On the other hand, the loss tangent slightly increases but it is always lower than unity evidencing a prevalent solid-like behaviour of the materials. This rheological behaviour can be attributed to the presence of the starch gel that is not thermoreversible [47] and on heating becomes only softer without melting. As a consequence, it can be speculated that the increase in temperature is not causing the rupture of the two-phase system (that is observed when the structured dispersing phase melts [2, 48]) or other macroscopic instabilities.

It is worth noting that the temperature ramp test does not evidence significant differences between the samples because the thermal behaviour is mainly related to the hydrogel characteristics.

3.2 Electric conductivity

Electric conductivity (κ) was investigated aiming at better understanding the microstructural characteristics of emulsified bigels [1]. Fig. 5 shows the variation of conductivity starting from E0 to E25. The reported results indicate a huge decrease in electric conductivity correspondent to the increasing organogels addition to the starting system. Since sample E0 is an O/W system, the gelled aqueous phase is continuous: this allows the system to conduce well the electric current thanks to the ions naturally present within the thermal water. When an oil phase is added to the system, no matter its rheological characteristics, the electric conduction of the final material is, obviously, reduced. This is because oil phases are natural electric insulants. Therefore, the reduction in conductivity can be attributed to the isolating action of the organogel. Going from sample E10 to E15, a further, stronger reduction in electric conductivity is found, and then an unexpected increase in sample E20 is observed, followed by a further decrease in E25. This trend seems to suggest that more complex changes than phase inversion are occurring within the system. In fact, phase inversion (i.e. the transition from O/W bigels to W/O bigels) should yield almost zero values of electrical conductivity (being the organogel an insulant), whereas experimental results seem to suggest a different spatial distribution of the phases, in such a way that the oil phase, even though present in a larger amount, seems to affect the conductivity less.

3.3 NMR-self diffusion results

The NMR self-diffusion method can distinguish continuous and dispersed phases based on the transport properties of the component molecules [24, 28]. Generally speaking, from the oil and water apparent self-diffusion coefficients it is possible to distinguish between oil-in-water (O/W), water-in-oil (W/O) or continuous type. In O/W emulsions the oil self-diffusion coefficient, D^{oil}, is much lower than water self-diffusion one, D^{w}, in W/O emulsions $D^{w} << D^{oil}$, while in continuous phase $D^{w} \sim D^{oil}$ [24]. However, solvation effects, incomplete segregation into domains or other inhomogeneities can affect the diffusion. In the special case of bigels is reasonable to believe that similar behaviors can be observed for W/O and O/W or bicontinuous bigel emulsions. In these conditions the diffusion can be written

$$D^{w} = f_i^{n} D_i^{w} + f_b^{n} D_b^{w} \quad (3)$$

where D_b^{w} is the diffusion of the bonded water molecules, while D_i^{w} , is the diffusion due to the inhomogeneities and will affect in proportion the fraction of molecules in the same framework f_i^{n} . Comparing with a multiple emulsion the water self-diffusion coefficient coming from the inhomogeneities correspond to the inner phase. Eq. 3 was used to analyse the data in terms of weighted D_b^{w} and D_i^{w} coefficients. Fig. 6 reports both water apparent self-diffusion coefficients and the relative fraction of molecules for all samples studied.

The black colour in Fig. 6 evidences the fraction of bonded water molecules with the corresponding diffusion coefficient. In sample E15, a strong decrease of the D_b^{w} coefficient can be observed, D_b^{w} is also the main diffusion coefficient of the system,

since the fraction of the inhomogeneities is very low at this organogel concentration. Increasing the organogel content the f_i^n grows and it may be related to domains of a secondary emulsion, further investigations are needed on this point.

Fig. 7 shows the oil self-diffusion coefficients as a function of organogel concentration, these data crossed with the previous ones provide a reasonable picture of the evolution of the system. Therefore, systems can be considered as organogel-in-hydrogel (O/W) at low fraction of organogels added to E0 (up to E10), since D^{oil}, is much lower than water self-diffusion, D_b^w. D_i^w has the same order of magnitude than D^{oil} for sample E20 and this can be related to a bicontinuous phase. However, E15 shows an inversion between D_b^w and D_i^w that could suggest a structural change potentially occurring when the highest fractions of organogel are added to the starting system; these structural changes could be attributed to the formation of a complex system, even including a phase inversion. Nevertheless, as already discussed in paragraph 3.2, sample E25 is still characterised by a conductivity value different from zero, suggesting that the system is more complex than a simple W/O one. In the light of this, contrast phase microscopy was also carried out to better understand the real microstructure of the system.

3.4 Contrast phase microscopy

With the aim of confirming the results obtained by NMR-self diffusion indicating a change in structural disposition of both phases forming bigel system, a further investigation of the mutual disposition of oil and aqueous phase was carried out with contrast phase optical microscopy. Fig. 8 shows the results for the investigated samples: E0 shows the typical microstructure of an O/W emulsion, and oil droplets within an aqueous phase can be easily recognised. Starting from sample E5, where organogel was

added in the lower fraction within the oil phase, a more complex microstructure becomes evident.

As a result of all tests carried out on these materials, it was speculated that the addition of the highest fractions of organogel mixed the starting emulgel leads to the formation of a complex matrix-in-matrix system where an organogel is distributed within a hydrogel containing, in turn, droplets of unstructured oil. This system is similar to that shown by Di Michele et al. [49].

These complex structures, different from a simple system where one phase is dispersed within another one, can explain the unexpected experimental results obtained by using the different analytical techniques.

4. CONCLUSIONS

The rheological and microstructural characteristics of cosmetic bigels, i.e two-phase systems where both phases are structured, [5, 7, 8] were investigated. Starting from a cosmetic cream basis, bigels were produced adding increasing fractions of a monoglycerides of fatty acids/olive oil organogel. A microstructural investigation was carried out on bigel samples using techniques already adopted in this area, such as rheological dynamic tests [7] and microscopy [14, 16]. Moreover, material microstructure and the relative position of aqueous and oily phases were investigated by using other experimental procedures, commonly adopted for emulsion characterisation [1, 24, 27] and, so far, not used in bigels analysis. Electrical conductivity was adopted to study the potential change in spatial distribution of phases that could occur when

organogel content increases, whereas Nuclear Magnetic Resonance (NMR), being a powerful non-invasive technique, was used to further analyse the bigel microstructure.

It is worth noticing that owing to the lack of previous works on bigels, carried out by using these techniques, more difficulties were found in data analysis and discussion.

Rheology was also used to investigate the bigel stability with dynamic temperature ramp tests [2] evidencing for all samples satisfying results probably because of the starch hydrogel characteristics.

The increasing addition of organogel fractions to the base emulsion caused the expected growth of samples consistency [34] (indirectly evaluated with parameter *A* of the weak gel model for the first time) up to a specific amount of organogel, correspondent to sample E10, after which a minimum value and a following new increase was found. This unexpected result was further investigated with other techniques and NMR self-diffusion data were discussed on the basis of results obtained for emulsions, owing to the lack of previous data on bigels, suggesting a transition from an O/W system to a more complex spatial arrangement of the phases, similar to that observed for bicontinous emulsions [28]. For the first time the NMR echo-decay analysis was adapted to bigel systems by using a bimodal exponential function.

Microscopy and electric conductivity seem to confirm this evidence suggesting the formation of a complex matrix-in-matrix system, which could be considered, probably, as a bicontinuous bigel at the highest fractions of organogel.

These results evidence that the addition of a structured oil phase to an existing O/W system can significantly modify the microstructure and, therefore, the macroscopic

properties. Probably, owing to the high consistency of both materials, it is very difficult to disperse the organogel within the existing aqueous phase. Therefore, a more complex material, where both phases seem entrapped one inside the other, is obtained. In this way, properties intermediate between those of O/W and W/O bigels can be obtained and, probably, tuned, depending on the ratio between the phases.

Since bigel structure is a result of an out-of-equilibrium process is consequently very hard to be predicted. In conclusion several insight to understand the bigel structure were given.

Nevertheless, further investigations should be carried out on simpler model bigels, by changing also the amount of hydrogel, to confirm the obtained results and to model the rheological properties of the bigel as function of both phases content.

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REFERENCES

[1] F.R. Lupi, D. Gabriele, B. de Cindio, M.C. Sanchez, C. Gallegos, A rheological analysis of structured water-in-olive oil emulsions, Journal of Food Engineering, 107 (2011) 296-303.

[2] F.R. Lupi, D. Gabriele, L. Seta, N. Baldino, B. de Cindio, R. Marino, Rheological investigation of pectin-based emulsion gels for pharmaceutical and cosmetic uses, Rheologica Acta, 54 (2015) 41-52.

[3] G. Houze, E. Cases, B. Colas, P. Cayot, Viscoelastic properties of acid milk gel as affected by fat nature at low level, International Dairy Journal, 15 (2005) 1006-1016.

[4] S. Satapathy, V.K. Singh, S.S. Sagiri, T. Agarwal, I. Banerjee, M.K. Bhattacharya, N. Kumar, K. Pal, Development and Characterization of Gelatin-Based Hydrogels, Emulsion Hydrogels, and Bigels: A Comparative Study, Journal of Applied Polymer Science, 132 (2015).

[5] K. Rehman, M.H. Zulfakar, Recent advances in gel technologies for topical and transdermal drug delivery, Drug Development and Industrial Pharmacy, 40 (2014) 433-440.

[6] H.M. Shewan, J.R. Stokes, Viscosity of soft spherical micro-hydrogel suspensions, Journal of Colloid and Interface Science, 442 (2015) 75-81.

[7] V.K. Singh, S. Ramesh, K. Pal, A. Anis, D.K. Pradhan, K. Pramanik, Olive oil based novel thermo-reversible emulsion hydrogels for controlled delivery applications, Journal of Materials Science: Materials in Medicine, 25 (2014) 703-721.

[8] V.K. Singh, I. Banerjee, T. Agarwal, K. Pramanik, M.K. Bhattacharya, K. Pal, Guar gum and sesame oil based novel bigels for controlled drug delivery, Colloids and Surfaces B: Biointerfaces, 123 (2014) 582-592.

[9] P. Aikens, S.E. Frieberg, Organized assemblies in cosmetics and transdermal drug delivery, Current Opinion in Colloid & Interface Science, 1 (1996) 672-676.

[10] L. Gilbert, C. Picard, G. Savary, M. Grisel, Rheological and textural characterization of cosmetic emulsions containing natural and synthetic polymers: relationships between both data, Colloids and Surfaces A: Physicochemical and Engineering Aspects, 421 (2013) 150–163.

[11] H. Masmoudi, Y. Le Dreu, P. Piccerelle, J. Kister, The evaluation of cosmetic and pharmaceutical emulsions aging process using classical techniques and a new method: FTIR, International Journal of Pharmaceutics, 289 (2005) 117-131.

[12] T.G. Mason, New fundamental concepts in emulsion rheology, Current Opinion in Colloid & Interface Science, 4 (1999) 231-238.

[13] G.J. Rhee, J.S. Woo, S.J. Hwang, Y.W. Lee, C.H. Lee, Topical oleo-hydrogel preparation of ketoprofen with enhanced skin permeability, Drug Development and Industrial Pharmacy, 25 (1999) 717-726.

[14] B. Behera, V.K. Singh, S. Kulanthaivel, M.K. Bhattacharya, K. Paramanik, I. Banerjee, K. Pal, Physical and mechanical properties of sunflower oil and synthetic polymers based bigels for the delivery of nitroimidazole antibiotic – A therapeutic approach for controlled drug delivery, European Polymer Journal, 64 (2015) 253-264.

[15] V.K. Singh, A. Anis, I. Banerjee, K. Pramanik, M.K. Bhattacharya, K. Pal, Preparation and characterization of novel carbopol based bigels for topical delivery of metronidazole for the treatment of bacterial vaginosis, Materials Science and Engineering: C, 44 (2014) 151-158.

[16] K. Rehman, M.C.I.M. Amin, M.H. Zulfakar, Development and Physical Characterization of Polymer-Fish Oil Bigel (Hydrogel/Oleogel) System as a Transdermal Drug Delivery Vehicle, Journal of Oleo Science, 63 (2014) 961-970.

[17] M.L. Johns, NMR studies of emulsions, Current Opinion in Colloid & Interface Science, 2009, pp. 178–183.

[18] M.A. Voda, J. van Duynhoven, Characterization of food emulsions by PFG NMR, Trends in Food Science & Technology, (2009) 533-543.

[19] I. Aslund, C. Cabaleiro-Lago, O. Soderman, D. Topgaard, Diffusion NMR for determining the homogeneous length-scale in lamellar phases, The Journal of Physical Chemistry B, 112 (2008) 2782–2794.

[20] A. Lutti, P.T. Callaghan, Undulations and fluctuations in a lamellar phase lyotropic liquid crystal and their suppression by weak shear flow, Physical Review E, 73 (2006) 011710.

[21] A. Lutti, P.T. Callaghan, Measurement of multilamellar onion dimensions under shear using frequency domain pulsed gradient NMR, Journal of Magnetic Resonance, 187 (2007) 251–257.

[22] L. Gentile, L. Coppola, S. Balog, K. Mortensen, G.A. Ranieri, U. Olsson, Phase coexistence in a dynamic phase diagram, ChemPhysChem, (in press).

[23] J. Plass, D. Emeis, Static and Dynamic NMR Studies on Cosmetic Emulsions, Magnetic Resonance in Colloid and Interface Science, Springer, Netherlands, 2002, pp. 375-382.

[24] B. Balinov, F. Mariette, O. Soderman, NMR studies of emulsions with particular emphasis on food emulsions, Encyclopedic handbook of emulsion technology, Marcel Dekker, New York, USA, 2003.

[25] F. Mariette, Investigations of food colloids by NMR and MRI, Current Opinion in Colloid & Interface Science, 14 (2009) 203-211.

[26] R. Angelico, A. Ceglie, G. Colafemmina, F. Delfine, U. Olsson, G. Palazzo, Phase behavior of the lecithin/water/isooctane and lecithin/water/decane systems, Langmuir, 20 (2004) 619-631.

[27] D. Gabriele, M. Migliori, R. Di Sanzo, C. Oliviero Rossi, S.A. Ruffolo, B. de Cindio, Characterisation of dairy emulsions by NMR and rheological techniques, Food Hydrocolloids, 23 (2009) 611-618.

[28] P. Kumar, K.L. Mittal, Handbook of Microemulsion Science and Technology, Marcel Dekker, New York, USA, 1999.

[29] O. Soderman, A. Lonnqvist, B. Balinov, NMR self-diffusion studies of emulsion systems. Droplet sizes and microstructure of the continuous phase, in: J. Sjoblom (Ed.) Emulsions, a fundamental and practical approach, Kluwer Academic, Netherlands, 1992.

[30] O. Soderman, B. Balinov, NMR self-diffusion studies of emulsions, in: J. Sjoblom (Ed.) Emulsions and emulsion stability, Marcel Dekker, New York, 1996, pp. 369-392.

[31] N. Hjorth, Traditional topical treatment of acne, Acta Dermato-Venereologica. Supplementum, Suppl 89 (1980) 53-56.

[32] P. Viola, M. Viola, Virgin olive oil as a fundamental nutritional component and skin protector, Clinics in Dermatology, 27 (2009) 159-165.

[33] F.R. Lupi, D. Gabriele, V. Greco, N. Baldino, L. Seta, B. de Cindio, A rheological characterisation of an olive oil/fatty alcohols organogel, Food Research International, 51 (2013) 510-517.

[34] F.R. Lupi, D. Gabriele, D. Facciolo, N. Baldino, L. Seta, B. de Cindio, Effect of organogelator and fat source on rheological properties of olive oil-based organogels, Food Research International, 46 (2012) 177-184.

[35] F.R. Lupi, D. Gabriele, B. de Cindio, Effect of Shear Rate on Crystallisation Phenomena in Olive Oil-Based Organogels, Food and Bioprocess Technology, 5 (2012) 2880-2888.

[36] F.R. Lupi, D. Gabriele, L. Seta, N. Baldino, B. de Cindio, Rheological design of stabilized meat sauces for industrial uses, European Journal of Lipid Science and Technology, 116 (2014) 1734-1744.

[37] N. Singh, J. Singh, L. Kaur, N.S. Sodhi, B.S. Gill, Morphological, thermal and rheological properties of starches from different botanical sources, Food Chemistry, 81 (2003) 219-231.

[38] S. Mazzulla, S. Sesti, A. Martini, V. Nicoletta, S. De Stefano, M. Morrone, G. Martino, Evaluation of natural reductants protecting red blood cell membranes against oxidative damage in psoriatic patients, Clinica Terapeutica, 162 (2011) E79-E84.

[39] F.R. Lupi, D. Gabriele, B. Cindio, Effect of Shear Rate on Crystallisation Phenomena in Olive Oil-Based Organogels, Food and Bioprocess Technology, 5 (2011) 2880-2888.

[40] L. Kilpatrick-Liverman, J. Mattai, R. Tinsley, J. Wu, Mechanisms of Skin Hydration, in: A.O. Barel, M. Paye, H.I. Maibach (Eds.) Handbook of Cosmetic Science and Technology, Informa healthcare USA, Inc., New York, USA, 2009, pp. 91-106.

[41] D. Gabriele, B. de Cindio, P. D'Antona, A weak gel model for foods, Rheologica Acta, 40 (2001) 120-127.

[42] S.J. Gibbs, C.S.J. Johnson, A PFG NMR experiment for accurate diffusion and flow studies in the presence of eddy currents, Journal of Magnetic Resonance, 93 (1991) 395–402.

[43] E.O. Stejskal, J.E. Tanner, Spin Diffusion Measurements: Spin Echoes in the Presence of a Time-Dependent Field Gradient, The Journal of Chemical Physics, **42** (1965) 288.

[44] L. Seta, N. Baldino, D. Gabriele, F.R. Lupi, B. de Cindio, The influence of carrageenan on interfacial properties and short-term stability of milk whey proteins emulsions, Food Hydrocolloids, 32 (2013) 373-382.

[45] H.A. Barnes, J.F. Hutton, K. Walters, An Introduction to rheology, Elsevier Science Publishers B.V., The Nederlands, 1989, pp. 19-23.

[46] N.H. Abdurahman, Y.M. Rosli, N.H. Azhari, B.A. Hayder, Pipeline transportation of viscous crudes as concentrated oil-in-water emulsions, Journal of Petroleum Science and Engineering, 90-91 (2012) 139-144.

[47] T. Kaper, M. van der Maarel, G.J.W. Euverink, L. Dijkhuizen, Exploring and exploiting starch-modifying amylomaltases from thermophiles, Biochemical Society Transactions, 32 (2004) 279-282.

[48] J.E. Moros, F. Cordobés, J.M. Franco, C. Gallegos, Enhancement of gel strength by application of thermal treatments in highly flocculated emulsions, Food Hydrocolloids, 17 (2003) 199-206.

[49] L. Di Michele, D. Fiocco, F. Varrato, S. Sastry, E. Eiser, G. Foffi, Aggregation dynamics, structure, and mechanical properties of bigels, Soft Matter, 10 (2014) 3633-3648.

FIGURES CAPTIONS

Figure 1 Scheme of investigated bigel preparation

Figure 2 Frequency sweep tests at 25°C in terms of G*(A) and loss tangent (B) for samples E0-E25. Experimental data (symbols) and weak gel model fitting (lines)

Figure 3 Weak gel model parameter in terms of network strength (A) and extension (B)

Figure 4 Temperature Ramp tests of samples E0 and E25 in terms of complex moduli and loss tangent versus temperature

Figure 5 Electric conductivity, κ, for samples E0-E25

Figure 6 Apparent Water Self-Diffusion Coefficients (D^w_b black diamond, D^w_i grey circle) obtained at 25 °C by using LED-PGSTE sequence (A) and the relative fraction of molecules (f^w_b black column, f^w_i grey column) with the relative diffusion coefficient by using eq. 1 (B).

Figure 7 Apparent Oil Self-Diffusion Coefficients obtained at 25 °C by using LED-PGSTE sequence.

Figure 8 Contrast phase microphotographs for samples E0-E25. Reference bar corresponds to 50 μm

TABLES CAPTIONS

Table 1 Composition of base sample E0

Table 2 Composition of organogel added to sample *E0*

Table 3 Samples ID and compositions

5: Figure 1

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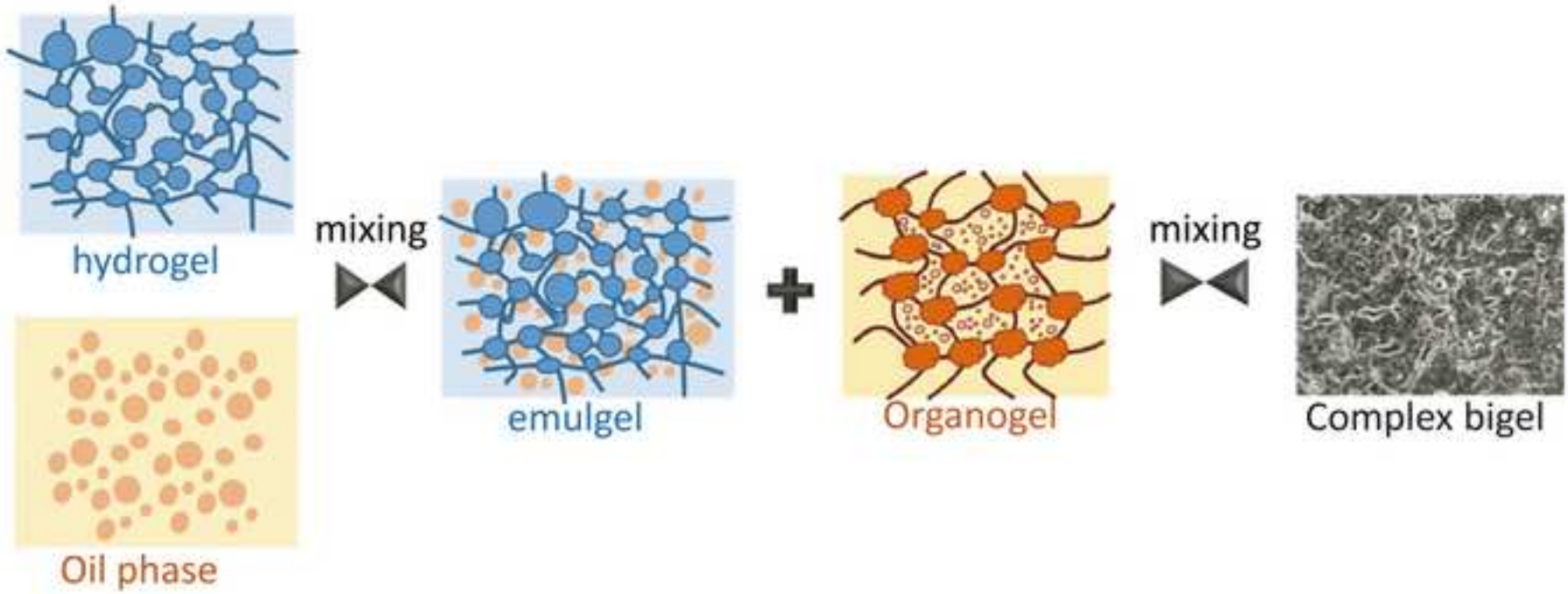

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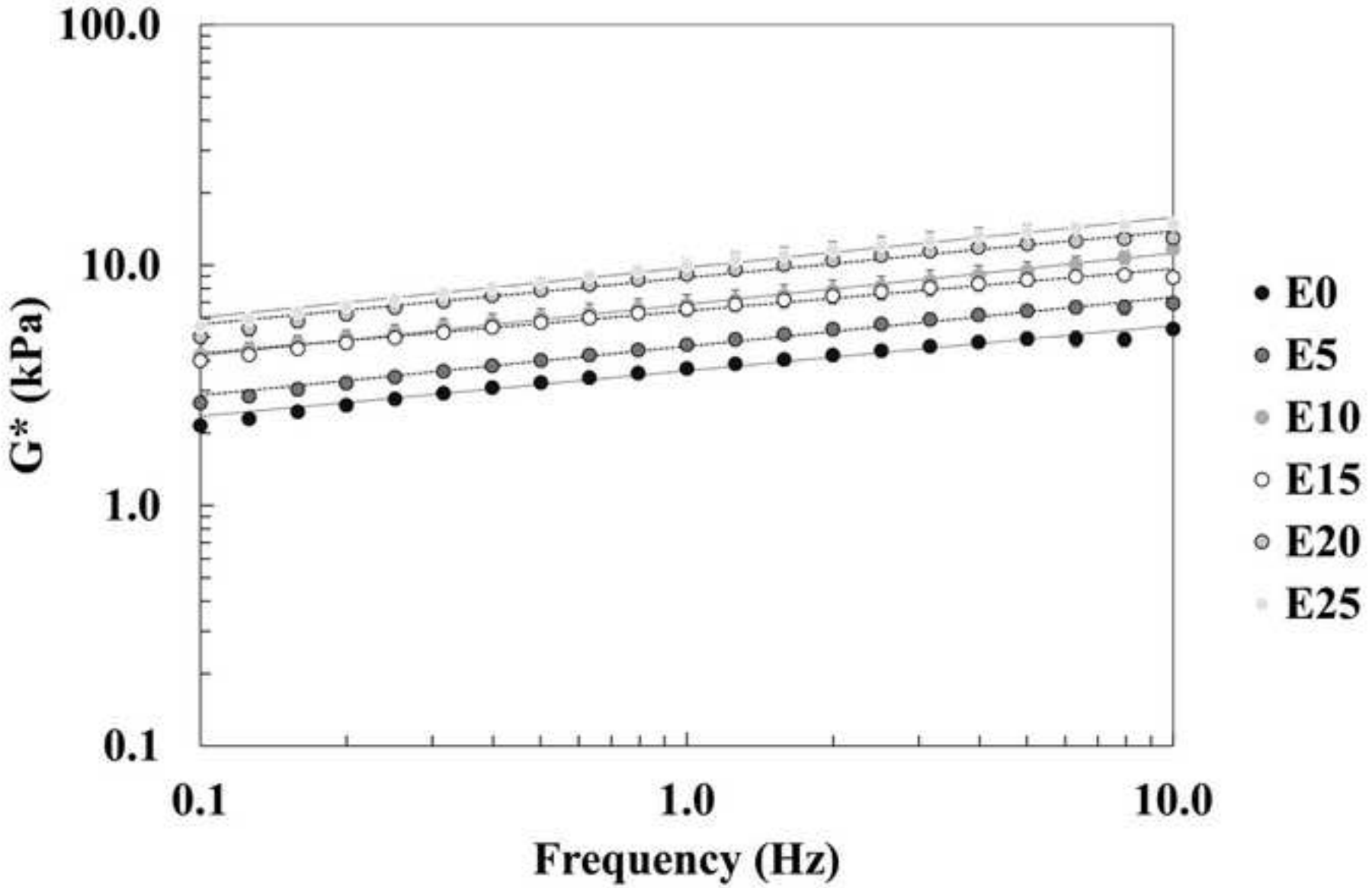

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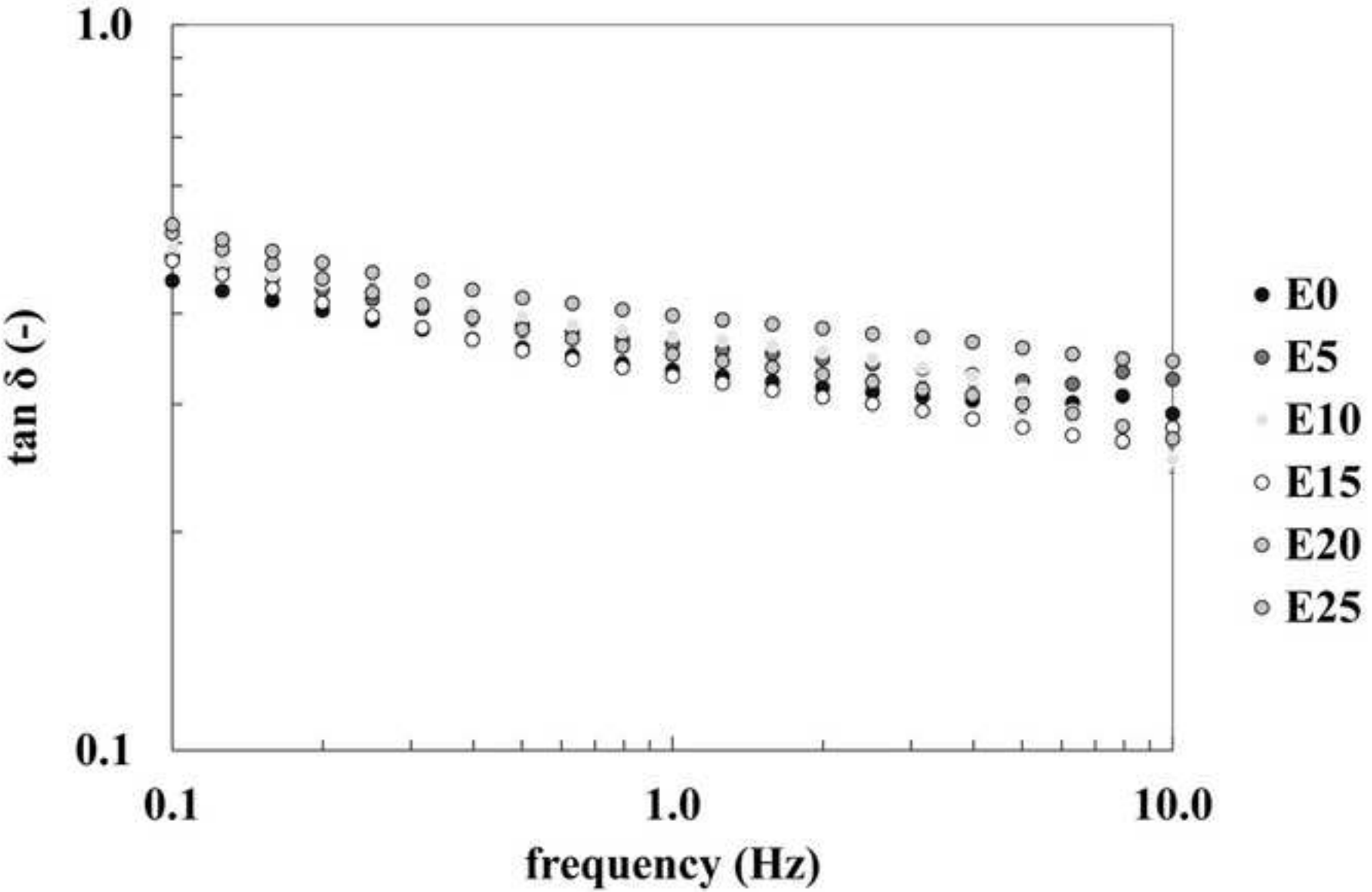

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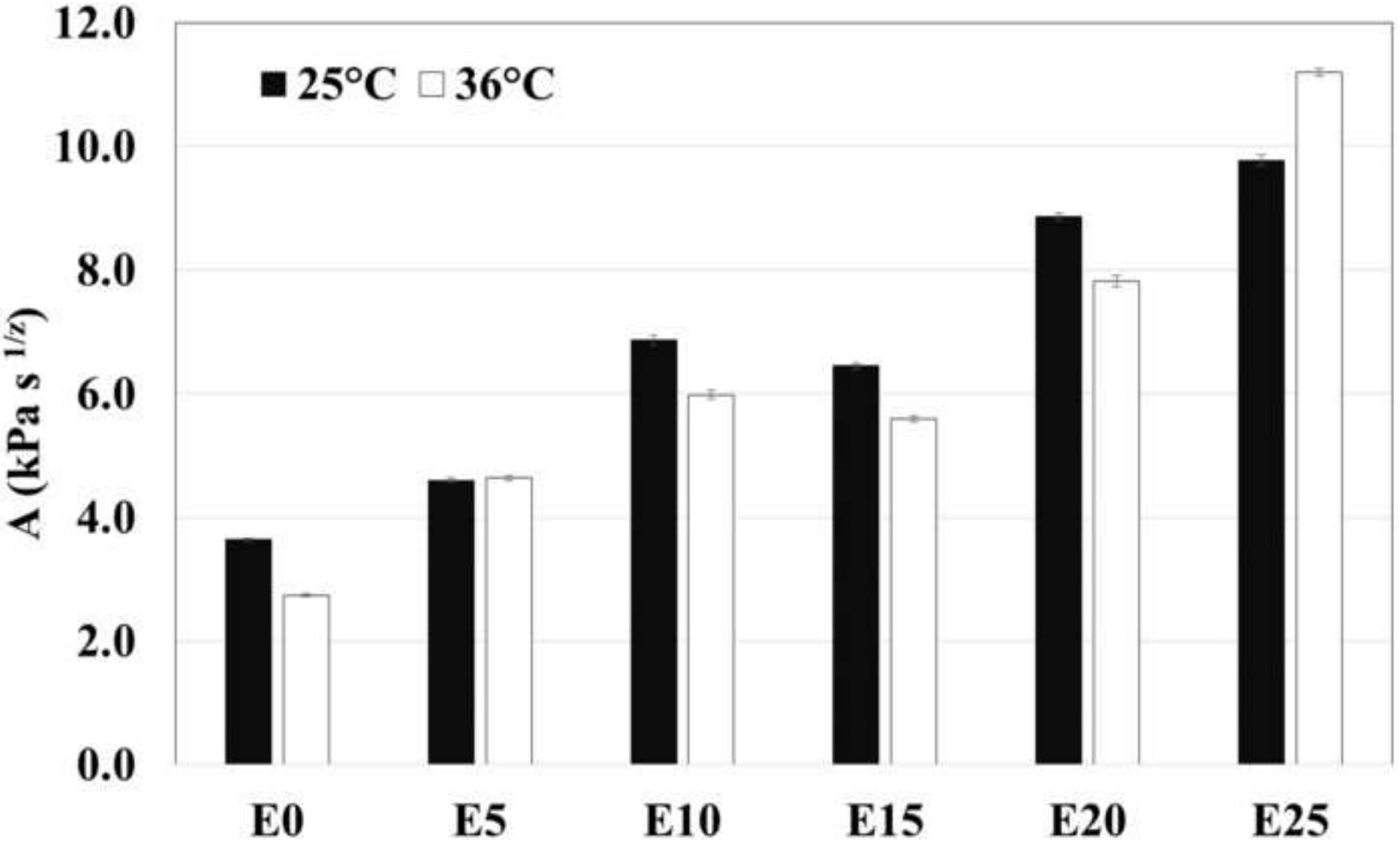

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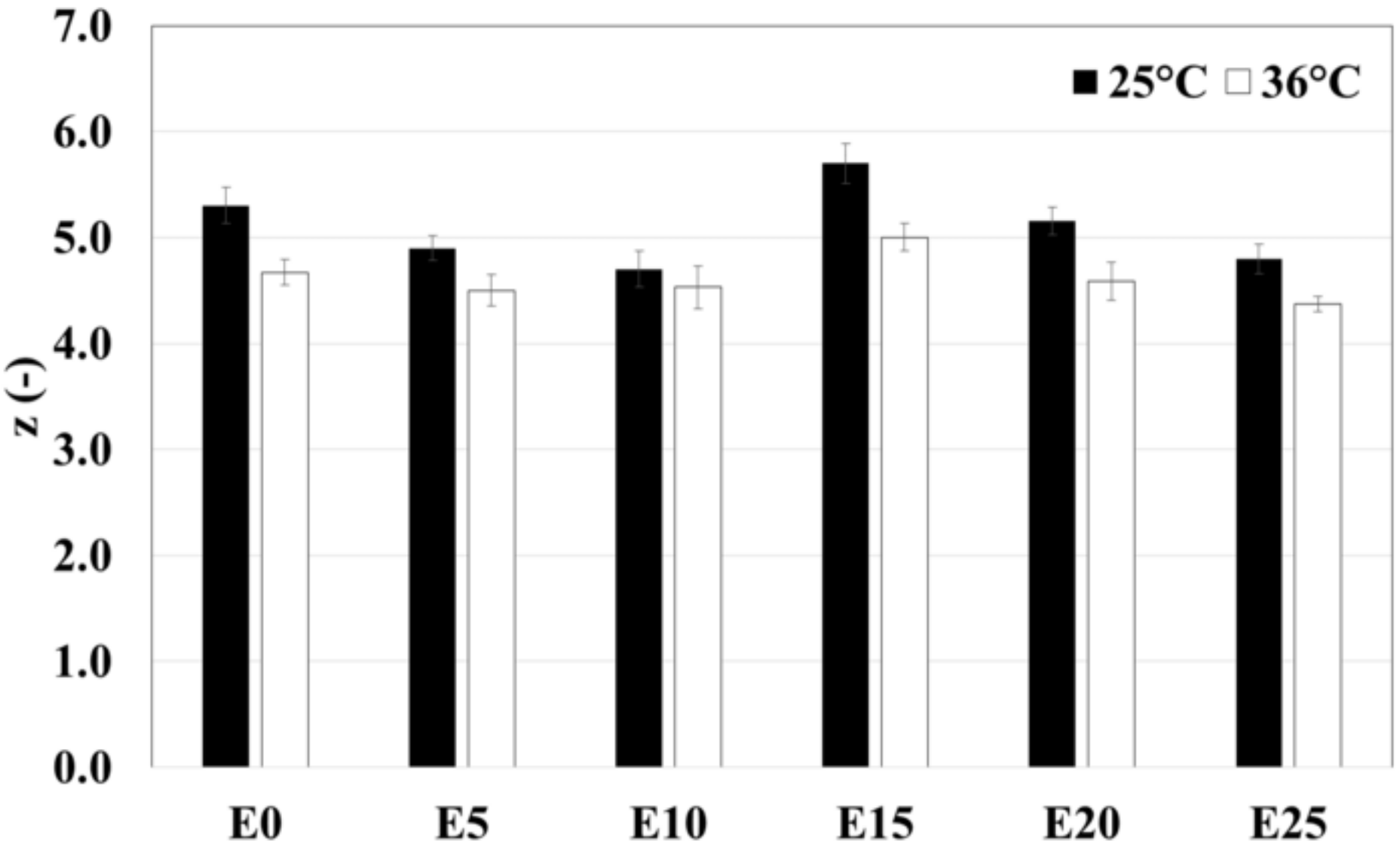

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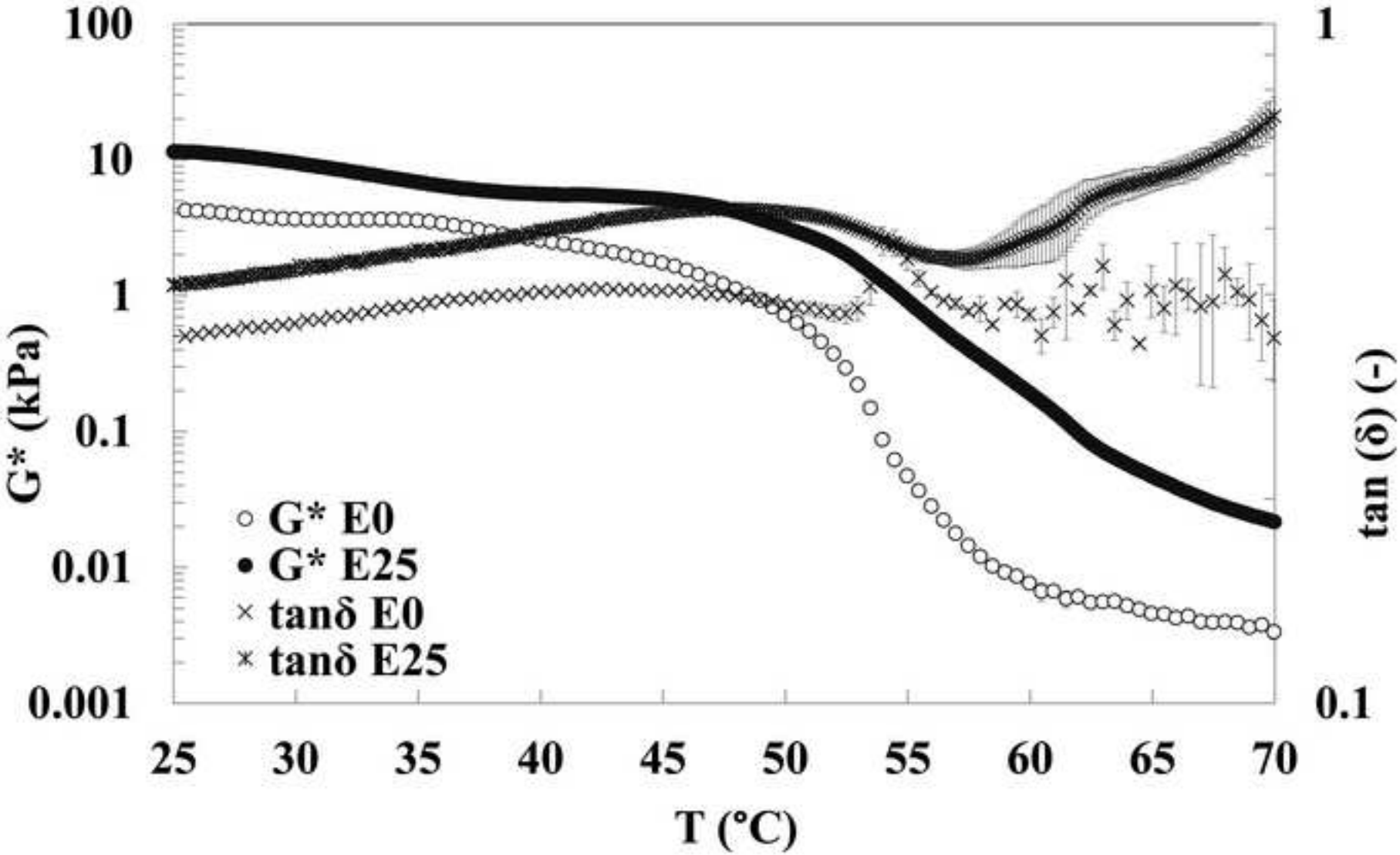

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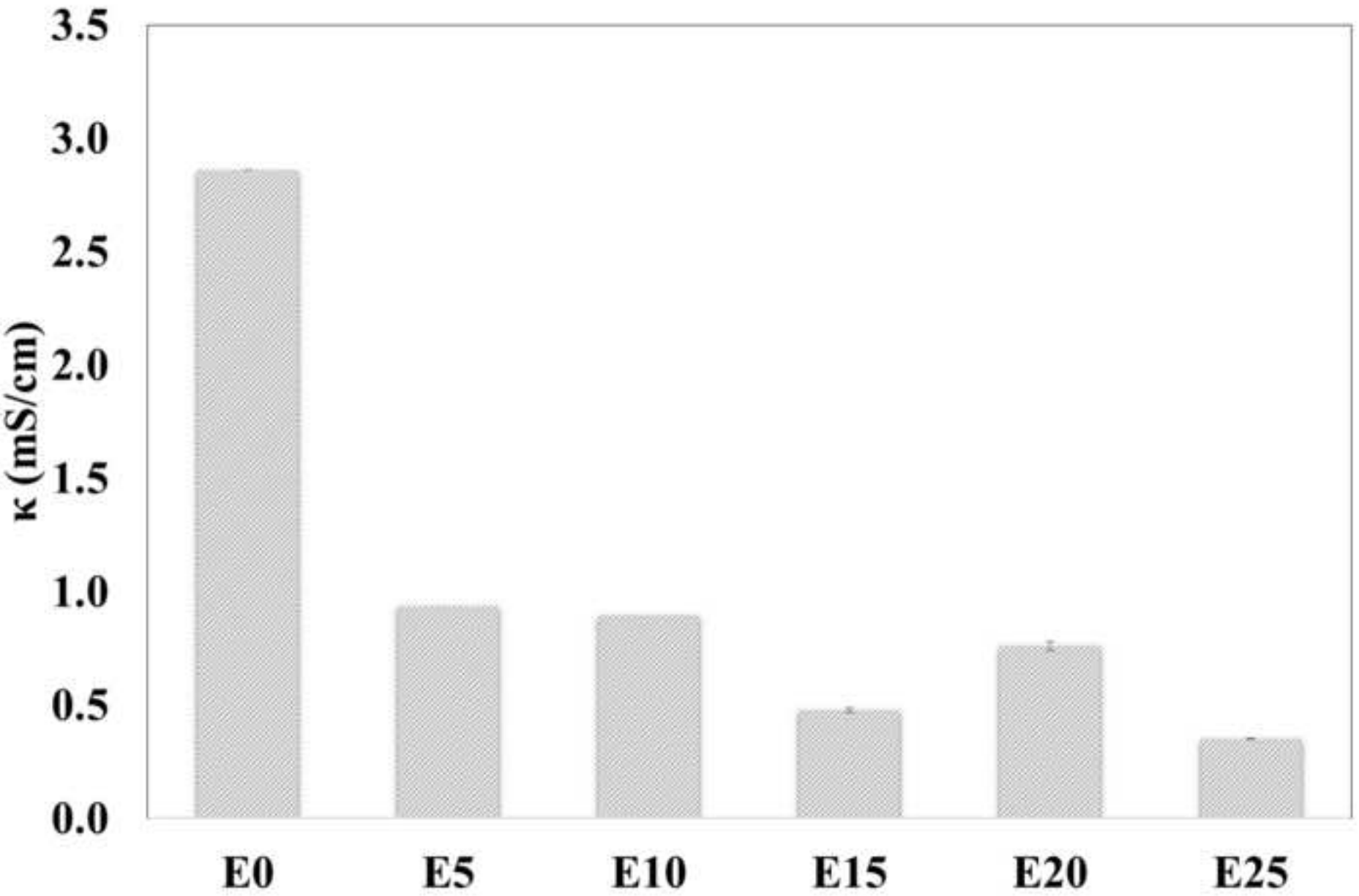

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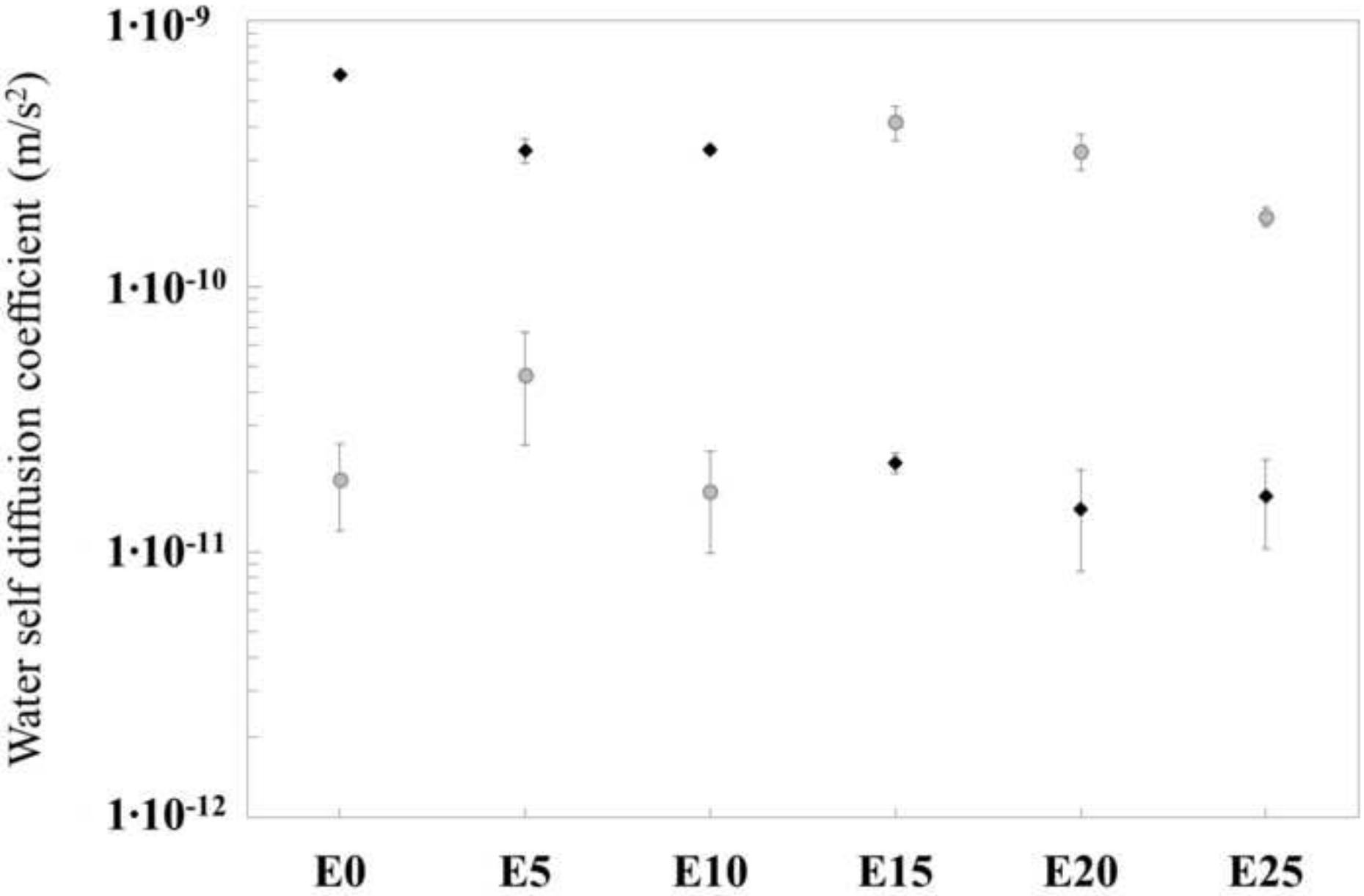

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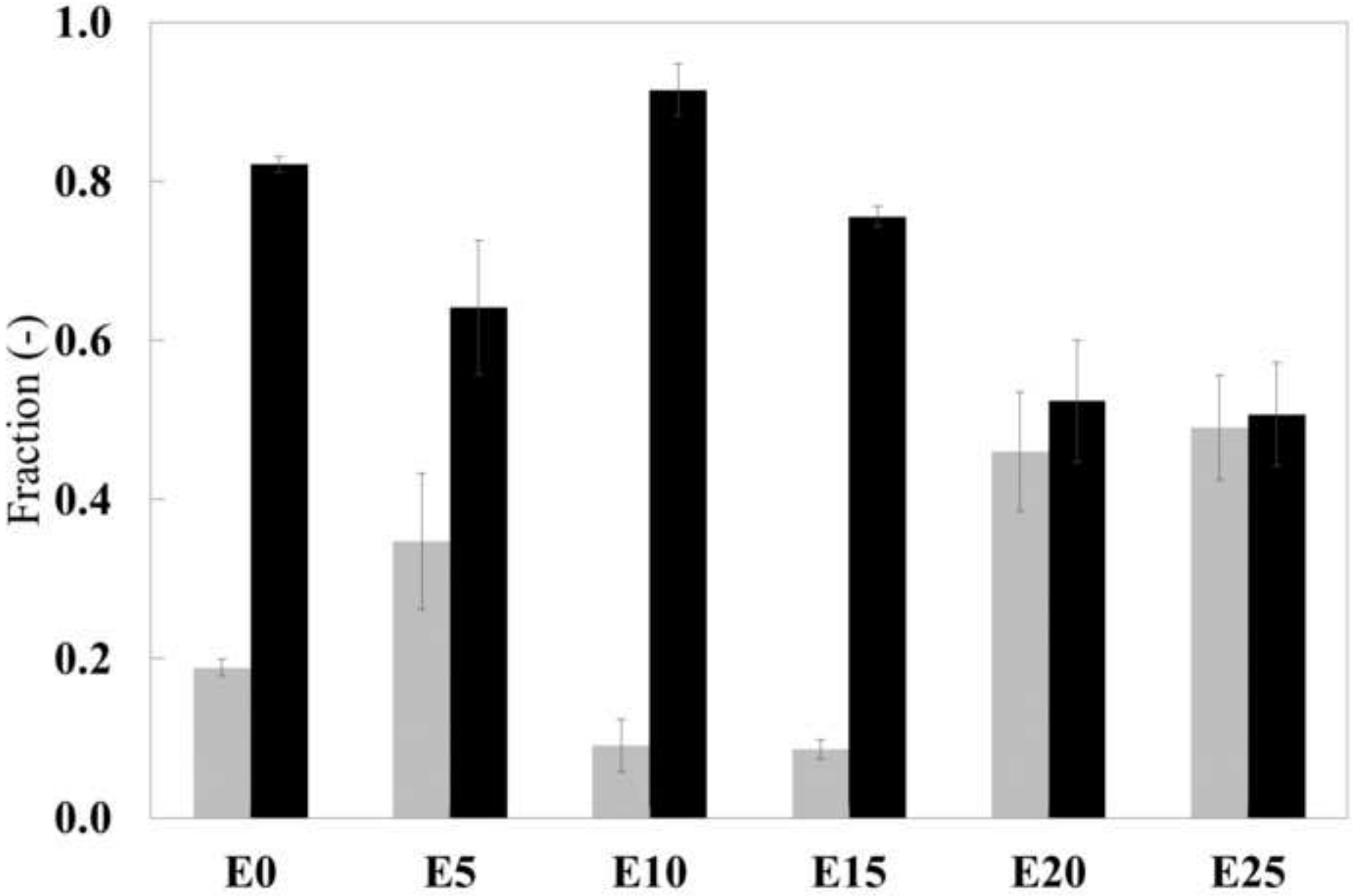

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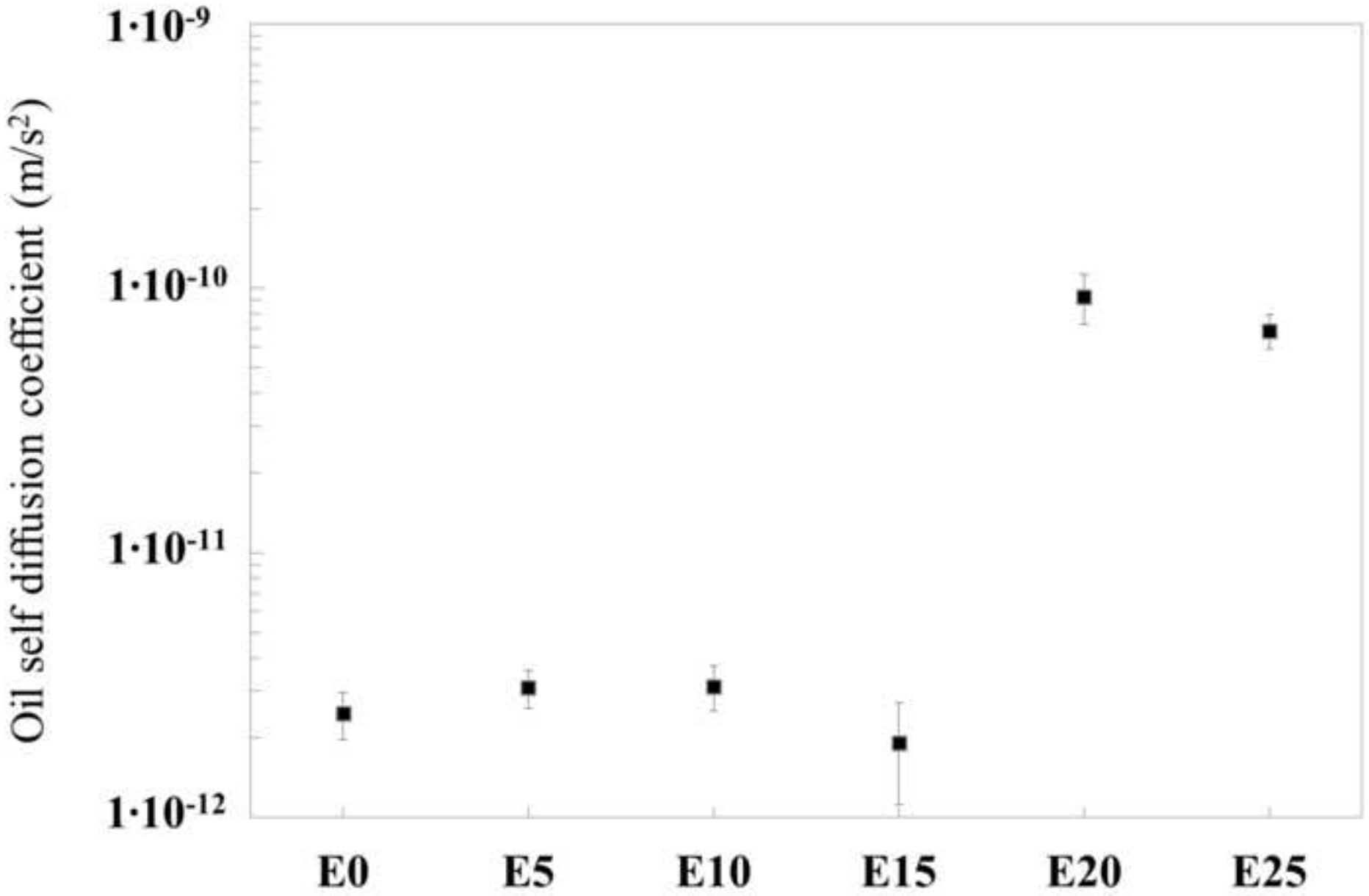

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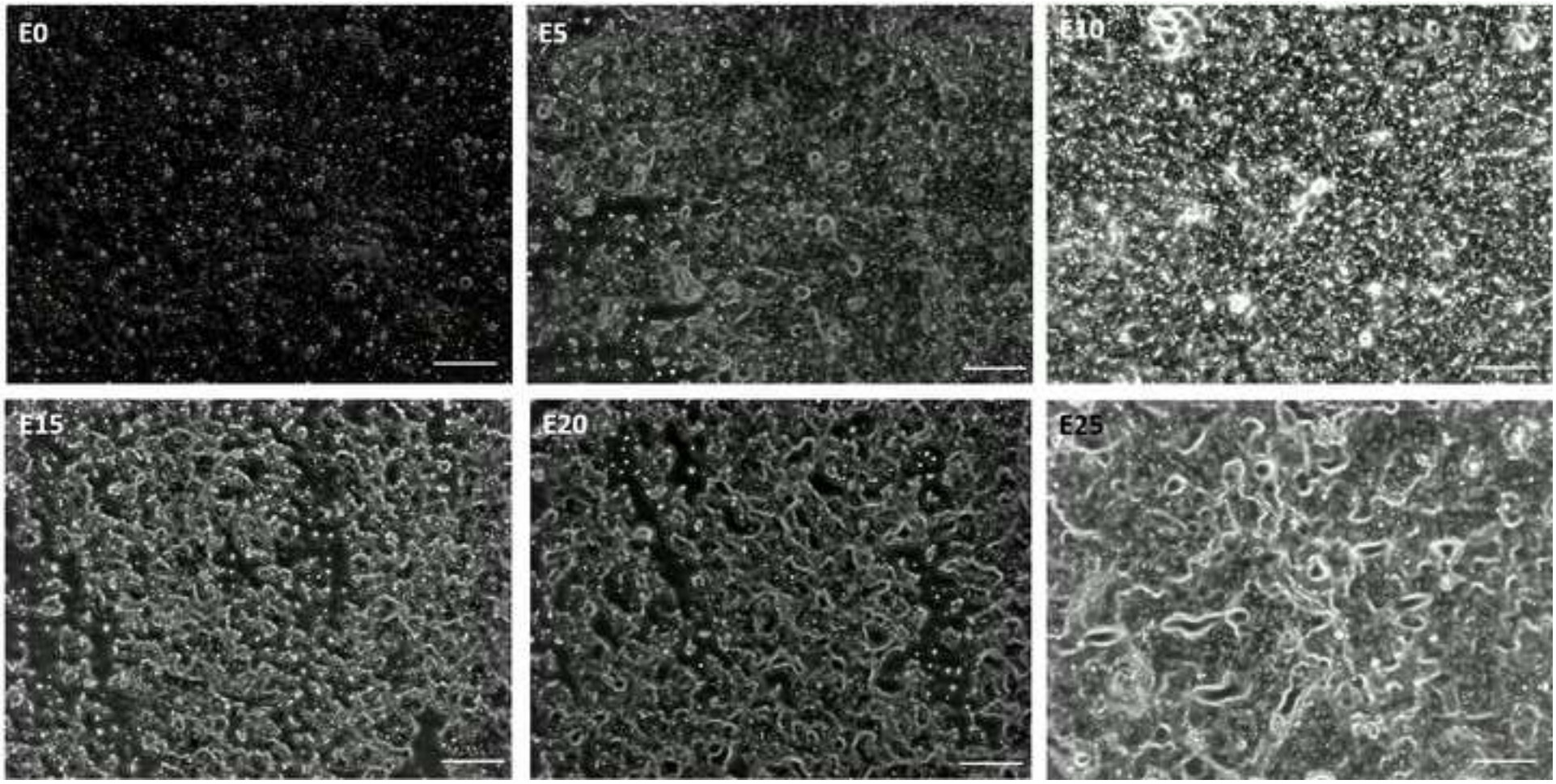

Ingredients	**Amount (w/w%)**
Aqueous phase	
Aqua (Sulphurous hyperthermal water)	64.08
Glycerin (Vegetable Glycerol)	2.5
Algae (Glyceric extract of algal microflora 1:1 in Sodium Benzoate 0.3%, Sodium dehydroacetate 0.3%, phenoxyethanol 0.7%, pH 4)	2.5
Potato starch modified (natural rheological modifier)	0.5
Sodium Ascorbyl Phosfate (anti-oxidant)	0.5
Phenoxyethanol (preservative)	0.7
Glycerin and Ethyl Lauroyl Arginate HCl (Aminat G) (preservative)	0.5
Tetrasodium Glutamate Diacetate (chelating agent GLDA) (Natural Chelating agent)	0.1
Oil phase	
Acemulgor Lam "V" (Potassium Palmitoyl Hydrolyzed Wheat Protein, Glyceryl Stearate, Cetearyl Alcohol) (Vegetable emulsifier)	9
Tegosoft CT (caprylic/capric Triglyceride) (Ester)	3
Prunus Amygdalus Dulcis Oil	3
Olea Europea Oil (Extra virgin olive oil)	10
Cethyl Alcohol (co-emulsifier)	2
Tocopheryl Acetate (anti-oxidant)	1
Ascorbyl Palmitate (anti-oxidant)	0.01
Citrus Arantium Dulcis Oil (Orange essential oil)	0.4
Citrus Medica Limonum Peel Oil (Lemon essential oil)	0.2
Cymbopogon Nardus Oil (Citronella essential oil)	0.01

Table 1

6: Table 2

Ingredients	Quantity (w/w%)
Myverol	15.56
Ascorbyl Palmitate	0.01
Algae	7.02
α-tocopherol	0.65
Extra Virgin Olive oil	76.76

Table 2

6: Table 3

Sample	Aqueous phase (w/w%)	Oil phase (w/w%)
E0	71.38	28.62
E5	68.00	32.00
E10	65.50	34.50
E15	63.50	36.50
E20	58.60	41.40
E25	55.10	44.90

Table 3