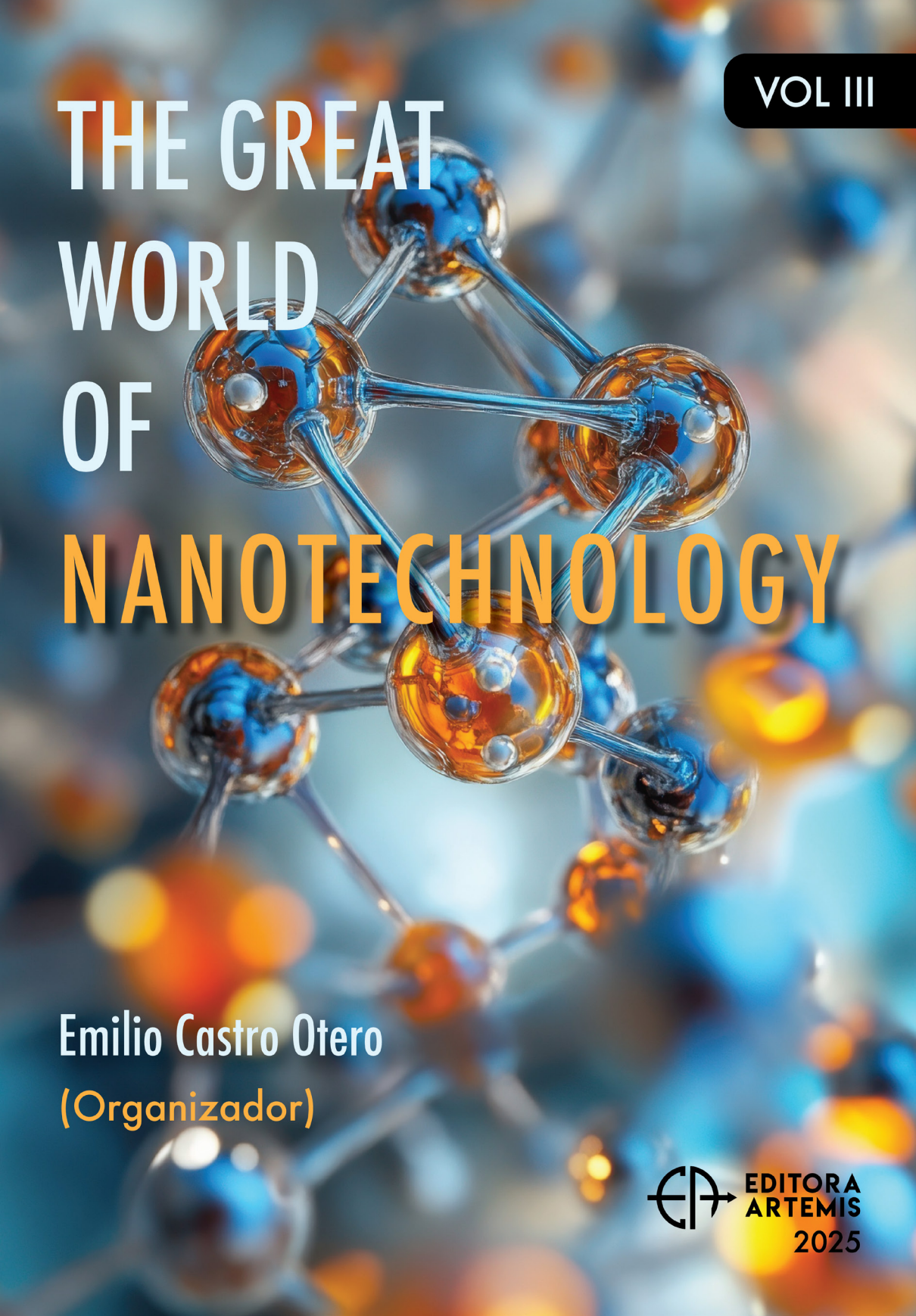
VOL III
THE GREAT WORLD OF NANOTECHNOLOGY
Emilio Castro Otero
(Organizador)
EDITORA ARTEMIS
2025

VOL III

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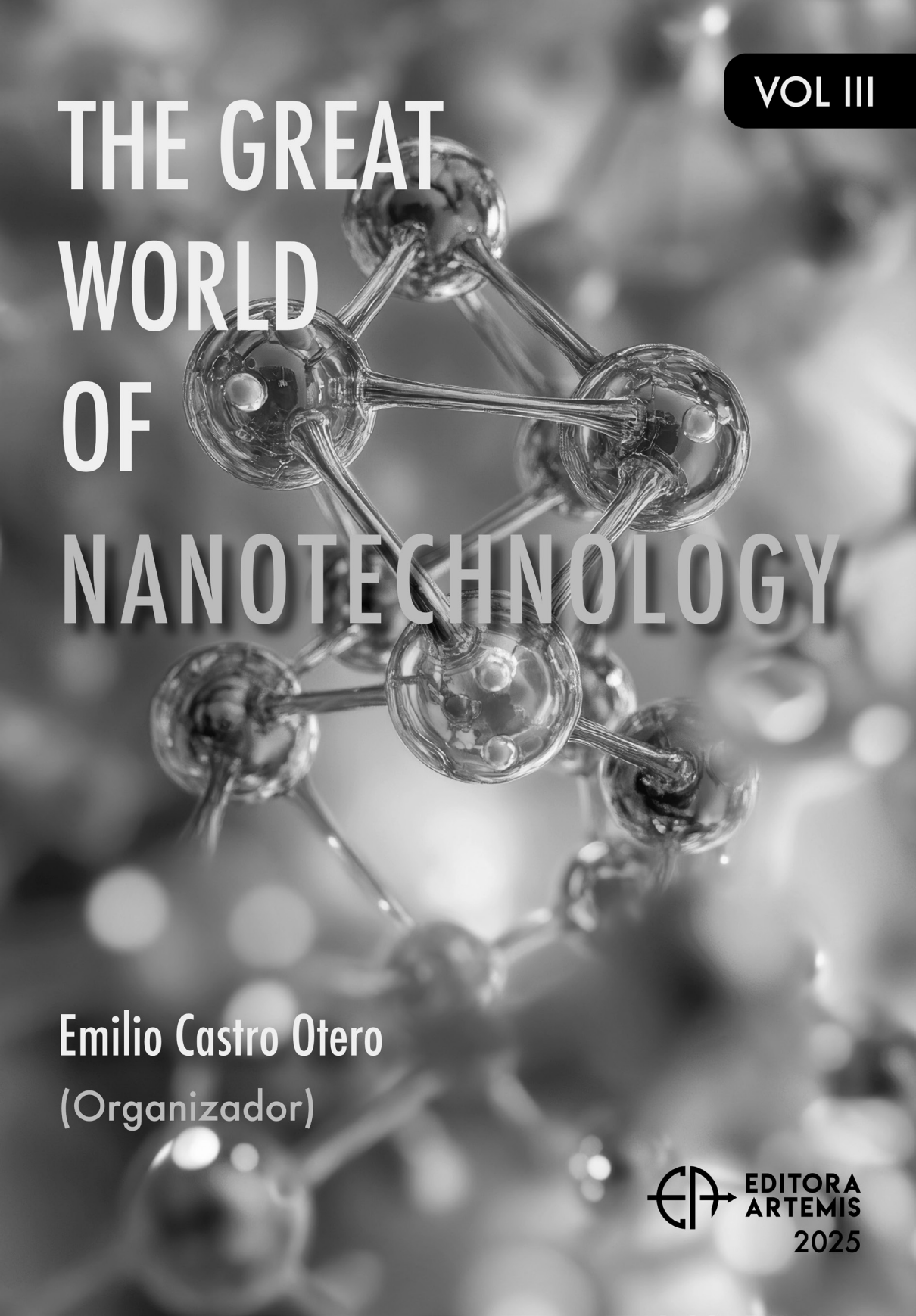

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FOREWORD

We are thrilled to present the third installment of ***The Great World of Nanotechnology***, a volume dedicated to exploring the cutting edge of nanotechnology applications, from the fundamental science of materials to their tangible impact on health, industry, and the environment. This book is meticulously structured into four thematic blocks, designed to guide the reader through the latest innovations that seek to solve crucial challenges in modern society.

The first pillar of this volume, ***Fundamentals of Nanomaterial Manufacturing and Characterization***, delves into the critical methodologies for creating high-quality nanostructures. Chapter 1 offers systematic research on the Effects of the Transfer Process on the Structure of Graphene Synthesized by Chemical Vapor Deposition. Complementing this, Chapter 2 examines Polymer Nanofibers via Airbrushing.

In ***Nanomaterials for Surface Engineering and Smart Sensors****, the chapters* explore how these nanostructures can be applied in functional devices and material protection. Chapter 3 presents advances in the development of sensors based on graphene functionalized with nanoparticles. Similarly, Chapter 4 addresses the electrodeposition of nanostructured metal coatings.

Nanotechnology in Biomedical and Pharmaceutical Applications brings three chapters focusing on the use of nanotechnology to improve human health. Chapter 5 evaluates the Cytotoxic Effect of Nanoemulsion with Pumpkin Seed Oil on Breast Cancer Cell Lines. In the field of tissue engineering, Chapter 6 describes the development of Alginate/Collagen Structures Enhanced with Conductive Nanoparticles (PEDOT) for the Regeneration of Small-Diameter Blood Vessels. Finally, Chapter 7 discusses the development of an antiseptic gel based on bioactive compounds encapsulated in nanoparticles.

Concluding our exploration, ***Nanotechnological Solutions for Environmental Remediation*** addresses the water pollution crisis. The final chapter describes the Removal of Arsenic from Groundwater Using Recycled Iron Nanoparticles through the development of a low-cost filter that uses iron nanoparticles.

We invite you to immerse yourself in reading The Great World of Nanotechnology. Vol. III, where science on the smallest scale translates into large-scale solutions. We sincerely hope that this compilation of advanced research will not only be of utmost interest to you, but also inspire new directions of study and application in this infinitely promising field.

Emilio Castro Otero

PRÓLOGO

É com grande entusiasmo que apresentamos a terceira edição de ***The Great World of Nanotechnology***, um volume dedicado a explorar a vanguarda das aplicações nanotecnológicas, desde a ciência fundamental dos materiais até o seu impacto tangível na saúde, na indústria e no ambiente. Este livro está meticulosamente estruturado em quatro eixos temáticos, concebidos para guiar o leitor através das mais recentes inovações que procuram resolver desafios cruciais na sociedade moderna.

O primeiro eixo deste volume, ***Fundamentos da Fabricação e Caracterização de Nanomateriais***, aprofunda as metodologias críticas para a criação de nanoestruturas de alta qualidade. O Capítulo 1 oferece uma investigação sistemática sobre os Efeitos do Processo de Transferência na Estrutura do Grafeno Sintetizado por Deposição Química de Vapor e o segundo capítulo examina as Nanofibras Poliméricas via Aerografia.

Em seguida, o eixo ***Nanomateriais para Engenharia de Superfícies e Sensores Inteligentes***, explora como essas nanoestruturas podem ser aplicadas em dispositivos funcionais e proteção de materiais. O Capítulo 3 apresenta os avanços no desenvolvimento de Sensores Baseados em Grafeno Funcionalizado com Nanopartículas. Paralelamente, e o Capítulo 4 aborda a Eletrodeposição de Revestimentos Metálicos Nanoestruturados.

O eixo ***Nanotecnologia em Aplicações Biomédicas e Farmacêuticas***, centra-se na utilização da nanotecnologia para melhorar a saúde humana. O Capítulo 5 avalia o Efeito Citotóxico da Nanoemulsão com Óleo de Semente de Abóbora em Linhagens de Células de Cancro da Mama. No campo da engenharia de tecidos, o Capítulo 6 descreve o desenvolvimento de Estruturas de Alginato/Colagénio Melhoradas com Nanopartículas Condutoras (PEDOT) para a regeneração de Vasos Sanguíneos de Pequeno Diâmetro. Finalmente, o Capítulo 7 expõe a Elaboração de um Gel Antisséptico Baseado em Compostos Bioativos Encapsulados em Nanopartículas.

Concluindo a nossa exploração, o eixo ***Soluções Nanotecnológicas para Remédios Ambientais,*** aborda a crise da contaminação da água. Este último capítulo descreve a Eliminação de Arsénico das Águas Subterrâneas Usando Nanopartículas de Ferro Reciclado, através do desenvolvimento de um filtro de baixo custo que utiliza nanopartículas de ferro.

Convidamo-lo a mergulhar na leitura de The Great World of Nanotechnology Vol. III, onde a ciência na escala mais pequena se traduz em soluções em grande escala. Esperamos sinceramente que esta compilação de pesquisas avançadas não só seja do seu interesse, mas também inspire novos rumos de estudo e aplicação neste campo infinitamente promissor.

Emilio Castro Otero

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ABSTRACT: Breast cancer has the highest mortality rate in women worldwide and current treatments have many side effects. Thus, nutraceuticals and nanomedicine aim to improve the quality of life and the effectiveness of conventional treatments. In this sense, this work aimed to evaluate the cytotoxic effect of a *Cucurbita moschata* pumpkin seed oil-loaded nanoemulsion (PSO-N) in metastatic

breast cancer cells (MCF-7). The oil was obtained by cold pressing and characterized by gas chromatography (GC). The PSO-N was prepared using a high-energy emulsification method. The PSO-N was characterized regarding Z-average, PdI, ZP, and pH. Also, a stability study was conducted for 3 months. A cytotoxicity assay was performed by MTT in concentrations of 500 - 3000 µg. mL^{-1}. It was identified a greater proportion of linoleic acid (51.55%). The nanoemulsion with the best stability parameters had HLB 13, Z-average = 149.99 ± 9.95 nm, PdI = 0.14 ± 0.03 and ZP of 22.39 ± 3.19 mV and the best storage condition is at 4 ºC. The nanoemulsion was cytotoxic for MCF-7 cells at a concentration of 500 µg.mL^{-1}. In view of the results, PSO-N has an adequate physical-chemical characteristics and a promissory activity for breast cancer treatment.
KEYWORDS: emulsions; inflammatory breast neoplasms; *cucurbita moschata*; toxicity tests.

EFEITO CITOTÓXICO DE NANOEMULSÃO CONTENDO ÓLEO DE SEMENTE DE ABÓBORA EM LINHAGENS DE CÉLULAS DE CÂNCER DE MAMA

RESUMO: O câncer de mama apresenta a maior taxa de mortalidade entre mulheres em todo o mundo, e os tratamentos atuais possuem muitos efeitos colaterais. Assim, nutracêuticos e a nanomedicina visam melhorar a qualidade de vida e a eficácia dos tratamentos convencionais. Nesse sentido, este trabalho teve como objetivo avaliar o efeito citotóxico de uma nanoemulsão contendo óleo de semente de abóbora *Cucurbita moschata* (PSO-N) em células de câncer de mama metastático (MCF-7). O óleo foi obtido por prensagem a frio e caracterizado por cromatografia gasosa (CG). A PSO-N foi preparada utilizando um método de emulsificação de alta energia. A PSO-N foi caracterizada quanto ao tamanho hidrodinâmico médio, PdI, ZP e pH. Além disso, um estudo de estabilidade foi conduzido durante 3 meses. O ensaio de citotoxicidade foi realizado pelo método MTT em concentrações de 500 a 3000 µg mL^{-1}. Foi identificada uma maior proporção de ácido linoleico (51,55%). A nanoemulsão com os melhores parâmetros de estabilidade apresentou HLB 13, Z-médio = 149,99 ± 9,95 nm, PdI = 0,14 ± 0,03 e ZP de 22,39 ± 3,19 mV, sendo a melhor condição de armazenamento a 4 °C. A nanoemulsão demonstrou citotoxicidade para células MCF-7 na concentração de 500 µg.mL^{-1}. Diante dos resultados, a PSO-N apresenta características químicas adequadas e atividade promissora para o tratamento do câncer de mama.
PALAVRAS-CHAVE: emulsões; neoplasias Inflamatórias da mama; *cucurbita moschata*; testes de toxicidade.

1. INTRODUCTION

Breast cancer is the most frequent cancer with the greatest causes of cancer death in women worldwide with an estimated 2.3 million new cases (11.7%), and is therefore a public health problem (Kashyap *et al*., 2022; Sung *et al*., 2021). Treatments for breast cancer include pharmacological treatment, surgery, chemotherapy or radiation and will depend on the stage of cancer (Rossi *et al*., 2018; Waks, Winer, 2019). Most of these

treatments have serious side effects, which decrease the patient's quality of life. This occurs because the drugs have molecules small enough to pass through the endothelium of several nonspecific regions (Vieira, Gamarra, 2016). In this sense, nutraceuticals and nanomedicine come together with conventional treatments to improve cancer therapies (Puttasiddaiah *et al*., 2022). Nutraceuticals are attractive because their reduced side effects, especially in cancer (Maiuolo *et al*., 2021), beneficial health effects and their reduced cost (Chauhan *et al*., 2013). Besides, nanomedicine appears to be a boost technology in medicine, through the improvement of treatments and diagnosis, using nanomaterials which can permeate and accumulate in solid tumors through a mechanism called enhanced permeability and retention effect (EPR) (Huang *et al*., 2021; Wu, 2021). This means that the tumor microenvironment provides an increased vascular permeability, because in inflamed regions or tumoral regions, cells are more spaced apart, unlike healthy cells, this allows nanoparticles to accumulate in these tumoral tissues (Farokhzad, Langer, 2009; Garbayo *et al*., 2020).

Cucurbita moschata is a species of pumpkin, belonging to the family Cucurbitaceae, widely cultivated and consumed in the world. Its seeds are generally considered to be agro-industrial residues, however, they have several bioactive compounds in their composition, being of great interest in health (Men et al., 2021; Veronezi, Jorge, 2012). They contain fatty acids, carotenoids, phenolic acids, tocopherols, flavonoids, minerals and vitamins (de Carvalho *et al*., 2012; Peiretti *et al*., 2017) being able to exercise the various biological activities already reported, such as antidiabetic, and cardioprotective action (Patel, 2013; Uuh-Narvaez *et al*., 2021).

In addition to these compounds, the literature describes the presence of a substance called moschatin, characterized as a type I ribosome inactivating protein (RIP I) in the mature seeds of *Cucurbita moschata*, which shows cytotoxic activity in human melanoma cells (Xia *et al*., 2003). It is reported the presence of highly oxygenated triterpene structures, called cucurbitacins, which have shown cytotoxicity, antitumoral, hepatoprotective and anti-inflammatory activity. In the seeds of *Cucurbita moschata*, there is the presence of cucurbitacin B, one of the main substances with cytotoxic activity (Montesano *et al*., 2018).

Therefore, we report herein the development of a pumpkin seed oil nanoemulsion, its long-term stability characterization and the evaluation of its cytotoxic effect in metastatic breast cancer cells.

2. MATERIAL AND METHODS

2.1. PUMPKIN SEED OIL EXTRACTION

For this work, fresh seeds of *Cucurbita moschata* (20°49'3,28"S and 45°8'1,31"W) were peeled off and subjected to cold pressing. The oil was subjected to centrifugation (Kasvi K14-4000) at 3000 rpm for 30 minutes. The yield was calculated by the ratio of the mass of the oil to the mass of the seed. This study was registered in the National System for Governance of Genetic Heritage and Associated Tradicional Knowledge (SisGen # AA91593).

2.2. CHARACTERIZATION OF PUMPKIN SEED OIL

Approximately 1mg of *C. moschata* seed oil was dissolved in 100 µL of a solution of sodium hydroxide 1 mol.L^{-1} in ethanol/water (95:5 v/v). After vortexing for 10 s, the oil was hydrolyzed through heating and vibration generated by electromagnetic waves at 30% power for 4 minutes. After cooling, 400 µL of 20% hydrochloric acid, 4µg NaCl and 600 µL of ethyl acetate were added. After vortexing for 10 s and standing for 5 min, an aliquot of 300 µL total of the organic layer was removed and dried by evaporation, thus obtaining free fatty acids (Sande *et al*., 2018). Subsequently, the free fatty acids were methylated with 100 µL BF3 / methanol (14%) by heating for 10 minutes in a water bath at 60 ºC, extracted in 500 µL of hexane and analyzed by Gas Chromatography. The analyzes were performed on an HP7820A Gas Chromatograph (Agilent) equipped with a flame ionization detector. A Supelcowax-10 30 m x 0.2 mm x 0.2 µm column (Supelco) with the following temperature gradient 150 ºC, 1 min, 10 ºC.min-1 to 240 ºC was used; injector (1/20 split) at 250 ºC and detector at 250 ºC. Hydrogen as carrier gas (6 mL.min^{-1}) and injection volume of 1 µL. Peak identification was done by comparison with FAME C14-C22 methylated fatty acid standards (Supelco #18917).

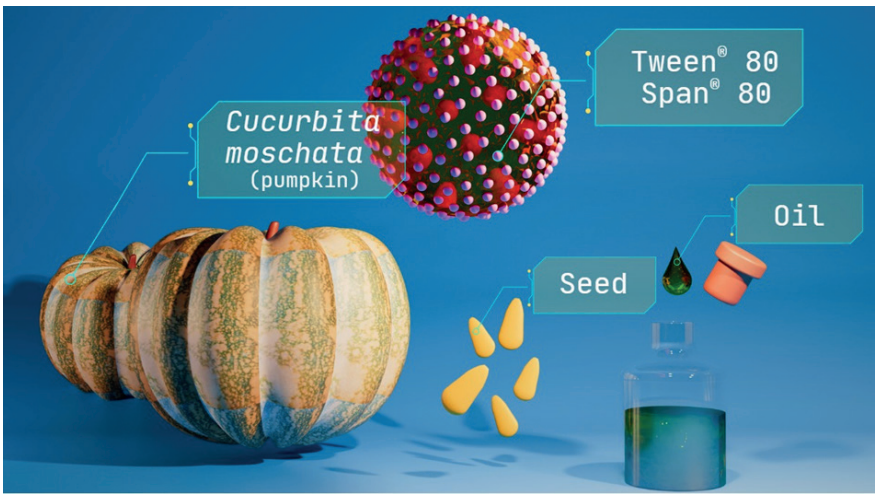
2.3. HLB DETERMINATION

Nanoemulsions with HLB 8.7; 10.9; 12 and 13 containing a percentage of 5% w/w oil, 5% surfactants and 90% water were prepared. The surfactants chosen for the preparation of the emulsions were sorbitan monooleate (Span® 80) and ethoxylated sorbitan monooleate (Tween® 80). After preparing the nanoemulsions, they were evaluated for their preliminary stability by submitting to centrifugations at 1000, 2000 and 3000 rpm for 15 minutes (Kasvi K14-4000). The nanoemulsion that presented the best macroscopic characteristics and did not have phase separation was selected for the next experiments.

2.4. PREPARATION OF NANOEMULSION

HBL 13 nanoemulsion was developed containing a percentage of 5% oil and 5% surfactants (Tween® 80 and Span® 80). All emulsions were prepared by the high energy method, in which the aqueous phase and the oil phase were prepared separately. After the preparation of each phase, the aqueous phase was slowly poured into the oil phase under magnetic stirring (MS300 Magnetic Stirrer) at 786 rpm at 50 ºC. The stirring was maintained until reaching room temperature. The emulsion was then subjected to ultrasonic processor at 20 kHz, 85% amplitude, for 5 minutes (Ultrasonics). An illustrative scheme of the preparation of the nanoemulsions is available in Figure 1.

Figure 1. Summary of the main steps concerning the methodology of the nanoemulsions preparation. Created with Blender 3D.



2.5. CHARACTERIZATION OF THE PHYSICAL-CHEMICAL PARAMETERS OF THE NANOEMULSION

The mean particle size (Z-average) and polydispersity index (PdI) were measured by Dynamic Light Scattering using a Zetasizer Nanoseries (Malvern Instruments, Worcestershire, United Kingdom). The analysis were performed at 25 °C, with a detection angle of 173°. The zeta potential (ZP) values were obtained by electrophoretic mobility (Zetasizer Nanoseries, Malvern Instruments, Worcestershire, United Kingdom). Prior to the analyses, samples were diluted in ultrapure water (1:400). The pH was directly determined in the samples (PH-2011, Etekcity, Anaheim, California, USA). The analyses were performed in triplicate.

2.6. STORAGE STABILITY STUDY

The nanoemulsion was stored in different 15 mL flasks, at temperatures of 4, 27 and 45 ºC for 0, 7, 15, 30, 60 and 90 days. After each time the samples were analyzed for the following parameters: Z-average, PdI, PZ and pH. The analyzes were performed in triplicates.

2.7. PREPARATION OF SUBSTANCES

From the three stocks: 10.000, 5000 and 1000 $\mu g.mL^{-1}$ the substances were diluted to the final concentration of 50, 100, 500, 1500 and 3000 $\mu g.mL^{-1}$. Then, the substances were diluted in Tween® 80, 1%, diluted in Dulbecco´s modified eagle medium (DMEM). For the oil control substance with ethanol (EtOH), the oil was diluted in 2% ethanol.

2.8. CYTOTOXICITY STUDY (MTT ASSAY)

The control and test formulations were submitted to sterilization by UV light for 15 minutes. Samples were prepared to the final concentration of 50, 100, 500, 1500 and 3000 $\mu g.mL^{-1}$, diluted in Tween® 80, 1%, at Dulbecco´s modified eagle medium (DMEM). For the oil control substance with ethanol (EtOH), the oil was diluted in 2% ethanol. MCF-7 and human fibroblast cells were seeded $1x10^{-4}$ cells/well in 96-well plates in the DMEM essential medium with L-glutamine (2mM) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin and maintained at 37 ºC in a 5% CO_2 in incubator (Thermo Scientific) After 24h of incubation, the cells were incubated with the test and control samples (nanoemulsion without the oil) at concentrations of 50, 500 1500 and 3000 $\mu g.mL^{-1}$. Additionally, free oil, oil with 2% ethanol, 2% ethanol and 2% DMSO (for a negative control) were applied to the cells. After 24 and 48 hours, the medium was discarded and 100 µL of MTT solution 5 $mg.mL^{-1}$ (Sigma) was added, according to the manufacturer's guidelines. Then, the plates were incubated at 37 ºC for 4 h. After the incubation time, the formation of formazan crystals was observed under an optical microscope, all medium was discarded and added 100 µL of 0.05 M HCl isopropanol solution for dissolution of the formazan crystals and optical density was measured at 570 nm and 645 nm in the spectrophotometer (Multiskan Go).

2.9. STATISTICAL ANALYSIS

The statistical analyzes were performed by using Graph Pad Prism 5.0. Analyzes of the stability study were carried out by comparing the different groups (Two-way ANOVA and Bonferroni) and in relation to the control group (T0) (Two-way ANOVA followed by the

Dunnett test). The MTT assay analyzes were performed comparing the different groups (Two-way ANOVA followed by the Bonferroni test). Values were presented as mean ± standard error of the mean. Statistical differences were considered significant at $p < 0.05$.

3. RESULTS

The yield percentage of pumpkin seed oil was 24.12% using cold pressing. The yield was calculated by the ratio of the mass of the oil to the mass of the seed. The chromatogram profile of pumpkin seed oil characterization (Table 1) revealed that the fatty acids most present in pumpkin seed oil were C18:2 (linoleic acid; 51.55%), C18:1 (oleic acid; 20.06%), C16:0 (palmitic; 15.66%) and C18:0 (stearic acid; 10.67%).

Table 1. The fatty acids profile of Curcubita moschata seed oil. (RT = Real Time; PSO = pumpkin seed oil-loaded).

Fatty acids	RT (min)	PSO (area)	PSO (%)
C12:0	3.137	12704	0.05
C14:0	4.308	44596	0.17
C16:0	6.315	4039395	15.66
C16:1	6.592	117485	0.46
C18:0	7.991	2753472	10.67
C18:1	8.185	5176699	20.06
C18:2	8.603	13301592	51.55
C18:3	9.107	63869	0.25
C20:0	9.587	146501	0.57
others		245752	0.56

To obtain the nanoemulsion with better physicochemical characteristics and greater stability, formulations with different HLB values were evaluated in terms of macroscopic appearance, particle size (Z-average), polydispersity index (PdI) and zeta potential (ZP) (Table 2). In this sense, the nanoemulsion with HLB = 13 presented the best parameters: Z-average 149.99 ± 9.95 nm, monodisperse population PdI = 0.14 ± 0.03 and ZP of 22.39 ± 3.19 mV.

Table 2. Characterization of the physical-chemical parameters of the nanoemulsions. (HLB = Hydrophilic-Lipophilic Balance; Z-average = Particle Size, PdI = Polydispersity Index; ZP = Zeta Potential).

Composition	Formulation	HLB	Microscopic aspect	Span 80 (%w/w)	Tween 80 (%w/w)	Z-avarage (nm)	PdI	ZP (mV)
	1	8.7	Increased sedimentation	59	41	196.73	0.24	-30.22

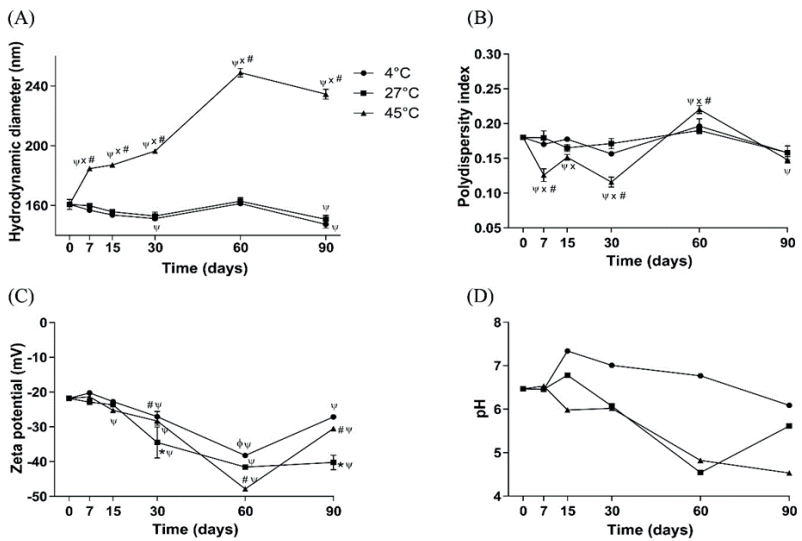
5% oil and 5% surfactants	**2**	10.9	Increased sedimentation	38	62	157.31	0.17	-12.51
	3	12	Low sedimentation	28	72	151.44	0.24	-23.61
	4	13	Low sedimentation	18	82	149.99	0.14	-22.39

The stability test carried out at 4 ºC, 27 ºC and 45 ºC revealed relative stability of the formulation at 4 ºC and 27 ºC.). A slight decrease in the mean Z was observed at 30 and 90 days at temperatures of 4 ºC and 27 ºC. The mean Z increased after 90 days at 45 ºC with a peak at 60 days with the particle measuring 245.4 nm. These results are available in function of time, as shown in Figure 2.

No significant difference was observed in the polydispersity index over time at 4 ºC and 27 ºC, but the polydispersity index also varied over the 90 days at a temperature of 45 ºC in relation to the 4 ºC and 27 ºC groups and in relation to the T0. However, during the 90 days, this parameter remained below 0.3 at all temperatures, guaranteeing the characteristics of a monodisperse population.

Furthermore, our results demonstrated variations in zeta potential at all temperatures over time. In this sense, the temperature that came closest to a suitable zeta potential parameter was 4 ºC, as it was the temperature that had the least variations and was close to ± 30mV.

Figure 2. Storage stability characterization at 4 ºC, 27 ºC and 45 ºC for 90 days. Evaluation of the (a) hydrodynamic diameter; (b) polydispersity index; (c) zeta potential; (d) pH. * $p < 0.05$ between groups 4 °C and 27 °C; ϕ $p < 0.05$ between 4 °C and 45 °C; # $p < 0.05$ between 27 °C and 45 °C; ψ $p < 0.05$: compared to T0.



pH decreased at all temperatures over time, but there was no statistical difference between groups. The results demonstrated a decrease in cell viability of more than 50% for MCF-7 cells at concentrations of 500, 1,500 and 3,000 µg.mL-1. However, concentrations of 1,500 and 3,000 µg.mL-1 also showed cytotoxicity in the control nanoemulsion. It is important to highlight that the nanoemulsion was not cytotoxic to fibroblasts at a concentration of 500 µg.mL-1, but it was also cytotoxic at concentrations of 1,500 and 3,000 µg.mL-1 in both the test and control groups.

4. DISCUSSION

According to the literature, pumpkin seed oil content varies between 42 to 54%(Murkovic *et al*., 2004). However, the work of Kabutey and collaborators obtained a percentage of 13.37% in yield by cold pressing of pumpkin seeds, which shows that we had a good yield taking into account the method used.

The variation in the oil content as well as the fatty acid profile may be due to differences in the cultivation of the plant and its varieties, stage of maturation, seed harvesting time and extraction method (Nyam *et al*., 2009). Our analyzes revealed a higher percentage of linoleic acid, which corroborates the results of Durante *et al*. (2014) who also observed a higher percentage of linoleic acid, being the main fatty acid, followed by oleic acid, in *Cucurbita moschata* pumpkin seed oil. The same was observed in the study by Puscas *et al*., 2022.

The higher linoleic acid content in the oil is attractive. A meta-analysis study suggests that dietary linoleic acid consumption is associated with a decreased risk of breast cancer, however, without any statistically relevant association (Zhou *et al*., 2016). Furthermore, in in vivo and in vitro models, dietary conjugated linoleic acid (CLA) was able to inhibit the development of carcinogenesis at multiple sites (Lee et al., 2005) and its activity delayed the growth of breast cancer cells. breast MCF-7. in an in vitro study (Bocca *et al*., 2010). From a chemical point of view, the high percentage of linoleic acid increases the risk of oil oxidation, which can lead to a decrease in the stability of the system (Rezig *et al*., 2012).

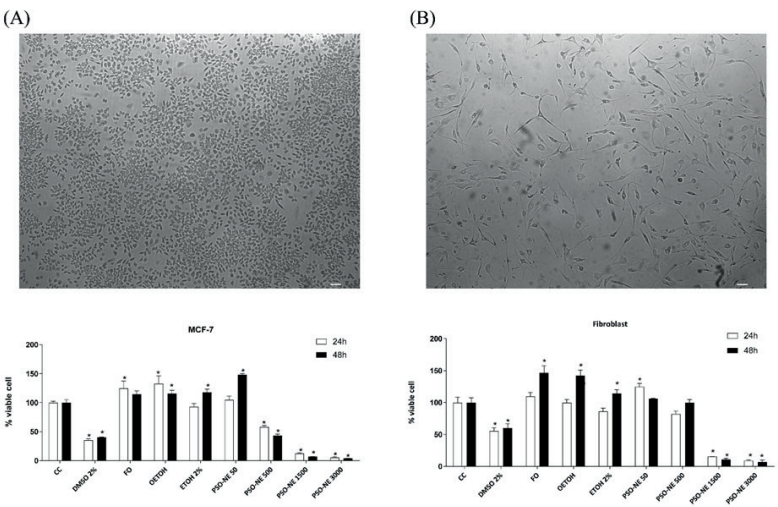
To obtain the nanoemulsion with better physicochemical characteristics and greater stability, formulations with different HLB values were evaluated in terms of macroscopic aspect, particle size (Z-average), polydispersity index (PdI) and zeta potential (ZP) (TABLE II). In this sense, the nanoemulsion with HLB = 13 presented the best parameters: Z-average 149.99 ± 9.95 nm, monodisperse population PdI = 0.14 ± 0.03 and ZP of 22.39 ± 3.19 mV. The literature indicates that nanostructures with an average

diameter close to 200 nm more easily penetrate the fenestrations of the tumor vascular epithelium (Liechty & Peppas, 2012); Danaei *et al*., 2018). Furthermore, nanoparticles that exhibit ZP close to ±30 mV exhibit greater colloidal stability (Roland *et al*., 2003); Kurpiers *et al*., 2020).

The pH can also be a parameter for monitoring the stability of the formulation, as changes in its values can indicate bacterial growth or chemical reactions. The pH decreased at all temperatures over time, but there was no statistical difference between the groups (Figure 2D), which in turn can be explained by oxidative reactions, generating free fatty acids that increase the negative charge of the system. Formulations with a high concentration of fatty acids are more susceptible to oxidation reactions, such as hydrolysis, changing pH values (Musakhanian *et al*., 2022) .

Over the 90 days of study, there was no break in the formulation, ensuring stability. At a temperature of 45 ºC, more parameters were changed over time, as expected, since many reactions and physicochemical changes occur at high temperatures. At temperatures of 4 ºC and 27 ºC, the nanoemulsion maintained its adequate physicochemical parameters. However, at 27 ºC a slight change in color was observed, losing the greenish characteristics of the oil and showing greater apparent sedimentation, in addition to a more pronounced variation in zeta potential in relation to 4 ºC. Thus, together, the best storage temperature for the pumpkin seed oil nanoemulsion was 4 ºC, which managed to keep its physicochemical characteristics closer to ideal.

Figure 3. Cell viability evaluated by MTT assay performed at 24 and 48h after pumpkim seed oil nanoemulsion treatment. The representative image of cell morphology and viability of (a) MCF-7 and (b) fibroblast. Statistical differences determined between the groups were determined according to ANOVA followed by the Bonferroni test * p <0.05. Scale bar: 100 µm. CC: cell control; FO: free oil; OETOH: oil diluted in 2% ethanol; ETOH: 2% ethanol; PSO-NE: pumpkin seed oil nanoemulsion; CT-NE: control nanoemulsion (without oil).



The cytotoxic potential of pumpkin seed oil nanoemulsion was evaluated using the MTT assay. The results demonstrated a decrease in cell viability of more than 50% for MCF-7 cells at concentrations of 500, 1,500 and 3,000 µg.mL-1 (Figure 3A). However, concentrations of 1,500 and 3,000 µg.mL-1 also showed cytotoxicity in the control nanoemulsion. It is important to highlight that the nanoemulsion was not cytotoxic to fibroblasts at a concentration of 500 µg.mL-1, but it was also cytotoxic at concentrations of 1,500 and 3,000 µg.mL-1 in both the test and control groups (Figure 3B).

This result demonstrates that the developed pumpkin seed oil nanoemulsion is cytotoxic to MCF-7 metastasis-derived breast cancer cells and not to fibroblasts (control cells). The biological activity of pumpkin seed oil nanoemulsion may be due to RIP, called moschatin, present in *C. moschata* pumpkin seeds, which also showed cytotoxic activity in M21 human melanoma cells (Chuan Xia *et al*., 2003)The other hypothesis related to the observed biological activity would be the presence of cucurbitacins in pumpkin seeds, which have cytotoxic and antitumor activity and specifically Cucurbitacin B, present in *C. moschata* pumpkin seeds (Montesano *et al*., 2018). Furthermore, Cucurbitacin B has been reported to inhibit hypoxia-inducing factor 1-α (HIF-1α), mainly associated with the progression of aggressive tumors (Ma *et al*., 2014).

Furthermore, the higher content of linoleic acid in the oil must have contributed to the observed cytotoxic activity (Bocca *et al*., 2010; Zeng *et al*., 2015). The cytotoxicity observed at concentrations higher than 1500 µg.mL-1 for both MCF-7 cell types and human fibroblasts may have been due to the surfactants present in the formulation. This result is expected, since there are reports in the literature about the cytotoxicity of surfactants, such as Tween® 80, used in this study (Arechabala *et al*., 1999).

5. CONCLUSION

The *Cucurbita moschata* pumpkin seed oil nanoemulsion at 4 ºC has an adequate physical-chemical characteristics and has a cytotoxic activity against MCF-7 cells. The findings demonstrate that the pumpkin seed oil nanoemulsion has a promising application as a nutraceutical for breast cancer treatment and it should be considered for further studies to investigate the antitumor mechanism involved and to validate its activity.

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ABOUT THE ORGANIZER

Emilio Castro Otero é professor da área de Bioengenharia na *Universitat Internacional de Catalunya*, Barcelona, Espanha. Pesquisador com ampla trajetória científica nas áreas de biomateriais, nanotecnologia, química física de polímeros e sistemas nanoestruturados aplicados à medicina regenerativa e à liberação controlada de fármacos. Sua produção abrange estudos fundamentais sobre auto-organização molecular, interações entre copolímeros e surfactantes, estabilidade proteica e desenvolvimento de nanopartículas e hidrogéis inteligentes, contribuindo de maneira significativa para a compreensão dos mecanismos físico-químicos que governam materiais avançados e sua aplicabilidade biomédica. Seu trabalho mais recente, publicado em Acta Biomaterialia (2023), aborda o papel angiogênico e imunomodulador de íons nas fases iniciais da regeneração óssea, consolidando sua atuação no campo dos biomateriais bioativos. Ao longo de sua carreira, publicou artigos de impacto em periódicos como Langmuir, Biomacromolecules, Journal of Biomedical Nanotechnology, Macromolecular Bioscience, Journal of Physical Chemistry B, Chemical Physics e Journal of Colloid and Interface Science, além de contribuir com capítulo de livro na área de nanomedicina, ampliando sua presença internacional na literatura científica.

Entre suas contribuições relevantes, destacam-se estudos sobre auto-organização de elastina recombinante e copolímeros responsivos à temperatura, síntese e caracterização de nanopartículas funcionalizadas, incluindo magnetita peguilada e nanocápsulas multifuncionais, e sistemas poliméricos capazes de encapsular e liberar fármacos de forma controlada, com foco em aplicações oncológicas e terapêuticas avançadas. Sua pesquisa também inclui extensas investigações termodinâmicas envolvendo surfactantes, copolímeros e sistemas micelares complexos, bem como análises detalhadas de interações entre proteínas e fármacos anfifílicos, contribuindo para o entendimento de processos de dobramento, estabilidade e agregação proteica. Com forte perfil interdisciplinar, o pesquisador tem colaborado com grupos internacionais de renome em bioengenharia, físico-química de materiais, nanotecnologia e biotecnologia aplicada, contribuindo para o desenvolvimento de estratégias inovadoras em engenharia de tecidos, medicina regenerativa e materiais biomiméticos.

Ao longo de quase duas décadas de produção científica contínua, Emilio Castro Otero consolidou uma carreira marcada pela investigação rigorosa, inovação metodológica e abordagem integrada da ciência dos materiais, oferecendo contribuições relevantes tanto para o avanço do conhecimento fundamental quanto para o desenvolvimento de soluções tecnológicas com potencial translacional na área biomédica.

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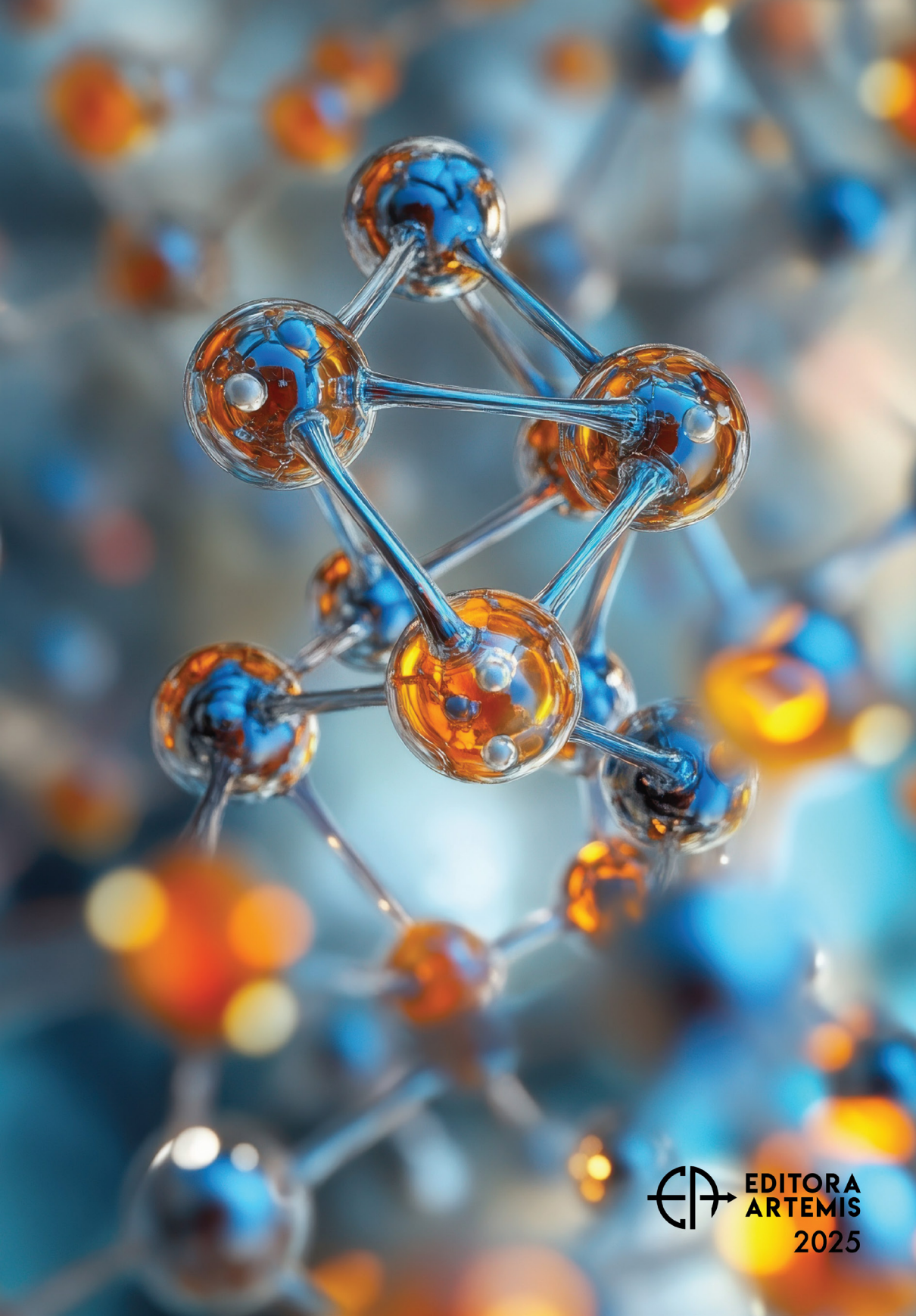

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