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# Multiple emulsions as carriers for the topical delivery of anti-inflammatory drugs

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**Abstract**
Radiation therapy can be adopted for many cancers, and it can damage healthy tissues and often induces skin lesions (pain/skin irritation/itchiness/dryness/swelling/redness). Many factors influence the adverse effects of radiotherapy, such as radiation dosage, dose frequency and fractioning, the area of skin exposed to radiation and treatment length. In this paper, multiple emulsions with a nonsteroidal anti-inflammatory drug-NSAID (diclofenac) were developed and evaluated for effective topical treatment of skin lesions following anti-cancer therapy. Multiple emulsions with different drop sizes were prepared in a Couette-Taylor flow contactor. High encapsulation efficiency ($>$ 90%) of diclofenac and high volume packing fraction of the internal droplets (0.54–0.96) were obtained. In addition, due to the presence of a polymer with adhesive properties – sodium carboxymethylcellulose, high emulsion stability ($>$ 60 days) was achieved. The emulsions displayed properties of shear-thinning fluids. The release study of diclofenac from a complex emulsion structure confirmed the possibility of modifying the release rates. The effectiveness of emulsion formulations was evaluated based on the viability tests of the fibroblast cell line irradiated with UV dose (15 J/m$^2$) and then treated with the emulsion with diclofenac. The results showed that the multiple emulsion-based formulations might be appropriate carriers for the topical delivery of NSAID drugs.

**Keywords**
multiple emulsion, skin lesions, nonsteroidal anti-inflammatory drugs, drug release, controlled-prolonged delivery

## 1. INTRODUCTION

One of the primary methods of many cancer treatments is radiotherapy. By using high doses of radiation, cancer cells are killed, or their growth is significantly slowed, due to damage to their DNA. However, radiation exposure usually triggers a similar response in both cancerous and normal tissue cells. This leads to direct damage to healthy skin and its deep tissue cells in the irradiated areas. After radiation therapy, about 85%–95% of cancer patients experience varying degrees of skin damage (Iacovelli et al., 2020; Yang et al., 2020). The common side effects of radiotherapy are pain, dryness, loss of elasticity, soft tissue fibrosis, capillary dilatation, ulceration, skin irritation, itchiness, dryness, swelling, redness and radiation dermatitis and irreversible damage to the endothelial cells of skin blood vessels (Iacovelli et al., 2020; Yang et al., 2020). Tissues become more susceptible to infection. Radiation-induced injury to healthy skin tissues can occur during and after radiation therapy. The overlapping side effects can lead to longer and more difficult treatment of the lesion, especially if it becomes cancerous. In addition, these side effects can vary in intensity depending on the total radiation/drug dose, the size of the single dose, and the area of skin exposed to radiation (Soto et al., 2020; Yang et al., 2020). The skin damage that occurs may limit the dose of radiation supplied and the treatment duration, which significantly impacts the outcomes of therapy. With the increasing morbidity of cancer and the extensive use of radiation in cancer therapy, the side effects of radiotherapy have become a severe problem. To treat radioactive skin damage, some drugs/active substances like corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), hyaluronic acid, triethanolamine, sucralfate, aloe, calendula extract in the form of a topical formulation are applied (Iacovelli et al., 2020; Zacher et al., 2008). They play a crucial role in treating skin lesions with variable success. Better efficacy of therapy is observed when topical treatment is implemented immediately after the beginning of radiotherapy (Iacovelli et al., 2020). Some of the benefits of topical drug delivery systems are the avoidance of the first pass effect, more selective delivery of the drug, ease of application, and avoidance of gastrointestinal incompatibility (Sharadha et al., 2020). Unfortunately, conventional topical drug delivery systems suffer from drawbacks, for instance topical therapeutic systems can induce adverse skin reactions. After direct skin contact with the active ingredient, some patients may experience skin irritation, contact dermatitis, and allergic reactions (Romita et al., 2019). In addition, drugs with a high molecular weight (over 500 Da) may not be easily absorbed through the skin. To minimise side effects, the active ingredients are administered topically to the affected areas using carriers. Various topical drug delivery systems, such as nanoemulsions, nanoemulgeles, lipid carriers, vesicular carriers, and different kinds of liposome, noisome, enzyme, DNA, viruses, and phospholipids-based carri-

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ers are the subject of intensive scientific study (Chen et al., 2020; Sharadha et al., 2020). In this paper, multiple emulsions were developed and evaluated as carriers of diclofenac sodium salt – a nonsteroidal anti-inflammatory drug (NSAID) as an effective formulation for the topical administration and treatment of radiation-induced skin lesions following anticancer radiotherapy. Multiple emulsions are complex structured systems ("drops in drops"). The functional structure of the emulsion enables the incorporation of one or more drugs (lipophilic and hydrophilic), enhancement of stability and bioavailability of encapsulated substances, prevention of interactions between substances, as well as with the external environment, and drug release in a controlled manner (Dluska et al., 2022a; Dluska et al., 2022b; McClements et al., 2007). These liquid-liquid dispersed systems have a broad range of possible applications, especially in pharmaceuticals and in medicine for the encapsulation/delivery/targeting/controlled release of active substances like drugs, living cells, antigens, cosmetics, and food (Dluska et al., 2019a; Klojdová et al., 2023; McClements et al., 2007). It is crucial that the properties of pharmaceutical formulations for topical applications remain constant over time. This means the proper selection of formulants, excipients and emulsion morphology, as well as the emulsion-based carrier preparation method, and the determination of storage conditions and shelf life of emulsions with the drug. In order to confirm that multiple emulsions with a drug were stable, stability tests were performed. A necessary feature of topical preparations is the controlled successive slow release of the active ingredient. This allows the product to be applied to the skin at greater intervals, increases patient comfort, and maintains a constant drug concentration for a period of time. If the entire dose of the drug contained in the carrier were to be released immediately, the drug concentration could exceed the therapeutic window (concentration range between the minimum and toxic concentrations) and lead to undesirable effects. The multiple-emulsion form of the drug makes it possible to prevent this adverse effect. Controlling the release of a drug from multiple emulsions is achieved through the drop size, structure, and physicochemical parameters of an emulsion (Dluska et al., 2019b; Dluska et al., 2022a; Markowska-Radomska and Dluska, 2012).

## 2. MATERIALS AND METHODS

### 2.1. Method of multiple emulsions preparation

The multiple emulsions with a nonsteroidal anti-inflammatory drug (diclofenac) were prepared by a one-step method in a continuous Couette-Taylor flow (CTF) contactor. Detailed information on the proposed preparation method can be found in the authors' previous papers (Dluska et al., 2022a; Dluska et al., 2022b; Markowska-Radomska et al., 2021). In brief, three emulsion phases (internal, membrane and external) flowed through the concentric gap between two coaxial cylinders, with the inner cylinder rotating and the outer cylinder remaining stationary. In the gap, intensive mixing occurred due to the presence of the rotational and axial flows. The helical flow creates favourable conditions for conducting processes in multiphase systems (large interfacial area, high mass transfer coefficients, also in highly viscous systems, the possibility of independent regulation of the mixing intensity and residence time) (Dłuska et al., 2007). This method allows the preparation of different types of emulsions (e.g., O/W/O, W/O/W, or O1/W/O2, W1/O/W2) depending on the composition of the phases and the operating conditions in the CTF contactor (Dluska and Markowska-Radomska, 2010; Markowska-Radomska and Dluska, 2016). The detailed composition of the emulsion phases is shown in Table 1.

Table 1. Composition of the emulsion phases.

| Emulsion phase | Composition |
|---|---|
| Internal | 1.91 wt.% sodium alginate, 0.24 wt. Pluronic P-123, 0.041 wt.% methanol, 0.0021 wt.% diclofenac sodium, distilled water |
| Membrane | 2 wt.% Span 83, soybean oil |
| External | 0.25 wt.% poloxamer 407, 0.20 wt.% sodium carboxymethylcellulose (CMC), 0.25 wt.% Pluronic P-123, 0.25 wt.% tween 80, distilled water |

All compounds used to prepare emulsions were supplied by Sigma Aldrich.

The rotational frequencies of the internal cylinder in the CTF contactor were respectively for ME1 emulsions: 1166 rpm; ME2: 925 rpm; ME3: 1264 rpm; ME4: 976 rpm. The liquid flow rates in the CTF contactor for emulsions ME1–ME4 were:

- the internal phase 20 $cm^3$/min,
- the membrane phase 40 $cm^3$/min,
- the external phase 60 $cm^3$/min.

### 2.2. Multiple emulsion characteristics, stability and rheology

The W1/O/W2 emulsions were evaluated for their characteristics (drop size and drop size distribution, volume packing fraction), stability (37 °C and 24 °C), and rheology (Rheolab QC, Anton Paar). Microscopic observations of the emulsions were conducted to determine their characteristics. The Olympus SC50 camera, Olympus CellSens software, and Olympus BX-60 optical microscope were used to capture the images. The samples were applied as a thin layer on microscope slides and photographed at different points to obtain an image of the entire surface of the layer. Drop diameters were found with Image Pro Plus 4.5 image analysis software (Media Cybernetics). For each multiple emulsion, at least 250 droplets of both the internal and membrane phases were measured.

Based on the obtained measurements, the distributions of droplet size, the arithmetic mean diameter ($d_{10}$), the Sauter mean diameter ($d_{32}$), the de Brouckere mean diameter ($d_{43}$) and polydispersity index (PDI) were determined for the membrane (denoted as "$D$") and internal (denoted as "$d$") phases. PDI was calculated as the de Brouckere mean diameter divided by the arithmetic mean diameter.

The stability of the emulsions was assessed based on microscopic observations of changes in the structure and the size of droplets of the internal and membrane phases over 60 days. The observation was confirmed for emulsions stored at a temperature of 24 °C and 4 °C. Emulsions were considered stable if the Sauter diameters of dispersed phases remained unchanged by more than 15% relative to the diameters of a fresh emulsion. In addition, observations were made on changes in the emulsion complex structure.

Rheological measurements were conducted using a rotational viscometer (RheolabQC, Anton Paar) with a measuring system of concentric cylinder geometry (gap length – 60 mm; gap size – 1.64 mm; cone angel – 120 °; the ratio of radii – 1.08). The tests were carried out at a temperature of 24 °C in the shear rate range of 50–2500 $s^{-1}$.

### 2.3. Drug release study

A study on drug release was carried out using the dialysis membrane method. Dialysis membranes with molecular weight cut-off (MWCO) of 14 kDa (Nadir®, Thermo Fisher) were employed in this procedure. The high MWCO value of the semi-permeable dialysis membrane allowed diclofenac to be transported from the membrane to the medium outside (Kim et al., 2021b) and, at the same time, retained emulsion droplets inside the membrane. The emulsion and PBS buffer (Phosphate Buffered Saline) of pH 7 were mixed at a volume ratio of 1:1 (2.5 $cm^3$:2.5 $cm^3$) and introduced into the dialysis bag. The prepared system was then placed in a falcon filled with the PBS buffer (to 50 $cm^3$). The PBS buffer with a pH of 7 was used to simulate conditions on inflamed skin (Auerswald et al., 2019). The release process was conducted at 37 °C. Samples of the external medium were collected over time and analysed to determine diclofenac salt content (spectrofluorimeter (Jasco, Model FP-6500), wavelength of 276 nm). To assess drug encapsulation efficiency, samples of the external phase of the fresh emulsions (just after preparation) were diluted in PBS buffer (1:1 – v/v), and immediately filtered through syringe filters (nylon, 0.45 µm, Bionovo), and then analysed (for methods of analysis see above). The encapsulation efficiency (EE %) was calculated as the difference between the total amount of drug added in the stream of the internal phase and the non-encapsulated drug determined in the external phase divided by the total drug added. Encapsulation efficiency, thus indicates the percentage of diclofenac salt successfully encapsulated into the emulsions.

### 2.4. Cell viability assay

Cell viability assays were conducted to assess the effectiveness of emulsion formulations in a simulated skin environment represented by the K21 fibroblast cell line. The fibroblasts K21 were irradiated (Crosslinker CL-1000) with UV doses of 15 J/$m^2$ (similar biological impact on cells as commonly used in a radiotherapy dose of ionizing radiation of 2 Gy (Castle et al., 2019; Han et al., 2014; Pan et al., 2013). In short, K21 cells were cultured in F-10 Nutrient Medium supplemented with fetal bovine serum (FBS-10%) + penicillin-streptomycin solution (Pen/Strep-1%) in 10 cm diameter dishes to the confluence of 80-90% (incubator: 37 °C and 5% $CO_2$). After 24 h, the medium was withdrawn from the cell culture. Then 5 $cm^3$ of PBS was added to the cells and exposed to 15 J/$m^2$ UV irradiation (Crosslinker CL-1000). After irradiation, PBS was replaced by 10 $cm^3$ of complete medium (F-10 + 10% FBS + 1% Pen/Strep) and cells were then cultured for 24h (incubator: 37 °C, 5% $CO_2$). The cells were then treated with the diclofenac-loaded emulsion with two concentrations: 25 µM and 75 µM (different concentrations were obtained by diluting the emulsion with a culture medium) and with diclofenac solution of the same concentration for comparative purposes. As a control sample, non-treated fibroblasts were used. Cell viability was measured after 24, 48, and 72 h of incubation (37 °C, 5% $CO_2$). For this purpose, cytotoxicity tests were conducted using the presto blue reagent – relative fluorescence was measured (the wavelength Ex/Em = 535/590 nm, DTX880 Multimode Detector, Beckman Coulter).

### 2.5. Statistical analysis

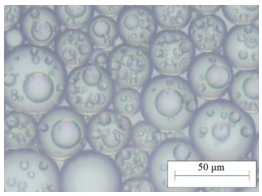
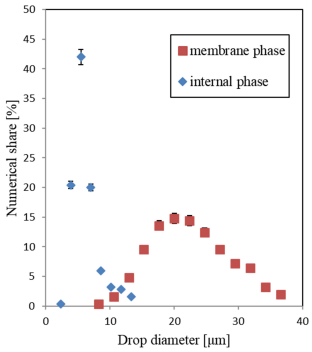
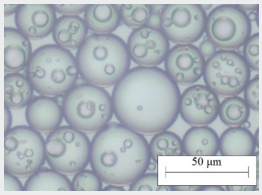
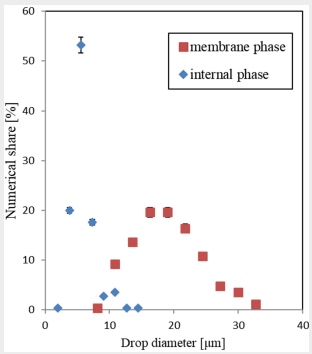
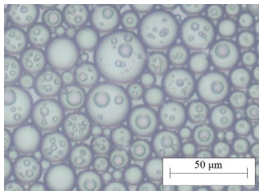
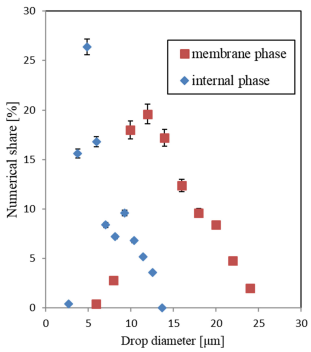
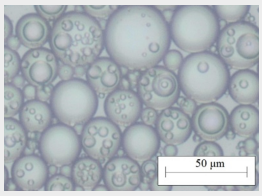
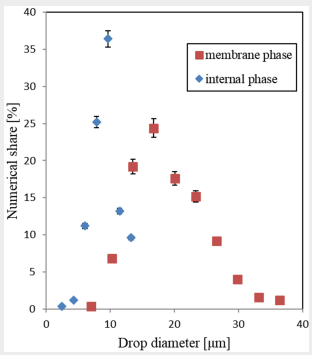
Each measurement/analysis was performed in triplicate and repeated three times. Results are presented as a mean ± standard error (SE).

## 3. RESULTS AND DISCUSSION

### 3.1. Assessment of multiple emulsions as carriers for the topical delivery of anti-inflammatory drugs

The paper proposes the multiple emulsions of W1/O/W2 (water in oil in water) type as carriers for effective encapsulation and delivery of diclofenac. Four stable multiple emulsions with diclofenac with a high volume packing fraction of the internal droplets (0.54–0.96) were obtained. The emulsions were prepared under different process parameters described in the experimental part (Section 2.1). The microscopic images of emulsion ME1 – ME4, emulsion characteristic parameters, and drop size distributions of emulsions are summarized in Table 2.

Table 2. Microscopic emulsion images, characteristics parameters, and drop size distributions.

| Emulsion | Microscopic image | The drop size distribution | Characteristics parameters |
|---|---|---|---|
| ME1 |   |   | $D_{10} = 22.5 \pm 1.1$ µm<br>$D_{32} = 25.7 \pm 1.3$ µm<br>$PDI_D = 1.2 \pm 0.1$<br>$d_{10} = 6.5 \pm 0.2$ µm<br>$d_{32} = 10.1 \pm 0.3$ µm<br>$PDI_d = 2.0 \pm 0.1$<br>$EE = 95.8 \pm 0.7\%$ |
| ME2 |   |   | $D_{10} = 19.1 \pm 0.6$ µm<br>$D_{32} = 22.0 \pm 0.7$ µm<br>$PDI_D = 1.2 \pm 0.1$<br>$d_{10} = 6.0 \pm 0.3$ µm<br>$d_{32} = 8.2 \pm 0.2$ µm<br>$PDI_d = 1.7 \pm 0.2$<br>$EE = 91.7 \pm 0.5\%$ |
| ME3 |   |   | $D_{10} = 15.1 \pm 0.5$ µm<br>$D_{32} = 18.0 \pm 0.5$ µm<br>$PDI_D = 1.4 \pm 0.2$<br>$d_{10} = 6.7 \pm 0.3$ µm<br>$d_{32} = 8.6 \pm 0.3$ µm<br>$PDI_d = 1.4 \pm 0.1$<br>$EE = 93.4 \pm 0.4\%$ |
| ME4 |   |   | $D_{10} = 19.2 \pm 1.0$ µm<br>$D_{32} = 22.9 \pm 1.2$ µm<br>$PDI_D = 1.3 \pm 0.1$<br>$d_{10} = 7.5 \pm 0.2$ µm<br>$d_{32} = 10.1 \pm 0.3$ µm<br>$PDI_d = 1.7 \pm 0.2$<br>$EE = 94.6 \pm 0.5\%$ |

The complex structure of the emulsion ME1–ME4 can be seen in microscope images presented in Table 2. The obtained emulsion structures have many droplets of internal phase in a droplet of membrane phase. The percentage of multiple droplets in the total droplet population is very high – over 97% for each emulsion. The Sauter diameters obtained for emulsions immediately after preparation ranged from 18.0 µm to 25.7 µm for the membrane phase drops

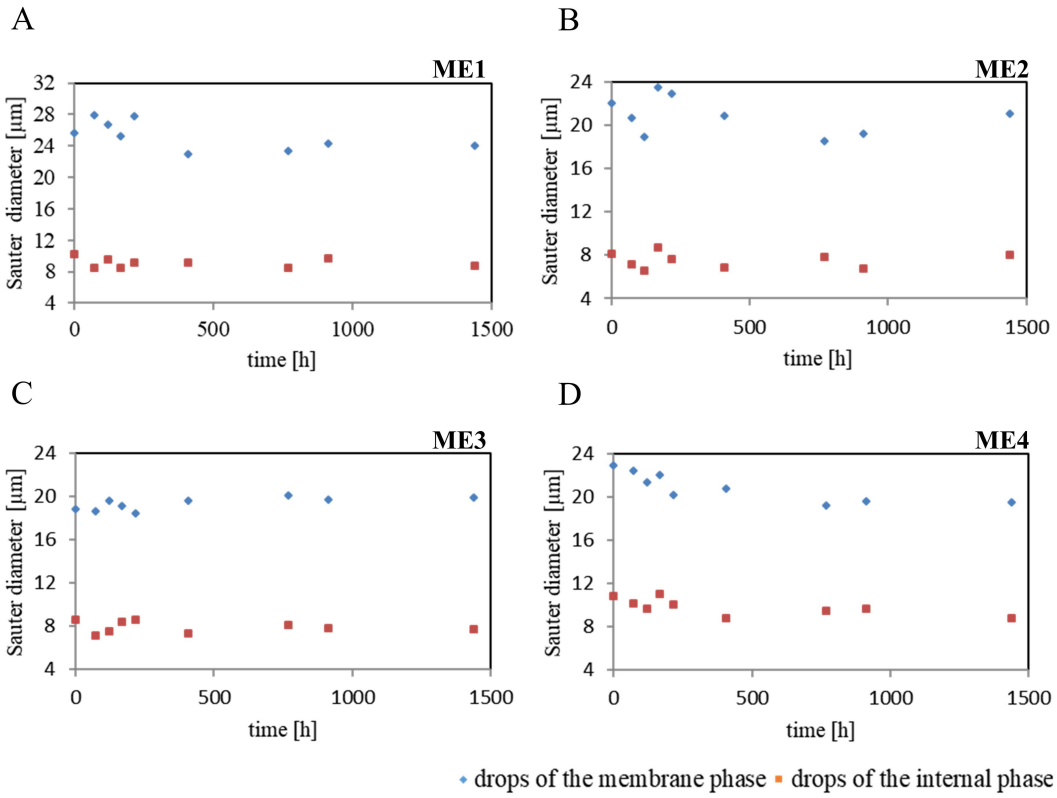



Figure 1. Changes in Sauter drop diameters of emulsions ME1 (A), ME2 (B), ME3 (C), ME4 (D) (results are presented as a mean of three independent measurements, the standard errors for the presented series were in the range of 2.1–8.3%).

and from 8.2 µm to 10.1 µm for the internal phase droplets. The ME1 emulsions were characterised by the highest mean diameters, and the ME3 by the lowest. All of the systems had relatively low polydispersity indexes (1.2 to 1.4 for the membrane phase and 1.4–2.0 for the internal phase). The NSAID drugs were encapsulated in the internal droplets of the emulsion. For all emulsions, high encapsulation efficiencies (over 90%) were obtained, Table 2.

Changes in the emulsion droplet diameters with time at 24 °C are presented in Figure 1. Emulsion stability assessments for samples stored during the time at temperatures of 24 °C and 4 °C are listed in Table 3.

The kinetic stability of the emulsion was evaluated on the basis of the measured mean values of the Sauter diameters. The maximum tolerable change in the diameters was assumed to be 15% relative to the initial value.

As shown in Fig. 1 the obtained multiple emulsions stored at 24 °C represented the ability to resist drop size change over up to 1500 h (about 60 days). Also, the multiple structures of emulsions remained unchanged, while for the temperature of 4 °C only emulsions ME1 were stable but over a shorter time (30 days – Table 3). For the other emulsions (ME2, ME3, ME4) droplet size changes were greater than 15% already during the shorter storage process. The stability tests performed made it possible to specify the storage conditions of the emulsions and their shelf life.

Table 3. Evaluation of the stability over time for two temperatures of storage.

| Storage conditions | | Stability of emulsions | | | |
|---|---|---|---|---|---|
| Temp. [°C] | Time [days] | ME1 | ME2 | ME3 | ME4 |
| 24 | 1 | ++ | ++ | ++ | ++ |
| | 7 | ++ | ++ | ++ | ++ |
| | 30 | ++ | ++ | ++ | ++ |
| | 60 | ++ | ++ | ++ | ++ |
| 4 | 1 | ++ | ++ | ++ | ++ |
| | 7 | ++ | ++ | + | + |
| | 30 | ++ | + | + | + |
| | 60 | + | + | – | – |

++ stable structure (change in the initial droplet size less than 15 %)
+ moderately stable (change in droplet size at the level of 15–25% of the initial size)
– unstable (change in droplet size more than 25% of the initial size)

The rheological properties including viscosity are among some of the more important physical attributes of the final dermatological emulsion. The obtained rheograms at 24 °C showed that the viscosity of the analysed emulsions ME1-ME4 decreased with shear rate increase, indicating a pseudoplastic (shear-thinning) non-Newtonian behaviour of fluids (Fig. 2).

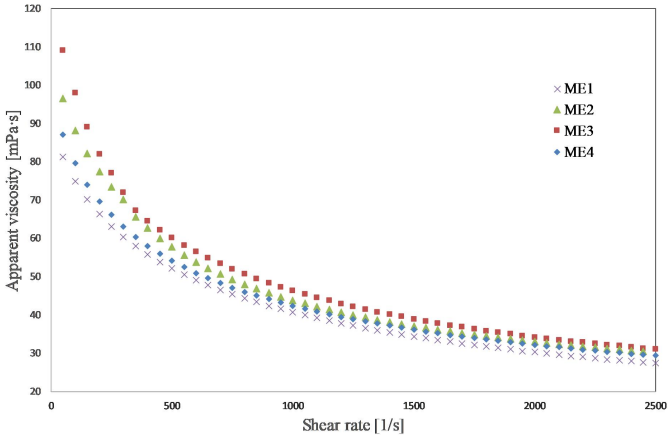



Figure 2. The apparent viscosity determined experimentally for emulsions ME1 (A), ME2 (B), ME3 (C), ME4 (D) (results are presented as a mean of three independent measurements, the standard errors for the presented series were in the range of 2.2–6.4%).

This characteristic is highly sought-after in pharmaceutical and cosmetic products intended for topical application (Suñer-Carbó et al., 2019). Such properties are favourable for easy, quick, and even distribution of the emulsion layer over the skin, as a result of which the skin is covered with a thin layer of preparation. This is especially important when the product is applied to damaged and painful skin.

The Power law rheological model (Eq. 1) was used for the quantification of the pseudoplastic behaviour of emulsions.

$$\mu = k \cdot \gamma^{n-1} \tag{1}$$

The consistency index ($k$) and flow behaviour index ($n$) can be determined based on viscosity measurements at varying shear rates. The method of least squares was used to determine the constants ($k$, $n$) in the power-law equation (Table 4).

Table 4. Consistency indexes and flow behavior indexes of prepared emulsions.

| Emulsion | ME1 | ME2 | ME3 | ME4 |
|---|---|---|---|---|
| $k$ [Pa·s$^n$] | 0.363 | 0.371 | 0.480 | 0.470 |
| $n$ | 0.677 | 0.682 | 0.640 | 0.654 |

For the emulsions ME1-ME4, the parameters k and n were correlated to the Power law model with a coefficient of determination (R2) ranging from 0.9944 to 0.9950. High values of squared Pearson correlation coefficients indicated that model predictions match experimental data very well.

## 3.2. Analysis of drug release kinetics from emulsion carriers

The release profiles of diclofenac from multiple emulsions of different sizes of membrane and internal phase droplets are presented in Figure 3.

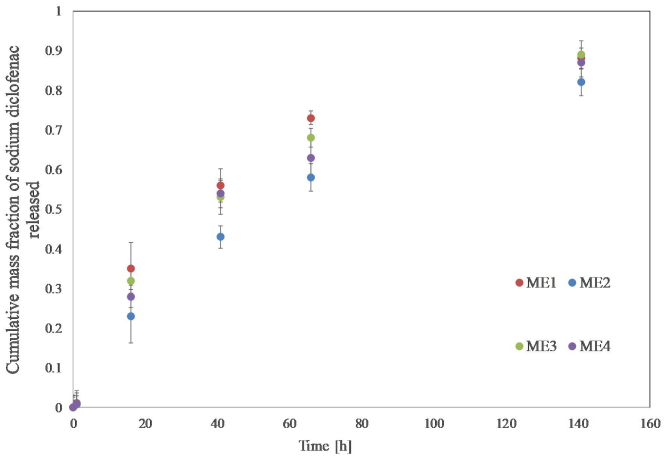



Figure 3. The time-dependent cumulative mass fractions of the anti-inflammatory drug (diclofenac) released from multiple emulsions ME1–ME4.

Over the course of the release process lasting 140 h, a significant amount of the drug encapsulated within the internal phase of the emulsion was released in a gradual manner, as shown in Figure 3. The amount of diclofenac released during this period ranged from 84% to 89% of the total amount (mass) encapsulated.

Figure 4A compares the drug release profiles of ME2 and ME3 emulsions differing in membrane phase droplet sizes (22.0 μm and 18.0 μm, respectively). The sizes of the internal droplets (8.2 μm for emulsions ME2 and 8.6 μm for ME3) and encapsulation efficiencies (91.7% and 93.4%, respectively) were similar. In the case of the ME3 emulsion having smaller membrane phase droplets, the amount of diclofenac released was greater than in the case of the ME2 emulsion with larger droplets. The drug release process occurred faster (on average by 7–10%) from the smaller droplets, which is related to the shorter diffusion path of the drug and larger interfacial area. On the other hand, comparing the amount of diclofenac released from ME2 and ME4 emulsions (Fig. 4B), similar in terms of membrane droplet size (22.0 μm and 22.9 μm, respectively) but differing in the size of the internal droplets (8.2 μm and 10.1 μm, respectively) the higher release rate (on average 5–11%) is related to a shorter diffusion path of the drug through the membrane phase from the bigger sized internal drops (ME4).

The release study of encapsulated substance confirmed the possibility of modifying the release rates. It was found that the releasing process can be controlled through the emulsion structure, which depends on the method and conditions of

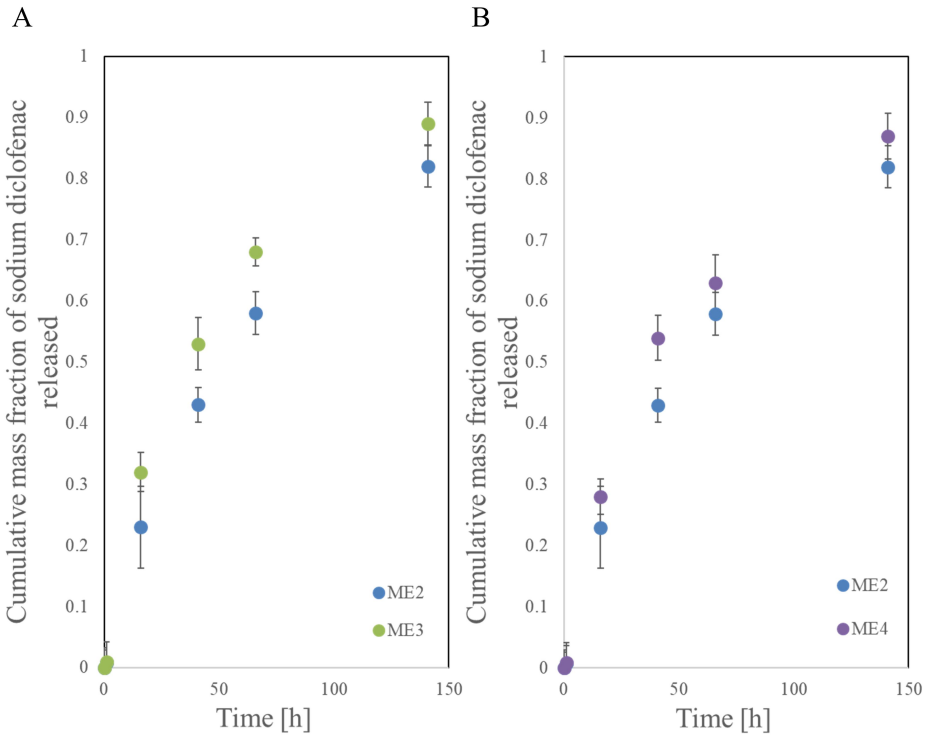



Figure 4. Comparison of time-dependent cumulative mass fractions of the anti-inflammatory drug (diclofenac) released from multiple emulsions ME2 and ME3 (A) and ME2 and ME4 (B) prepared in the CTF contactor.

preparation, the composition of the emulsion phases and its physicochemical parameters.

The release profiles obtained indicate that this process occurred in a controlled manner. Delivery of the drug from the emulsion-based carrier occurred slowly thus the process was prolonged. Such release characteristics are favorable for dermatological preparations because they do not expose the patient to high drug concentrations of the whole dose that could potentially have harmful effects on the skin (Kim et al., 2021a).

### 3.3. Evaluation of the effectiveness of emulsion formulations

In order to assess the effectiveness of the emulsion form of diclofenac, viability tests were carried out on K21 human skin fibroblast cells. Emulsions containing diclofenac at doses of 25 μM and 75 μM were added to the irradiated cells. Changes in the viability of cells treated with the ME1 emulsion are shown in Table 5. Also, the drug solution of the same concentration was added to the system with the irradiated cells to compare our proposed novel therapy with the classical one.

After irradiating the control cell sample, a significant decrease of cell proliferation was found. Adding diclofenac-loaded emulsions to the irradiated cells, a gradual increase of cell viability was observed (on average by 20–88%). The effect was dose-dependent and greater with the higher dose of diclofenac. Moreover, cell viability was increased after incubating the human fibroblast (K21 cell line) with an emulsion-based carrier of diclofenac. Better results were found, i.e. about 30 % higher viability of irradiated skin cells (K21) treated with the drug in emulsion than for a drug solution representing a classical formulation for the same drug concentration.

## 4. CONCLUSIONS

These results showed that the multiple emulsion might be appropriate carriers for the topical delivery of NSAID drugs. The emulsion drop size was found to be the control parameter of the release process rate. The drug release rate was high at the first stage of the release process, allowing the active concentration to be reached, maintained continuously for a period of time and slowly decreased. This is a desired effect as it enables the patient to receive a constant dose, and the drug concentration stays within the therapeutic window for longer. Due to the extended-release profile provided by the emulsion carrier, the NSAID level did not reach the toxic concentration, which could result in adverse effects on the skin.

The emulsions remained kinetically stable for 60 days while being stored at room temperature (24 °C), which makes them convenient to store. Refrigerating the samples did not help preserve their characteristics but rather destabilised them faster.

Table 5. The viability of K21 cells after irradiation (UV doses of 15 J/m$^2$) and treatment with a drug encapsulated in multiple emulsions and diclofenac solution in doses of 25 μM and 75 μM.

| Cell viability [%] | | | | | |
|---|---|---|---|---|---|
| Time [h] | Control (non-treated cells) | Cells treated with diclofenac solution | | Cells treated with diclofenac-loaded emulsion ME1 | |
| | | diclofenac: 25 μM | diclofenac: 75 μM | diclofenac: 25 μM | diclofenac: 75 μM |
| 24 | 43 ± 1.1 | 51 ± 7.3 | 61 ± 4.7 | 63 ± 8.1 | 76 ± 5.2 |
| 48 | 24 ± 0.9 | 58 ± 5.8 | 62 ± 5.6 | 78 ± 7.4 | 88 ± 5.1 |
| 72 | 7 ± 3.9 | 60 ± 4.2 | 65 ± 3.8 | 87 ± 3.4 | 95 ± 4.6 |

The appropriate drug delivery profile suggests that the emulsion formulation described in this work has the potential to be used in the treatment of inflammations resulting from exposure of the skin to X-ray radiation. After subjecting the irradiated fibroblasts to the multiple emulsions containing NSAID, a significant increase in cell viability by 88% was found compared to non-treated cells. Also, the cell viability increased by 30% on average, compared to the classic preparation (drug solution). This effect was dose-dependent and did not occur within the control sample (non-treated cells). In addition, emulsions as shear thinning fluids ensure easy and accurate distribution on a given area of the patient's skin without irritating the painful area of the skin. The above-mentioned properties of the prepared double emulsions make them promising topical carriers not only of anti-inflammatory drugs but also other active substances.

Ongoing advancements in topical and transdermal delivery bring forth novel technologies that allow precise dosing control, targeted delivery to specific areas, and improved absorption through the skin. This expands the variety of therapeutic substances that can be administered through skin application.

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## SYMBOLS

$d_{10}$ arithmetic mean diameter, μm
$d_{32}$ Sauter mean diameter, μm
$d_{43}$ de Brouckere mean diameter, μm
*EE* encapsulation efficiency, %
*h* time, h
*k* consistency index, Pa·s$^n$
*n* flow behaviour index
PDI polydispersity index

*Greek symbols*

$\gamma$ shear rate, 1/s
$\mu$ apparent viscosity, Pa·s

*Subscripts*

*D* membrane phase drops
*d* internal phase drops

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