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MULTIFUNCTIONAL EMULSION STRUCTURES FOR ENCAPSULATION AND MODIFIED RELEASE OF ACTIVE INGREDIENTS

WIELOFUNKCYJNE STRUKTURY EMULSYJNE DO ENKAPSULACJI I MODYFIKOWANEGO UWALNIANIA SKŁADNIKÓW AKTYWNYCH

A b s t r a c t

The paper presents the results of encapsulation of biologically and chemically active ingredients, such as living cells and drugs within multiple emulsions and release co-encapsulated drugs with the rate controlled by physicochemical properties of emulsions. The influence of process parameters in a Couette-Taylor flow bioreactor on the obtained emulsions structures, rate and mechanisms of release and the possibility to modify the release profiles have been discussed and presented.

Keywords: multiple emulsions, encapsulation process, release rate and mechanism

S t r e s z c z e n i e

Praca dotyczy wyników badań enkapsulacji substancji biologicznie i chemicznie czynnych takich jak leki i żywe komórki w emulsjach wielokrotnych oraz procesu ko-uwalniania z szybkością kontrolowaną parametrami fizykochemicznymi emulsji. W pracy przedyskutowano wpływ parametrów procesowych w bioreaktorze z przepływem Couette'a-Taylora na otrzymywane struktury emulsji oraz szybkość i mechanizm procesu uwalniania oraz możliwość modyfikacji krzywych uwalniania.

Słowa kluczowe: emulsje wielokrotne, proces enkapsulacji, szybkość i mechanizm uwalniania

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1. Introduction

Multiple emulsions are defined as dispersed systems having structures of droplets in a drop (Fig. 1). The smaller droplets of one liquid (internal phase) within larger drops of a second immiscible liquid (membrane phase) are themselves dispersed in a continuous phase, which has the same composition as the smaller droplet, or a different one. Due to their compartmentalised internal structure, multiple emulsions present many advantages over simple O/W or W/O emulsions for encapsulation, such as the ability to carry both polar and non-polar molecules, and better control in releasing therapeutic molecules [1-5]. They combine the properties of both types of simple emulsions and have the potential to encapsulate a large number of different ingredients e.g. cosmetics, drugs, living cells and incompatible materials, and protect active substances from the environment [1, 4, 6]. These dispersed systems offer a wide range of possible applications for cosmetics, food or the pharmaceutical industries, especially for the encapsulation and controlled release of active ingredients. Double emulsions ($F_1/F_2/F_3$) represent the simplest structures among multiple emulsions, Fig. 1.

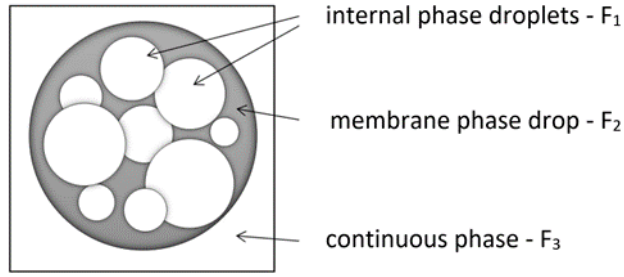

Fig. 1. The structure of double emulsion $F_1/F_2/F_3$

Encapsulation is the process of entrapping biologically/chemically active substances or in general particles of solid or droplets of liquid material or bubbles of gas within a protective membrane to produce capsules/drops in the micro/nanometre to millimetre range. There are two encapsulation processes, matrix in case dispersing active substances within membrane material or core shell when surrounding/coating with a continuous film of polymeric material is considering. The matrix particle allows a manufacturer to encapsulate more than one active ingredient within one particle, while the core shell is ideal for protecting an active ingredient where humidity and moisture or aggressive agents are present. The method of encapsulation can be divided into chemical, physiochemical, electrostatic and mechanical processes. Chemical processes include the interfacial and *in situ* polymerisation methods. Physiochemical processes include coacervation phase separation, multiple emulsion, meltable dispersion and powder bed methods. Mechanical processes include the air-suspension method, pan coating, and spray drying, spray congealing, micro-orifice system and the rotary fluidisation bed granulator method. Material or materials closed can be released for a specified time at a predetermined rate, depending on the process conditions for release-controlled or modified release.

Systems with a modified release of active agents are defined as systems providing a modify rate, profile or place where the release occurs, in comparison to conventional dosage forms of drugs administered through the same route [1, 4, 6]. The main objective of applying modified release systems is to achieve a constant concentration of active ingredients over a therapeutic time. Among systems with a modified profile of release, the following types can be distinguished: delayed, sustained and pulsatile release. The most widespread presentations of modified release drugs include oral, parenteral and transdermal dosage forms. Oral dosage forms are the most commonly used by patients due to their convenient administration. Obtaining drugs with a modified profile of an active agent release involves employing different settings concerning dispersed systems like suspensions, emulsions, aerosols, liposomes or micellar/lamellar structures. However, micro/nanoparticles that have undergone coating or incorporation are most frequently applied to a modified release profile.

Multiple emulsions are an example of a dispersed system applied in the modified release of an active agent. There are two main mechanisms of release: simple or facilitated diffusion or fragmentation of multiple emulsion, i.e. breakage of multiple drops and formation of simple emulsion [6]. In addition, the release process can be controlled by both of the mentioned mechanisms. In majority of drug release systems, the release is limited by mechanisms like dissolution, osmosis, diffusion or chemical reaction [1]. They can take place simultaneously or at different stages of the release process.

A literature overview has shown that the issue of the simultaneous encapsulation of two or more different active ingredients in the multiple emulsion, and their release has not been fully reported and explained yet.

The purpose of the work was to investigate a simultaneous encapsulation of two active ingredients, such as two drugs or living cells within double emulsions and process of release of co-encapsulated drugs.

2. Emulsification and encapsulation process in a Couette-Taylor flow biocontactor

The aim of the experimental research was to examine the process of biological and chemical ingredients encapsulation in double emulsions. Methods: Double emulsions of two types $W_1/O/W_2$ and $O_1/W/O_2$ were prepared by simultaneous emulsification and encapsulation in liquid-liquid Couette-Taylor flow (CTF) bioreactor/biocontactor, Fig. 2 (annular gap width of 1.5 and 2,5 mm and length of 400 mm).

$W_1/O/W_2$ emulsions consisted of alginic acid aqueous solution (inner phase) with living cells (Human Embryonic Kidney – HEK) and a cryoprotectant (sucrose), paraffin (membrane phase), distilled water (outer continuous phase) with appropriate surfactants (Tween and Span) added to each phase. The process parameters included: the rotational frequency of the inner cylinder of the CTF biocontactor: 540 rpm, an annular gap width of 2.5, the ratio of the volumetric flow rates of phases: internal to continuous: 0.25÷0.5 and internal to membrane: 0.5÷1.0, concentration of living cells: one million cells per cm^3.

$O_1/W/O_2$ emulsions contained liquid paraffin as oil phases with an addition of two model active agents (phenyl salicylate and benzoic acid) to inner oil phase and aqueous gelatine solution as a membrane water phase. The process of emulsification and co-

encapsulation of model drugs into $O_1/W/O_2$ has been successfully carried out under conditions corresponding to the rotational frequency of the inner cylinder of the CTF contactor: 1550÷1900 rpm, an annular gap width of 1.5, the ratio of the volumetric flow rates of phases: internal to continuous: 0.1÷0.5 and internal to membrane: 0.2÷0.5, concentration of drugs in the inlet stream for phenyl salicylate: 9.25 and 10 wt%, for benzoic acid: $2 \div 9.1 \cdot 10^{-3}$ wt%.

The details of the preparation technique of multiple emulsions in the CTF contactor and micro-encapsulation procedure were discussed in our earlier publications [6, 7]. The CTF contactor creates suitable hydrodynamic conditions for conducting mass transfer processes in multiphase systems (high interfacial area and mass transfer coefficients) [7÷9].

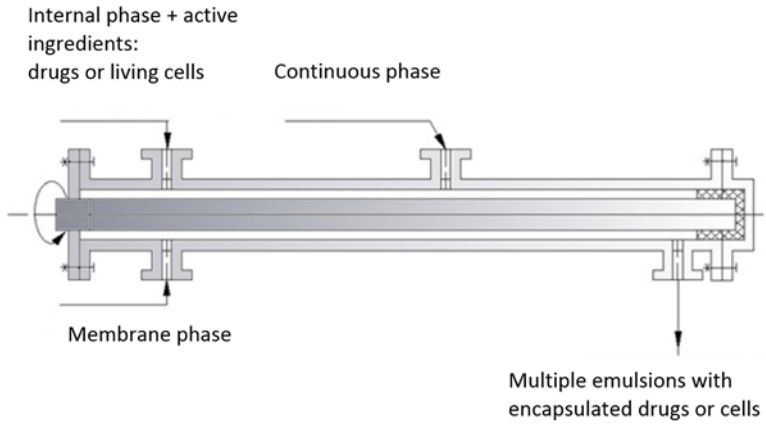

Fig. 2. Liquid-liquid Couette-Taylor flow biocontactor/bioreactor for forming double emulsion $W_1/O/W_2$ and $O_1/W/O_2$ and encapsulating of active ingredients

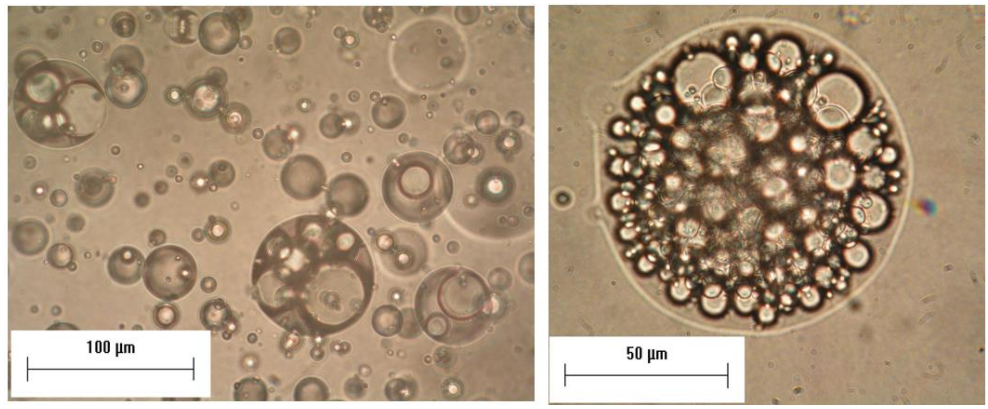

Fig. 3. The representative photos of multiple emulsions $O_1/W/O_2$ with co-encapsulated of two hydrophobic drugs (phenyl salicylate and benzoic acid) within internal paraffin droplets: phenyl salicylate (9.25 wt%) and benzoic acid $2.68 \cdot 10^{-3}$ wt%: left – at time t = 0, right – at t = 46.22 h.

Hydrodynamic conditions in the CTF biocontactor: rotational frequency of inner cylinder: 1622 rpm; flow rates of the liquid phases: internal/membrane/continuous = 50 /15 /100 cm^3/min

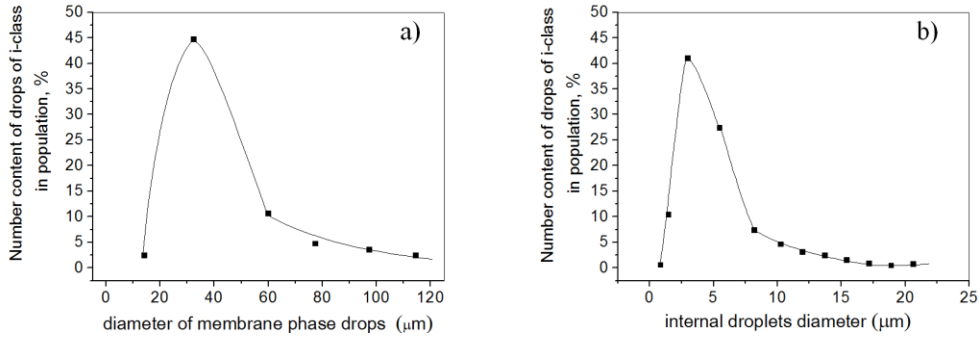

Fig. 4. The drop size distribution of double emulsions $O_1/W/O_2$ with two hydrophobic drugs encapsulated presented in Fig. 3: a) membrane phase drops, b) internal droplets

The shear stresses in a Couette-Taylor Flow apparatus are reduced by one to two orders of magnitude compared to a stirred tank with similar power input per unit volume and stirrer diameter being equal to the rotor of CTF [7]. This is a result of an increase in the area subjected to a constant maximum shear defined by friction drag on the larger surface of cylinder in the CTF device.

The structures of double emulsions with two hydrophobic drugs being encapsulated into internal droplets and drop size distribution are presented in Figs. 3 and 4.

The structures of double emulsions with living cells encapsulated within the internal droplets and the drop size distribution are shown in Figs. 5 and 6.

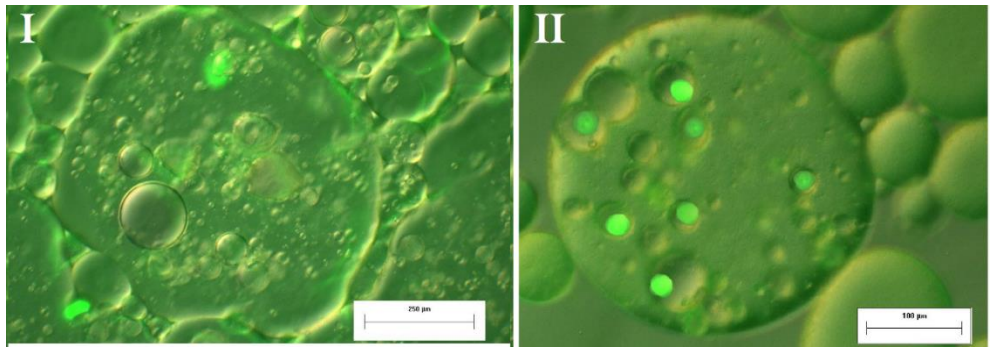

Fig. 5. The fluorescence microscope photos of multiple emulsions $W_1/O/W_2$ with encapsulated of living cells (HEK- Human Embryonic Kidney) within internal alginate droplets – I and additionally with sucrose as a cryoprotectant in the internal droplets – II:
Hydrodynamic conditions in the CTF biocontactor: rotational frequency of inner cylinder: 540 rpm; flow rates of the liquid phases: internal/membrane/continuous = 30 /30 /60 cm^3/min

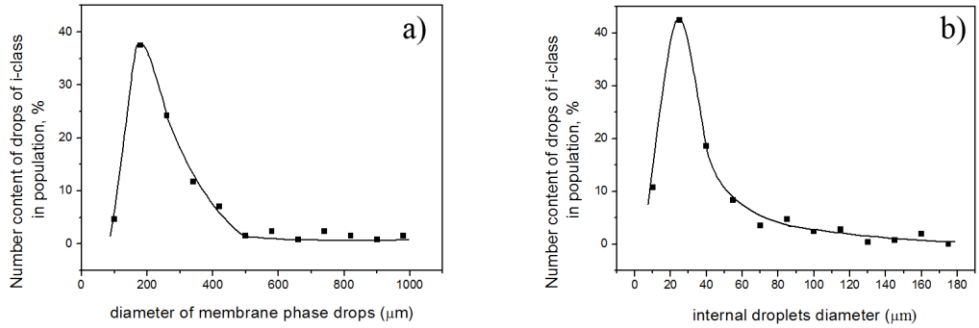

Fig. 6. The drop size distribution of double emulsions $W_1/O/W_2$ with living cells (HEK) encapsulated within internal alginate droplets shown in Fig. 5: a) membrane phase drops, b) internal droplets

3. The rate and mechanism of simultaneous release of two active ingredients

The experimental release study involved the influence of parameters of the emulsification and encapsulation process in a CTF biocontactor and the conditions of the release process (mixing intensity) on the release rates of two entrapped model active agents from internal paraffin droplets to the external paraffin phase of emulsions. The release experiments have been carried out in the stirred tank under a stirring frequency of 150 and 250 rpm at a temperature of 37°C.

The process of simultaneous encapsulation of phenyl salicylate and benzoic acid in the internal phase of $O_1/W/O_2$ double emulsions in the CTF contactor has shown an ability to create modified release by forming different structures of emulsions. The best double emulsions have been those obtained from a solution of drug concentration: 10 wt % of phenyl salicylate and $2.91 \cdot 10^{-3}$ wt % of benzoic acid in a contactor with an internal cylinder frequency of 1802 rpm [10]. These double emulsions $O_1/W/O_2$ with co-encapsulated hydrophobic have been characterised by a high encapsulation efficiency, the highest stability of internal and membrane phase drops and a two-step release profile of phenyl salicylate. The influence of preparation conditions in the CTF contactor, an initial concentration of benzoic acid and the conditions in the release environment on the kinetics of release process are demonstrated in Figs. 7 ÷ 9. The release profiles have been presented in the form of a dependence of cumulative mass fraction of the released ingredient on time.

Simultaneous release of both active substances from double emulsions (prepared under different hydrodynamic and process conditions in the CTF contactor) has shown that the release process occurring in the stirred tank is governed by a diffusion mechanism. The release mechanism has been verified during experiments performed in the stirred tank by comparing change in the drop size using microscopic image analysis. Since the diameter of the membrane phase drops and internal droplets remain unchanged, consequently, the basic release mechanism responsible for the release was unchanged too.

The comparison of the release profiles of emulsions with one (phenyl salicylate) and two (phenyl salicylate + benzoic acid) active agents has shown that the modified pattern, i.e. two-step release kinetics of phenyl salicylate has been a result of the presence of the second ingredient (Figs. 7, 8).

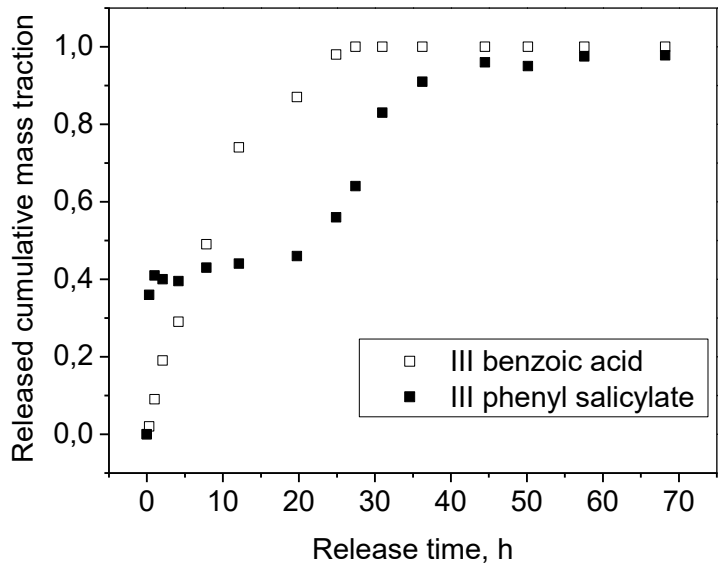

Fig. 7. Release profiles of both encapsulated drugs: benzoic acid and phenyl salicylate from double emulsions III formed under conditions in the CTF contactor: rotational frequency of inner cylinder 1802 rpm, initial concentration of benzoic acid: $9.09 \cdot 10^{-3}$ wt% and phenyl salicylate 10 wt%

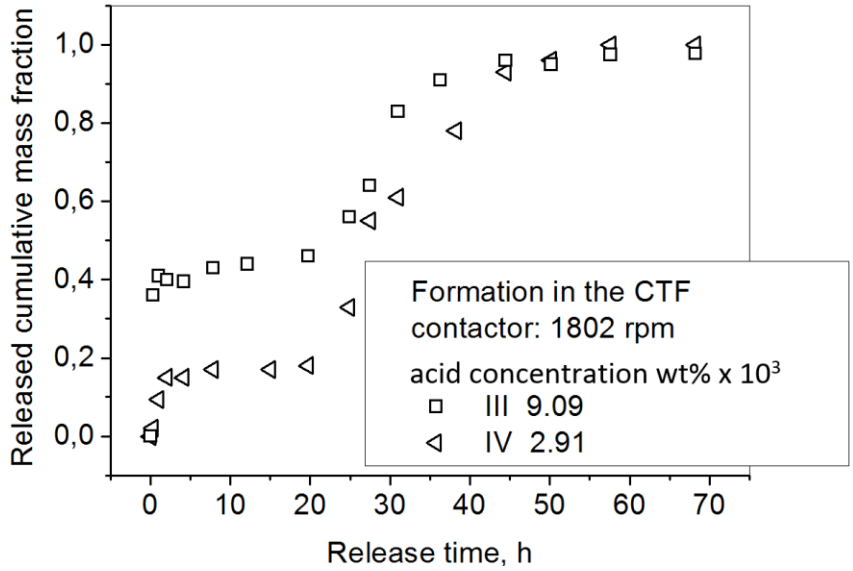

Fig. 8. The influence of an initial benzoic acid concentration on the release rate of phenyl salicylate from double emulsions III and IV formed in the CTF contactor

Addition of the second ingredient affects the time required to completely release the basic encapsulated ingredient, i.e. phenyl salicylate. The longest period of time required for a complete release of phenyl salicylate was observed for emulsions, which have been prepared under the highest rotational frequency of inner cylinder in the CTF contactor. Increasing the mixing intensity of the release medium caused a faster release of the encapsulated drugs (Fig. 9).

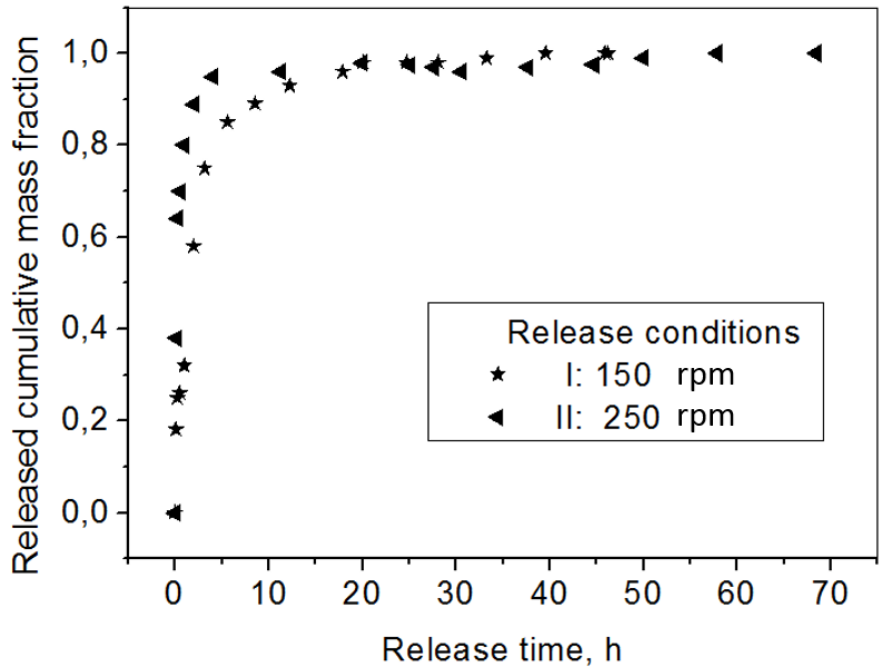

Fig. 9. The influence of the mixing intensity of environment on the release rate of phenyl salicylate from double emulsions I and II formed under conditions in the CTF contactor: rotational frequency of inner cylinder: 1622 rpm; initial concentration of benzoic acid: $2.68 \cdot 10^{-3}$ wt% and phenyl salicylate: 9.25 wt% – emulsions I, initial concentration of benzoic acid: $9.09 \cdot 10^{-3}$ wt% and phenyl salicylate 10 wt% – emulsions II

4. Summary

The study showed that it is possible to encapsulate biologically and chemically active ingredients, such as living cells and two different drugs in double emulsion $W_1/O/W_2$ or $O_1/W/O_2$ using the CTF contactor. Multiple emulsions with living cells can be further considered as carriers of biological material and a microenvironment for banking and storing cells for therapeutic purposes.

The release study involved the simultaneous release of two entrapped model active ingredients (two hydrophobic drugs) from the internal droplets to the external continuous phase of emulsions of

different structures proved that it is possible to modify the release kinetics, i.e. the rate and type of release profiles. The release of active ingredients from multiple emulsions can be controlled through the physicochemical parameters of emulsions and the size of internal droplets and drops forming a liquid-permeable membrane separating the internal droplets from the external environment. A desirable modified type of the release profile would be achieved by adding a few active ingredients into the internal droplets.

Research on simultaneous encapsulation and release of a few substances that have been made until now demonstrates new potential applications of multiple emulsions as systems for controlled/modified drugs release in targeted therapies. However, the issue of simultaneous release of a few active agents from multiple emulsions requires an extension of experimental investigations and mathematical modelling of the process.

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A c k n o w l e d g m e n t s

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