

# Stability evaluation of emulsion-based topical preparations: a valuable potential of dynamic-mechanical thermoanalysis (DMTA) test as a rapid rheological alternative to conventional freeze-thaw test

Nebojša Cekić<sup>1,2,\*</sup>, Sanela Savić<sup>1,2</sup>, Snežana Savić<sup>3</sup>

<sup>1</sup> DCP Hemigal, Research and Development Sector, Tekstilna 97, 16000 Leskovac, Serbia

<sup>2</sup> Faculty of Technology in Leskovac – University of Niš, Bulevar oslobođenja 124, 16000 Leskovac, Serbia

<sup>3</sup> Department of Pharmaceutical Technology and Cosmetology, University of Belgrade – Faculty of Pharmacy, Vojvode Stepe 450, 11221 Belgrade, Serbia

\*Corresponding author: Nebojša Cekić, email: nesafarm@icloud.com

---

## Abstract

The assessment of stability in emulsion-based topical preparations can be approached through real-time monitoring and/or accelerated methods, drawing predictions from pertinent stability-related physicochemical parameters. Ensuring the robustness and durability of topical products during storage, transport, and application necessitates thorough stability testing. However, due to the diversity of emulsion types and their intended use, there is no universal standard test, placing the liability on formulators/manufacturers to tailor appropriate assessments. Notably, topical emulsions, particularly cosmetic variants, often exhibit impressive stability with extended shelf lives. Nonetheless, evaluating their stability and decision-making remain challenging and time-consuming in industrial contexts. This underscores the demand for alternative testing protocols that expedite stability assessments and predict emulsion-based product stability accurately. This article comprehensively surveys literature, enriched with practical insights, exploring core mechanisms behind emulsion stability and prevention of instability. The discussion encompasses diverse approaches to stability assessment, revealing methodologies and parameters under examination during testing. Particular focus is placed on the dynamic-mechanical thermoanalysis (DMTA) method explored as a rapid, rheologically-based alternative to the conventional freeze-thaw test, emphasizing its usefulness for expediting the stability evaluation of emulsion-based topical preparations.

**Key words:** topical emulsions, physical stability, accelerated stability test, rheological tests, shelf life

---

[doi.org/10.5937/arhfarm73-46319](https://doi.org/10.5937/arhfarm73-46319)

## Introduction

Webster's dictionary defines stability as "resistance to chemical change or to physical disintegration" (1). Within the realm of emulsion-based topical products, the concept of stability should be interpreted not merely as a static state of being 'fixed' or 'unlikely to change,' as per the dictionary definition. Instead, it should be viewed through the lens of 'managed, recorded, and permissible change' (2). Therefore, it becomes imperative to contextualize this dictionary definition temporally, wherein a product constancy should endure consistently from inception to completion. Hence the necessity arises for assessing stability, a process aimed at delineating a product shelf life and gauging the product resilience under the market conditions prevalent during its sale and utilization. It is worth noting that the concept of shelf life transcends mere storage duration (shelf time); rather, it encompasses the entirety of the product lifecycle — commencing from production, extending through storage and distribution, and culminating at the cessation of user interaction. Stability assessment can be achieved through real-time monitoring or by making predictions based on pertinent stability-related physical parameters. Regardless of whether these assessments are conducted in real time or using accelerated methods, it is imperative to perform tests that guarantee the durability and soundness of topical preparations under conditions aligned with their storage, transportation, and application. In general, these tests also encompass aspects such as chemical stability, microbiological resilience, and the compatibility between product contents and their containers. While chemical alterations and microbiological breakdown can indeed influence product stability, the focus of this paper pertains to stability in terms of alterations in one or multiple physical attributes over a designated timeframe.

Taking into account their diversity in appearance, formulation composition and application, emulsions (macro-, micro- or nano) have normally presented a true challenge to the formulators and manufacturers responsible for assuring and maintaining quality, safety and efficacy of these products. In addition to the complex nature of topical emulsion formulations, the diverse range and the varying, relatively uncertain, conditions under which they may be stored and used, make the assessment of stability of emulsion products an intricate task, especially the prediction of their long-term stability. In practice, there exists no universally standardized test to assess the stability of all types of emulsion products. The responsibility often falls upon the formulator or manufacturer to determine the appropriate tests tailored to the specific product (3-7). Beyond the analysis of active pharmaceutical or cosmetic ingredients and the assessment of microbiological and chemical stability, the evaluation of physical stability is of paramount significance. This entails appraising the resistance of emulsion products to phenomena like creaming, sedimentation, coalescence, and phase separation. Such evaluations are pertinent in particular for emulsions of the oil-in-water (O/W) or water-in-oil (W/O) varieties. Given the remarkably extensive interfacial area inherent in these systems, the microstructure of dispersions is thermodynamically unstable (7, 8). However, through the utilization of emulsifiers and thickeners, emulsions can achieve a state of certain kinetic stability, which persists for a defined duration, desirably encompassing at least their shelf life. It is

imperative to underscore the critical distinction between the thermodynamic and the kinetic stability of an emulsion. The implications of this differentiation will directly influence the choice of stability evaluation tests that are employed. Thermodynamic instability informs us about the fundamental likelihood of a destabilization process taking place. Meanwhile, kinetics sheds light on the pace at which the state of dispersion is undergoing change, and the speed at which a product might deviate from its designated specifications (5, 7). In this context, destabilization occurrences are anticipated in cases involving thermodynamically unstable entities, such as conventional emulsions and nanoemulsions. Conversely, microemulsions, which are characterized by inherent thermodynamic stability, can still undergo destabilization, but typically as a result of the ingredients ratio change (due to water evaporation, for example), chemical degradation of their components, or a breakdown caused by microbiological activity.

The current article presents a comprehensive survey of the literature, combined with thorough practical insights, concerning the fundamental mechanisms responsible for achieving the sought-after stability of emulsions and preventing instability. It delves into a multitude of approaches for assessing the stability of products based on emulsions, while elucidating the methodologies employed and the parameters scrutinized throughout the stability assessment process. Particular emphasis is placed on the development and utilization of the dynamic-mechanical thermoanalysis (DMTA) method. This method is explored as a rapid, rheologically-based alternative to the conventional freeze-thaw test. The article further deliberates on its feasibility as a means for expediting the stability evaluation of topical preparations reliant on emulsion bases.

### **Regulatory aspects of stability testing**

The stability data prerequisites for human pharmaceuticals within the European Community (EC) are established upon a framework of Directive and Regulation requisites. These standards are further augmented by a set of advisory guidelines that have been formulated and ratified via the International Conference on Harmonization (ICH) protocols. In cases where no pertinent ICH initiative exists, the Committee for Proprietary Medicinal Products (CPMP) takes the lead in developing guidelines. These requisites encompass various categories, including novel drugs and their corresponding finished products, as well as established active ingredients and the products they are incorporated into. To conduct stability testing on new drug substances and products, the ICH Stability Guideline Series Q1A-F were formulated (9-14). The guidelines incorporate the notion of climatic zones I - IV and the corresponding conditions for long term and accelerated stability testing, including temperature, humidity and study duration (Table I).

According to the ICH Q1A (R2) Guideline (9), samples undergo monitoring for what is termed "significant change", a term encompassing various factors. This includes instances where there is a potency reduction of 5% from the initial batch analysis of the finished product, any degradation product surpassing its predetermined acceptance threshold, the product deviating from its pH limits, dissolution performance straying beyond the specified range, or failure to meet acceptance criteria for attributes like

appearance, physical characteristics, and functionality tests. Notable factors among these include color alteration and phase separation (9). Moreover, there are instances where a change in the emulsion vehicle might not be easily observable on a macroscopic level, yet it can still have a detrimental impact on the functionality of the active pharmaceutical ingredient (API). While there are well-defined criteria for assessing product stability linked to alterations in the assay of the API, the criteria pertaining to the physical stability of the emulsion-based vehicle appear somewhat less precise. This pattern appears to extend to the realm of cosmetics as well. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products (Cosmetics Regulation EC 1223/2009) does not offer explicit guidelines for stability criteria, except for the requirement that cosmetics must be safe for human health during typical or reasonably foreseeable usage conditions (15). Stability considerations for emulsion-based cosmetics might only display influence in specific instances, with sun protection products standing out as a conspicuous example. Hence, in order to address the crucial query of "what level of change is deemed acceptable", it becomes essential to establish stability criteria within specifications tailored to each specific product. These criteria delineate permissible boundaries for deviations from the original properties as they were at the time of production.

**Table I** Stability testing protocols for drug substances/products according to ICH guidelines (9, 26) - general case

**Tabela I** Protokoli za ispitivanje stabilnosti lekovitih supstanci/preparata prema ICH smernicama (9, 26) - uobičajeni uslovi čuvanja

| Stability study | Climatic zone                               | Storage conditions |                |                  |
|-----------------|---|--------------------|----------------|------------------|
|                 |   | Temperature        | Humidity       | Minimum duration |
| Long term       | I (Temperate climate)                       | 21°C ± 2°C         | 45% RH ± 5% RH | 12 months        |
|                 | II (Subtropical and Mediterranean climates) | 25°C ± 2°C         | 60% RH ± 5% RH | 12 months        |
|                 | III (Hot, dry climate)                      | 30°C ± 2°C         | 35% RH ± 5% RH | 12 months        |
|                 | IVa (Hot, humid climate)                    | 30°C ± 2°C         | 65% RH ± 5% RH | 12 months        |
|                 | IVb (Hot and very humid climate)            | 30°C ± 2°C         | 75% RH ± 5% RH | 12 months        |
| Accelerated     |   | 40°C ± 2°C         | 75% RH ± 5% RH | 6 months         |

## **Stability of emulsion-based topicals**

Emulsion stability can be characterized as the ability to maintain the original state of dispersion in accordance with predetermined stability criteria throughout a designated duration, while subjected to specified or foreseeable conditions of storage and usage (4). To assess stability, it is essential to conduct a comprehensive stability study aimed at establishing the product shelf life and gauging its resilience when exposed to the prevailing market conditions under which it is both sold and employed. It is important to note that the concept of shelf life extends beyond mere storage duration (shelf time). It encompasses the entirety of the product lifespan, spanning from production and storage to distribution and culminating at the point of end-user utilization. Hence, shelf life of an emulsion-based product can be defined as the timespan during which it remains seemingly unchanged or exhibits variations that fall within the limits stipulated by the product specifications. In essence, it signifies that the product retains adequate stability, and any instances of destabilization that do arise fall within acceptable thresholds (4, 5). The recommendations for conducting stability studies on pharmaceutical topicals are predetermined, as elucidated in the preceding section. In the following section, we will delve into the specific intricacies of stability assessment, with a particular emphasis on cosmetics formulated using emulsion bases. It is worth noting that many of the fundamental principles discussed are also applicable to pharmaceutical topical products.

The primary goal of a stability study is to evaluate the comprehensive and holistic stability of a product ("total overall stability"), encompassing both its intrinsic stability and compatibility with its packaging, often referred to as "product-container compatibility" (6). This evaluation serves as the basis for predicting, estimating, and subsequently confirming the product shelf life. Additionally, the study aids in recommending suitable storage conditions and, in some cases, ensuring customer safety.

Two primary approaches exist for conducting stability studies to predict the shelf life of products. These approaches significantly differ in terms of time frame and the conditions under which they subject the products to stress. The first method involves conducting stability tests under standard storage conditions, often referred to as "shelf-test" or "long-term stability test" (6). The second method involves accelerated stability tests, which intentionally subject the products to stress conditions to expedite the evaluation process. Particularly for highly stable products, which cosmetics frequently fall under, assessing shelf life can become a time-consuming aspect of new product development. Consequently, reliance on accelerated stability tests becomes crucial. These tests are performed under conditions that expedite the acquisition of stability-related insights in the most efficient manner. The long-term physical stability of new formulations can be evaluated through various physical, physicochemical, rheological, and chemical measurements. However, it is important to note that methods of this nature cannot be used to precisely calculate shelf life in terms of months or years (8). Stability testing operates as a forward-looking procedure that relies on data collected from products stored under conditions intended to hasten changes that might occur during market (or 'normal') circumstances (4, 5). Like other predictive methods, stability testing is not absolute but rather entails a certain likelihood of

success. This likelihood is lowest when relying on short tests performed under conditions that induce high acceleration, as this increases the risk of detecting changes that would not transpire under actual market conditions. In such instances, the test ceases to merely accelerate changes found under 'normal' conditions. The probability of success rises as test conditions align more closely with market conditions, and the duration of the test increases. Nevertheless, all predictive methods retain a success probability that falls short of 100%. In this context, a stability test is inherently a process that takes at least as long as the actual shelf life of the product. Therefore, it remains imperative to consistently maintain reference products under standard storage conditions (“shelf-test”) and assess them at specified intervals for changes. This process serves not only to confirm and reassess previously derived shelf life estimates, but also to potentially extend the product expiration period as well. The determination of when to grant a so-called 'stability clearance' hinges on the specific product, as well as the available information about related products and market experience with similar items with known stability profiles (6).

### ***Accelerated test conditions***

Accelerated testing involves subjecting the product to various external stress factors. These primarily include elevated temperature, low temperature, and cyclic temperature changes. Commonly utilized temperature regimes for accelerated stability testing of emulsion-based topical preparations are presented in Table II. Additionally, physical forces such as gravity, achieved by employing centrifugation or vibration, humidity variations, and exposure to light can also be applied during the stability testing process. This process involves monitoring quality parameters at specific time intervals and comparing them with those of a reference or standard product with a well-established stability profile in the market, if feasible (5, 6).

**Table II** Commonly applied temperature regimes for accelerated stability testing of emulsion-based topical preparations

**Tabela II** Uobičajeni temperaturni režimi za ubrzano ispitivanje stabilnosti emulzionih preparata za topikalnu primenu

| <b>High temperature</b> | <b>Low temperature</b> | <b>Cyclic temperature changes<br/>(one cycle)</b> |
|-------------------------|------------------------|---|
| 37 ± 2°C                | 5 ± 2°C                | RT* (24 h)/ -5 ± 2°C (24 h)                       |
| 40 ± 2°C                | -5 ± 2°C               | 40°C (24 h)/ 4 ± 2°C (24 h)                       |
| 45 ± 2°C                | -10 ± 2°C              | 45°C (24 h)/ -5 ± 2°C (24 h)                      |
| 50 ± 2°C                |                        | 50°C (24 h)/ -5 ± 2°C (24 h)                      |

\*RT – room temperature

\*RT – sobna temperatura

The fundamental principle of accelerated testing at elevated temperatures is to expedite changes that would typically take place at room temperature. However, the chosen elevated temperature should not induce alterations that would not naturally occur under standard storage conditions (6). For example, it should not induce phenomena such as phase inversion in emulsions stabilized with nonionic surfactants or compromise the physical integrity of products designed to melt at body temperature. This underscores the importance of understanding the specific system under investigation. In practical applications, temperatures exceeding 50°C are seldom employed. The extent of acceleration can be evaluated by comparing the magnitude of changes that occur at, for example, 45°C and other elevated temperatures, with those observed in samples stored at room temperature. Assessing samples stored at elevated temperatures can be roughly estimated based on the assumption of a two-fold acceleration for every 10°C temperature rise. However, even under optimal conditions, this remains a rather rough approximation (6). The use of control products is crucial for precisely this reason, as they provide an essential reference point for interpreting the outcomes of product stability and product-container compatibility tests. Control products offer a standard against which test results can be compared, aiding in the accurate interpretation of data and enhancing the reliability of the overall assessment. This approach helps identify potential changes in the product attributes under accelerated conditions and enables the prediction of its stability under normal storage conditions.

The Arrhenius equation finds relevance solely in probing the chemical stability of individual compounds within simple solutions. Consequently, its applicability holds limited value in cosmetic stability tests. Accurately estimating the level of acceleration over market conditions necessitates knowledge of the average temperature prevailing in the market. For instance, if the ambient temperature is 20°C, storing a product at 45°C for three months might approximate 6 to 12-18 months at room temperature. However, this approximation is not universally consistent. In markets with higher temperatures, storage at 45°C implies relatively less acceleration over the conditions that the product might encounter in the market. Therefore, tests must be extended over a sufficiently long period to gather data that can be used to draw conclusions about shelf life under such conditions. This is due to the fact that temperature conditions are not advised to exceed 45-50°C (6).

Whenever feasible, it is advisable to employ a minimum of two of the elevated temperatures mentioned in Table II, ensuring they are suitably spaced apart. This practice enhances the capacity to comprehend and quantify changes that occur across distinct temperature levels. The typical duration for maintaining samples at high temperatures during accelerated testing is around three months, but this period can be readily extended up to a year if needed (5, 6, 16).

Regarding low-temperature limits (Table II), the temperature range of  $5 \pm 2^\circ\text{C}$  could be utilized for storing reference samples. Conversely, the lower range ( $-5 \pm 2^\circ\text{C}$  or  $-10 \pm 2^\circ\text{C}$ ) is well-suited for evaluating the consequences of short-lived deviations from the typical storage conditions, which might occur during transportation or shipping processes.

The duration of this low-temperature testing is comparable to that of high-temperature testing (5, 6).

Temperature cycling, often referred to as the freeze/thaw (F/T) test, involves subjecting samples to alternating temperature conditions at regular intervals. The number of cycles can vary, while the typical temperature limits for the cycles are shown in Table II. The standard number of cycles is 6, spanning 12 days. This could extend to 18 days if an intermediate cycle at room temperature is incorporated. The F/T testing is particularly valuable for assessing the stability of emulsions or creams, as it provides insight into how these products perform under varying temperature conditions. This method can often offer more revealing information compared to simply subjecting samples to constant elevated temperatures. It simulates the real-world scenario of temperature fluctuations, which can impact product stability in practical usage.

An alternative to the F/T testing method is the DMTA test. DMTA serves as a high-performance substitute for accelerated freeze-thaw stability testing, capable of producing comparable data within a matter of hours. This method offers a quicker and more efficient way to assess the stability of emulsions or creams under temperature cycling conditions (3, 17). A more comprehensive explanation of the DMTA test will be provided in the subsequent sections of this text.

### ***Photostability***

Exposure to light can lead to various changes in products, encompassing modifications in color, odor, ingredient degradation, and potential shifts in packaging characteristics, including changes in its physical integrity and appearance. Products that are likely to encounter light exposure in the market, especially those packaged in clear or semi-transparent containers, should undergo light exposure testing. The objective is twofold: first, to ascertain the impact of light exposure on the unprotected product (in transparent packaging); and second, to assess the effects on the packaging itself, such as discoloration and stress cracking.

A challenge associated with the photostability test is quantifying the degree of exposure that the test samples have received and understanding the extent and type of light that actual marketed products are subjected to. The light exposure experienced by products can vary depending on factors like location, season, weather, and the specific type of light source, be it daylight (filtered by windows) or artificial room light (fluorescent, incandescent). Furthermore, while natural daylight changes with geographic location, season, and atmospheric conditions, artificial light sources degrade over time, leading to alterations in the intensity and composition of the emitted light. As a result, simulating the effects of light exposure can be complex and challenging to accelerate artificially (6).

One approach to mitigating this challenge involves storing control samples of similar products known to maintain satisfactory stability under market conditions. These control samples are then compared to the test samples, which are either kept in darkness or wrapped in aluminum foil. If the test samples exhibit light stability comparable to the



control samples, it is highly likely that they will demonstrate stability under actual market conditions. However, interpreting the results becomes more intricate when test samples are less stable than the corresponding controls. In such cases, these samples may still remain stable under the conditions present in the market (6).

The photostability testing procedures generally involve subjecting samples to window-filtered north-facing daylight. This can be conducted by placing the products in both borosilicate glass vials and inside the containers in which they are marketed simultaneously. For reference purposes, the same tests are conducted using a reference/standard product in borosilicate glass vials as well as within its original container which might contain stabilizers (6).

Photostability testing can be performed in climate chambers or light cabinets as well. Insights and guidance from the pharmaceutical field, such as those provided by the ICH Q1B photostability guideline (10, 18, 19), can offer valuable reference points when designing and implementing light exposure testing for cosmetic products.

### ***Stability study types***

There are four distinct types of stability studies, each varying in study design, objectives, and test conditions (20, 21).

#### ***Preliminary stability test***

A preliminary stability test, often referred to as a screening test, is primarily aimed at screening and selecting formulations during the product development phase. Unlike other types of stability studies, its primary goal is not to provide data for shelf-life estimation. Instead, formulation candidates are subjected to extreme temperatures for a shorter period, typically ranging from 15 to 30 days. The objective here is to observe any potential interactions among ingredients and detect early signs of instability. Samples are evaluated within inert containers, commonly glass, although containers made of materials intended for the product market packaging could also be used. The frequency of sample evaluations can vary based on factors such as experience, product specifications, unique characteristics of specific formulation components, or the type of preserving system employed.

Generally, evaluations are conducted at the study initiation and throughout the entire duration of the test. The standard set of parameters for evaluation comprises organoleptic parameters (such as appearance, color, and texture) and physicochemical parameters like pH value, conductivity, rheological properties, centrifugation behavior, particle size and size distribution, and, in some cases, the monitoring of active ingredient concentration. Whenever possible, it is highly recommended to include a reference product with a known stability profile for the relevant market(s) in which the product will be marketed. During the preliminary stability test, the assessed parameters are compared against samples stored in a refrigerator or at room temperature, often kept in the dark (20, 21). This comparison helps to identify any deviations or changes in the tested parameters and offers valuable insights into the formulation stability under these conditions.

### *Accelerated stability test*

An accelerated stability test is a predictive study designed to estimate a product stability, useful lifespan, and its compatibility with the primary container. This test is also useful when significant changes are expected to occur in the product ingredients, manufacturing process, primary container material, or when new equipment or outsourced manufacturing is involved. It is typically carried out on the development phase batches, pilot batches, initial production batches, and other relevant scenarios. Unlike the preliminary stability test, accelerated testing employs less extreme conditions. During this test, samples are subjected to various conditions for a period usually lasting at least 90 days. However, the test duration could be extended to six months or even a year, contingent upon factors such as the nature of the product and the climate conditions in the target market. The samples are placed in inert containers, usually glass, but should also be tested within the containers used for market packaging. The conditions applied include heating in ovens/chambers, cooling in refrigerators and light irradiation (if applicable, considering its potential effects on color, odor, and ingredient degradation). It is advisable to expose samples to multiple temperature conditions, representing the diverse environments they could encounter (6, 20, 21).

The evaluation frequency can vary depending on factors like product characteristics, formulation components, and preserving systems used. However, a recommended evaluation schedule includes assessments after 24-72 hours and then on the 7th, 15th, 30th, 60th, and 90th day. If the study is extended, monthly evaluations are recommended until its completion. While it is not possible to prescribe a single examination schedule suitable for all products and situations, such a schedule would help determine the number of samples required for the test. It is wise to store a surplus of samples beyond the calculated number, allowing for repeated tests in case unexpected results arise. This approach ensures that the stability evaluation remains robust and comprehensive (6, 20, 21).

In addition to the standard organoleptic and physicochemical parameters, the evaluation set also includes microbial parameters, tests for barrier properties of the packaging, and compatibility between the product and the primary container. Microbiological parameters assessment encompasses microbial count, challenge test of the preserving system, including the assay of preservatives, and preservatives assaying conducted prior to the test and upon its conclusion. For evaluating the barrier properties of the packaging system and compatibility with the primary container, the product weight loss/gain is evaluated. The difference between test samples in the primary packaging and control samples stored in impermeable containers is compared. Testing at elevated humidity is less certain to solely accelerate changes at 'normal' storage conditions compared to tests at elevated temperatures. The effect of elevated humidity depends on the specific hazard – for instance, ingress of water vapor could accelerate changes, while loss of water or volatile constituents might be delayed. The *vice versa* is also true – measuring weight loss at lower relative humidity (RH) values might produce false positive data compared to what would occur under normal storage conditions (6). If tested

in a climate chamber at 40°C and 75% RH, the water loss ratio would be greater for lower RH values (very dry climate), resulting in a linear relationship to some extent. ICH Q1A offers guidelines for deriving the water loss rate at the measured (alternative) relative humidity compared to the reference relative humidity (9). Assessing the water vapor barrier properties of a container at 37°C and 80% RH can represent an acceleration of 8-10 times over 20°C and 80% RH. This is based on the vapor pressure of water at 37°C and 20°C, respectively, assuming a doubling of the rate of diffusion of water vapor per 10°C temperature rise. If there is no means to monitor or control RH, it is suggested to determine weight loss/gain at normal storage conditions (“long-term test”) to ensure accurate and meaningful results (6).

The evaluation of compatibility between the product and the primary container is greatly influenced by the type and material of the primary packaging. Various phenomena are closely monitored during this assessment, including absorption (of preservatives, for example), leaching of constituents of the container by the product, overall migrations, corrosion, failure of label adhesive and exfoliation of laminates, loss of physical integrity (twisting, bulging, cracking), closure system change/loss of function, valve function, etc. The compatibility evaluation takes into account a wide array of potential interactions between the product and its packaging, ensuring that the packaging preserves the product integrity and safety throughout its intended shelf-life. Once again, including a reference product with a known stability profile for the relevant market(s) is highly recommended. By comparing the tested parameters against samples stored in a refrigerator or at room temperature (kept in the dark), deviations or changes can be accurately identified (4-6, 20).

#### *Long-term stability test*

The long-term stability test, often referred to as a shelf test, involves storing samples under normal storage conditions, usually at room temperature. The samples are placed on a shelf without being subjected to additional stressors. This type of test is significant for confirming, re-evaluating, and potentially extending previously determined shelf-life decisions for a product. The minimum duration of the shelf test is guided by the initially predicted shelf-life. It is recommended to carry out the test for at least the duration of the predicted shelf-life. The frequency of analyses during the test should be determined based on factors like the nature of the product, the number of batches produced, and the estimated expiry date. The set of parameters for evaluation in this test is typically similar to those used in previous tests, including organoleptic parameters, physicochemical parameters (pH value, conductivity, rheological parameters, particle size distribution, etc.), microbial parameters (microbial count and challenge tests), and assessment of product-container compatibility and barrier properties of the packaging.

Continuing the follow-up process is advisable if the goal is to extend the product expiry period beyond what was initially established (6, 20, 21).

### *Transportation test*

The transport stability test focuses on evaluating the product resistance to the conditions it may encounter during transportation and handling. These conditions can include elevated temperatures in cargo holds, low temperatures in airplanes, humidity, vibration, pressure, and impacts. There are two main approaches to conducting this type of test:

1. Samples are exposed to the actual conditions experienced during transportation using various means such as trucks, airplanes, trains, or ships. This test assesses the performance of the primary packaging, secondary packaging (if applicable), and the formulation itself. Data loggers are often employed to monitor and record the conditions that the samples are exposed to throughout the transportation process.
2. Samples are subjected to conditions and equipment that mimic different modes of transportation and their variations. While this test cannot perfectly replicate actual transportation conditions, it provides an approximation of how the packaging and formulation might perform during actual transportation conditions. Simulation conditions typically include vibration, pressure, drop tests, and variations in temperature and humidity (20, 21).

A rheological oscillatory test could be utilized to characterize the product behavior when exposed to vibrations during transportation. This test involves conducting frequency sweeps with a deformation strain just outside the linear-viscoelastic region (LVER) to assess how the product responds to vibration frequencies. Frequency sweeps and their application will be explained in more detail later in the paper (22).

The transport stability test is crucial to ensuring that the product packaging can withstand the rigors of transportation and that the formulation remains stable and safe throughout the journey to its destination.

### *In-use stability test*

In addition to formal stability studies, it is crucial to emphasize the significance of in-use stability testing, especially when it comes to topical emulsion products packaged in multi-dose containers, which is often the norm. Such products, due to frequent opening and closing, inherently carry the risk of quality deterioration post-initial use. Issues such as physicochemical degradation, contamination, and microbial growth pose concerns, making the handling, usage, and storage of such products vital for patient and consumer safety (23, 24).

The primary objective of in-use stability studies is to establish a suitable timeframe during which a multiple-dose drug product can maintain its stability within predefined physical, chemical, and microbiological specifications. This entails retaining the specified quality, safety, and effectiveness once the container has been opened (23, 24). Established guidelines, including those by the European Medicines Agency in 2001 (25) and the World Health Organization in 2018 (26), underscore that in-use stability testing serves

the dual purpose of providing information for labeling, determining storage conditions and the post-opening in-use period for multi-dose products, as well as defining the shelf life after opening (referred to as the in-use shelf life) for gaining marketing approval of the drug product (25, 26).

It is important to note that the practical simulation of the actual use of topical emulsion-based products (in liquid or semi-solid form) involves consideration of several relevant factors. These include the type and capacity of the container (whether it is a bottle, tube, jar, pump, or flexible dispenser), the quantity and frequency of product use in real-life scenarios, the method of product withdrawal, duration of use, application site, environmental conditions during usage (such as temperature, humidity, and light exposure), as well as the expected behavior of the end user. These variables make in-use stability testing for topical emulsion products a particularly challenging endeavor (23, 24).

### ***Sample preparation and selection of samples***

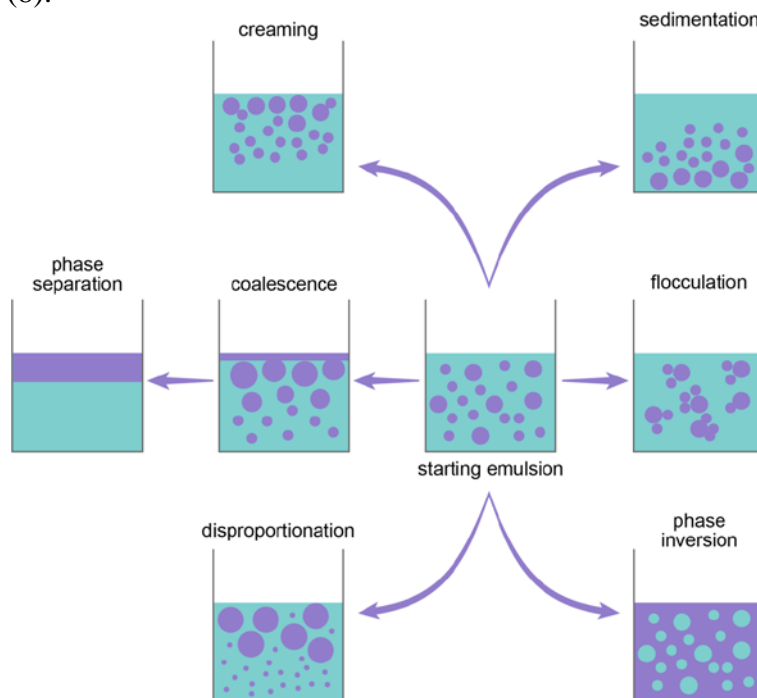
To effectively distinguish between the inherent stability of the product itself and the compatibility of the product with its packaging, it is recommended that samples used for stability evaluation be placed in neutral and transparent glass flasks. These flasks should have lids that ensure a tight seal, preventing the escape of gases or vapors into the environment. It is important to take care during the sample placement to avoid incorporating air into the product, as this could impact the results. When placing samples in the test containers, it is recommended not to fill the container to its total volume. Leaving approximately one fourth of the container capacity as head space allows for potential gaseous exchanges. This consideration is especially relevant in cases where products may undergo changes due to gas evolution or other reactions. In situations where there is a known incompatibility between the formulation components and glass (as seen with Silica Silylate in “dry liquid” concept products), an alternative container material must be chosen to prevent undesirable interactions (20, 21).

Stability testing should involve packaging that is made of the exact same material(s) and is as similar as possible in all other aspects to the packaging intended for marketing the product. If the product is intended to be marketed in various types of packaging, it is advisable to conduct stability tests for each packaging type. Similarly, when dealing with a range of package sizes, it is recommended to test the product in the smallest container as it often presents the most challenging conditions for stability (6).

### **Emulsion destabilization phenomena**

Destabilization phenomena in emulsions are complex processes that can lead to changes in the state of the dispersion. These processes are crucial to understand when designing stability studies and interpreting the observed changes in emulsions. Figure 1 provides an overview of various phenomena that can transform the state of dispersion. These changes can directly induce shifts in the continuous and/or dispersed phase, resulting in irregularities within the dispersion itself, such as variations in concentration

or phase separation. It is important to note that every alteration in the dispersion state is intricate and cannot be easily explained. Moreover, multiple processes often occur concurrently (8).



**Figure 1. Principal emulsion destabilization phenomena**

**Slika 1. Osnovni fenomeni destabilizacije emulzija**

Creaming is the event that does not entail a complete emulsion breakdown, but rather the separation of the emulsion into two distinct phases due to density differences between the dispersed and continuous phase. One of these phases, referred to as the cream, contains a higher concentration of the dispersed phase compared to the other. For emulsions characterized by low viscosity and smaller droplet sizes ( $< 0.7 \mu\text{m}$ ), the natural separation driven by earth gravity is mitigated by the random movement of particles known as Brownian motion (7). Creaming is the primary mechanism through which the dispersed phase begins to separate in an emulsion, and it often serves as a precursor to the subsequent process of coalescence. The rate at which creaming (or settling in the case of dispersed phases denser than the continuous phase) occurs can be approximated using the Stokes' equation. The sign of the density difference is negative in the case of creaming (for an O/W emulsion), but positive for sedimentation or settling (for a W/O emulsion). Furthermore, the Stokes' equation highlights that creaming is hindered by certain factors: a small droplet radius, a highly viscous continuous phase, and a minimal density difference between the oil and water phases (27).

Flocculation can be broadly described as the process in which droplets come together to form three-dimensional clusters, without the actual merging (coalescing) of

the droplets themselves. Notably, each individual droplet retains its distinct structure and remains as an entirely separate entity within the cluster. During processes such as creaming/sedimentation and flocculation/agglomeration, the initial distribution of droplet sizes remains relatively unchanged at an individual droplet level. In such instances, attractive forces between droplets are separated only by a thin film of the continuous phase. However, droplets also have a tendency to grow over time, which leads to a decrease in the overall interfacial energy present within their interfaces. Consequently, the distribution of droplet sizes tends to shift towards larger sizes as storage time progresses (during shelf life). This growth can occur through coalescence, where smaller droplets merge to form larger ones, resulting in an increase in the average droplet size. In more extreme cases, this process can lead to complete separation of the phases and the collapse of the emulsion structure (8, 28).

Another mechanism responsible for the change in droplet size distribution is Ostwald ripening, often referred to as disproportionation. In this process, larger droplets grow at the expense of smaller ones due to the diffusion of molecules from the dispersed phase of smaller droplets to that of larger droplets. This diffusion is driven by the difference in capillary pressure between the droplets. Smaller droplets, with higher capillary pressure, release their molecules to the larger droplets, leading to a further size increase of the larger droplets. It is crucial to emphasize that while the pressure difference between small and large droplets serves as the driving force for diffusion, the rate of diffusion is influenced by the solubility of the dispersed phase in the continuous phase. A higher volume of the dispersed phase results in greater relative vapor pressure and, consequently, higher solubility. The droplet radius also contributes to this phenomenon, as smaller radii correspond to greater solubility. There are methods to effectively mitigate Ostwald ripening to an acceptable degree. For instance, in W/O emulsions, if soluble substances are added to the aqueous dispersed phase - particularly those with low molecular weight like salts or sugars, the concentration of these substances will increase through Ostwald ripening. This increase in concentration results in higher osmotic pressure within the smaller droplets, which counteracts the capillary pressure differences. Consequently, water molecules diffuse from smaller to larger droplets through the continuous phase until the system reaches a metastable equilibrium. At this point, further Ostwald ripening is suppressed, and the emulsion achieves a state of stability against further disproportionation (8, 29).

Phase inversion is a phenomenon in which the roles of the continuous and dispersed phases within an emulsion spontaneously switch. This transition occurs based on the system specific properties, volume ratio, and energy input. Unplanned phase inversion can lead to the formation of coarse emulsions in certain scenarios. This often occurs when combining emulsion phases in a manner where the continuous phase is gradually introduced to the dispersed phase during mixing. Phase inversion can be particularly observed when utilizing ethoxylated nonionic emulsifiers that, beyond a certain temperature, cease to hydrate further and instead begin acting as emulsifiers for the opposite type of emulsion (W/O emulsion). This occurrence can be harnessed to achieve

finely dispersed inner phases, as seen in concepts like phase inversion temperature (PIT), where the polarity of the dispersed phase also plays a significant role (30).

In addition, there are other events that can lead to destabilization. These include changes caused by factors like irradiation, thermal or chemical degradation of individual components, or microbiological breakdown. The impact of these events might culminate in the eventual phase separation of the dispersion, following any of the previously mentioned pathways. These complexities highlight the intricate nature of dispersion destabilization and the multifaceted factors that contribute to it (8).

Physical forces, like centrifugal acceleration, can significantly hasten concentration changes or phase separation (depicted in the upper portion of Figure 1) in a selected dispersion. This is because phase separation processes such as sedimentation, flotation, or creaming are influenced by the force of gravity. In contrast, certain physicochemical phenomena, including Ostwald ripening and phase transitions (illustrated in the lower part of Figure 1), cannot be expedited by applying physical forces. However, coalescence and flocculation (found in the middle section of Figure 1) can be accelerated through either physical or thermal means (8).

Given that elevated temperature is a commonly employed factor in accelerated stability testing, it is important to elucidate the potential mechanisms of emulsion destabilization associated with higher temperatures. In addition to the expected increase in sedimentation and creaming due to decreased viscosity, several specific phenomena like Ostwald ripening, phase inversion, coalescence, and flocculation can be accelerated by elevated temperatures. This approach is rooted in the concept of a moderate energy barrier that maintains the stability of the dispersion by preventing colliding particles from getting close enough to interact through van der Waals forces and adhere to each other. By raising the temperature, particles acquire higher kinetic energy, augmenting the likelihood of overcoming this barrier and promoting the aggregation or coalescence of particles (8).

Yet another destabilization factor often employed during stability testing is freezing and F/T testing. Depending on the emulsifier system employed and type of emulsion, several physicochemical phenomena occurring during frozen storage could contribute to the observed impact of F/T cycling on emulsion stability. The following factors collectively might contribute to changes in the stability of emulsions subjected to F/T cycling:

1. When emulsions are placed in a freezer, water crystallization occurs. As more water crystallizes, the droplets are forced closer together. In this process, there might not be enough free water to fully hydrate the droplet surfaces, promoting interactions between droplets.
2. Ice crystallization leads to an increase in the ionic strength of the non-frozen aqueous phase surrounding the emulsion droplets. This heightened ionic strength may encourage interactions between droplets.



3. Ice crystals formed during freezing could potentially penetrate the oil droplets and disrupt their interfacial membranes. This disruption may make the droplets more susceptible to coalescence.
4. Cooling could cause some of the fat within the emulsion droplets to crystallize. This crystallization might encourage partial coalescence, as a fat crystal from one droplet could penetrate the membrane of another droplet (31, 32).

### **Parameters and methodologies in stability testing**

Parameters for evaluation and stability metrics encompass the characteristics of the dispersion state or behavior that need to be monitored in accordance with the specific desired product attributes. These parameters are heavily contingent on the nature of the product under study and the specific ingredients employed in its formulation. Some typical parameters that are monitored include (6, 20, 21):

1. Organoleptic parameters: appearance, color, odor, sensory attributes such as the product texture and how it feels to the touch;
2. Physicochemical parameters: pH value, conductivity, droplet size and size distribution, rheological parameters (viscosity, viscoelastic properties), centrifugation properties, density, turbidity, detection of structures that stabilize systems (e.g., liquid crystals under polarized light), water percentage and water activity, concentration of active ingredients;
3. Microbiological parameters: microbial count, challenge test of the preserving system (assesses how well the preservatives maintain microbial control), concentration of preservatives throughout the test.

It is worth noting that solely relying on challenge tests might not always be adequate. Some degradation products of antimicrobials could possess notable antimicrobial activity themselves, but might also be more toxic. Similarly, depending only on preservative assays after the test might not provide a complete picture. The presence of preservatives could be sufficient in quantity but rendered ineffective due to interactions with nonionic surfactants. Moreover, some preservatives might be absorbed by plastic containers, leading to their removal from the product over time. Therefore, a comprehensive approach is required to thoroughly assess the stability and quality of the product (6).

Gathering the requisite data for stability assessment and shelf-life projection entails the utilization of a range of methodologies, typically encompassing sensory evaluations, particle sizing, and methods such as pH measurement, conductivity analysis, centrifugation (including multisample centrifugation), microscopic examination (employing image analysis), and rheological testing. Some of the other techniques employed for determination of various aspects of emulsion stability, not so frequently used in industrial setting, include nuclear magnetic resonance (NMR) spectroscopy – to investigate droplet size and size distribution (33, 34), Fourier transform infrared (FTIR) spectroscopy – to assess chemical modifications (35), differential scanning calorimetry

(DSC) – to quantify fat crystal properties and emulsion susceptibility to partial coalescence (36). When monitoring the stability of an emulsion system, it is advised to use techniques working with the final product rather than with isolated parts of the system or after dilution of the system, in order to avoid changes of the real structure/state of the emulsion, so that clear conclusions about emulsion stability could be made (37).

### ***Visual observation***

The visual inspection of changes in emulsion organoleptic characteristics such as appearance, color, odor or sensory attributes (texture) during storage is the simplest way to directly obtain information about the (in)stability of emulsions (5, 20). The visual observation is also the easiest method to characterize the gravitational separation (creaming/sedimentation) in emulsions: after placing in the transparent test-tube, emulsion is tenderly agitated to ensure an initial homogeneity of the system (but avoid any breakdown of aggregates and change in the droplet size distribution), left for a certain period of time or more frequently exposed to centrifugation to induce creaming/sedimentation, and afterwards the height of formed different layers (Figure 2) is measured to calculate creaming index. It is also possible to use a digital camera to take photographs of an emulsion over time, which could be further analyzed with software image analysis to quantify changes in the emulsion appearance during storage (36). However, a known limitation of the visual method in determining the extent of creaming/sedimentation is that it is not always easy for the human eye to accurately observe the gravitational separation of the stored emulsion, especially when the boundaries between separated layers, if there are any, are not clearly visible (e.g., diffusive boundaries or optically opaque layers) (33, 36).



**Figure 2. Visual observation of emulsion destabilization phenomena**  
**Slika 2. Vizuelno opažanje fenomena destabilizacije emulzija**

### ***Optical profiling***

To obtain information about the creaming/sedimentation rate, which requires knowing the change in droplet concentration (or sometimes droplet size) as a function of emulsion height over time, an optical profiling method based on the propagation of light waves through the emulsion, also referred to as turbidimetry, can be applied (33, 36). Automated analytical instruments relying on the measurement of backscattered and transmitted light as a function of sample height are commercially available (e.g., Turbiscan), and the turbidimetric method is considered a simple, rapid, quantitative and reproducible technique to determine emulsion stability against gravitational separation (36, 38).

In order to predict the long-term stability of emulsions from measurements done within a short time, an accelerated optical profiling method may be used (7, 36). There are instruments such as multisample analytical centrifuges which employ photometrical measurement of turbidity or transmission intensity over entire sample height while the emulsion is subjected to a defined centrifugal force in order to accelerate the potential destabilization processes. Phenomena such as creaming, sedimentation, flocculation, coalescence or even Ostwald ripening can be observed within a short time frame by using this multisample analytical centrifugation (7).

### ***pH value measurements***

Hydrolysis of the emulsion components such as triglycerides of the oil phase, phospholipids, or active substances can lead to the formation of degradation products (e.g., acid species like free fatty acids, lysophospholipids, glycerophospholipids) which may induce alterations, usually a reduction, in the pH values of (nano)emulsions. Therefore, the measurement of emulsion pH values over storage or upon an accelerated test by directly immersing a pH electrode into the emulsion sample can provide useful information about potential chemical and microbiological changes which may compromise the stability and quality of the final emulsion product (34, 39).

### ***Electrical conductivity measurements***

Characterization of emulsion creaming/sedimentation can also be achieved by direct immersion of electrode into the emulsion sample at different positions of the emulsion container (for example, near the top, in the middle, near the bottom) and monitoring the changes in electrical conductivity at a particular height over time (35, 36); by employing a suitable theoretical model or calibration curve, the obtained electrical conductivity values can be converted to droplet concentration, providing information on the emulsion stability against gravitational separation (36).

Measurements of emulsion electrical conductivity can also be regularly used to monitor phase inversion in an emulsion (35, 36). Indeed, O/W emulsions have an aqueous continuous phase characterized by a high electrical conductivity; on the other hand, W/O emulsions contain oil continuous phase with low electrical conductivity. Therefore, in the

case of phase inversion of an O/W emulsion to a W/O emulsion, a significant reduction in electrical conductivity can be observed (36).

### ***Centrifugation method***

Sedimentation/creaming instability of emulsions can be accelerated by centrifuging the emulsion sample at a standardized acceleration, time and temperature (usually 3000 rpm, 15-30 min, 2 cycles in the case of conventional emulsions), often with the reference/standard sample. Upon centrifugation, the physical stability of centrifuged sample is visually evaluated. The centrifuging test produces stress in the sample, simulating an increase in the force of gravity and increasing the mobility of the particles, thus anticipating possible instabilities. These changes may appear in the form of precipitation, creaming, separation of phases, caking, or coalescence, among others (5).

The special type of centrifugation, designated as multisample centrifugation, combines the acceleration of the destabilization process experienced due to storage at higher temperature and acceleration by centrifugation. Besides higher temperature, centrifugation along freeze-thaw-cycles is very powerful to detect destabilization in a freshly formulated dispersion having a tendency to coalesce. By providing information about the rate of sedimentation and creaming, this method can potentially be utilized to calculate the shelf-life of the product (7).

### ***Microscopy***

Various types of microscopy, including optical microscopy, electron microscopy (scanning, transmission, confocal laser scanning microscopy or atomic force microscopy, can be successfully employed to characterize emulsion microstructure, morphology, state of dispersion, as well as various instability phenomena, particularly droplet flocculation in emulsions. Optical microscopy is for sure the most widely used, simple, inexpensive, readily available, powerful tool for direct qualitative or quantitative characterization of the emulsion droplets. Coupled with a digital camera, computer and software for image analysis it can provide important information about the flocs, such as their size, morphology and internal packing (33, 36). Since the individual dispersed droplets or aggregated droplets can be directly observed, microscopy technique can distinguish between different types of droplet aggregation processes, namely between coalescence or Ostwald ripening (characterized by the growth of individual droplets) versus flocculation (characterized by the association of a number of droplets) (36). As discussed in section 4 ('Emulsion destabilization phenomena'), the destabilization phenomena of creaming/sedimentation and Ostwald ripening in emulsions are significantly affected by the size of the inner phase particles. Hence, assessing both the droplet size and its distribution in emulsions is a crucial parameter. Various techniques exist for sizing (which will be detailed in the following sections), but when dealing with typical emulsions, image analysis emerges as a primary and effective option.

Microscopy tools can also be used to (i) monitor for the variations in droplet size distribution during storage by determining the number and size of emulsion droplets (e.g.,

in the majority of cases O/W emulsions are polydisperse, characterized by a wide droplet size distribution; on the other hand, in order to have a good long-term shelf-life, W/O emulsions have to be relatively monodisperse, with particle size ranging from 1-3  $\mu\text{m}$ ); (ii) monitor for appearance/disappearance of the crystals (e.g., API, UV filters); (iii) monitor for emulsion stabilizing structures (e.g., some loss of viscosity after accelerated aging coupled with the loss of such structures strongly indicates instability) (33, 36).

### ***Particle size analysis***

Droplet size and size distribution are among the most important characteristics of emulsion systems and represent fundamental parameters in the evaluation of emulsion stability; in other words, the increase in droplet size is the first indicator of emulsion instability. By measuring the droplet size and size distribution using instrumental particle sizing techniques, destabilization phenomena such as droplet flocculation, coalescence, or Ostwald ripening can be monitored (36). Light scattering techniques are by far the most widely used techniques for the characterization of emulsion droplet size and size distribution (33). Among them, dynamic light scattering, also referred to as photon correlation spectroscopy, is particularly useful for measuring fine droplets ranging from a few nanometers to several micrometers (nano-size range), providing information about the hydrodynamic mean particle diameter and polydispersity index (34). In the case of a wider size range, which is usually the case with most topical emulsions, or when a small fraction of larger emulsion droplets/flocs should be detected, a rapid, accurate, non-destructive laser diffraction technique can be employed (33). There are several instruments available, including Malvern Mastersizer particle size analyzers, operating in the range from a few nanometers to several millimeters; data about volume weighted diameters  $d(0.5)$ ,  $d(0.9)$ ,  $d(0.99)$ , and  $D[4,3]$  can be provided (40).

The limitation of both mentioned light scattering methods is the need to dilute samples before measurement in order to avoid multiple scattering, which may modify the extent and nature of droplet flocculation (33, 36). Recently, back-scattering techniques based on the use of optical fibers have been introduced, allowing the measurement of concentrated samples (without previous dilution) (33). Furthermore, unlike microscopic examination, by using the common light scattering particle sizing techniques it is difficult to directly distinguish whether the larger particles presented in the emulsion are individual droplets or flocs. The best practice would therefore be to combine these methods in the characterization of emulsion stability (36).

### ***Surface charge analysis***

Besides droplet size and size distribution, the droplet surface charge is another important characteristic of emulsions and a representative indicator of emulsion stability. It is usually determined by electrophoretic light scattering technique, which measures the electrophoretic mobility of emulsion droplets using devices such as Malvern Zetasizer, which is then converted to the zeta potential. High absolute zeta potential values, usually greater than 30 mV, can induce electrostatic repulsion between droplets and hence

improved stability of the emulsion system against coalescence (34, 41). A change in emulsion zeta potential values during storage, coupled with a change in the pH values of same samples, may indicate a certain emulsion instability (e.g., due to hydrolytic degradation of formulation components) (40, 42). It is essential to take into account that the dilution of an emulsion sample required for zeta potential measurement could potentially impact the reliability of the data obtained. This influence can arise from factors such as varying conductivity, when using pure distilled water for dilution, or potential compromise of the physical stability of charged emulsion droplets, when employing an electrolyte solution for emulsion dilution (34).

### ***Rheological tests***

The most commonly used rheological tests involve continuous rotational testing. These tests aim to achieve various objectives, such as determining the flow behavior by obtaining flow curves or viscosity curves of the samples. This approach also provides viscosity values at specific shear rates and establishes the yield point (yield stress), which is the minimal shear stress value at which material initiates flowing. The yield point is frequently considered an "indicator" of dispersion stability, as it signifies the ability to suspend and thus to prevent the coalescence of the dispersed phase particles. While these data may meet the requirements of quality control (QC), a fundamental problem with this method is that it necessitates the destruction of the material structure in order to obtain data (through flow). A plethora of stability-related insights can be obtained by analyzing the sample while it is in a rest state, where solely elastic deformations occur, enabling the characterization of its viscoelastic properties. Two oscillatory tests, namely amplitude and frequency sweeps, are utilized to conduct these analyses (22).

#### *Amplitude sweeps*

Amplitude sweeps are isothermal oscillatory tests conducted at varying amplitudes while maintaining a constant frequency. Since stable dispersions and gels form a three-dimensional network of intermolecular interaction forces, when the data is graphed, the resulting curves for  $G'$  and  $G''$  often exhibit constant plateau values within the LVER at low amplitude values. The storage modulus  $G'$  [Pa] represents the measurement of deformation energy stored by the sample during the shear process, indicating its reversible deformation behavior and elastic properties. On the other hand, the loss modulus  $G''$  [Pa] measures the deformation energy consumed by the sample during the shear process, which is subsequently dissipated as "viscous heating."  $G''$  thus represents the viscous behavior of the test material (22).

In the LVER, amplitude sweeps provide insights into the structural (viscoelastic) characteristics of the sample as follows:

1. The presence of a gel or solid is indicated if  $G' > G''$ . This suggests a certain firmness and stability in such dispersions.
2. The presence of a liquid, fluid, or sol is indicated if  $G'' > G'$ . In this case, the viscous behavior dominates over the elastic behavior in the LVER, and

dispersions' stability cannot be expected. Even highly viscous materials with entangled molecule chains but lacking a consistent chemical or physical network-of-forces exhibit this behavior. Such materials are typically not stable at rest, as they exhibit slow flow over time, albeit at a very low flow velocity (22).

From the amplitude sweep graph, two additional important parameters can be obtained: the yield point and the flow point. The yield point, also known as yield stress, signifies the upper limit of the LVER in terms of shear stress. As long as stresses below the yield point are applied, the internal structure remains relatively unchanged, resulting in reversible viscoelastic behavior within this range. The flow point, on the other hand, represents the point at which the crossover occurs, with  $G'$  equaling  $G''$ . This signifies the transition from a gel-like state, where  $G' > G''$ , to a liquid state, where  $G'' > G'$ . To achieve a flow point, the pre-condition  $G' > G''$  must be met.

Yield stress is often favored by scientists for material analysis and assessing structural strength, as it helps avoid irreversible structural changes by staying within the LVER limit. On the other hand, practical users tend to prefer the flow point for evaluation, as it indicates the point at which the internal structure sufficiently breaks down, leading to material flow (22).

#### *Frequency sweeps*

While amplitude sweeps provide valuable insights into the structural (viscoelastic) characteristics of dispersions, they may not accurately simulate behavior at rest since these tests are typically conducted at a constant angular frequency of  $\omega = 10$  rad/s. This frequency is not representative of very slow motion or the state at rest. To truly evaluate a material at rest, frequency sweeps are performed. These tests involve varying the frequency while keeping the amplitude constant. The chosen amplitude value corresponds to the limiting value of the LVER in terms of shear strain.

Prior to conducting a frequency sweep, an amplitude sweep is carried out to ensure that measurements are within the LVER. Frequency sweeps are isothermal tests used to explore time-dependent deformation behavior by simulating short-term behavior with high frequencies and long-term behavior with low frequencies. They are particularly useful for assessing the stability of dispersions at rest and during long-term storage. Additionally, frequency sweeps help to get insights into issues like sedimentation, settling, flotation, syneresis, and phase separation (22).

To analyze behavior at rest, it is recommended to determine the  $G'$  value at an angular frequency of  $\omega = 0.01$  rad/s. It is crucial to note that the  $G'$  value at rest is not the same as the "yield point," which is a shear stress value, despite both being measured in the same unit (Pa). Based on numerous experiments, some general guidelines for  $G'$  values determined at  $\omega = 0.01$  rad/s can be outlined under specific conditions:

1.  $G' \geq 10$  Pa – indicates a certain level of dispersion or gel stability.
2.  $G' \leq 1$  Pa – suggests insufficient stability for practical applications.

3.  $G'$  values in between – further testing is recommended, possibly involving determination of yield stress and flow stress through amplitude sweeps.

These practical guidelines are based on the assumption that specific pre-conditions are fulfilled, including testing conducted within the LVER and the existence of a gel-like character where  $G' > G''$  (22).

### **Dynamic-mechanical thermoanalysis (DMTA) test as a high-performance alternative for accelerated freeze-thaw stability testing**

As previously discussed, a commonly utilized method for assessing the stability of emulsions, especially those susceptible to coalescence, is the freeze-thaw test. This procedure involves subjecting samples to cycles of cooling/freezing and conditioning/thawing over a 24-hour period, constituting one cycle of the test. Variations of this test can involve different temperature extremes, cycle completion times, inclusion of room temperature cycles, and the number of cycles (with a recommended minimum of six cycles). The goal of this test is also to replicate the anticipated market conditions, reflecting expected storage and usage scenarios. Familiarity with the ICH guidelines on climatic zones for stability testing conditions can aid in designing an appropriate test protocol (3, 17).

Additionally, it has been emphasized that rheological characterization is a frequently employed instrumental method to assess the physical properties of emulsion products during stability testing. This technique offers valuable insights into the impact of various factors such as formulation variables, shear forces during filling processes, temperature fluctuations, and time variations on the stability of emulsions as thermodynamically metastable systems. Alongside traditional viscosity measurements (viscosity and flow curves) and yield point determination, understanding the viscoelastic properties of the samples is crucial. To achieve this, oscillatory tests, specifically amplitude and frequency sweeps, are conducted. In cases where temperature variations are introduced during these oscillatory tests, they are referred to as dynamic-mechanical thermoanalysis (DMTA) tests (3, 17, 43).

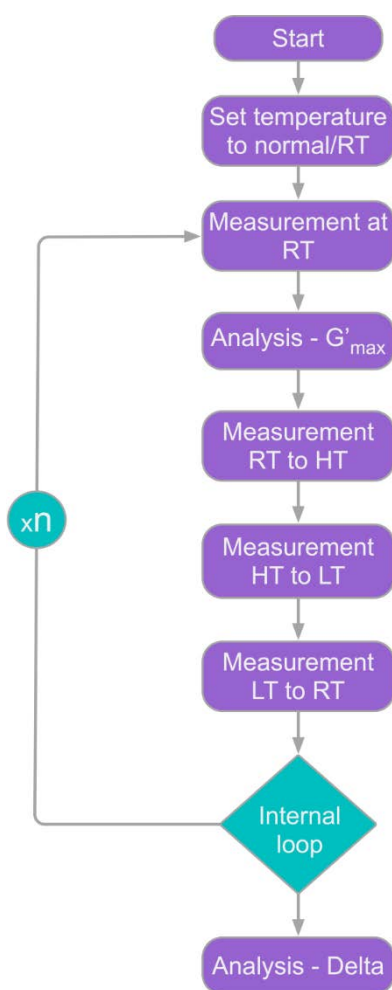
Cosmetic emulsions are known for their remarkable stability, often featuring shelf lives spanning months to years. For that reason, the evaluation of their stability can present a bottleneck in the development of new products (8). This holds particularly true in the realm of research and development (R&D), where expediting the formulation of novel emulsion products is crucial. Similarly, in QC departments, saving time in the process of either disqualifying or approving samples from product batches with borderline specifications is paramount (3, 17).

In our previous work, we established a promising correlation between stability data obtained from the DMTA test and the results from the F/T test conducted within a stability chamber. In essence, we demonstrated that the DMTA test could significantly compress the timeline required for a stability assessment of this kind. This innovative approach condensed the typical span of several days or even weeks needed for the F/T test to just



a matter of hours. This acceleration in testing time can significantly enhance the efficiency of high formulating turnover and facilitate swift decision-making processes in both R&D and QC settings (3, 17, 43).

DMTA tests are performed in the LVER for the relevant formulations, utilizing an angular frequency of  $\omega = 10$  rad/s. Consequently, it is essential to conduct at least an amplitude sweep for the emulsions under examination. Basically, the DMTA test is designed to track the variation in storage modulus  $G'$  during each successive cycle relative to the  $G'$  value obtained in the initial measurement. These comparisons allow for the calculation of the delta value, which serves as an indicator of the structural alteration in the tested formulation. Figure 3 provides a simplified representation of the standard test setup and parameters used for conducting a DMTA test.

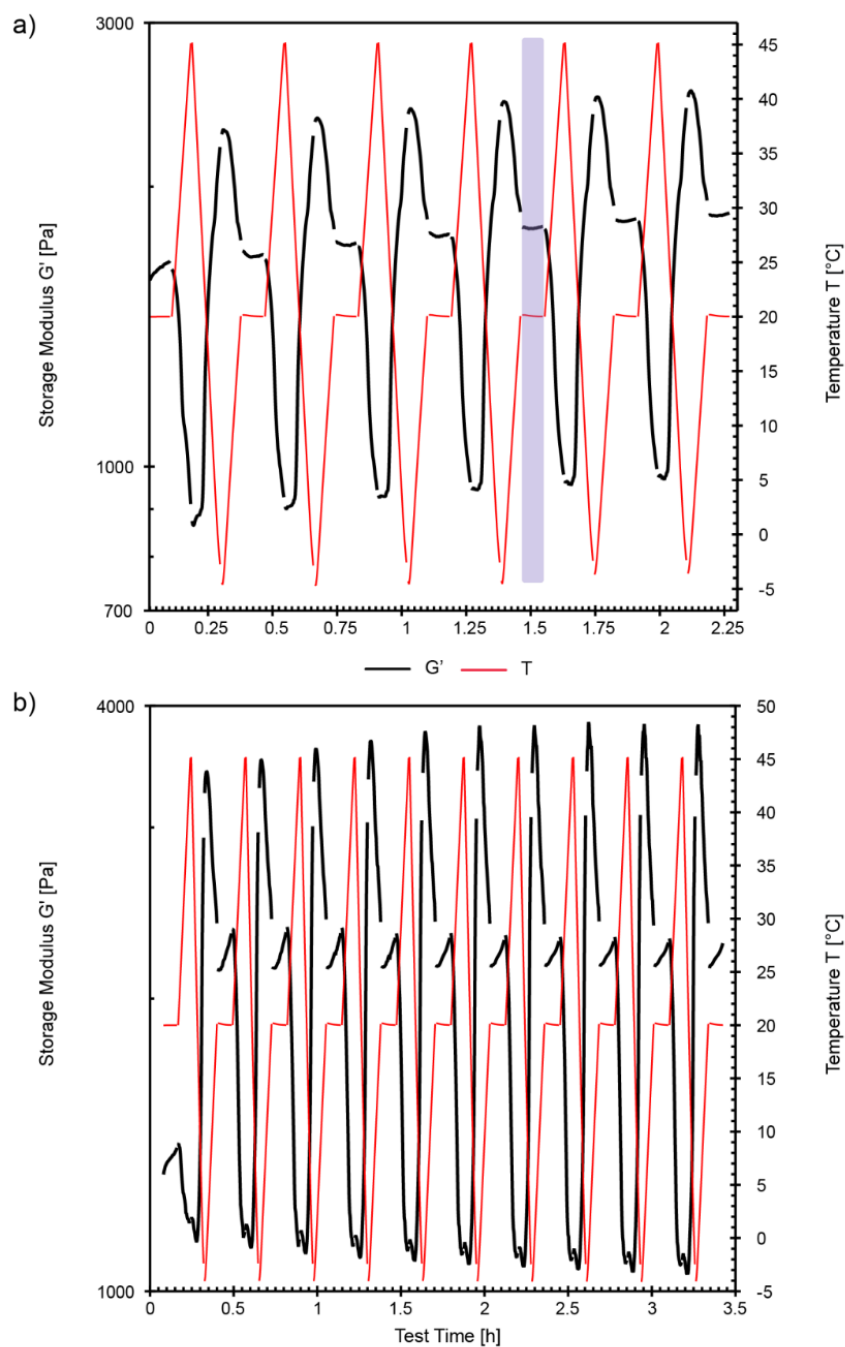


**Figure 3. Simplified test definition of the DMTA test; RT – room temperature, HT – high test temperature, LT – low test temperature, n – number of test cycles**  
**Slika 3. Pojednostavljeni prikaz definicije DMTA testa; RT – sobna temperatura, HT – visoka temperatura testa, LT – niska temperatura testa, n – broj ciklusa testa**

In terms of hardware, the essential requirement is an air-bearing rheometer, equipped with a suitable geometry system such as parallel plates, cone-plate, or coaxial cylinders. The choice of geometry depends on the specific emulsion type, including factors like consistency and dispersed phase particle size being analyzed. To ensure accurate results and prevent structural changes caused by water evaporation, all testing systems utilized should include suitable evaporation blockers. An air-bearing rheometer is essential due to the minute deflection of the measuring tool within the range below the yield point. This requires highly sensitive instruments with a high resolution for torque and deflection angle values. Using an air-bearing device ensures that the measurement is carried out within the reversible elastic deformation range, maintaining accuracy and reliability (22).

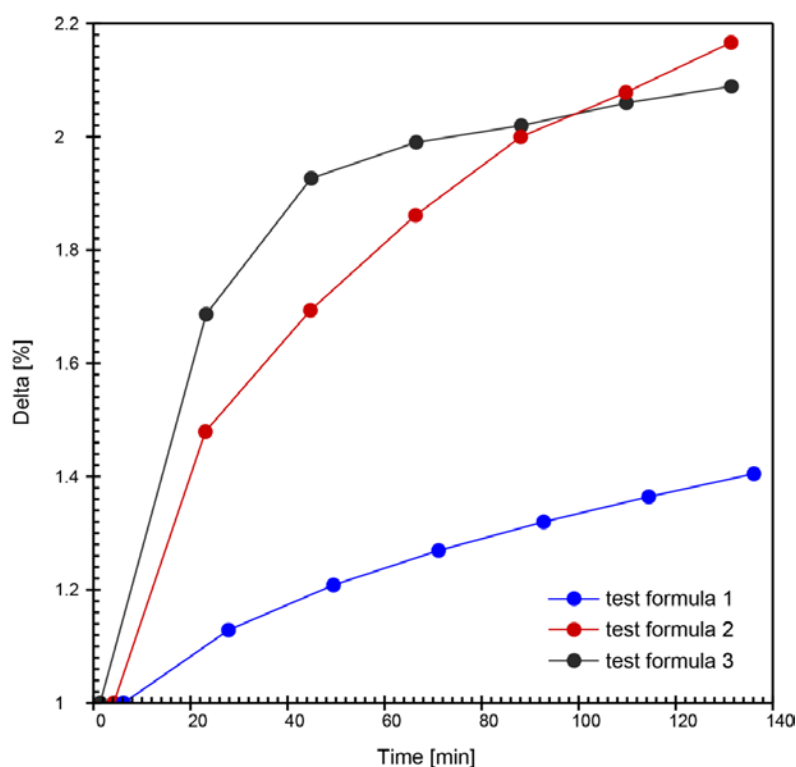
The rheometer must be operated using software that allows manual programming of each stage of the test. Referring to Figure 3, the test begins by setting the sample temperature to room temperature. Once equilibrium is reached, the measurement of the storage modulus is taken. The software then analyzes the data to determine the maximal storage modulus value,  $G'_{max}$ , which serves as the initial reference for subsequent comparisons ( $G'_0$ ). The temperature is then cycled from the chosen high temperature, through the selected low test temperature, and back to room temperature. At each cycle, measurements are taken and the subsequent  $G'_{max}$  analysis is conducted ( $G'_n$ ). This temperature cycling,  $G'$  measurement at room temperature, and the analysis step are repeated for the desired number of cycles, constituting a single freeze-thaw cycle. Notably, this approach allows for continuous monitoring of structural changes throughout the temperature variations, offering a distinct advantage compared to the traditional freeze-thaw test, which typically only provides information about the final change after the entire test is completed. A typical output of the DMTA test is depicted in Figure 4, illustrating the change in storage modulus concerning temperature and time. Figure 4a showcases a standard scenario with a number of cycles equivalent to the typical six cycles of the freeze-thaw test. The shaded purple region signifies one measurement at room temperature/ $G'_{max}$  analysis cycle within the test. Conversely, due to the test's brief duration, a greater number of cycles can be conducted to obtain more data for higher-resolution comparisons of challenging formulations (Figure 4b).

Once all cycles are completed, the program is designed to calculate the delta value for each cycle ( $G'_n/G'_0$ ). The plotted delta values for various formulations could serve to select the formulation with the least structural change in research and development scenarios, or to assess the delta change against predefined specification values for the tested product within quality control (Figure 5). All of these evaluations can be achieved within a few hours, due to the accelerated nature of the test.



**Figure 4.** Change of storage modulus ( $G'$ ) in DMTA test as a function of temperature change for representative formulations; a) six test cycles, b) ten test cycles; purple region – region of measurement and  $G'_{\max}$  analysis at room temperature

**Slika 4.** Promena modula elastičnosti ( $G'$ ) u DMTA testu prikazana u funkciji promene temperature za odabrane formulacije; a) šest test ciklusa, b) deset test ciklusa; ljubičasto označeni region predstavlja jedan ciklus merenja i određivanja  $G'_{\max}$  na sobnoj temperaturi



**Figure 5.** Change of storage modulus  $G'$  (expressed as structural change delta) of selected test emulsions, acquired from DMTA tests

**Slika 5.** Promena modula elastičnosti  $G'$  (izražena kao strukturna promena delta) za odabrane emulzije proizvode, dobijena iz DMTA testa

## Conclusion

Stability testing serves as a predictive tool, leveraging data from products stored under conditions that are anticipated to accelerate changes comparable to those in market settings. Like all predictive methodologies, it possesses an inherent degree of probability rather than absolute certainty. Hence, an evaluator's expertise, grounded in comprehensive knowledge of the tested system, and familiarity with the extent of acceleration mimicked by the test conditions in relation to market conditions, plays a pivotal role in enhancing the accuracy of conclusions drawn. Particularly for highly stable products, such as cosmetics, the evaluation of shelf life often becomes a bottleneck in new product design. As a remedy, accelerated stability tests, including the freeze-thaw test, are commonly employed. However, dynamic-mechanical thermoanalysis (DMTA) offers a significant edge in terms of time efficiency. By compressing the typical duration of F/T testing from days or weeks to a matter of hours, DMTA presents an innovative temporal advantage. Moreover, DMTA enables the continuous monitoring of structural changes across temperature fluctuations, providing an added dimension of insight compared to the traditional freeze-thaw test, which typically yields information only at the test conclusion.

## Acknowledgments

This research was funded by the Ministry of Science, Technological Development and Innovation, Republic of Serbia, through Grant Agreement with the Faculty of Technology in Leskovac – University of Niš No: 451-03-47/2023-01/ 200133 and the University of Belgrade – Faculty of Pharmacy No: 451-03-47/2023-01/ 200161.

## References

1. Merriam-Webster's Collegiate Dictionary. 11th ed. Springfield, Massachusetts: Merriam-Webster Inc.; 2004. 1623 p.
2. Matthews BR. Regulatory aspects of stability testing in Europe. *Drug Dev Ind Pharm.* 1999;25(7):831-56.
3. Cekic ND, Savic SM, Savic SD. Dynamic-mechanical thermoanalysis test: a rapid alternative for accelerated freeze-thaw stability evaluation of W/O emulsions. *Drug Dev Ind Pharm.* 2019;45(12):1896-906.
4. International Organisation for Standardisation (ISO). ISO/TR 13097:2013(en) – Guidelines for the characterization of dispersion stability. 2013.
5. International Organisation for Standardisation (ISO). ISO/TR 18811:2018(en) – Cosmetics — Guidelines on the stability testing of cosmetic products. 2018.
6. International Federation of Societies of Cosmetic Chemists (IFSCC). The fundamentals of stability testing (IFSCC Monograph No. 2). Weymouth: Micelle Press; 1992.
7. Badolato GG, Aguilar F, Schuchmann HP, Sobisch T, Lerche D. Evaluation of long term stability of model emulsions by multisample analytical centrifugation. In: Auernhammer GK, Butt HJ, Vollmer D, editors. Surface and interfacial forces – from fundamentals to applications. Progress in Colloid and Polymer Science; vol 134. Berlin, Heidelberg: Springer; 2008.
8. Lerche D, Sobisch T. Direct and Accelerated Characterization of Formulation Stability. *Journal of Dispersion Science and Technology.* 2011;32(12):1799-811.
9. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Harmonised Tripartite Guideline Q1A (R2): Stability testing of new drug substances and products. 2003.
10. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Guideline Q1B: Stability testing: Photostability testing of new drug substances and products. 1996.
11. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Guideline Q1C: Stability testing for new dosage forms. 1996.
12. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Guideline Q1D: Bracketing and matrixing designs for stability testing of new drug substances and products. 2002.

13. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Guideline Q1E: Evaluation of stability data. 2003.
14. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). ICH Guideline Q1F: Stability data package for registration applications in climatic zones III and IV. 2006.
15. Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. 2009 (current consolidated version: 16/08/2023).
16. Cosmetics Europe. Guidelines on stability testing of cosmetic products. 2004.
17. Cekic ND, Savic SM, Savic SD. Dynamic-mechanical thermoanalysis test as a high-performance alternative for accelerated freeze-thaw stability testing: a case study of O/W emulsions. *Drug Dev Ind Pharm.* 2020;46(3):505-20.
18. Huynh-Ba K. Handbook of stability testing in pharmaceutical development: regulations, methodologies, and best practices. New York: Springer; 2008. 389 p.
19. Baertschi SW, Alsante KM, Tønnesen HH. A critical assessment of the ICH guideline on photostability testing of new drug substances and products (Q1B): Recommendation for revision. *J Pharm Sci.* 2010;99(7):2934-40.
20. Rieger MM. Stability testing of macroemulsions. *Cosmetics and toiletries.* 1991;106:59-69.
21. ANVISA National Health Surveillance Agency. ANVISA Cosmetics Products Stability Guide. Quality in Cosmetic Series. 2004.
22. Mezger T. The Rheology Handbook. Hannover: Vincentz Network; 2020. 528 p.
23. Lee H, Jin M, Jeon W-J, Kim H, Jung M, Yoo H, et al. Regulatory and safe-use considerations related to stability after opening of nonsterile dosage forms. *J Pharm Investig.* 2022;52(3):319-29.
24. Chavda H. In-use stability studies: guidelines and challenges. *Drug Dev Ind Pharm.* 2021;47(9):1373-91.
25. European Medicines Agency (EMA). Note for Guidance on in-use stability testing of human medicinal products. 2001.
26. World Health Organization (WHO). TRS 1010 – Annex 10: WHO guidelines on stability testing of active pharmaceutical ingredients and finished pharmaceutical products. WHO Technical Report Series 1010, 2018.
27. Particle Sciences – Drug Development Services. Emulsion stability and testing. Technical Brief; vol. 2. 2011.
28. Tadros TF. Flocculation of emulsions. In: Tadros TF, editor. Emulsions: formulation, stability, industrial applications. Berlin/Boston: Walter de Gruyter GmbH; 2016; p. 113-28.
29. Tadros TF. Ostwald ripening in emulsions and its prevention. In: Tadros TF, editor. Emulsions: formulation, stability, industrial applications. Berlin/Boston: Walter de Gruyter GmbH; 2016; p. 129-40.
30. Tadros TF. Phase inversion and its prevention. In: Tadros TF, editor. Emulsions: formulation, stability, industrial applications. Berlin/Boston: Walter de Gruyter GmbH; 2016; p. 161-72.
31. Aoki T, Decker EA, McClements DJ. Influence of environmental stresses on stability of O/W emulsions containing droplets stabilized by multilayered membranes produced by a layer-by-layer electrostatic deposition technique. *Food Hydrocolloids.* 2005;19(2):209-20.

32. Ghosh S, Coupland JN. Factors affecting the freeze–thaw stability of emulsions. *Food Hydrocolloids*. 2008;22(1):105-11.
33. Tadros TF. Characterization of emulsions and assessment of their stability. In: Tadros TF, editor. *Emulsions: formulation, stability, industrial applications*. Berlin/Boston: Walter de Gruyter GmbH; 2016; p. 173-96.
34. Klang V, Valenta C. Lecithin-based nanoemulsions. *J Drug Deliv Sci Technol*. 2011;21(1):55-76.
35. Masmoudi H, Dréau YL, Piccerelle P, Kister J. The evaluation of cosmetic and pharmaceutical emulsions aging process using classical techniques and a new method: FTIR. *Int J Pharm*. 2005;289(1-2):117-31.
36. McClements DJ. Critical review of techniques and methodologies for characterization of emulsion stability. *Crit Rev Food Sci Nutr*. 2007;47(7):611-49.
37. Zografu G. Physical stability assessment of emulsions and related disperse systems: a critical review. *J Soc Cosmet Chem*. 1982;33:345-58.
38. Alade OS, Mahmoud M, Al Shehri DA, Sultan AS. Rapid determination of emulsion stability using turbidity measurement incorporating artificial neural network (ann): experimental validation using video/optical microscopy and kinetic modeling. *ACS Omega*. 2021;6(8):5910-20.
39. Bernardi DS, Pereira TA, Maciel NR, Bortoloto J, Viera GS, Oliveira GC, et al. Formation and stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. *J Nanobiotechnology*. 2011;9:44.
40. Đorđević SM, Radulović TS, Cekić ND, Randelović DV, Savić MM, Krajišnik DR, et al. Experimental design in formulation of diazepam nanoemulsions: physicochemical and pharmacokinetic performances. *J Pharm Sci*. 2013;102(11):4159-72.
41. Mitri K, Shegokar R, Gohla S, Anselmi C, Müller RH. Lipid nanocarriers for dermal delivery of lutein: preparation, characterization, stability and performance. *Int J Pharm*. 2011;414(1-2):267-75.
42. Isailović T, Đorđević S, Marković B, Randelović D, Cekić N, Lukić M, et al. Biocompatible nanoemulsions for improved aceclofenac skin delivery: formulation approach using combined mixture-process experimental design. *J Pharm Sci*. 2016;105(1):308-23.
43. Savić SM, Cekić ND, Savić SR, Ilić TM, Savić SD. 'All-natural' anti-wrinkle emulsion serum with *Acmella oleracea* extract: A design of experiments (DoE) formulation approach, rheology and in vivo skin performance/efficacy evaluation. *Int J Cosmet Sci*. 2021;43(5):530-546.

# **Procena stabilnosti emulzionih preparata za topikalnu primenu: vrednost dinamičko-mehaničkog termoanalitičkog (DMTA) testa kao brze reološke alternative konvencionalnom testu smrzavanje-odmrzavanje**

**Nebojša Cekić<sup>1,2,\*</sup>, Sanela Savić<sup>1,2</sup>, Snežana Savić<sup>3</sup>**

<sup>1</sup> DCP Hemigal, Sektor za istraživanje i razvoj, Tekstilna 97, 16000 Leskovac, Srbija

<sup>2</sup> Tehnološki fakultet u Leskovcu – Univerzitet u Nišu, Bulevar oslobođenja 124, 16000 Leskovac, Srbija

<sup>3</sup> Katedra za farmaceutsku tehnologiju i kozmetologiju, Univerzitet u Beogradu – Farmaceutski fakultet, Vojvode Stepe 450, 11221 Beograd, Srbija

\*Autor za korespondenciju: Nebojša Cekić, e-mail: nesafarm@icloud.com

---

## **Kratak sadržaj**

Procena stabilnosti emulzionih preparata za topikalnu primenu može se sprovesti praćenjem promena u realnom vremenu i/ili primenom ubrzanih metoda, te predviđanjem stabilnosti i roka trajanja proizvoda na osnovu merenja relevantnih fizičko-hemijskih parametara tokom ispitivanja. Kako bi se obezbedila robusnost i dugoročnost emulzionih proizvoda za kožu tokom čuvanja, transporta i primene, neophodno je sprovesti pažljivo isplanirano, opsežno ispitivanje stabilnosti. Međutim, imajući u vidu različite tipove emulzija i njihovu namenu, ne postoji univerzalni standardni protokol za ispitivanje stabilnosti, što formulatore/proizvođača čini odgovornim kada je u pitanju izbor odgovarajućeg testa i metodologije. Evidentno je da emulzije za topikalnu primenu, a posebno kozmetičke emulzije, često pokazuju visoku stabilnost sa dugim rokovima upotrebe. S druge strane, procena stabilnosti ovakvih emulzija i donošenje odgovarajućih odluka i dalje ostaje izazov u industrijskom okruženju i zahteva dosta vremena, što nameće potrebu za alternativnim protokolima koji omogućavaju ubrzano ispitivanje, ali i uspešno predviđanje stabilnosti emulzionih proizvoda. Prikazani rad daje sveobuhvatni pregled literature prožet praktičnim pogledima na ključne fenomene odgovorne za stabilnost emulzija, zatim daje uvid u različite pristupe za procenu njihove stabilnosti, uključujući metodologije koje se koriste i parametre koji se prate tokom ispitivanja. Rad u poseban fokus stavlja dinamičko-mehanički termoanalitički (DMTA) metod kao brzu reološku alternativu konvencionalnom testu smrzavanje-odmrzavanje, posebno ističući primenljivost metoda za ubrzano ispitivanje stabilnosti emulzionih preparata za topikalnu primenu.

**Ključne reči:** emulzije za kožu, fizička stabilnost, ubrzano ispitivanje stabilnosti, reološki testovi, rok upotrebe