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Impact of Simulated Microgravity on Nanoemulsion Stability – A Preliminary Research

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Abstract Purpose: This project includes an analysis of nanoemulsions in microgravity simulation. Based on the understanding of these simulations, we can design and produce nanoemulsion drugs stable enough to go on the mission to space. **Methods:** Oil in water nanoemulsions were formulated using 30% oil and 70% water phase. A total of five nanoemulsions: control, carbamazepine, diclofenac sodium, fenofibrate, and melatonin were prepared via sonication method. The average viscosity of the emulsions was 33.3 ± 6.5 cP and the average pH was 6.27 ± 0.62 . These nanoemulsions were characterized for particle size distribution and zeta potential before and after 1, 2, 3, 4, and 7 days in microgravity simulation by using a three-dimensional Clinostat. **Results:** Before microgravity simulation, the control, carbamazepine, diclofenac, fenofibrate, and melatonin had an average particle size of 254.1, 202.3, 909.3, 221.1, and 226.9 nm, respectively. From day 1 to day 7 in microgravity simulation, the control, carbamazepine, diclofenac, fenofibrate and melatonin nanoemulsions decreased in particle size by 25.5, 4.4, 137.7, 7.9, and 0.6 nm, respectively. The zeta potential of all nanoemulsions were in the range of -64.3 to -68.0 mV, with exception of diclofenac. **Conclusion:** Throughout 7 days in microgravity simulation, all of the nanoemulsions remained stable and decreased in particle size. Future research must be done on the stability of nanoemulsions containing different drugs and evaluating the drug stability using High Performance Liquid Chromatography analytical method. It is also essential to simulate microgravity for a longer period of time in order to truly determine its effect on drug stability.

Keywords: nanoemulsions, simulated microgravity, stability

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1. Introduction

The continuous advancement of pharmaceutical technology has allowed the development of novel dosage forms. The major problem associated with the development of medications is that a high percentage of drugs have low bioavailability, mainly due to insolubility in biological fluids, polymorphism and stability issues [1]. These problems might be accentuated when an astronaut has to take a medicine in space, due to a lack of gravity. Nanotechnology provides an alternative to overcome some of the described limitations by reducing the drug particle size to nanoscale. This might increase drug solubility, but it does not address polymorphism. Various nanoscale delivery systems are described in the literature, including nanoemulsions. In such a system, the drug is solubilized, improving its bioavailability and avoiding polymorphism issues. Nanoemulsions are dispersions of nanoscale droplets formed by shear-induced disruption. The present

convention for nanoscale materials includes structures having length scales in the range of 1 to 300 nm [2,3]. Among the various methods reported for the production of nanoemulsions, the ultrasonic agitation of a premixed emulsion of microscale droplets is the most common. In this method, a vibrating solid surface agitates the premixed emulsion at ultrasonic frequencies, typically 20 kHz or larger, and high power, causing extreme shear and cavitation that breaks up droplets. High-power ultrasonic devices include focusing horns and pointed tips. According to the Derjaguin–Landau–Verwey–Overbeek theory, the total potential (VT) of interaction between the particles, is the somatory of the repulsive interaction (VE) and attractive interaction (VA), i.e., $(VT = VE + VA)$, shows a primary minimum, a repulsive barrier, and a secondary minimum. Flocculation in the primary minimum is assumed to be irreversible due to the strength of the van der Waals interaction at short distances. The onset of destabilization occurs when the maximum of the repulsive barrier of the potential is equal to zero ($VT = 0$

and $\partial_{V_T} / \partial r_{ij} = 0$, where r_{ij} is the distance between the particles i and j). Nonzero repulsive barriers provide kinetic stability to the system with respect to irreversible flocculation [20]. According to [2], extreme shear can be used to transform microscale emulsions into nanoemulsions through microfluidic and ultrasonic processes. Since the ultrasonic field is typically inhomogeneous, in most ultrasonic schemes it is necessary to recirculate the emulsion through the region of high power so that all droplets experience the highest shear rate. Reasonably uniform droplet size distributions at dilute concentrations can be obtained if the emulsion is recirculated many times in the described way [2]. A large number of factors must be controlled to make a reproducible, stable emulsion. These include selecting an appropriate composition, controlling the order of addition of the components, and applying the shear in a manner that effectively disrupts the droplets [2]. A major problem in emulsion science and technology concerns stability control. Though mixtures of surfactants and other additives are widely utilized as controlling agents, the link between the physical chemistry of the droplet interface and the collective properties of the emulsion have been established only qualitatively, so that stabilization criteria used in technical practice are mainly empirical [5].

Microgravity research is applicable to biotechnological, chemical, and physical processes. Its value has been shown by the American National Aeronautics and Space Administration (NASA) and also by international researchers in recent years. The surveys include: evaluating the growth and virulence of microorganisms in order to assist in the isolation of genes relevant to the development of vaccines [6]; growing tissues of different species, since they develop three-dimensionally in microgravity, thereby improving the reliability of the effect of the drugs tested [7]; producing crystals in microgravity and characterize the structure of the molecule and assess the success of the molecule under development [8]. Research in microgravity is primarily essential to evaluate the impact of gravity on biological processes and organisms, but knowing the behavior of pharmaceutical formulations is also a matter of high relevance. However, research in the near-Earth orbit is severely constrained by the limited number of flight opportunities. Ground-based simulators of microgravity are valuable tools for preparing spaceflight experiments, but they also facilitate stand-alone studies and thus provide additional and cost-efficient platforms for gravitational research. Such simulators do not abolish the 1g force of gravity, but instead either randomize the direction of gravity with respect to the sample over time (omnilateral stimulation — clinostat principle) or compensate the gravity force by creating a counteracting force (magnetic levitation). On the ground, only drop towers are able to provide real free-fall conditions for a period of seconds [9]. Space research and space visits for common man are no longer just a dream. Such trips open an entirely new area of research and introduce challenges of drug delivery for the space travelers. In this context, the aim of this research was the evaluation of nanoemulsion stability on microgravity simulation using a Clinostat – 3D. Based on this understanding, we can produce nanoemulsion drugs stable enough to permit an application on space missions.

2. Methodology

Oil-in-water nanoemulsions were prepared with soyabean oil, lecithin, methylparaben, propylparaben, pluronic® F68, glycerin, PEG 400 and purified water. All components were from Sigma and were accurately weighed. The actual amount of each component is not shown because a patent application is pending. All the oil phase ingredients were mixed and stirred till a clear solution was obtained. A similar procedure was performed for water phase ingredients. The water and oil phase were emulsified in amber bottles using the XL-2000 Sonicator (Misonix, NY) for three 15 minutes periods. Five different nanoemulsions were prepared: control (no drug), carbamazepine (20 mg/mL), diclofenac sodium (15 mg/mL), fenofibrate (9.6 mg/mL), and melatonin (1 mg/mL). All the used drugs were purchased from Sigma (USA). The average viscosity of the emulsions was 33.3 ± 6.5 cP and the average pH was 6.27 ± 0.62 . A Clinostat-3D was used to provide microgravity simulation, which is endowed by means of continuous random changes of orientation, relative to the gravity \hat{s} vector. This gravity \hat{s} vector is generated by a combination of two different movements that can generate effects comparable to the direction for seconds or longer. The rotation effect of true microgravity when the changes are faster than the object response time to gravity and never has a constant \hat{s} of both axes, was fixed to 1.6 rpm. To perform microgravity simulation, the samples (three of each) were transferred to 1.5 mL eppendorf tubes, completely filled without air. All tubes were covered with aluminum foil to avoid light degradation.

Tested nanoemulsions were characterized for particle size distribution and zeta potential at day zero and after 1, 2, 3, 4, and 7 days in microgravity simulation. Particle size and zeta potential of nanoemulsions were evaluated by Mastersizer® 2000 (Malvern, UK) and Zetasizer® (Malvern, UK), respectively.

3. Preliminary Results

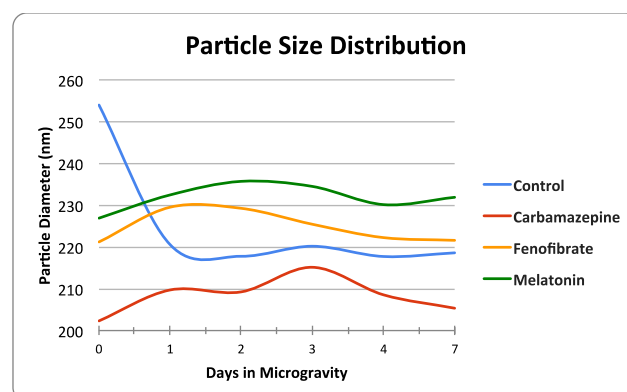


Figure 1. Particle size distribution of control, carbamazepine, fenofibrate, and melatonin nanoemulsions after 0, 1, 2, 3, 4, and 7 days in simulated microgravity

Before microgravity simulation, the control, carbamazepine, fenofibrate, melatonin, and diclofenac nanoemulsions displayed an average size of 254.1, 202.3, 221.1, 226.9, and 909.3 nm, respectively. As seen in Figure 1, fenofibrate and carbamazepine increased by 10

nm after day 1 in microgravity, while the control group reduced by approximately 37 nm. A smaller modification in droplet size was observed in the melatonin nanoemulsion. In the same period, diclofenac-containing nanoemulsion increased its particle size to 1073 nm, but decreased again after 7 days of clinorotation to 935.3 nm (Figure 2). From day 1 to day 7 in microgravity simulation, the control, carbamazepine, diclofenac, fenofibrate and melatonin nanoemulsions decreased their droplet size by 14.0%, 1.8%, 0.25%, 3.0% and 2.2%, respectively.

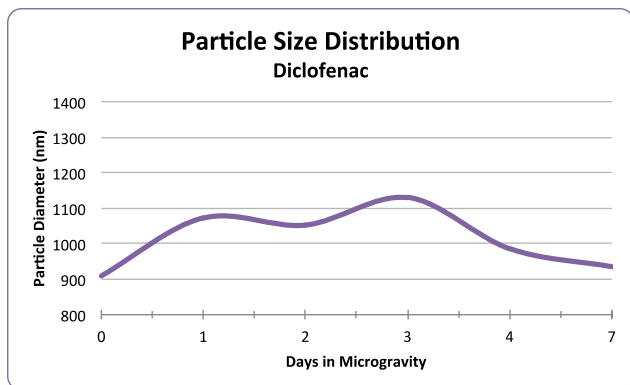


Figure 2. Particle size distribution of diclofenac nanoemulsion after 0, 1, 2, 3, 4, and 7 days in simulated microgravity

The zeta potential of all nanoemulsions varied within -61.3 to -68.0 mV (Figure 3) along the 7 days, with the exception of diclofenac, which zeta potential value changed from -74 mV to -78 mV (Figure 4).

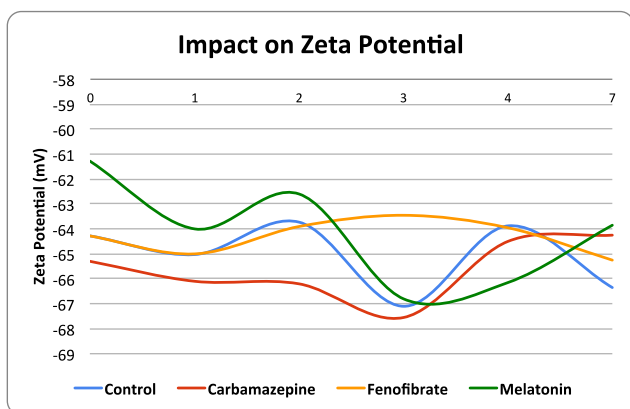


Figure 3. Zeta potential of control, carbamazepine, fenofibrate, and melatonin nanoemulsions after 0, 1, 2, 3, 4, and 7 days in simulated microgravity

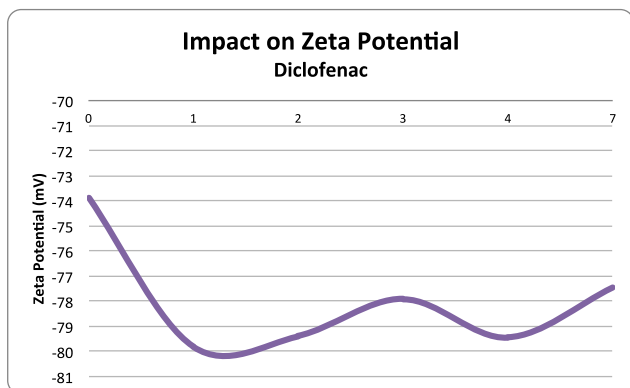


Figure 4. Zeta potential of diclofenac nanoemulsion after 0, 1, 2, 3, 4, and 7 days in simulated microgravity

4. Discussion

Nanoemulsions are well-characterized drug delivery systems with varied applications in the pharmaceutical field, including intravenous, oral, and ocular drug delivery. They have demonstrated to reduce drug side effects, while improving the associated pharmacological effects [12].

Microgravity simulation has shown its importance in the area of nanotechnology. The synthesis of nanopowders and nanoparticles along with the evaluation of nanoemulsion stability are some of the processes that have been investigated in microgravity and are significant to the development of nanotechnologies [9]. In fact, this provides data on the behaviour of drug delivery systems intended for use by space travelers, or even in space life, expected to occur in the future. It is well-known that there are many physiological changes in those under a spatial experience. Headache, backache, sleep disturbance, stress and fatigue and among the most common complaints. Considering that the majority of medicines used by space travelers are intended to relieve those symptoms, the drugs formulated in this work in the form of nanoemulsions were selected accordingly. Taking into account the high cost of space missions, models validated on Earth are used for a preliminary assessment of these formulations. Thus, in this study, a Clinostat -3D was used to simulate microgravity.

According to [3], only particles below 300 nm are considered nano scale materials. The obtained data suggest that diclofenac did not form a nanoemulsion using the considered base formulation. Diclofenac was added into the base formulation in form of salt, which can be the reason of not forming a nanoemulsion as expected, even before clinorotation. This suggests the need to change the base nanoformulation for the association of this drug. Using the same base nanoformulation and encapsulating fenofibrate, we found opposite results. In fact, after 7 days in simulated microgravity using a clinostat-3D the particle size increased from 225 nm to 300 nm, while zeta potential dropped from -59 mV to -65 mV in day 1 to day 2 clinorotation and returns to -59 mV after 7 days in the clinostat. Despite changing particle size in the fenofibrate nanoemulsions of different forms, and that one of them reached the nano-scale limit, the zeta potential returned to the initial value, suggesting that clinorotation keeps nanoformulation stability.

As mentioned above, the tested drugs were selected based on their interest in spatial pharmacy in our research group. Melatonin (N-acetyl-5-methoxytryptamine) is a hormone produced by various animals and plants [14]. In humans, melatonin has the main role in regulating sleep and its levels are known to increase in quiet environments. It also acts as an antioxidant, influencing the recovery of skin cells exposed to UV radiation [15]. This means that melatonin may help space travelers with sleeping difficulties, as well as it might provide protection from high radiation exposure. In this sense, the guarantee of a stable melatonin formulation for this group of individuals is essential. Melatonin nanopowders (nanoparticles and nanoemulsions) were previously reported [23], displaying a size from 134 to 325 nm (no information provided on zeta potential). In our research, nanoemulsions presented a mean droplet size of 226.9 nm, and a zeta potential of -65mV.

Polymorphism of drugs has been extensively studied in recent decades. Studies made by [16] showed that glassy samples of poly(methylmethacrylate) (PMMA) spheres at high volume fraction failed to crystallize after more than a year on Earth and crystallize fully in less than two weeks in microgravity. This suggests that gravity masks or alters some of the intrinsic aspects of colloidal crystallization. Also, according to [10], the crystals grown in microgravity show improved crystal habits, smoother faces, greater crystallinity, better optical quality and larger void volumes than the materials grown on Earth. Thus, the authors believe that there is a clear distinction between the covalent bonds in semiconductor materials, which are not significantly affected by microgravity, and the weaker forces (like those that determine the structure of proteins over length scales of around 0.3–0.4 nm), which are more susceptible to the dynamic disturbances that operate in crystallization on Earth. Studies [4] mentioned that crystal growth is governed by pure diffusion only causing slowdown of the crystals significantly. As the size and mass of particles increases, the rate of particle diffusion drops. The typical diffusion rates of micron-size particles is approximately $10^{-10} - 10^{-9} \text{ cm}^2/\text{s}$, which is $\sim 104 - 105$ times lower than for small drug molecules and $\sim 102 - 103$ times lower than that of proteins. Therefore, the movement of large particles would be affected significantly more by interfacial forces than by passive diffusion. On this basis, the microgravity environment should eliminate several possible effects that render analysis of nanofluidic phenomena more difficult at standard gravity.

Carbamazepine can be found in the market in the form of tablets, capsules and suspensions, all items for oral administration. Considering the modifications that can happen in microgravity environment in solid particles, it is important to develop a formulation that could be used in other routes of administration. This drug presents different polymorphs, each showing different dissolution and bioavailability profiles [17]. As psychiatric illness is known to be triggered by great stress, such as that certainly felt by astronauts in many occasions, the use of a stable carbamazepine nanoemulsion would be a relevant pharmaceutical achievement. In our experiments, carbamazepine nanoemulsions presented in an initial droplet size of 221.1 nm. This increased by 10 nm after one day in clinorotation, and returned almost to initial size after 7 days in the Clinostat-3D. The change in zeta potential was minimal, suggesting the stability of the nanoemulsions system in microgravity simulation for a week. Kelmann et al. [18] prepared carbamazepine parenteral nanoemulsions through spontaneous emulsification method. As in our study, they used soybean oil and lecithin, and obtained a droplet size of 150 nm and a potential zeta of -40mV . It is well known that droplet size in a nanoemulsion is highly dependent on used excipients and applied method. Therefore, this difference in particle size is possibly due to different methodologies between both studies. The authors further reported the occurrence of precipitation in their carbamazepine nanoemulsions, as a result of polymorphic transition. In our study, this analysis has still to be done, as well as a quantitative analysis.

Fenofibrate is used in patients with cholesterol disorders (hypercholesterolemia type IIa and IIb) and / or

in patients with elevated triglyceride levels (endogenous triglycerides type IV or associated types IIb and III). Like all the mentioned drugs, it is poorly soluble in water. Thus, the incorporation into nanoemulsions increases its solubility and consequently improves bioavailability [1]. According to [18], both cholesterol and triglyceride levels can increase from stress. Therefore, the evaluation of fenofibrate nanoemulsion stability in microgravity is of fundamental importance.

Sodium diclofenac is a very good non-steroidal anti-inflammatory drug and it is very effective in many kinds of pain [19]. Topical diclofenac is well tolerated and is associated with fewer side-effects than other topical non-steroidal anti-inflammatory drugs (NSAIDs). It also has a lower rate of gastrointestinal complications than other oral NSAIDs. Diclofenac nanoemulsions prepared by spontaneous emulsification were previously reported in the literature [21]. The mean diameter was 443 nm when 10 mg diclofenac were used and a zeta potential of -30 mV was described. These results showed the feasibility of preparing diclofenac-containing nanoemulsions, thus suggesting that we need to optimize our formulation regarding the method or the proper characteristics of the formulation.

5. Conclusions

Zeta potential and particle size are important parameters to evaluate the stability of nanoemulsions. Throughout the 7 days in microgravity simulation, all of the nanoemulsions presented a decrease in droplet size, although in most cases only a very slight reduction was observed (up to 3%). Zeta potential values also suffered only minimal changes, certainly devoid of physiological impact. Therefore, in general it was considered that the formulations remained stable for the one week period of study. Nevertheless, considering the limited experimental results published in this field, future research must be directed to broaden the evaluation of the stability of nanoemulsions containing different drugs. Evaluating drug stability using High Performance Liquid Chromatography or other robust analytical methods should be considered as well. It is also essential to consider simulating microgravity for a longer period of time to determine if there is a time limit to the drug stability.

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