**PRELIMINARY ANALYSIS OF EMULSION-BASED FORMULATIONS
CONTAINING PUMPKIN SEED OIL AND HEMP SEED OIL
FOR INTERNAL USE**

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With a long tradition in pharmaceutical design, emulsions are functional formulations that can maintain their adaptive power connected with the new formulation requirements. Hence, this study proposed preliminary assays concerning the obtaining of natural emulsions for oral administration, incorporating pumpkin seed oil and hemp seed oil as oil phases, with lecithin as emulsifying agent. Using emulsification method, O/W and W/O emulsions were prepared and characterized from a stability point of view considering organoleptic parameters, conductivity properties followed by an extensive superficial analysis by fitting two different goniometric approaches like contact angle and pendant drop models. The emulsions obtained were stable, homogeneous, their properties being reflected by composition. Conductivity values confirmed the type of emulsions, completing their profile. Superficial analysis revealed that lecithin can sustain a proper stability due to a variation of surface tension values around 25 mN/m. The mean contact angle values ranging between $31.87 \pm 0.51^\circ$ and $44.01 \pm 5.48^\circ$ defined an adequate wettability, being correlated with the internal structure. To conclude, this preliminary study offered important data concerning the stability of some emulsions for oral delivery, accessing natural biocompatible components. On this way, it can be created multifunctional systems with nutritional value, but also special vehicles designed for drug delivery.

Keywords: emulsions, vegetable oils, superficial analysis

INTRODUCTION

Defined as classical coarse dispersions, with multiple applications in pharmaceutical domain, emulsions are colloidal metastable systems composed of two immiscible phases, usually an oil and an aqueous phase (Leal-Calderon *et al.*, 2007). The internal phase will be dispersed in a continuous phase and stabilized ideally with an emulsifier which is recognized for its contribution to the diminishing of the interfacial tension at the contact of the two phases being characterized by superficial and electrical properties (Liu *et al.*, 2020). Simple ternary systems will be resulted, being known as O/W and W/O emulsions (Leal-Calderon *et al.*, 2007). Thus, the emulsifier is considered the key element with a major impact for emulsion stability, by avoiding phase separation phenomena, influencing the quality profile and the biopharmaceutical properties of the final formulation (Costa *et al.*, 2019). In this direction, soy lecithin is a natural and safe biocompatible emulsifier with a high content of phospholipidic fractions, where phosphatidylcholine is dominant, presenting amphiphilic properties due to the presence of positive and negative charges (van Hoogevest and Wendel, 2014). It is appreciated for its stabilization power in emulsion manufacturing, largely selected in the formulation of oral preparations on a concentration domain between 1-15%, as a function of each phase concentration and the intended type of emulsion, combined or not with other emulsifiers (Traynor *et al.*, 2014, Ponphaiboon *et al.*, 2018). To create multifunctional emulsions, vegetable oils as natural by-products can be considered a good option in order to ensure the generation of a personalized system, due to their

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biocompatibility and their potential therapeutic outcomes which are studied for centuries and correlated with the relief of some imbalances of the human body normal functions (Górecki *et al.*, 2016). Pumpkin seed oil can be a potent antioxidant due to its composition based on fatty acids and carotenoids, with physiological effects at cellular level. Particular actions are found to be appreciated in the cure of the urinary tract infections with a specific extent on the cure of benign prostatic hyperplasia (Alhakamy *et al.*, 2019). Being rich in omega-3 and omega-6 fatty acids with a high amount of vitamin E, hemp seed oil has multiple benefits for human body, based on anti-inflammatory and antioxidant actions which are implied in the prevention of cardiovascular disease, metabolic ailments or degenerative disorders (Miculková *et al.*, 2017).

Considering these statements, this preliminary study aimed to obtain and evaluate a group of O/W and W/O emulsions for oral delivery, using lecithin as an emulsifier and two vegetable oil phases, namely pumpkin seed oil and hemp seed oil. It was observed the impact of the emulsifier for each type of formulation with repercussions on physical appearance and stability parameters.

MATERIALS AND METHODS

Materials

Liquid soy lecithin was purchased from Amitex (Amitex Agro Product Private Ltd, India), organic certified pumpkin seed oil and hemp seed oil were supplied from NaturalniProdukti.com. Distilled water was used as aqueous phase.

Preparation of O/W and W/O Emulsions

Four formulations, as it can be observed in Table 1, were prepared using 10-50% oil phase, being distinguished here pumpkin seed oil and hemp seed oil. Water volume was adapted in order to obtain O/W or W/O emulsions, selecting lecithin 6-10% as an emulsifier. Using emulsification method, 100 mL of each emulsion were prepared with respect to the following preparation method: the oil phase was measured in a graduated cylinder. Lecithin was weighed at the analytical balance, then placed in a proper mortar and triturated with small amounts of oil phase until the primary emulsion will be formed. In the last step, the water gently warmed was added in small portions until 100 mL of emulsion were obtained. The obtained emulsions were shortly coded as: E1, E2, E3 and E4, being further remembered in the present paper.

Table 1. Composition of O/W and W/O emulsions

Sample	Pumpkin seed oil (% v/v)	Hemp seed oil (% v/v)	Lecithin (% m/v)	Distilled water (% v/v)
E1	10	-	10	80
E2	-	10	10	80
E3	50	-	6	44
E4	-	50	6	44

Organoleptic Analysis

The organoleptic analysis was performed after preparation and over seven days. In

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this case, for each formulation were followed characteristics like appearance, consistency, fluidity, homogeneity, color and the presence of instability clues like phase separation. The dilution test was realized to confirm structural modifications in the internal structure of emulsions considering two ratios of dilution 1:4 and 1:10 which were compared with the primary system.

Conductometry Analysis

Conductivity analysis was carried out using Consort C931 conductivity meter (De Bruyne Instruments, Belgium) designed as a multifunctional platform which was planned for various determinations, being equipped with a platinum electrode calibrated with a saline potassium chloride solution. The determinations were performed in triplicate.

Evaluation of Superficial Properties

For the evaluation of superficial properties considering the free superficial energy and contact angle measurements, CAM-101 apparatus, equipped with a Hamilton syringe and a C 209-30 needle was used (KSV Instruments Ltd., Finland). Young equation was fitted with two models of analysis: the contact angle (CA) and pendant drop methods. The measurements were performed in triplicate.

RESULTS AND DISCUSSION

The O/W emulsions formulated with pumpkin seed oil and hemp seed oil were kept their stability over a week from the preparation time. It was observed a homogeneous appearance with a semi-fluid structure which was correlated with the composition of the systems. The yellow color was specific and attributed to the components: the oil phase and the emulsifier. It can be mentioned that O/W emulsion with pumpkin seed oil had an intense color than one with hemp seed oil and can be associated with its composition rich in carotenoids. On the other hand, the W/O systems had similar characteristics, considering the aspect and the color parameters, excepting their consistency which was highly viscous and correlated with an elevated amount of oil phase of 50% which was also responsible for some oxidative phenomena visualized in the superior portion of the vials. In the formulation process for future studies, the attention must be awarded to the addition of proper antioxidants.

After the completion of dilution test, were obtained several data about structural behaviour of O/W and W/O emulsions, offering preliminary information that were correlated with the future experimental results. Thus, it was observed that O/W emulsions E1 and E2 exhibited a good stability after the addition of water fractions. The color intensity was slightly yellow without an intense appearance. No phase separation was observed and the homogeneity was proper. On the opposite, in the case of W/O emulsions, was noted an initiation of phase separation after several minutes of examination, thus, these systems are more susceptible to experience instability events.

Conductivity evaluation is essential for internal structure analysis of fluid colloidal dispersions. On this way, according to Table 2, was confirmed the emulsion type for each sample, obtaining the mean values of conductivity ($\mu\text{S}/\text{cm}$), after samples measuring in triplicate, at 25°C at 7 and 14 days after preparation, the values being between 397.66-1081.00 $\mu\text{S}/\text{cm}$ and 604.00-1326.66 $\mu\text{S}/\text{cm}$, respectively. An increase in conductivity after 800 $\mu\text{S}/\text{cm}$ was specific for O/W emulsions E1 and E2 which

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contained a high amount of aqueous phase of 80%, while the inversed type represented by E3 and E4 had lower values being correlated with the incorporation of a large concentration of oil phase.

Table 2. Conductivity values for the O/W and W/O emulsions prepared with pumpkin seed oil and hemp seed oil tested at 25°C and mean values $\pm$ SD at 7 days and 14 days from preparation time

Emulsion	Mean values of conductivity $\pm$ SD (μ S/cm) after 7 days	Mean values of conductivity $\pm$ SD (μ S/cm) after 14 days	Emulsion type
E1	934.66 $\pm$ 1.59	1111.00 $\pm$ 10.00	O/W
E2	1081.00 $\pm$ 4.58	1326.66 $\pm$ 10.40	O/W
E3	397.66 $\pm$ 5.13	712.33 $\pm$ 6.42	W/O
E4	401.00 $\pm$ 2.64	604.00 $\pm$ 19.15	W/O

It is important to note that the inclusion of lecithin as an emulsifier will influence the conductivity due to its amphiphilic structure, with cationic and anionic charges. Thus, the main components that will influence the electrical properties of the emulsions are water molecules and the emulsifier.

Considering the superficial properties, the formulated emulsions E1, E2, E3 and E4 were analysed by applying goniometric principles. The superficial tension was determined using the equation Young (Popa *et al.*, 2013):

$$\gamma_{SG} = \gamma_{SL} + \gamma_{LG} \cdot \cos \theta \quad (1)$$

The equation was correlated with two representative models that can offer valuable information considering the surface properties and peculiarities of the internal structure for both O/W and W/O emulsions: the pendant drop and contact angle models. Thus, in Table 3, are presented results specific for both types of determinations, while in Figure 1, it can be depicted a variation of mean superficial tension values in time (9 s) for each emulsion and also the variation of the mean values of contact angle in time (4 s). The mean values of surface tension had a similarity over the analysis for both type of emulsions, being a mark of their stability. It can be considered that lecithin as a potent emulsifier will create at the oil/water interface a monomolecular layer with the aim to decrease the interfacial tension, forming an electrical barrier that keeps the particles dispersed in the continuous medium.

Table 3. Results of the mean values $\pm$ SD after superficial assays for O/W and W/O emulsions prepared with vegetable oils and lecithin

Emulsion	Surface tension σ_M (mN/m) Pendant drop model	Surface tension σ_M (mN/m) Contact angle model	Contact angle ($^\circ$)
E1	25.14 $\pm$ 0.97	32.21 $\pm$ 5.11	40.46 $\pm$ 4.89
E2	23.46 $\pm$ 0.60	24.40 $\pm$ 2.88	32.05 $\pm$ 2.01
E3	23.32 $\pm$ 0.37	25.23 $\pm$ 2.92	31.87 $\pm$ 0.51
E4	25.35 $\pm$ 2.34	26.69 $\pm$ 2.53	44.01 $\pm$ 5.48

Figure 1. Graphical representations for superficial parameters:
a) Cumulative profiles showing the variation of superficial tension (mN/m) versus time (s) for O/W and W/O emulsions E1, E2, E3 and E4
b) Cumulative profiles showing the variation of contact angle for O/W and W/O emulsions expressed as mean contact angle ($^{\circ}$) as a function of time (s)

The contact angle model applied in the case of each emulsion, gave information concerning the wettability properties, exploring the hydrophilic or hydrophobic behaviour of O/W and W/O formulations. Using this model, in Figure 2 are proposed a series of determinations with the most representative results, where it can be observed that the O/W emulsions E1 and E2 are characterized by a high wettability, being observed changes in the contact angle value on a period of 4 seconds. In the case of E2, where the contact angle keeps its value around 35° , wasn't observed major changes. In the case of W/O emulsions E3 and E4 with 50% oil phase, a slightly hydrophobic character of the droplets was dominant for E4, with significant modifications of hysteresis from 55.69° to 28.40° . Considering the placement of contact angle values in the domain of $0-90^{\circ}$ for each formulation it was experienced an incomplete wettability, being deducted that the adhesion forces at the solid surface are superior than cohesion ones, but not as powerful to generate a complete wettability to reach a 0° contact angle. As a final note, the hydrophilicity will be increased for the emulsions in the following order, from the most hydrophobic through the most hydrophilic structures: $E4 > E1 > E2 > E3$.

Figure 2. Pendant drops and evolution of contact angle for O/W and W/O emulsions from 1 s to 4 s: case a) contact angle variation for emulsion E1 with pumpkin oil; b) contact angle variation for emulsion E2 with hemp seed oil; c) contact angle variation for emulsion E3 with pumpkin seed oil, and d) contact angle variation for emulsion E4 with hemp seed oil

CONCLUSIONS

It can be appreciated that the emulsions prepared with vegetable oils are functional colloidal dispersions with a complex pattern which was analysed in the present preliminary study. In this direction, were obtained stable, homogeneous systems, with a proper appearance which was correlated with the composition. O/W emulsions had a superior stability than W/O type, due to a large amount of lecithin selected in the formulation. The conductivity evaluation confirmed the type of each emulsion. The results of superficial analysis proved the lecithin influence in the diminishing of surface tension, in order to promote stable systems, as well as the wettability behaviour which was characterized by contact angles under 90°, exhibiting the dynamics occurred between hydrophilic and hydrophobic forces at a solid surface. Based on their properties, the designed emulsions can be attractive systems with biocompatible components for oral administration, being a suitable option for drug formulation.

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