Construction Of Ternary Phase Diagram for Three-Component System (Oil-Water-Surfactant) As A Preliminary Step Before Formulating a Nanoemulsion

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Abstract

The objective of the study was to investigate the behavior of ternary phase diagrams consisting of water, oil, and surfactant. The researchers aimed to identify and characterize the different phases obtained from combinations of oil and surfactant/cosurfactant mixtures (S-mix), including conventional emulsions, gel/viscous phases, and transparent/translucent nanoemulsions (NEns). To achieve this objective, the researchers constructed ternary phase diagrams using the water titration method at room temperature. They gradually added and mixed water into the system, and after each addition, they visually analyzed the resulting phases. The study utilized *Opuntia Ficus indica* seed (OFIS) oil as the oil phase, Tween-80 as the surfactant, PEG-400 as a co-solvent, and different proportions of S-mix (surfactant mixture), along with water as the third phase. By varying the ratios of the S-mix (which included Tween-80 and another surfactant), the researchers aimed to understand the effects on phase behavior, including the formation of emulsions, gels, and NEns. Specifically, they tested ratios of 1:0.5, 1:1, 1:11.5, 1:2, 1:2.5, and 1:3 for the S-mix composition. The study found that combinations of surfactant and co-surfactants (Tween 80: PEG-400) with OFIS oil resulted in high gel or viscous phases. Based on the ternary phase diagrams, the ratio of the S-mix (1:1) was identified as more suitable for formulating NEns, providing high stability and a large area of the NEn region. This finding suggests that the 1:1 ratio of the S-mix could be used to optimize the formulation and stability of NEns. Overall, the study contributes to the understanding of oil-water-surfactant systems, ternary phase diagrams, and their applications in emulsion and NEn formulations. The findings provide insights into the behavior and composition of these systems, facilitating the development of improved emulsion and NEn products.

Keywords: Emulsion, Nanoemulsion, *Opuntia Ficus indica*, Phase diagram, Ternary,

Introduction:

A ternary graph (triangle plot), is a graphical representation used to show the compositions of systems composed of three species.[1] It is a barycentric plot where the ratios of the three variables are depicted as positions in an equilateral triangle. In a ternary plot, the proportions of the three variables (a, b, and c) must sum to a constant, usually represented as 100%. This means that a + b + c = K, where K is the constant for all substances being graphed. Since the three proportions cannot vary independently and there are only two degrees of freedom, knowing two variables is sufficient to determine the position of a sample's point on the graph. For example, if a and b are known, c can be calculated as K - a - b. The ternary plot allows us to graphically represent the intersection of all three variables in two dimensions. It is particularly useful when studying the mutual solubilities of liquids in a two-phase system, such as water and oil in contact with a surfactant.[2] The ternary plot helps determine the compositions of the different phases and understand their behavior. According to the phase rule, a single phase in a three-component system can possess four degrees of freedom, which are the temperature, pressure, and compositions of two of the three components. However, in the graphical representation of the ternary plot, it is often necessary to hold temperature and pressure constant due to the difficulty of graphically representing multiple variables. This special form of the phase rule applies to two-component systems of constant pressure. By constructing and analyzing the ternary phase diagram, valuable information can be gained about the behavior and compositions of the three-component system under study. The ternary plot provides a visual representation that simplifies the understanding of complex interactions between the components.[3]

Nanoemulsion (Nen) refers to a thermodynamically stable, transparent, or translucent dispersion of oil droplets in water (oil-in-water NEn) or water droplets in oil (water-in-oil NEn), with droplet sizes typically in the nanometer range (10-200 nm). NEns are characterized by their small droplet size, high stability, and enhanced bioavailability.[4] The formation of NEns involves the use of surfactants and co-surfactants to reduce the interfacial tension between oil and water phases, allowing the formation of fine droplets. The surfactants adsorb at the oil-water interface, forming a protective layer around the droplets to prevent coalescence and aggregation. Co-surfactants are often used to enhance the solubilization of the oil phase and promote the formation of small droplets. NEns have attracted significant attention in various industries, including pharmaceuticals, cosmetics, and food, due to their improved stability, enhanced solubility, and increased bioavailability of active ingredients. They offer advantages such as improved drug delivery, better sensory properties, and increased absorption. The formulation and stability of NEns depend on several factors, including the type and concentration of surfactants and co-surfactants, the ratio of oil to water, and the preparation method employed (such as high-pressure homogenization or ultrasonication). The selection of suitable components and optimization of formulation parameters are crucial to achieve stable and desirable NEn systems. NEns have a wide range of applications, including drug delivery systems, topical formulations, food and beverage products, and cosmetic and personal care products. Their small droplet size and improved stability make them effective carriers for lipophilic and hydrophilic active ingredients, allowing for enhanced performance and targeted delivery.[5]

In this preliminary study, the objective is to investigate the impact of the oil and S-mix ratio on the formation of a large NEn region. The study focuses on utilizing OFIS oil as the oil phase and varying proportions of S-mix, which consists of Tween 80 and PEG-400. By altering the ratio of the S-mix components, the study aims to identify the optimal combination that results in a significant area of the NEn region in the ternary phase diagram. The NEn region represents the composition range where stable and transparent/translucent NEns can be formed. The researchers will likely prepare different formulations with varying ratios of Tween 80 to PEG-400 in the S-mix.[6] They will then analyze the resulting ternary phase diagrams and observe the size and stability of the NEn region. The specific ratios tested may include different weight ratios or volume ratios, such as 1:0.5, 1:1, 1:11.5, 1:2, 1:2.5, and 1:3. By determining the optimal oil and S-mix ratio, the study aims to provide insights into the formulation of NEns using Opuntia Ficus indica seed (OFIS) oil and the surfactant mixture (Tween 80 and PEG-400). The results will contribute to understanding the factors influencing NEn formation and can guide future formulation development for applications in various industries such as pharmaceuticals, cosmetics, and food.[7]

Materials and methods

Materials

Opuntia Ficus indica seed oil, tween-80, and PEG-400 were obtained from Fischer Scientific Deionized water was used throughout the experiments. All other chemicals utilized in the experiments were analytical grade.

Methods:

Construction of ternary phase diagrams

In this study, ternary phase diagrams were constructed to investigate the formation of NEns using OFIS oil, Tween-80 as a non-ionic surfactant, and PEG-400 as a co-surfactant. The purpose was to identify the appropriate ratio of surfactant and co-surfactant that would result in a large existence area of the NEn phase.[8] The experimental procedure involved mixing Tween-80 with different ratios of PEG-400, namely 1:0.5, 1:1, 1:11.5, 1:2, 1:2.5, and 1:3. These mixtures served as the surfactant and co-surfactant components in the ternary phase diagrams. OFIS oil was mixed with aliquots of each surfactant-co-surfactant mixture at room temperature (25 °C). To construct the phase diagrams, water was added gradually to the oil-surfactant-co-surfactant mixtures while vigorously stirring with a vortex mixer. The water titration method was used, where the addition of water increased the water content in the system and decreased the concentration of the surfactant-co-surfactant mixture. This process was performed at room temperature. After each water addition, the resulting mixtures were visually checked and categorized as clear NEns, emulsions, or gels. The observed behavior of the mixtures determined the phase in which they were placed on the phase diagram. The points representing the samples were marked on the phase diagram, and the area covered by these points was considered as the region where NEns exist. Quantities of all three phases, namely oil, surfactant-co-surfactant mixture, and water, were determined as percentages by weight (w/w). By systematically varying the ratios of surfactant and co-surfactant and observing the resulting phases on the phase diagram, the study aimed to identify the specific combination of components that would lead to a larger existence area of the NEn phase. This information would contribute to the understanding and formulation of stable and desirable NEn systems for various applications.[9]

Table 1: The proportions of components used in the study

Component (volume)			**Component (%)**		
OFIS Oil	**S-mix (1:0.5)**	**Water**	**OFIS Oil**	**S-mix**	**Water**
1	9	85.6	1.05	9.41	89.54
2	8	70.2	2.49	9.98	87.53
3	7	55.2	4.60	10.74	84.66

4	6	40.8	7.87	11.81	80.31
5	5	31.2	12.14	12.14	75.73
6	4	18.2	21.28	14.18	64.54
7	3	9.5	35.90	15.38	48.72
8	2	4.2	56.34	14.08	29.58
9	1	1.3	79.65	8.85	11.50
	S-mix (1:1)				
1	9	89.2	1.01	9.07	89.92
2	8	65.2	2.66	10.64	86.70
3	7	41.2	5.86	13.67	80.47
4	6	25.2	11.36	17.05	71.59
5	5	10.3	24.63	24.63	50.74
6	4	5.4	38.96	25.97	35.06
7	3	2.1	57.85	24.79	17.36
8	2	1.2	71.43	17.86	10.71
9	1	0.5	85.71	9.52	4.76
	S-mix (1:1.5)				
1	9	87	1.03	9.28	89.69
2	8	65.2	2.66	10.64	86.70
3	7	51.2	4.90	11.44	83.66
4	6	39.2	8.13	12.20	79.67
5	5	20.8	16.23	16.23	67.53
6	4	11.3	28.17	18.78	53.05
7	3	5.8	44.30	18.99	36.71
8	2	1.2	71.43	17.86	10.71
9	1	0.4	86.54	9.62	3.85
	S-mix (1:2)				
1	9	89.2	1.01	9.07	89.92
2	8	79.6	2.23	8.93	88.84
3	7	66.4	3.93	9.16	86.91
4	6	52.3	6.42	9.63	83.95
5	5	35.2	11.06	11.06	77.88
6	4	20.8	19.48	12.99	67.53

7	3	7.6	39.77	17.05	43.18
8	2	3.4	59.70	14.93	25.37
9	1	1.2	80.36	8.93	10.71
S-mix (1:2.5)					
1	9	84.6	1.06	9.51	89.43
2	8	70.5	2.48	9.94	87.58
3	7	61.5	4.20	9.79	86.01
4	6	45.2	7.25	10.87	81.88
5	5	33.2	11.57	11.57	76.85
6	4	24.5	17.39	11.59	71.01
7	3	19.8	23.49	10.07	66.44
8	2	10.6	38.83	9.71	51.46
9	1	7.5	51.43	5.71	42.86
S-mix (1:3)					
1	9	85.6	1.05	9.41	89.54
2	8	75.4	2.34	9.37	88.29
3	7	61.2	4.21	9.83	85.96
4	6	55.2	6.13	9.20	84.66
5	5	44.7	9.14	9.14	81.72
6	4	33.5	13.79	9.20	77.01
7	3	24.5	20.29	8.70	71.01
8	2	19.6	27.03	6.76	66.22
9	1	11.6	41.67	4.63	53.70

Polarized light microscopy

During the experimental process, a visual inspection was conducted after each addition of water to the mixture of oil, surfactant, and co-surfactant. The purpose of this inspection was to determine the characteristics of the resulting samples and classify them into different phases. The samples were categorized as NEns if they appeared as transparent or translucent liquids that could flow easily. These NEns exhibited isotropic behavior, meaning they had a uniform composition throughout. On the other hand, samples that appeared as milky or turbid liquids were identified as emulsions. These emulsions indicated the presence of dispersed droplets within a continuous phase.[10] Additionally, if the samples did not show a change in the meniscus (the curved surface at the edge of a liquid) after tilting them to an angle of 90°, they were categorized as gels. Gels are semi-solid materials with a three-dimensional network structure. To further verify the nature of the NEn samples, cross-polarized light microscopy was employed. Isotropic substances, such as NEns, do not reflect polarized light. Consequently, when observed under cross-polarized light, NEn samples would appear dark, confirming their isotropic nature. By visually inspecting the samples and distinguishing between NEns, emulsions, and gels, the researchers could assess the formation of different phases and determine the behavior of the system under study.[11]

Results and discussion

A ternary phase diagram is a valuable tool in identifying phase boundaries and regions within a ternary mixture composed of oil, water, and surfactant/co-surfactant. It helps visualize the existence of biphasic or monophasic regions, as well as the nature of the phases within these regions, such as non-isotropic or liquid crystalline.[12] When dealing with systems involving four or more components, the ternary phase diagram is commonly used to depict the relationships and compositions of these components. Each corner of the diagram represents a binary mixture of two components, such as surfactant/co-surfactant, surfactant/water, oil/drug, and water/drug mixtures. The concentrations at each corner of the ternary phase diagram correspond to 100% concentrations of the respective component.

In this study, three types of system combinations were developed using Tween-80, PEG400, and water, with specific ratios of surfactant (S) to co-surfactant (CoS). The ternary phase diagrams were then analyzed to investigate the effect of using OFIS oil with the various surfactant/co-surfactant mixtures on the formation of stable NEns.

By studying the ternary phase diagrams, the researchers aimed to understand how the interactions between the oil, water, and surfactant/co-surfactant components, as well as their specific ratios, influenced the formation and stability of NEns. This information is crucial for optimizing the formulation of stable NEn systems in various applications.

The ternary Phase Diagram for Water, OFIS Oil, and S-mix was shown in Figure 1.

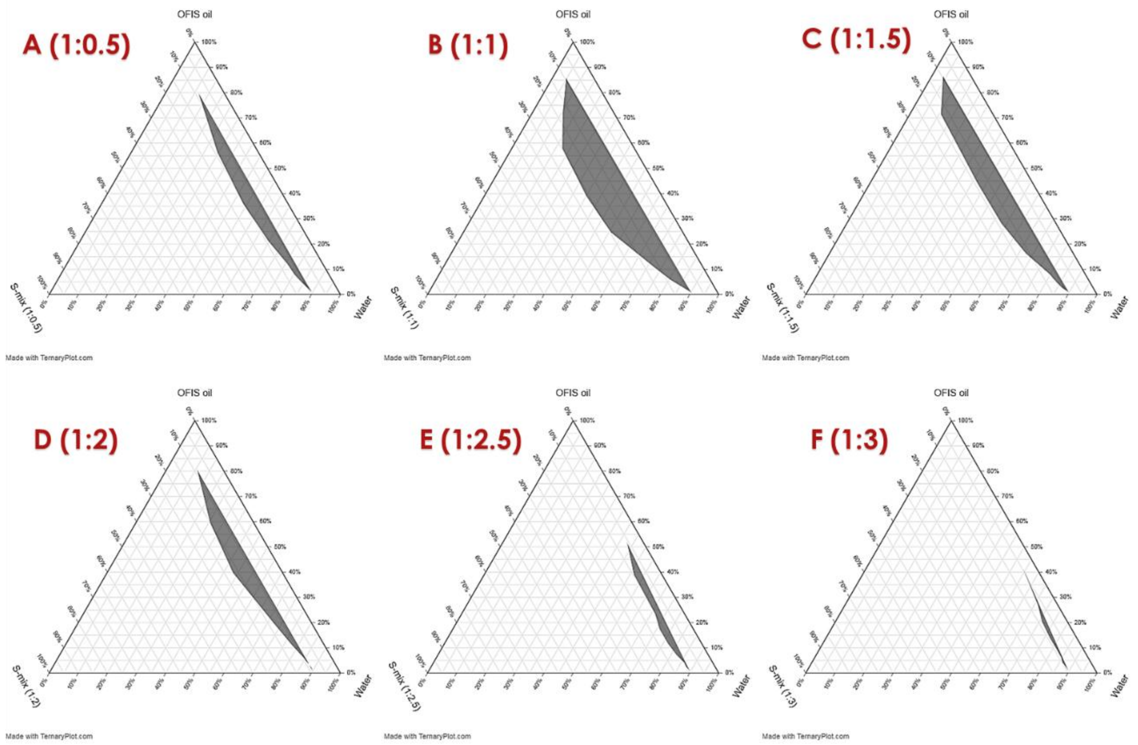

Figure 1: Ternary phase diagrams composed of OFIS oil/Tween 80/PEG400/water with SS-mix ratios (A) 1:0.5, (B) 1:1, (C) 1:1.5, (D) 1:2, (E) 1:2.5, and (F) 1:3; The shaded region (one phase-region of miscibility) is Nano emulsion area

The calculation for Determination of % Oil, Water, and S-mix

The following procedure s adopted for the calculation for the determination of the phases ratios

$W_1=V_1\times\sigma_1$

$W_2=V_2\times \sigma_2$

$W_3=V_3\times \sigma_3$

Where,

V_1, V_2, and V_3 are the Volume of Water, Oil & S-mix respectively.

σ_1, σ_2, σ_3 are the densities of Water, Oil & S-mix respectively.

$$\%\text{ of Water } = \frac{W1}{(W1+W2+W3)}X100$$

$$\%\text{ of Oil} = \frac{W2}{(W1+W2+W3)}X100$$

$$\%\ \text{of surfactant} = \frac{W3}{(W1+W2+W3)} X100$$

Results and discussion

The ternary phase diagram shown in Figure 1 represents the possible conditions of water, oil, and surfactant in a system. The curve depicted on the diagram separates two distinct regions: the region above the curve, which represents a one-phase system (region of miscibility), and the region below the curve, which represents a two-phase system (region of immiscibility). In the region of miscibility (above the curve), the water, oil, and surfactant are fully soluble and form a single phase. This indicates that the components are well-mixed and homogenous, resulting in a homogeneous solution or dispersion. In contrast, the region of immiscibility (below the curve) represents a two-phase system. This means that the water, oil, and surfactant are not fully soluble and separate into two distinct phases. Typically, this occurs when the surfactant concentration or the oil-to-water ratio exceeds a certain limit, leading to the formation of separate oil-rich and water-rich phases. The curve on the ternary phase diagram serves as a boundary between these two regions, indicating the conditions under which the system transitions from a one-phase to a two-phase state. It provides valuable information about the miscibility and phase behavior of the components in the system. By analyzing the ternary phase diagram, researchers can determine the optimal compositions and ratios of water, oil, and surfactant to achieve a desired phase behavior and stability in various applications, such as emulsion formulation, NEn development, or nanoparticle synthesis.

Conclusion

Indeed, the phase diagram study provides valuable insights into the role of surfactant, co-surfactant, and oil in determining the properties of the system at different compositions. By analyzing the phase behavior and emulsification areas, the study allows us to understand the influence of the surfactant and co-surfactant structure on the interfacial properties and stability of the system. In the study, the S-mix with a 1:1 ratio of Tween 80 and PEG-400 demonstrated a high emulsification area compared to other ratios. This finding suggests that this specific surfactant/co-surfactant combination is well-suited for creating and stabilizing NEns. The balanced properties of Tween 80 and PEG-400 in this ratio likely contribute to their efficient coverage at the oil-water interface, resulting in a larger area of stable NEn formation. The insights gained from this study have significant implications in various industries, including the pharmaceutical industry, cosmetics, and personal care products. NEn delivery systems are of great interest in pharmaceutical formulations as they can improve the solubility, bioavailability, and targeted delivery of drugs. The knowledge obtained from this study can aid in formulating effective NEn-based drug delivery systems. Similarly, in the cosmetics and personal care industry, NEns are utilized for the delivery of active ingredients, enhancing product stability, and improving sensory attributes. Understanding the impact of surfactant and co-surfactant structure on NEn formation allows for the development of optimized formulations with desired properties.

Acknowledgments

None

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