Investigation on the Impact of Cholesterol Structure on W/O/W Structure

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Abstract

W/O/W multiple emulsions, due to their unique "two-membrane and three-phase" structure, can enhance the encapsulation efficiency of active ingredients and improve product stability in cosmetics, thus garnering significant attention. Cholesterol and its derivatives not only possess skincare effects but also play a role in stabilizing the emulsion interface. However, their specific impact on the stability of W/O/W emulsions remains unclear.This study focuses on this aspect, aiming to provide a scientific basis for the formulation design of cosmetics and the application of functional ingredients, thereby facilitating the development of more efficient and stable cosmetic products. In the experiments, polarizing microscopy was employed to observe the structure and assess the particle size distribution. Environmental conditions were simulated through room temperature, high-temperature constant temperature, thermal cycling, and rapid centrifugation tests. Evaluation criteria included phase separation, appearance, odor changes, and encapsulation efficiency (determined by measuring conductivity changes to assess the encapsulation effect and stability of the W/O emulsion). The findings reveal that the addition of cholesterol and its derivatives can significantly enhance the stability of W/O/W composites. The use of lipophilic surfactants with a low HLB value (≤5) is conducive to the formation of a stable system. However, an excessively high IOB value (＞0.2) of the oil phase can compromise the stability of the system under high-temperature conditions. Moreover, excessive addition of cholesterol and its derivatives can affect the encapsulation stability of the W/O/W composite at low temperatures.This study offers valuable guidance for optimizing the formulation of W/O/W emulsions and highlights the potential of cholesterol and its derivatives as interfacial stabilizers in multiple emulsion systems.

Keywords:

W/O/W multiple emulsion; Cholesterol and its derivatives; Stability; Encapsulation efficiency

1. INTRODUCTION

In the field of cosmetics, water-in-oil-in-water (W/O/W) multiple emulsions have gained significant attention due to their unique structure and functionality. W/O/W multiple emulsions can effectively encapsulate active ingredients, enhancing product stability and bioavailability[1]. Their development history can be traced back to the mid-20th century. With advancements in emulsification technology, the concept of multiple emulsions was gradually proposed and applied in cosmetics. The uniqueness of W/O/W emulsions lies in their "three-phase, two-membrane" structure, which not only encapsulates active ingredients of different properties but also effectively isolates and protects these ingredients, prolonging their action time.

Cholesterol and its derivatives play a crucial role in cosmetics. They not only possess moisturizing and skin-nourishing effects but also serve as emulsifiers or stabilizers, improving product texture and stability. Cholesterol can enhance the interfacial stability of emulsions, preventing flocculation and coalescence. Additionally, the incorporation of cholesterol can improve the uniformity of emulsion particle size, thereby enhancing product stability and encapsulation efficiency[2-3].

However, the specific impact of cholesterol-based structures on W/O/W emulsions remains unclear. Research on the effects of cholesterol and its derivatives on the performance of W/O/W emulsions is of great significance, as it will aid in further optimizing cosmetic formulations and improving product stability and efficacy.

This study aims to delve into the influence of cholesterol and its derivatives on the stability of W/O/W emulsions, providing a scientific basis for cosmetic formulation design and the application of functional ingredients. Through this research, we hope to develop more efficient and stable cosmetics to meet consumers' demand for high-quality cosmetic products.

2. MATERIALS AND METHODS

2.1 Preparation method[4-5] as shown in the following figure：

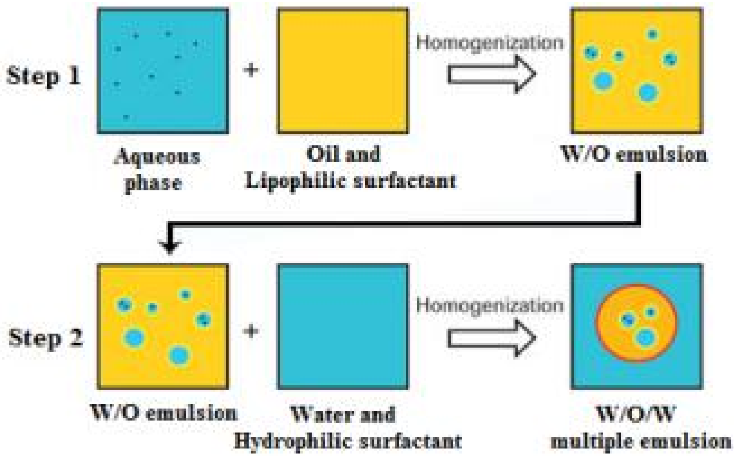


Fig 1: Two step emulsification process

2.2 Evaluation methods

Structural Evaluation: The W/O/W multiple emulsions were observed using a polarizing microscope for structural characterization, and the particle size of the W/O/W multiple emulsions was assessed based on microscopic photographs.

Stability Testing: Samples were subjected to stability testing at room temperature (25°C) for 90 days, 48°C for 90 days, 5 cycles of 45°C ↔ -15°C temperature alternation, and rapid centrifugation by LUM GmbH (45°C, 4000 rpm, 5 hours).

Evaluation Criteria

Layer Separation Evaluation: ○ No separation, × Separation.

Appearance Evaluation: ○ No change, △ Slight change (viscosity thinning), × Change (separation, emulsion breaking, phase inversion, etc.).

Odor Evaluation: ○ No change, × Change (abnormal odor: such as oxidized oil smell, etc.).

Encapsulation Efficiency (EE) Evaluation: To confirm the encapsulation efficiency and stability of the W/O emulsion within the prepared W/O/W multiple emulsions, the leakage of NaCl from the inner aqueous phase was assessed by monitoring changes in the electrical conductivity of the W/O/W multiple emulsions to evaluate the encapsulation efficiency and stability of the W/O emulsion within the W/O/W multiple emulsions.

2.3 Human Skin Hydration Evaluation

Test Location: Inner left forearm.

Test Parameters: Transepidermal water loss (TEWL) and stratum corneum water content.

Test Products: Sample 1 (the formulated multi - action moisturizing cream) and Sample 2 (a commercial beauty cream).

Test Instrument: Corneometer CM 825.

Number of Subjects: 5.

2.4 Main Materials

Cholesterol and sterol derivative materials include: Cholesterol, Phytosteryl Macadamiate, Phytosteryl/isostearyl/cetyl/stearyl/behenyl dimer dilinoleate.

The emulsifier selected for the external aqueous phase is the Tween 80 system.

3. RESULTS AND DISCUSSION

3.1 The Impact of Cholesterol and Sterol Derivatives on the Stability of W/O/W Emulsions

Table 1 The Stability Evaluation of a~e Groups W/O/W Emulsions

Group		a	b	c	d	e
LUM GmbH	Phase Separation	○	○	×	×	○
25℃	Appearance	○	○	△	×	○
	Odor	○	○	○	○	○
48℃	Appearance	○	○	×	×	×
	Odor	○	○	×	×	×
cycle test	Appearance	○	○	×	×	×
	Odor	○	○	×	×	×

a：2% Phytosteryl Macadamiate；b：0.1% Cholesterol + 1% Phytosteryl Macadamiate；c：Without cholesterol and its derivatives; d: 2% Phytosteryl/isostearyl/cetyl/stearyl/behenyl dimer dilinoleate；e：2% COPERNICIA CERIFERA (CARNAUBA) WAX；All other components are the same

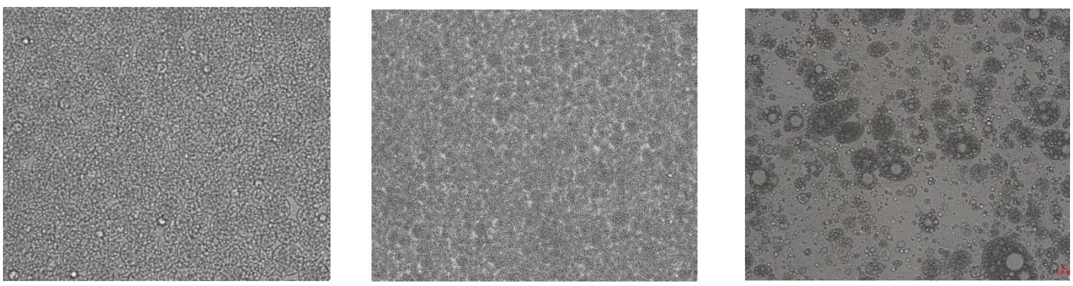


Fig 2: Micrographs of the W/O/W structure for Group a after accelerated testing for 3 months.(Left: 25°C, 500×; Center: 48°C, 200×; Right: -18°C, 200×)

3.2 The Influence of Hydrophile-Lipophile Balance (HLB) Value of Oil-Soluble Surfactants on Stability

Table 2: The Stability Evaluation of f~g Groups W/O/W Emulsions

Group		f	g
LUM GmbH	Phase Separation	○	×
25℃	Appearance	○	×
	Odor	○	×
48℃	Appearance	○	×
	Odor	○	×
cycle test	Appearance	○	×
	Odor	○	×

f：Polyglyceryl-6 polyricinoleate（HLB=3.2）; g：Polyglyceryl-3 diisostearate（HLB=5.5）; All contain 0.1% Cholesterol + 1% Phytosteryl Macadamiate.

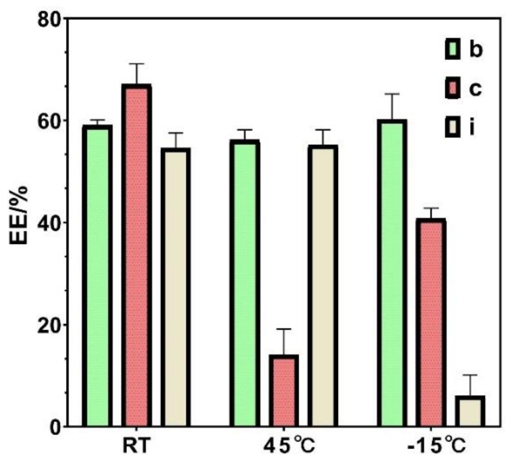
3.3 The Influence of Oil Phase Type (IOB Value) on Stability

Table 3: Results of the Oil Phase's Influence on Stability

Group		b	h
LUM GmbH	Phase Separation	○	×
25℃	Appearance	○	△
	Odor	○	○
48℃	Appearance	○	×
	Odor	○	×
cycle test	Appearance	○	△
	Odor	○	○

b: Isononyl Isononanoate（IOB=0.2）; h：Triethylhexanoin（IOB=3.6）; Other conditions being the same

3.4 The Impact of Sterol and Derivative Ratios on Encapsulation



Efficiency

b: 0.1% Cholesterol + 1% Phytosteryl Macadamiate;　c: Without cholesterol and its derivatives;　i: 0.5% Cholesterol + 3% Phytosteryl Macadamiate

Fig 3: Results of Encapsulation Efficiency Test

3.5 Human Skin Hydration Test Results

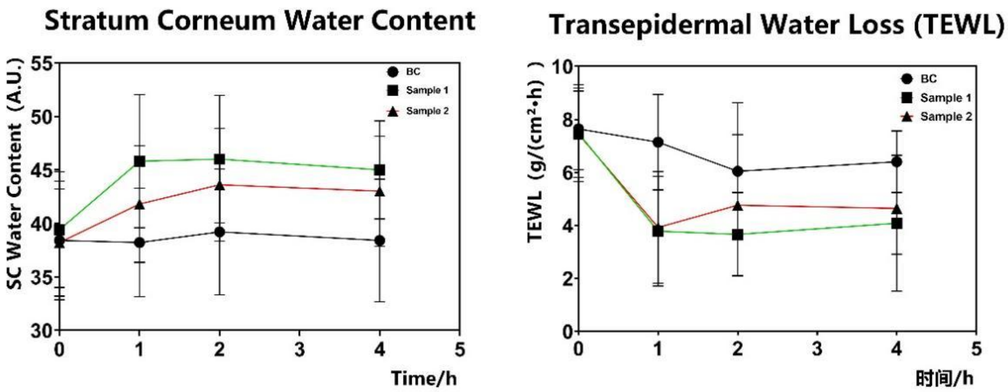


Fig 4: Results of Human Skin Hydration Test

The effects of cholesterol and sterol derivatives on the stability of W/O/W (water-in-oil-in-water) compositions are presented in Table 1 and Figure 1. All groups exhibited a distinct W/O/W structure upon initial preparation. However, as indicated by the test results in Table 1, the W/O/W compositions of groups a and b passed the stability evaluation tests, while those of groups c to e did not. This suggests that the addition of cholesterol and/or sterol derivatives in this system enhances the stability of W/O/W compositions, but there is still selectivity in the choice of sterol derivatives. Furthermore, as inferred from the comparison of group e, the stability mechanism is unrelated to an increase in melting point.

To further investigate the factors influencing the stability of multiple systems containing cholesterol and/or sterol derivatives, the HLB (Hydrophilic-Lipophilic Balance) value of lipophilic surfactants and the IOB (Ionization of Oil Bond) value of the oil phase were explored[6-7], with results presented in Tables 2 and 3. Under fixed conditions of the external phase, a lower HLB value of the lipophilic surfactant is more conducive to forming a stable W/O/W composition. The optimal HLB value of the lipophilic surfactant in this W/O/W composition is ≤5. However, this study only employed polyglycerol-type lipophilic emulsifiers, and other types of emulsifiers were not explored. The W/O/W composition in group h of Table 3 failed the stability evaluation test, indicating that a higher IOB value of the oil phase is detrimental to the stability of this system's W/O/W composition, particularly under high-temperature accelerated stability conditions. Therefore, the optimal IOB value of the oil phase in this system's W/O/W composition is ≤0.2. Overall, a greater polarity difference among the three phases tends to enhance stability.

Additionally, encapsulation efficiency is an important indicator for evaluating the quality of W/O/W emulsion formulations[8-9]. A high encapsulation efficiency typically indicates a more reasonable preparation process and formulation design, resulting in higher-quality formulations. Experimental results in Figure 3 show that the encapsulation efficiency of the W/O emulsion in the W/O/W composite of group b ranges from 50-60%. After 3 months of high- and low-temperature testing, its conductivity did not change significantly, indicating no leakage of NaCl electrolyte from the inner aqueous phase. In contrast, the encapsulation efficiency of the W/O/W composite in group c significantly decreased after high- and low-temperature testing. Data from group i indicate that increasing the content of cholesterol and sterol derivatives in the W/O primary emulsion maintains stable encapsulation efficiency under high-temperature testing but decreases to 6.1% under low-temperature testing, suggesting that excessive cholesterol and/or sterol derivatives are detrimental to the low-temperature stability of W/O/W composites. Therefore, the W/O/W composite in this system demonstrates good long-term stability in terms of appearance, multiple structures, and W/O emulsion encapsulation.

Multiple emulsions formulations typically offer good moisturizing effects, particularly in water - locking and oil - phase moisturizing[10]. Sterol compounds, being endogenous in the human body, can provide excellent moisturizing benefits to the stratum corneum. Human skin moisturizing tests were conducted. As shown in Figure 4, compared to a blank control group, the stratum corneum and epidermal water content of five Asian adult subjects significantly improved 1, 2, and 4 hours after applying samples 1 and 2 to the inner forearm. This indicates that under the test conditions, sample 1 increased skin moisture content. Moreover, compared to a commercial W/O/W multiple product, this system's W/O/W complexes demonstrated superior moisturizing and water - locking performance, aligning with expectations.

4.CONCLUSION

This study delved into the role of cholesterol and its derivatives in W/O/W emulsion systems. Results showed they significantly enhanced emulsion stability, and positively affected the encapsulation efficiency and moisturizing properties. Nevertheless, optimizing these properties requires precise adjustment of the emulsifier, oil phase, and the ratio of cholesterol and sterol derivatives. In -vivo skin moisturizing tests indicated that the developed W/O/W emulsion outperformed commercial counterparts, effectively boosting skin hydration which lasted for an extended period. This highlights its high efficacy and market competitiveness.

Overall, the research offers a scientific basis for W/O/W emulsion formulation in cosmetics. By fine - tuning the types and amounts of cholesterol derivatives, and selecting suitable emulsifiers and oil phases, more efficient and stable cosmetic products can be created. Future research could explore combining different cholesterol derivatives and testing them on diverse skin types and under various environmental conditions to meet a wider range of consumer needs.

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