IFSCC 2025 full paper (IFSCC2025-302)

"Glyco-san:

A Multifunctional Chitosan-Based Technology for Enhanced Cleanser Performance"

Takehiko Kasai [1], Ayano Takeda [1], Tatsushi Isojima [1], Yuichi Ikeda [1], Rina Mogaki [1], Olivia Isard [1], Masanori Orita [1], Anne Potter [2], Abel Messavussu [2], Christelle Batout [2], Cynthia MORAIN [2], Mariko Hasebe [3], Richard Chen [4], Brave Wang [4], Nariyoshi Yoshioka [4], Emiko Kato [1], Satoko Banno [1]

[1] L'Oreal Research & Innovation, Kawasaki, Japan; [2] L'Oreal Research & Innovation, Aulnay-sous-Bois, France; [3] L'Oreal Research & Innovation, Clark, USA; [4] L'Oreal Research & Innovation, Shanghai, China

1. Introduction

In the cosmetics market of recent years, there has been a growing demand for high-quality, multifunctionality, and sustainability. Cleansers are no exception. While conventional cleansers excel in cleansing property, this very strength presents a challenge that they often remove naturally occurring emollient oils or beneficial ingredients that are designed to stay on the skin, along with dirt. Furthermore, while foam stability is preferred for usability, it has been difficult to incorporate into cleanser formulations and to retain on the skin a large quantity of oils, which act as anti-foaming agents. [1]. Methods to retain these beneficial ingredients have been proposed [2-3], but such attempts remain ongoing challenges.

The "Glyco-san" technology addresses this challenge by using the electrostatic complexation (Figure 1(a)) of the bio-based materials, chitosan and rhamnolipids, to emulsify and encapsulate oils (Figure 1(b)).

In this study, we attempted to use this Glyco-san technology to develop a cleanser formulation that can retain active ingredients and necessary emollient oils on the skin. To understand the mechanism, we analyzed the internal structure of the wet foam. In addition to the green nature of components, Glyco-san technology, which utilizes the inherent electrical charge of the component materials, enables formulation without the use of organic solvents or heating processes. As such, Glyco-san is in line with the principles of green chemistry, making it sustainable and environmentally friendly.

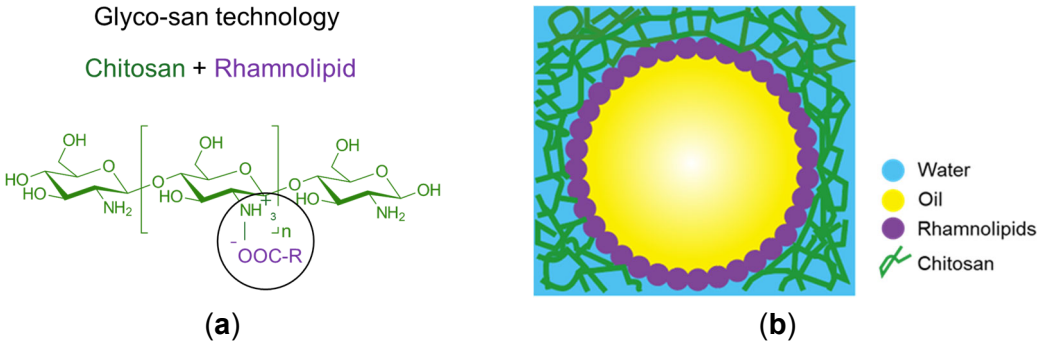


Figure 1: (a) Glyco-san technology (Electrostatic interaction of chitosan and rhamnolipids); (b) Conceptual diagram of the Glyco-san

2. Materials and Methods

2.1 Materials

Agaricus bisporus derived chitosan (M.W. 110kDa), was obtained from KitoZyme (Herstal, Belgium). A 45% rhamnolipids solution was obtained from Evonik Goldschmidt (Essen, Germany). Isostearic acid from Croda (Yorkshire, UK) was used as the fatty acid, and diisopropyl sebacate from Stéarinerie Dubois (Boulogne Billancourt, France) were used as oil. Disodium cocoyl glutamate (and) sodium cocoyl glutamate were obtained from Ajinomoto (Tokyo, Japan).

2.2 Preparation of Glyco-san formulations

The model cleanser formulations with or without Glyco-san (Table 1) were prepared as follows. Chitosan was dispersed in water by mixing (Labolution from Primix, Awaji, Japan) and then dissolved by adding lactic acid (pH 5). Fatty acid and oils were added to the solution, followed by disodium cocoyl glutamate (and) sodium cocoyl glutamate and rhamnolipids. The process is designed to be environmentally friendly. No organic solvent or heating are used. Microscopic observation was performed to confirm the formation of Glyco-san. In addition, an emulsifying property of Glyco-san was assessed by visual observation. The composition of Glyco-san was analyzed by Cryo-TOF-SIMS (Time-of-Flight Secondary Ion Mass Spectrometry, TOF-SIMS 5, IONTOF Japan, Yokohama, Japan).

Table 1 Cleanser formulations (wt.%)

INCI	Glyco-san	Without Glyco-san
WATER	87.8	90.0
CHITOSAN	0.2	0.0
LACTIC ACID	0.5	0.5
ISOSTEARIC ACID	1.0	1.0
DIISOPROPYL SEBACATE	5.0	5.0
GLYCOLIPIDS (RHAMNOLIPIDS)	0.5	0.5
DISODIUM COCOYL GLUTAMATE (and) SODIUM COCOYL GLUTAMATE	5.0	5.0

2.3 Observation, Structural Analysis, and Evaluation of Foam

The foamability and foam stability of cleanser formulations with and without Glyco-san were evaluated using a foam analyzer (Dynamic Foam Analyzer DFA100 from KRÜSS). To compare the formulations, 75 g of each solution was used in the presence of an anti-foaming agent, 1 g of silicone oil. Air was introduced to generate foam, and the changes in foam height were monitored for 600 seconds.

Foam viscoelasticity measurements were performed using a rheometer (Rheometer DHR-2 from TA Instruments). The test was conducted at 25 °C using a 40 mm, 1 ° cone plate, with an oscillation amplitude ranging from 0.01% to 1000%.

Observations of foam were conducted using fluorescence microscopy (BZ-X710 from KEYENCE). Pyranine was used to visualize the hydrophilic components and Nile Red was used to visualize the oils. For structural analysis of the foam, high-resolution 3D X-ray microscope (nano3DX from Rigaku) was used. The X-ray source was Cu, operated at a tube voltage of 40 kV and a tube current of 30 mA. An S-CMOS camera served as the detector.

To confirm the composition of the foam, the Cryo-TOF-SIMS analysis was conducted.

2.4 Observation and Evaluation of Deposition

Cleanser formulations with Glyco-san and without Glyco-san were prepared with a fluorescent dye, Pyranine (green). The prepared 12.5 mg sample was applied to the left half of a PMMA plate (HD6 from Helioplate). Water was sprayed onto the plates for washing, and the plates were observed under a fluorescence microscope. To quantify the rhamnolipids contained in the deposition, analysis was performed using HPLC (ACQUITY UPLC H-Class system from Waters). To confirm the composition of the deposition, Cryo-TOF-SIMS analysis was made.

2.5 Evaluation of cleansing property

To evaluate the cleansing property of Glyco-san, 1 mg/cm^2 of liquid foundation on the market was applied to a PMMA plate (SPFM ASTER-PA01 from Shiseido IRICA Technology INC., Japan). After drying, 1 mg/cm^2 of the formulation of Glyco-san or without Glyco-san was then applied and observed after washing with tap water for 10 seconds.

3. Results

3.1 Formation of Glyco-san

When 0.2 wt.% chitosan solution and 0.5 wt.% rhamnolipids solution were mixed, a complex of chitosan-rhamnolipids was formed through electrostatic interaction and subsequently precipitated in water (data not shown). When such a complex is used for emulsification (Glyco-san, Table 1), it effectively forms a complex at the water-oil interface and contributes to emulsion stability. It is suggested that the Glyco-san complex encapsulates oil and contributes to emulsion stability by electrostatically adsorbing chitosan at the surfactant (rhamnolipids) emulsified interface (Figure 1(b)). On the other hand, the emulsion without Glyco-san was unstable.

3.2 Observation, Structural Analysis and Evaluation of Foam

Foam analyzer:

The foam properties of cleanser formulations with and without Glyco-san were investigated by the foam analyzer in the presence of an anti-foaming agent. Figure 2 shows the changes in foam height as a function of time. The formulation with Glyco-san showed a slightly higher maximum foam height than that without Glyco-san. The foam heights of both cleanser formulations decreased progressively. After 270 seconds, the cleanser formulation with Glyco-san maintained the level of foam for a long time, while the cleanser formulation without Glyco-san progressively reduced the foam height. This suggests that the Glyco-san can help stabilize the foam.

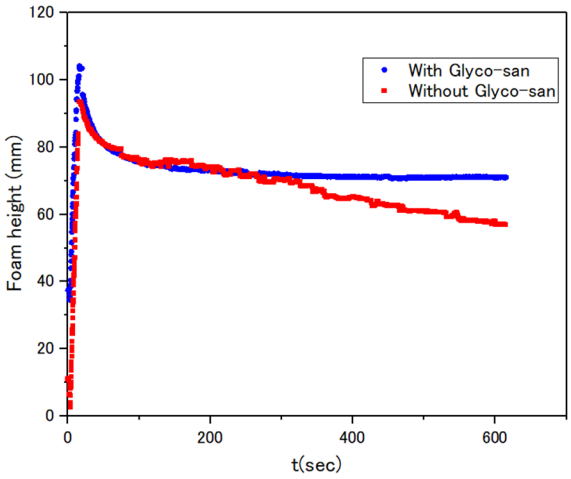
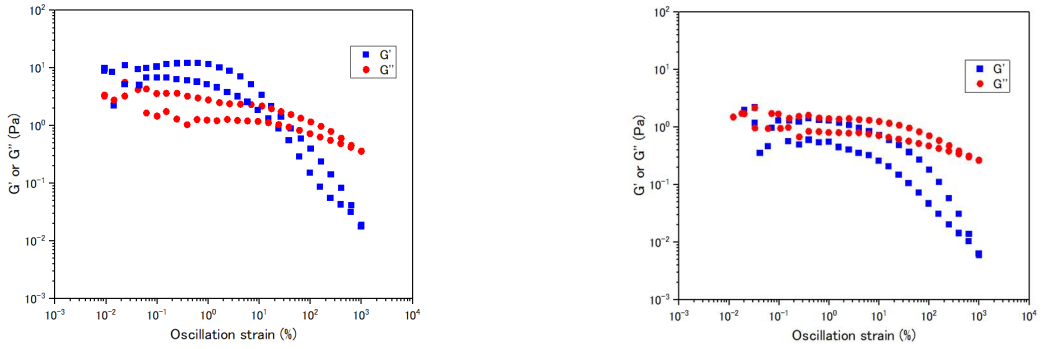


Figure 2 Evolution of the foam height: (Blue) With Glyco-san; (Red) Without Glyco-san

Foam viscoelasticity:

The foam properties of the cleanser formulations were analyzed by viscoelasticity measurement. The cleanser formulation with Glyco-san showed higher storage modulus (G') and loss modulus (G") values than those without Glyco-san (Figure 3(a-b)), indicating that Glyco-san foam has more elastic structure than the foam without Glyco-san. The foam of the cleanser formulation with Glyco-san exhibited a higher G' than G" in the linear viscoelasticity region (Figure 3(a)), suggesting that the foam had solid-like property. The foam of the cleanser formulation with Glyco-san showed good recovery under an oscillating strain (Figure 3(a), blue dots), implying that the foam had a high elasticity which avoided its disruption. On the other hand, the cleanser formulation without Glyco-san showed the foam had liquid-like property (Figure 3(b)).

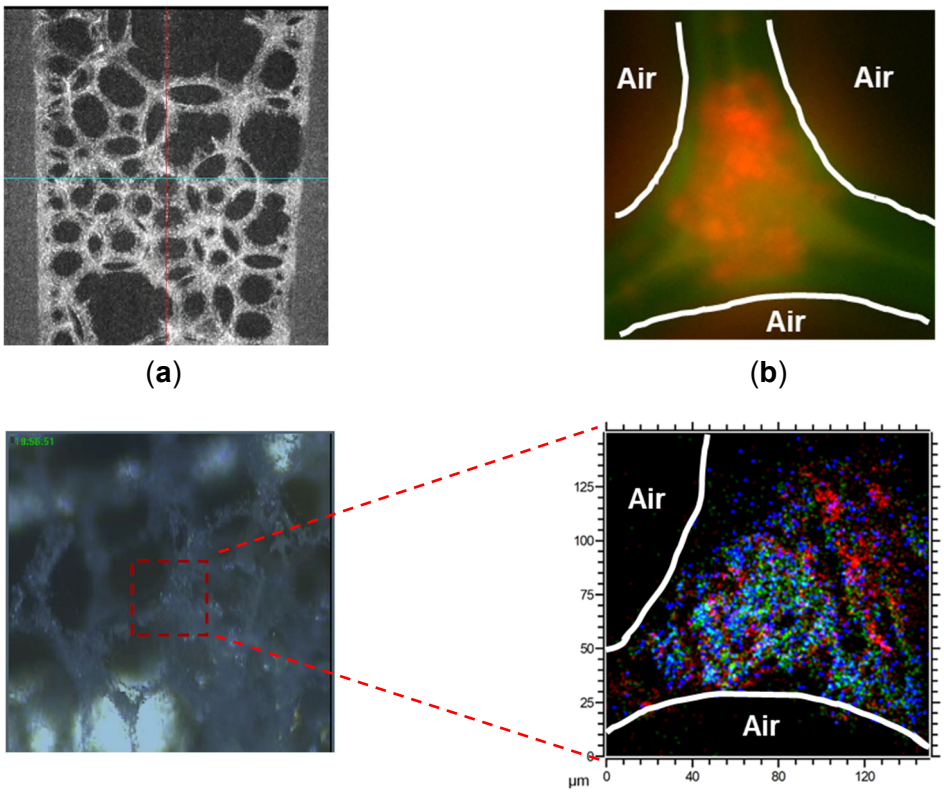


(**a**) (**b**)

Figure 3 Foam viscoelasticity: (a) with Glyco-san; (b) without Glyco-san

Foam image analysis (X-ray CT, Fluorescence microscope and Cryo-TOF-SIMS):

The X-ray CT image showed that high density materials were present in the liquid films of the foam of Glyco-san formulation (Figure 4(a)). Under the fluorescence microscope stained with Pyranine and Nile Red, the signals of hydrophilic (green) and hydrophobic (red) materials were observed (Figure 4(b)). The results of Cryo-TOF-SIMS showed the presence of CN^-, $C_4H_6NO_2^-$, $C_5H_5NO_3^-$ and $C_{13}H_{23}O_4^-$, indicating that the components of Glyco-san, chitosan and diisopropyl sebacate, are present in the liquid films and Plateau borders of the foam (Figure 4(d)).



(**a**) (**b**)

(**c**) (**d**)

Figure 4 Image analysis: by (a) X-ray CT image of the foam with Glyco-san; (b) Fluorescence microscopy image of Glyco-san complex in Plateau border; (c) Picture of the foam analysis area taken by the Cryo-TOF-SIMS internal camera; (d) Cryo-TOF-SIMS image of Plateau border of the foam with Glyco-san (Green: Chitosan, Blue: Rhamnolipids, Red: Oil (Diisopropyl sebacate))

3.3 Observation and Evaluation of Deposition

The deposition on half PMMA plate was observed by fluorescence microscopy and its components were analyzed. Fluorescence microscopy revealed a deposition of the Glyco-san formulation after washing (Figure 5). The Glyco-san formulation showed 1.8-fold higher deposition compared to that without Glyco-san, according to the quantitative HPLC analysis of rhamnolipids (data not shown). Subsequently, Cryo-TOF-SIMS analysis confirmed that the deposition contains chitosan (Figure 6(a)), rhamnolipids (Figure 6(b)), and diisopropyl sebacate (Figure 6(c)).

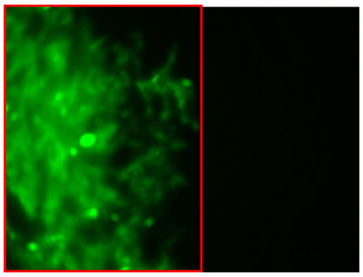
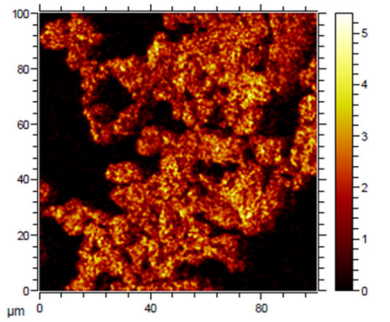
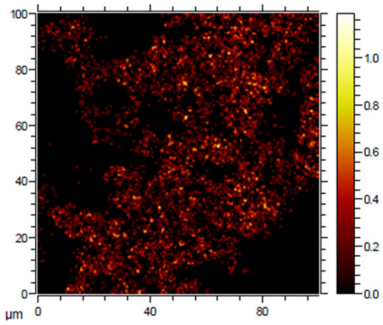


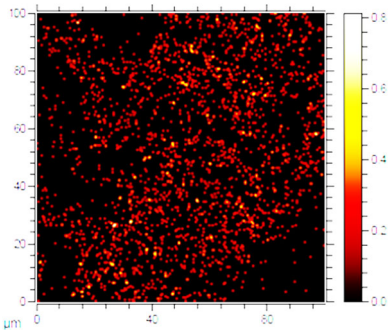
Figure 5 Fluorescence microscopy observation of deposition after washing. Green: Hydrophilic component (Chitosan, Rhamnolipids etc.)



(a)



(b)

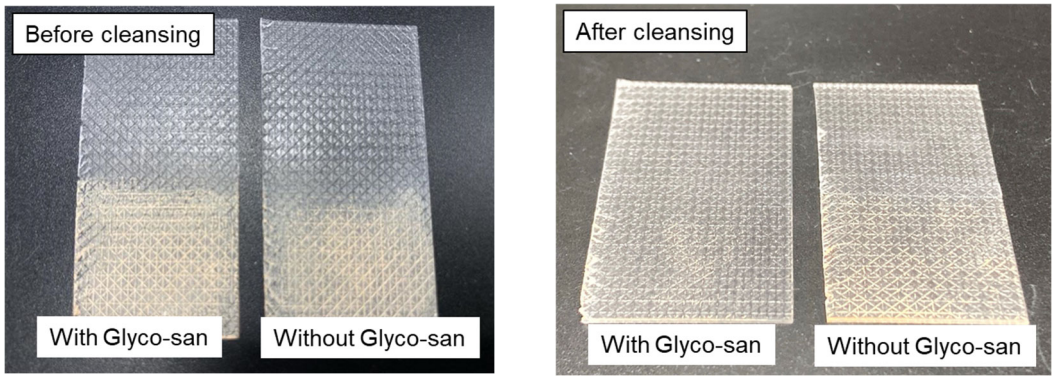


(c)

Figure 6 Cryo-TOF-SIMS observation of deposition after washing: (a) Chitosan; (b) Rhamnolipids; (c) Oil (Diisopropyl sebacate)

3.4 Evaluation of Cleansing property:

The results of the cleansing test are shown in Figure 7. A comparison of the cleansing property of the Glyco-san formulation (Figure 7(b) left) and the formulation without Glyco-san (Figure 7(b) right) revealed less residual foundation with the Glyco-san formulation, demonstrating its superior cleansing property.



(**a**) (**b**)

Figure 7 Cleansing property of Glyco-san (left) and without Glyco-san (right):
(a) Before cleansing; (b) After cleansing

4. Discussion

This research aims to develop a cleanser that provides high moisturizing and skin-protective functionality after cleansing. A primary challenge in traditional formulations of cleansers has been the difficulty of retaining active ingredients and naturally inherent emollient oils on the skin after cleansing. As mentioned before, Glyco-san technology addresses this by utilizing the electrostatic complexation of chitosan and rhamnolipids, and subsequent precipitation in water. We attempted to use this mechanism for making deposition incorporating oils onto the skin.

As Glyco-san is an insoluble material with localized hydrophilic and hydrophobic groups, it is thought to be adsorbed on the interface between water and oil. The Glyco-san formulation enabled successful oil emulsification and encapsulation. During this process, the anionic surfactants (rhamnolipids) are adsorbed onto the water-oil interface, while the cationic groups of the chitosan interact with the anionic groups of the rhamnolipids through electrostatic interactions, forming Glyco-san complex.

The formulation with Glyco-san maintained foam stability for a longer period (Figure 2). This emulsification/encapsulation by Glyco-san is thought to contribute to foam stabilization by capturing the oils, which would otherwise act as an anti-foaming agent. The viscoelastic measurement results showed that the foam with Glyco-san exhibited higher G' (storage modulus) and G" (loss modulus) values compared to the foam without Glyco-san, indicating a more elastic foam structure (Figure 3(a-b)). Specifically, Glyco-san demonstrated solid-like or "gel-like" behavior in linear viscoelastic region measurements, where G' > G" (Figure 3(a)). It also exhibited a smaller hysteresis loop, suggesting minimal structural breakdown of the foam when subjected to oscillatory strain. In contrast, the foam without Glyco-san displayed

liquid-like behavior with G'' > G' and a larger hysteresis loop (Figure 3(b)), indicative of foam collapse. The gel-like behavior of foams containing Glyco-san is hypothesized to be caused by the formation of an interconnected network between the anionic surfactants and the complex.

X-ray CT of Glyco-san foam revealed the presence of the high-density materials within both the liquid films and Plateau borders (Figure 4(a)). Analysis by fluorescence microscopy revealed that the material consisted of hydrophilic components that fluoresce green and hydrophobic components that fluoresce red (Figure 4(b)). The result of Cryo-TOF-SIMS reveals that the existence of Glyco-san components, chitosan, rhamnolipids, and oils (Figure 4(d)). These observation results clearly demonstrate the localization of Glyco-san within both the liquid films and Plateau borders of the foam. It is thought that this Glyco-san forms a network at the interface, increasing foam viscoelasticity and thereby strengthening the liquid film of foam. As a result, the Glyco-san formulation demonstrated superior foam stability compared to the formulation without Glyco-san.

We consider that the Glyco-san complex present in the foam film inhibits drainage, contributing to foam stability. Furthermore, these complexes accumulate at the Plateau borders, forming larger complexes, which enhances deposition on the skin [4].

The insoluble complex of Glyco-san in the foam easily deposits onto the skin after washing (Figure 5). Cryo-TOF-SIMS analysis of the deposition suggests that Glyco-san effectively encapsulates and retains oil during the deposition process (Figure 6). The results of cleansing tests revealed that the Glyco-san formulation demonstrated superior cleansing performance against foundation compared to the formulation without Glyco-san (Figure 7). We suppose that Glyco-san forms high elastic foam which efficiently removed dirt with mechanical force.

One characteristic of a superior cleanser is the stability of its foam. Stabilizing a fine, dense foam leads to an improved user experience. On the other hand, one of the aims of this study is to increase the deposition of oil. A large quantity of oil needs to be introduced, but as oil is also known as an anti-foaming agent, introducing it in large amounts is difficult. However, Glyco-san technology has solved this problem and deposited active ingredients, which are chitosan, rhamnolipids and emollient oils.

In addition to these functionalities, chitosan, a primary component of Glyco-san is a naturally occurring cationic polymer containing amino groups [5], has been reported to have antibacterial and anti-inflammatory properties. We also confirmed its antibacterial activities against *S. aureus* and *C. acnes*, and its anti-inflammatory activities *in vitro* on the activated keratinocytes (data not shown). Combined with the biofilm disrupting activity of rhamnolipids [6], chitosan's antimicrobial and anti-inflammatory properties may further increase the potential for targeted skin benefits. By combining these two ingredients, the development of highly functional cleansers is anticipated.

5. Conclusion

With Glyco-san, we have successfully developed a cleanser that overcomes the challenge of conflicting performance, namely cleansing performance and retention of necessary materials such as emollient oils and beneficial ingredients. Glyco-san technology demonstrated an

enhanced foam stability, cleansing property, and increased deposition of beneficial ingredients on the skin.

The superior functionality of Glyco-san lies in its ability to simultaneously provide a comfortable user experience and address specific skin concerns. Glyco-san technology represents a significant contribution to the advancement of cleanser formulations, holding the potential for creating more effective cleansing products. This cleanser incorporates chitosan, known for its antibacterial and anti-inflammatory effects. Therefore, future research should focus on further verifying the long-term effects and clinical benefits of Glyco-san.

One particularly interesting aspect of the Glyco-san technology used in this study is that it utilizes natural materials and a complex formation method based on the intrinsic potential charges of these materials. The preparation process does not require organic solvents or heating processes. Therefore, Glyco-san is a sustainable technology that aligns with the principles of green chemistry and environmental awareness.

Glyco-san technology is promising for various applications, by varying the anionic materials to be paired with chitosan and the oil components.

6. References

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