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Short Communication

Formulation and *in vitro* Evaluation of a Cosmetic Multiple Emulsion from Olive Oil

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Abstract. Stability of multiple emulsion prepared using olive oil was evaluated at different storage temperatures. The formulation remained stable at lower temperatures for 28 days without any phase separation. Significant changes in the pH were observed in the formulation, kept at 8 °C from the 5th day onwards, in the electrical conductivity at 40 °C from 14th day onwards and in the globule size, at 40 °C and 40° C + 75% RH condition from 24 h onwards.

Keywords: Multiple emulsion, olive oil, globule size, stability.

Multiple emulsion systems are novel complex systems (Vyas and Khar, 2004) finding potential biopharmaceutical applications, including prolonged drug delivery systems (Nakhare and Vyas, 1997), protection of active substances (Silva-Cunha *et al.*, 1997), as sorbent reservoir in drug over-dosage treatment, as adjuvant vaccines etc. (Antimisiaris *et al.*, 1993) as well as in cosmetics (Gallarate *et al.*, 1994) and house hold products.

However, multiple emulsions are inherently unstable especially if natural oil is used. Olive oil contains a number of fatty acids and sterols. In this study, stability of an emulsion containing olive oil was observed; the latter was used because of its cosmetic benefits (British Pharmacopoeia, 2004). Abil-EM90 was used as lipophilic emulsifier and Tween-80, as hydrophilic emulsifier.

Accelerated stability studies were performed on the formulation for 28 days at 8 °C, 25 °C, 40 °C as well as at 40 °C + 75% relative humidity (RH) at different time intervals. The data so obtained was evaluated by applying the statistical tests.

After heating of the oil phase (olive oil + lipophilic emulsifier) and aqueous phase (water + magnesium sulphate) at 72 °C $\pm$ 1 °C, the two were mixed and stirred and cooled to room temperature (primary emulsion). Next, Tween 80 and water were added and homogenized (formulation).

The possible mechanisms for deterioration of multiple emulsions include swelling of internal drops, rupture of the oil layer or coalescence of the oil globules or internal water droplets (Florence and Whitehill, 1982). Electrolytes promote an osmolar balance between the internal and external aqueous phases and also increase the stability of multiple emulsions by formation of a rigid interfacial layer between oil and aqueous phase (Grossiord and Seiler, 1998).

The freshly prepared primary emulsion and the formulation were white in colour. There was no change in colour of primary emulsion and the formulation or major liquefaction or phase separation at 8 °C, 25 °C, 40 °C and at 40 °C + 75% relative humidity (RH), till 28 days. The formulation at 40 °C showed slight liquefaction after 14 days and the sample at 40 °C + 75% RH, after 21 days.

There was a tendency of phase separation in the samples of formulation at 40 °C after a period of 14 days which increased with time but in sample at 40 ºC + 75% RH condition, phase separation was observed after a period of 21 days which persisted till the end. This indicated that the formulation was stable up to a period of 28 days at lower temperature.

No phase separation after centrifugation was seen in any of the samples of primary emulsion, kept at different storage conditions upto 21st day but slight phase separation on centrifugation was seen on 28th day of observation in the samples kept at 40 °C and 40 °C + 75% RH condition.

In the case of formulation, no phase separation after centrifugation was seen in any of the samples kept at 8 °C and 25 °C till the end of 28th day. But slight phase separation on centrifugation was seen in the sample kept at 40 °C on 21st day and more phase separation on centrifugation was observed on 28th day. While in the sample kept at 40 °C + 75% RH condition, slight phase separation on centrifugation was seen on 21st day of observation, which persisted up to 28th day.

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The pH of the sample of formulation kept at 8 °C, 25 °C, 40°C + 75% RH remained constant for 24 h and then increased continuously (Fig. 1). The increase in pH might be due to the production of basic by-products by any of the ingredients of the formulation or release of hydroxyl ions from glass containers.

ANOVA at 5% level of significance, showed that the change in pH of the formulation was significant at different levels of time and temperature. By using LSD technique it was found that most significant change in pH was observed in the formulation kept at 8 °C from 5th day up to the end of the study.

Increase in the electrical conductivity of multiple emulsions is due to the transfer of electrolytes entrapped in the inner aqueous phase to external aqueous phase and decrease in electrical conductivity is due to the transfer of electrolytes which are lost into external aqueous phase during the process of manufacture towards internal aqueous phase.

In this study, electrical conductivity of freshly prepared formulation was 0.0 µS/cm which increased with the passage of time and no conductivity difference was seen in any of the samples up to 72 h. The electrical conductivity of sample of formulation kept at 8 °C, 25 °C and 40 °C + 75% RH condition, increased continuously from the 7th day onwards and that kept at 40 °C increased continuously from 5th day (Fig. 2). Increase in conductivity might be due to the diffusion of electrolytes from internal aqueous phase to external aqueous phase.

ANOVA at 5% level of significance, revealed that the change in electrical conductivity of formulation was significant at different levels of time and temperature and by using LSD technique, it was found that the most significant change in pH was observed in the formulation kept at 40 °C and from14th day till the end of the study.

The increase or decrease in the globule sizes indicates the process of instability (Florence and Whitehill, 1982). Globule sizes of all the samples of formulation kept at different storage conditions increased continuously from 21.83 µm, which was in the range of average globule size of multiple emulsions (Florence and whitehill, 1982), from 1st h after preparation till the end of observation period i.e., 28 day (Fig. 3).

By using two-way analysis of variance (ANOVA) techniques at 5% level of significance, it was found that the change in globule size of formulation was significant at different levels of time and temperature. Insignificant changes were observed in the sample of formulation kept at 8 °C.

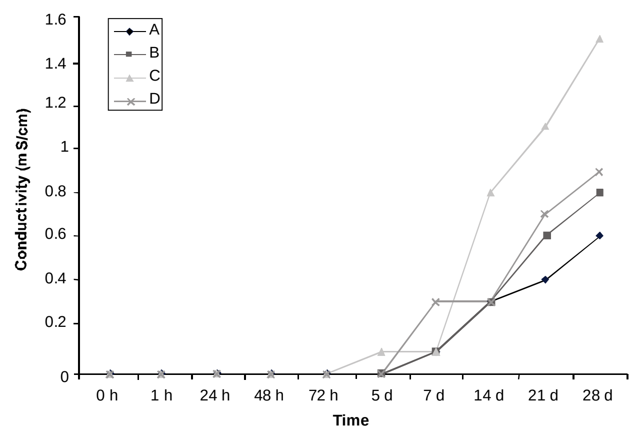

Fig. 2. Conductivity values of formulation kept at different storage conditions, A = 8 °C; B = 25 °C; C = 40 °C; D = 40 °C + 75% RH.

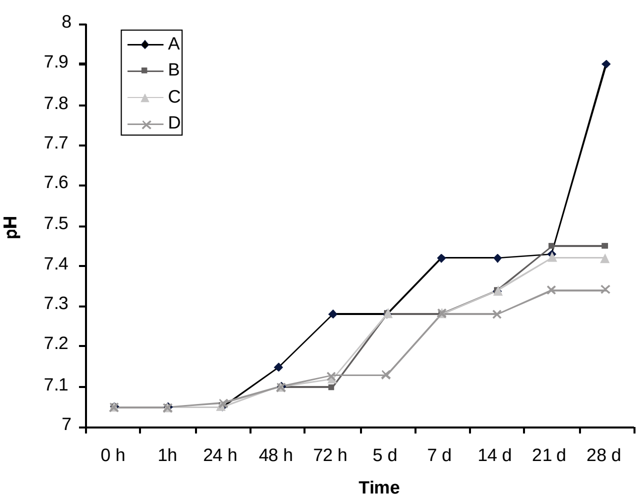

Fig. 1. pH values of formulation kept at different storage conditions, A = 8 °C; B = 25 °C; C = 40 °C; D = 40 °C + 75% RH.

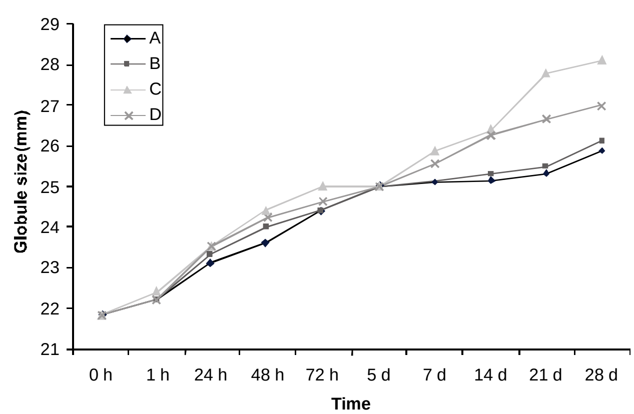

Fig. 3. Globule sizes of formulation kept at different storage conditions, A = 8 °C; B = 25 °C; C = 40 °C; D = 40 °C + 75% RH.

Conclusion

A stable w/o/w emulsion can be formulated by using olive oil in a concentration of 22% with 4% Abil.

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