Studying and Preparing Stable Emulsions of Water in Oil and Creating Montanide Oil Adjuvant for Making Oil Vaccines like Newcastle

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Abstract : In many drugs, emulsion-based processes are being widely used. Due to the different phenomena, emulsions are inherently and thermodynamically unstable and thus studying the stability of emulsions is very important, especially in pharmaceutical emulsions. Regarding the fact that pharmaceutical emulsions contain a wide range of physical and chemical properties like the stability, thus in present study, stabilizing of water in oil emulsion and creating Montanide oil adjuvant in making oil vaccines such as Newcastle is undertaken. In a 6-month period of time the ration of stable emulsion were determined. The results of the experiments showed that the most suitable ratio of making such an adjuvant is the ratio of 72:28 (Oil : Antigen) and the ratio of 11:1 (Oil : Emulsifier).
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1. Introduction

Currently emulsions technology enjoys a special and recognized status in chemical industries.Regarding the extensive application of emulsions in different industries, their study from the chemical and physical viewpoint is very important. Todays, emulsions technology has got very wide and the diversity of the emulsions has increased their complexity. One of the main and most important applications of the emulsions can be found in pharmaceutical industries. Emulsions are systems that are formed by the dispersal of at least one non-mixing liquid in another liquid. Such systems are thermodynamically unstable. Their stability can be increased by adding materials such as surfactants (Bibette,1996) . Emulsions would appear in W/O form or O/W form or in W/O/W mixed form or vice versa (Swarbrick J, 2000).

Creating a film between stabilizer surfaces, the surfactants prevent the particles to re-attach to each other (Kennedy E,1998). Several factors play role in determining the type of the emulsion among which one can refer to the nature of emulsifier materials, value, HLB, the ratio of phases and the viscosity of the phases (Ansel H& Allen L,1999). Quantitative assessment of HLB was first done by Griffin. The scale of hydrophilicity or lipophilicity of non-ionic surfactants can be obtained by this criterion (Eccleston G,1992). Clotting and integrity are both a type of aggregation of the drops to attach to each other and make a bigger drop. Now the density of continual phase, the phenomena of sedimentation and creaming will happen in emulsions on the basis of the density difference of the mass that is resulted by the process of clotting or integration; so that if the density of continual phase is less than the density of the mass, then the phenomenon of the sedimentation will occur. Otherwise, the phenomenon of creaming will occur (M. Robins, 2000). The process of integration occurs when the available drops in emulsion are getting close to each other and create an aggregate; i.e. the primary drops are converted to each other after the attachment. Indeed the protective membrane that is made by the emulsifier over the surface of the drop is disjointed and the synthesis of the drops gets possible. Thus the velocity of the process of the integration in the system depends on the nature, performance, geometric structure, and the functionality of the emulsifier in making the protective membrane of the drops in emulsion (Morientz, 1956).

The effective parameters on the stability of the emulsion include density, viscosity, and the distribution of the size of the drops in emulsion. Regarding the fact that these parameters are changed in some conditions like the temperature and time (Roberto, A & Buffo, Gary A, 2002) and (Buffo, G, 2001), since the value of the used emulsifiers and volume of the water and oil phases can change the emulsion properties like its stability, and since these factors are effective in the therapeutic effects of pharmaceutical emulsions, this study has been designed to investigate the effect of such factors on the stability of the emulsion. In this research, a wide scope of the water and oil phases has been studied.

2. Materials and Methods

In this research twin 80, arlacelA , sampler, lab tube, digital homogenizer, liquid paraffin, antigen V4, and viscometer were used. The selected method for preparing emulsions was as follow: in each lab

tube a different percent of liquid paraffin with approximately 6% of the mixture of two twin 80 and arlacel A were added; then antigen V4 (water phase) was smoothly added to oil phase. After the process of adding water phase to the oil phase was finished, mixing process begins to assure the complete integration. Then after the mentioned integration, the samples were used to investigate the superficial viscosity in the temperature of the laboratory environment using viscometer.

3. Results

Regarding the experiments of this research, the parameter of the temperature for all samples (161 samples) were considered fixed for the temperature of the laboratory environment. The time was studied at 5 levels from o1 week to 6 months. The results of the study on the prepared formulations in order to investigate the effect of water phase and oil phase and the volume of the surfactants are shown in table 1. It is to be mentioned that 161 formulas were prepared and studied, but since the prepared emulsions showed the highest rate of stability in a specific scope of the ratio of water phase and oil phase. In this paper we have sufficed to mention the results of this scope of ratio of water phase and oil phase. Table 1 shows the results of the investigation of prepared emulsions in different ratios of water phase and oil phase and fixed ratios of emulsifier. As it is obvious in the table, in the sample formulations of No. 35 to No. 45 in which the scale of the water phase is gradually increased but all have a fixed amount of emulsifiers, the highest rate of stability belongs to the sample formulation No. 41. Additionally, any increase in the water phase leads to the decrease in the amount of the viscosity. These samples were made of the combination of different percentages of water and oil phases and emulsifier.

The stability of each of these samples was studied after 1 week, 1 month, 2 months, 4 months, and 6 months. Finally, the sample No. 41 (formed by a combination of 28.125% water phase and 65.625% of oil phase, and 3.125% of arlacel A emulsifier and twin 80 emulsifier) was recognized as the most stable emulsifier.

Table 1. Results of prepared emulsions in different ratios of water phase, oil phase and fixed ratios of emulsifier

Sample No.	Oil phase percentage	Water phase percentage	Twin 80 percentage	Arlasel A percentage	Stability duration
35	68.440	25.310	3.125	3.125	1 week
36	67.970	25.780	3.125	3.125	1 week
37	67.500	26.250	3.125	3.125	1 month
38	67.030	26.720	3.125	3.125	1 month
39	66.560	27.190	3.125	3.125	2 months
40	66.090	27.660	3.125	3.125	4 months
41	65.625	28.125	3.125	3.125	6 months
42	65.155	28.595	3.125	3.125	4 months
43	64.690	29.060	3.125	3.125	2 months
44	64.220	29.530	3.125	3.125	1 month
45	63.750	30.000	3.125	3.125	1 month

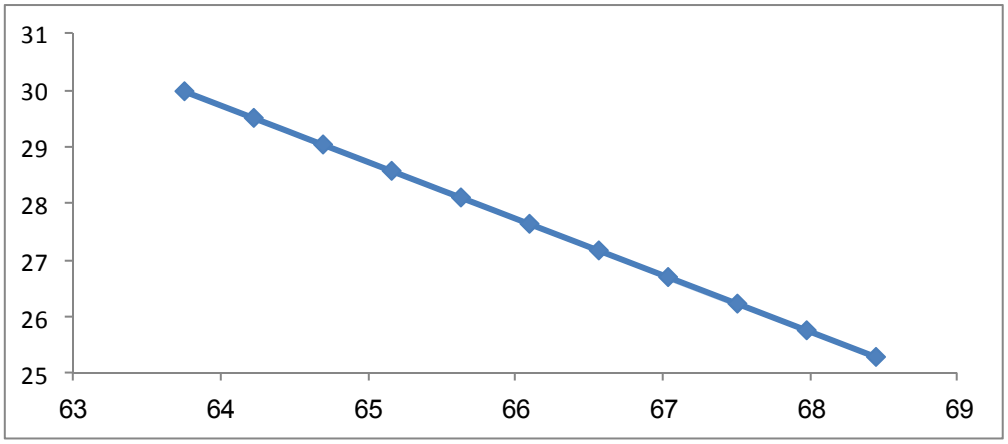

Figure 1. Ratio of the percentage of water phase (Y) to oil phase (X) in samples No. 35 to 45

4. Discussion and conclusion

The stability of the emulsions depends on the quality of raw materials, formulation, and building technology of the emulsion. In designing and making any desirable vaccine, apart from the needed precise in choosing the quality and quantity of the needed antigen in order to make enough immunity response to increase the effectiveness and functionality of the vaccine, it is extremely important to be careful in choosing the adjuvant. Some important factors such as formulation and the structure of the antigen are involved in choosing the suitable adjuvant, where in this research we studied and compared the different formulations and their difference in the stability of the emulsion.

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