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INCLUSION COMPLEXATION OF VITAMIN A PALMITATE WITH ß-CYCLODEXTRIN IN AQUEOUS SOLUTION.

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ABSTRACT

Study of the inclusion complex between vitamin A palmitate and ß-cyclodextrin in aqueous solution was performed to determine the stoichiometry and the association constant of the complex by the phase solubility diagram and fluorescence intensity measurements.

INTRODUCTION

Vitamin A palmitate, as other retinoids (1), plays an important role in the prevention of chemical carcinogenesis. Cyclodextrin, used to improve solubility and stability of drugs could also improve the bioavailability of this molecule.
The aim of this work is to prove the existence of an inclusion complex in aqueous solution between vitamine A palmitate and ß-cyclodextrin. So, phase solubility diagram and fluorescence intensity of vitamin A palmitate with ß-cyclodextrin were used to define important parameters such as stoichiometry and affinity constant.

MATERIALS AND METHODS

1) Phase solubility diagram

Solubility studies were carried out according to the method of Higuchi-Connors (2). Excess amounts of vitamin A palmitate were added to

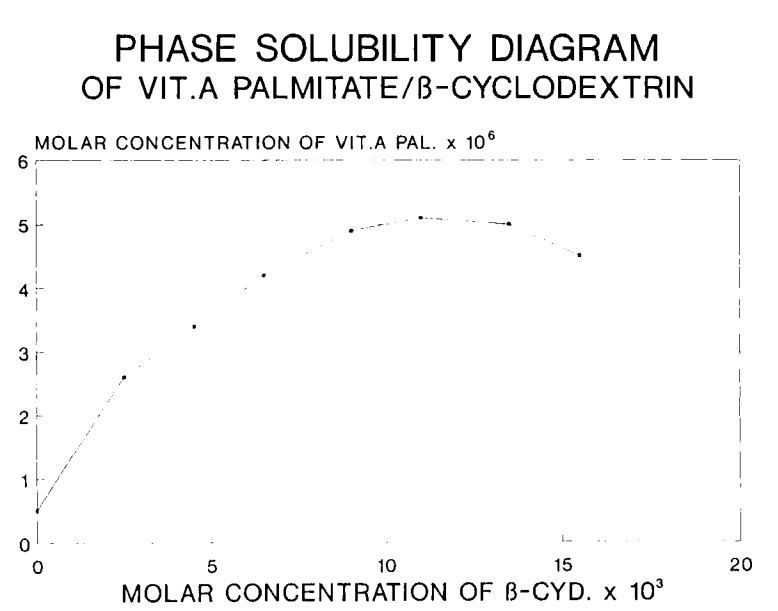

FIGURE 1

aqueous solutions containing different concentrations of ß-cyclodextrin (Roquette, France) and stored for a day, without stirring and protected from light. This is necessary to avoid the formation of vitamin micro-emulsion (3) and its decomposition. The filtered solutions were analyzed spectophotometrically, with a UV-2101 PC SHIMADZU connected to an AT 386 computer, to define the solubility characteristics.

2) Fluorescence

Fluorescence spectra were taken using a SLM-48000 spectrofluorimeter (Urbana, Illinois, USA). According to Agbaria and Gill (3), an ethanolic vitamin A palmitate solution, in a round bottom flask, was dried as a thin film by rotary evaporation, and the vacuum was maintained for 30 min. after complete evaporation, to remove the residual solvent. Distilled water was then added into the flask, incubated for 6 hours, and filtered to eliminate vitamin micro-emulsion. The filtered solutions, ready to be used, gave an approximate vitamin A palmitate concentration of 5×10^{-7} M .
A constant volume of this solution was mixed with different volumes of a saturated ß-cyclodextrin solution (corresponding to a concentration of 1.6×10^{-2} M) and these mixtures were then adjusted to 3 ml by adding of an appropriate volume of distilled water. Concentration of ß-cyclodextrin in these final solutions ranged from 2.6×10^{-4} M to 4.0×10^{-3} M.

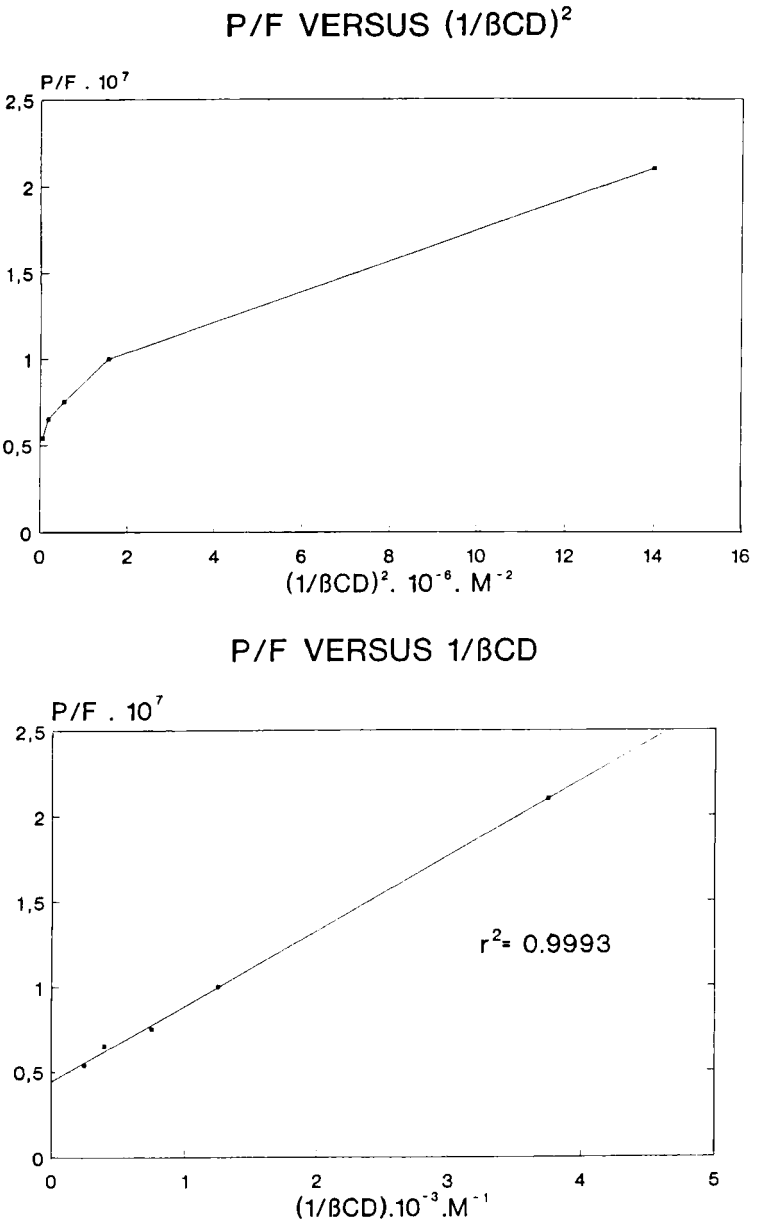

FIGURE 2
Double reciprocal plots of the
Vitamin A palmite/ß-cyclodextrin complex

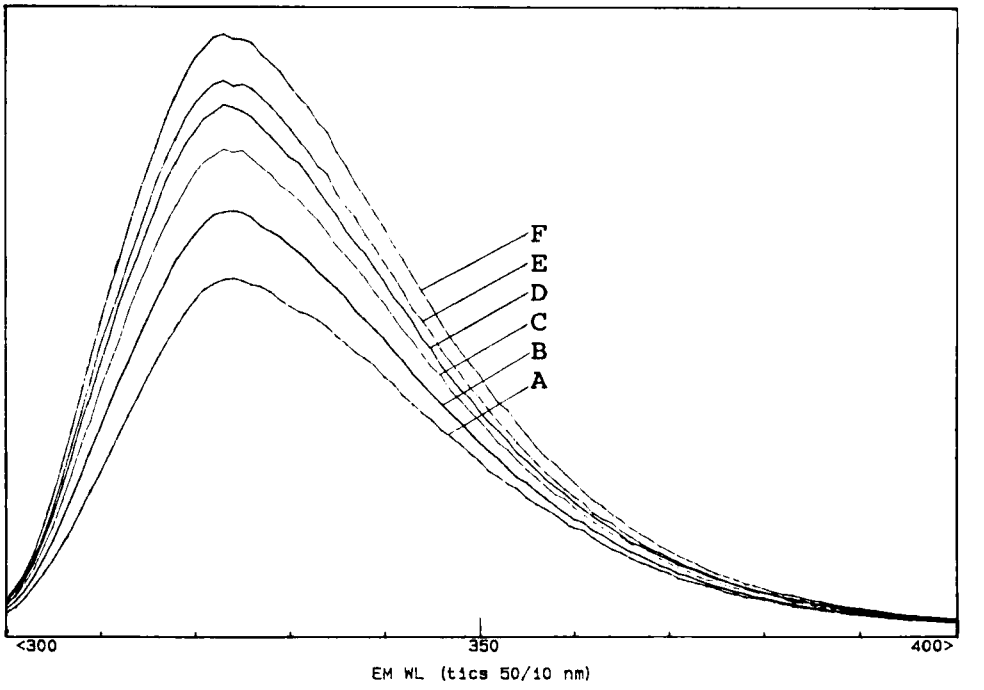

FIGURE 3
Fluorescence spectra of aqueous vitamin A palmitate solutions (3.33×10^{-7} M±) at various concentrations of ß-cyclodextrin: (A) without ß-CD; (B) 0.26×10^{-3} M ß-CD; (C) 0.79×10^{-3} M ß-CD; (D) 1.32×10^{-3} M ß-CD; (E) 2.64×10^{-3} M ß-CD; (F) 3.96×10^{-3} M ß-CD.

Fluorescence spectra were then determined at the exciting wavelengh of 290nm, which corresponds to the absorption maximum.

RESULTS AND DISCUSSION

1) Solubility study

Figure 1 shows the phase solubility diagram of vitamin A palmitate/ß-cyclodextrin system. The Bs-type solubility curve suggests an 1:2 molar ratio of the complex for high concentration of ß-cyclodextrin. The theoretical stability constant, calculated from the right side of the curve, is 1.88×10^{2}. The same type of solubility curve was also obtained with the values of the fluorescence spectra performed using the same solutions.

2) Fluorescence analysis

The stability constant for low concentration of ß-cyclodextrin is calculated by the classical method of the double reciprocal plot derived from the following equation (4) :

$$\frac{[A]_0}{F_{ACD}} = \frac{1}{Keq\ G\ Q_{ACD}\ [CD]_0^{\alpha}} + \frac{1}{G\ Q_{ACD}}$$

where $[A]_0$ and $[CD]_0$ are the initial analytical concentration of vitamin and ß-cyclodextrin, Q_{ACD} is the quantum yield of the complex, G is a constant characteristic of the fluorophore and instrumental parameters, F_{ACD} is the fluorescence signal of the complex and Keq is the equilibrium constant. The exponent α might be 1 or 2, depending on the considered stoichiometry. α=1 when the vitamin-ß-cyclodextrin molar ratio model is 1:1 and α=2 when the molar ratio model is 1:2.
Plotting $[A]_0/F_{ACD}$ versus $1/[CD]^{\alpha}$ (figure 2), it is possible to determine the stoichiometry and the affinity constant of the complex. When α=1, a straight line is obtained; in contrast, with α=2 the result is no more a straight line. This means that the 1:1 molar ratio is the model to apply. So, at low concentrations of ß-cyclodextrin, only an 1:1 complex is present, with a calculated stability constant of approximately 2.2×10^{-3}. In figure 3 are reported the fluorescence spectra, from which the fluorescence values, applied to the equation, were taken out.

CONCLUSION

The existence of an inclusion complex between vitamin A palmitate and ß-cyclodextrin in aqueous solution is confirmed. Fluorescence measurements show that, at low concentrations of ß-cyclodextrin, 1:1 molar ratio is only present. 1:2 vitamin-ß-cyclodextrin complex begins to form only at high concentrations of ß-cyclodextrin.

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