Physicochemical Studies on the Composition and Stability of Metal-Gossypol Complexes. I. Fe^{+++} Gossypol Complex

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Abstract

The complex formation of Fe^{+++} with gossypol has been investigated at constant ionic strength 0.15. The formation of only one complex, metal: gossypol (1:1) is detected by Job's method of continuous variation, conductometric titration and chemical analysis of the isolated complex. It appears that the two perihydroxyls are the most plausible sites where the ferric ions are chelated. The pK-value as calculated by the method of Turner and Anderson is 6.75 and free energy change of combination is −9.208 kcal.

Introduction

THE IRON-GOSSYPOL combination has been of interest in that it has been proved nontoxic to mice (1). Withers and Brewster (2) have demonstrated that ferric ammonium citrate is an antidote to cottonseed meal toxicity. Several workers (3–8) have used ferrous sulfate to render cottonseed meal nontoxic to nonruminants.

Though the reaction between iron and gossypol has thus been practically employed for some time, little attention has been given to the systematic study of this reaction. A few preliminary reports (9–12) indicate the possibility of complex formation between ferric ions and gossypol. The present communication deals with physicochemical studies on the composition and stability of Fe^{+++}-gossypol complex. The work yields data on the binding of metal ions to gossypol, thus throwing light on the basic facts in regard to bleaching of cottonseed oil. In addition, it indicates the role of ferric ions in detoxification of cottonseed meal.

Experimental

Gossypol (as the acetic acid complex) supplied by the Southern Regional Research Laboratory, New Orleans (USA) was used throughout these investigations. A stock solution of potassium gossypolate in 10% acetone was prepared as recommended by Jonassen and Demint (13). Ferric chloride was used as a source of metal ions. A.R. potassium chloride was dissolved in doubly distilled water (distilled in all glass apparatus) and used to maintain constant ionic strength.

Conductometric titrations were carried out on a Leeds and Northrup conductivity bridge with a dip-type cell. Optical density measurements at 330–450 mμ were made on a Beckman DU spectrophotometer using hydrogen and tungsten lamps as light sources for ultraviolet and visible region, respectively. Lambert-Beer's law was found to apply throughout the whole range of gossypol concentration. During conductivity titrations and spectrophotometric measurement, a constant ionic strength of 0.15 was maintained by adding the requisite amount of potassium chloride solution. All measurements were carried out at room temperature (25C).

Fe^{+++} gossypol complex was isolated by mixing equal volumes of equimolar solutions of ferric chloride and potassium gossypolate, kept for 3 hr in the dark under inert atmosphere. When the color (olive green) was fully developed, the mixture was evaporated on a water bath. The precipitate was filtered, washed free of chloride, recrystallized twice from acetone and dried in a vacuum desiccator. The complex was ignited at 750C heated to constant weight and weighed as Fe_2O_3.

Results and Discussion

Chemical analysis of the complex gives an average 9.7% of iron. Assuming a 1:1 combination the theoretical value of iron per 100 g of complex would be 9.78 g. An inflection in conductometric titration (Fig. 1) is obtained when an equal mole ratio of gossypol and ferric ions is present. These two observations clearly indicate that one mole of ferric ions combines with one mole of gossypol.

Having determined the combining ratio of iron-gossypol interaction, Job's (14) method of continuous variation is employed. Equimolar solutions are mixed (10×10^{-6} M of ferric chloride and potassium gossypolate) and optical density is measured from 330 to 450 mμ. The results are depicted in Figure 2 (*A*, *B*, *C*) where the difference in optical density (Δ O.D.) of the complex and pure gossypol is plotted against the mole fraction of gossypol. An inflection is obtained at 0.5 again indicating a 1:1 combination (15).

The stability constant is calculated by the method of Turner and Anderson (16) by comparing the data drawn from the two curves (Fig. 2 *A* and *B*).

The formation of complex ions can be represented by the equation

$$n\ A + m\ B \rightleftharpoons \cdot\ A_n\ B_m$$

in which A is a metallic ion and B an organic molecule or an anion. If *a* and *b* are the initial concentration of A and B and x is the equilibrium concentration of the complex, then the equilibrium constant

$$K = \frac{x}{(a - x)(b - x)}$$

The value of *x* can be computed by comparing the data of two curves (Fig. 2 *A* and *B*) at a given optical density. These curves with different initial concentrations of the reactants have the same value of *x*. Thus

$$x = \frac{(a_1\ b_1) - (a_2\ b_2)}{(a_1 + b_1) - (a_2 + b_2)}$$

For two mixtures having the same optical density (0.35), the values of a_1, a_2, b_1, b_2 and x are found to be 8.2×10^{-6} M, 3.0×10^{-6} M, 1.8×10^{-6} M, 2.0×10^{-6} M and 1.75×10^{-6} M, respectively. The equlibrium constant is estimated to be 5.661×10^6 and the free energy change of combination ($\Delta F = -RT \ln K$) is −9.208 kcal.

The pK of Fe^{+++} gossypol complex thus calculated comes out to be 6.75. This indicates that the complex is fairly stable and dissociates very slightly in solution. It appears that the ferric complex is slightly less stable than the ferrous complex, since the reported pK-value for Fe^{++}-gossypol complex (13) is 7.6. If we assume with Jonassen and Demint (13)

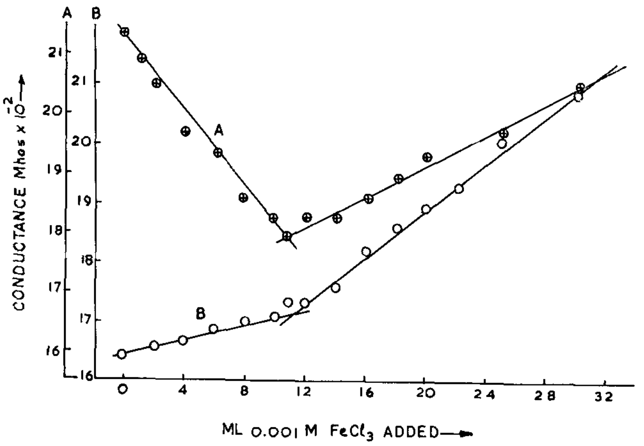

FIG. 1. Conductometric titration of (B) 100 ml of 0.0001 *M gossypol in* 0.0002 M KOH *against* 0.001 M $FeCl_3$ and (A) control consisting of 100 ml of 0.0002 M KOH with 0.001 M $FeCl_3$.

that the perihydroxyls are the plausible sites for binding of metal ions (17), then there would be a striking similarity between mode of combination of iron in both its oxidation states with gossypol. The pK values of the two complexes, differing only by 0.85, strongly support this viewpoint.

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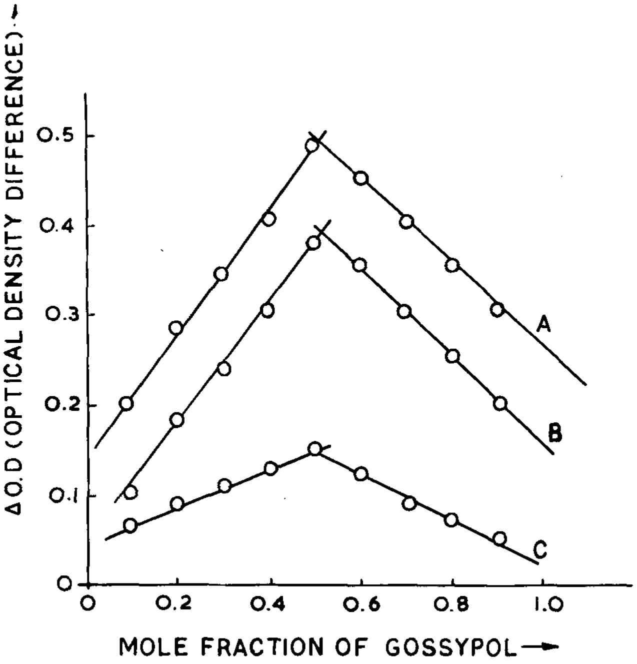

FIG. 2. Changes in optical density with varying mole fractions *of ferric chloride and gossypolate ions*. Curve *A*: 10 × 10^{-6} M initial concentration of reactants, at 360 mμ. Curve *B*: same as *A* but diluted twice. Curve *C*: for 10 × 10^{-6} M initial concentration of reactants, at 380 mμ.

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