

Application of Gossypol Acetic Acid as a Reagent For Iron (Iii) Ions

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ABSTRACT. This article presents the advantages of utilizing gossypol and its derivatives as reagents for iron (III) (Fe (III)) ions. A novel spectrophotometric method has been developed for the determination of Fe (III) using gossypol derivatives in the presence of a universal buffer solution. Optimal conditions have been identified, and the composition and stability constants of the Fe (III) complex with gossypolacetic acid have been determined.

Key words: Gossypol, Gossypol acetic acid (GAA), Analytical reagent, Fe (III) ions, Spectrophotometric method, Isomolar series, Calibration curve

INTRODUCTION

It is well known that various metals, including Fe, Co, Zn, Cu, Ca, and Mg, are essential elements for both plants and the human body. These metals play a vital role in numerous biochemical processes. Many biologically active substances derived from plants require the presence of metal ions for their activation, and specific metal ions can activate proteins and enzymes.

Organic substances containing complex groups of atoms are particularly valuable for analytical purposes. These groups facilitate the formation of chelate compounds with metal ions, which often exhibit intense color and low solubility in water, making them easily extractable with non-aqueous solvents.¹

One such organic substance is gossypol, which is a yellow toxic pigment found in cottonseeds. Its chemical formula is 2,2'-di-(1,6,7-trioxy-3-methyl-5-isopropyl-8-naphthaldehyde).² The gossypol molecule consists of dimeric naphthalene rings connected by two carbon atoms at the 2,2' position, as well as six hydroxyl groups attached to the 1,1', 6,6', and 7,7' carbon atoms, respectively. Additionally, aldehyde functional groups are present at the 8,8' carbon atom positions, contributing to specific physical and chemical properties³ (Fig. 1).

Consequently, depending on the metal being complex and the reaction conditions, gossypol can form complex compounds with various compositions and structures. The gossypol molecule has four alkyl groups: two methyl groups and two isopropyl groups at positions 3 and 5, respectively. These alkyl groups constitute the lipophilic part of gossy-

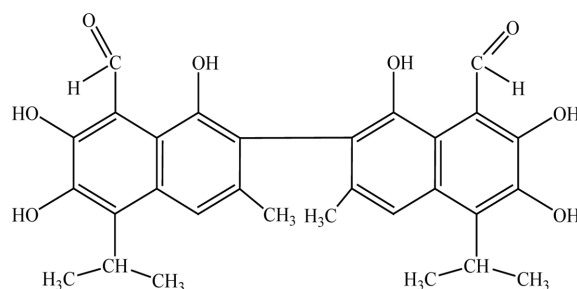


Figure 1. Structural formula of gossypol.

pol (Fig. 2), while the other side of the naphthalene ring, which contains the hydroxyl and aldehyde groups, is the lipophobic part of the molecule.

This rationale offers a logical explanation for the reactivity of the gossypol molecule. However, despite a significant number of studies focusing on the structure and properties of gossypol and its derivatives, there is a lack of research on the complex compounds, composition, and stability of gossypol-metal complexes in aqueous solutions. An urgent issue is the selection of selective ligands (particularly for platinum group metals) based on the physicochemical analysis of the factors influencing complexation selectivity, such as the type of coordinating group, the nature of the donor atom in gossypol, and the acidity and ionic composition of solutions.⁴⁻⁵ Gossypol, a complex pigment found in cotton seeds, poses color-related issues in extracted oil and, more importantly, increases its toxicity. Consequently, the addition of soluble iron salts to mitigate the toxicity of post-fat products in animal husbandry affects livestock feed-

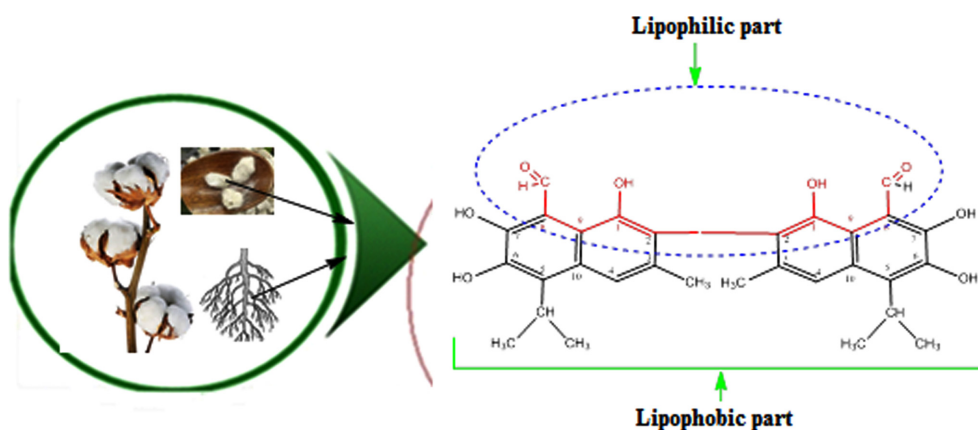


Figure 2. Lipophilic and lipophilic part of the gossypol molecule.

ing. The reduction in toxicity and nutritional value of nutrients is attributed to the coordination of iron ions with gossypol in the seeds.⁶⁻⁷ The aim of this study is to investigate the complexation of iron with gossypol acetic acid (GAA) and explore the practical application of this reaction for the spectrophotometric determination of iron in natural substances.

EXPERIMENTAL PART

Research Methods

The research employed various methods, including pH-meter, spectrophotometry, and direct quantification methods using spectrophotometric measurements, specifically the calibration graph method.

Chemical Reagents, Materials, and Equipment

The researchers used chemically pure reagents (ch.p.) and pure for analysis (p.f.a.) qualifications. The following materials and equipment were used.

Working solutions of iron were prepared by dissolving precisely weighed portions of salt $FeCl_3 \cdot 6H_2O$ (chemically pure qualification) in distilled water, then transferred into a 250 ml volumetric flask and diluted to the mark.

Ethyl alcohol for preparing the initial HAA solution. Reagent purification by double recrystallization in a water-ethanol solution. Organic solvents of chemically pure (ch.p.) qualification or pre-purified by distillation, with purity monitored by boiling point.

Paper ascending chromatography to verify the purity of the reagent. UV/Vis spectrophotometer Optizen-III for recording absorption spectra of the Me-R complex solutions. Glass electrode pH-meter KSL-1100-1 for monitoring solution acidity.

RESULTS AND THEIR DISCUSSION

Gossypol has a complex labile molecule, for which two different tautomeric and conformational transformations are possible. One of the manifestations of the polyfunctionality of gossypol is the possibility of its existence in aldehyde-lactol and benzoid-quinoid equilibria, which can play an essential role in the interaction of the gossypol molecule with cell components. Thus, if the aldehyde form readily interacts with enzymes containing free amino groups, primarily with the formation of a Schiff base, then the lactol form can react due to hydrogen bonds and hydrophobic interaction, and its further participation in the reaction is determined by the conditions of a shift in equilibrium towards the formation of aldehyde and quinoid forms.⁵ To study the tautomeric forms of gossypol and its derivatives, we used 1H and ^{13}C NMR spectroscopy.⁸⁻¹³

The presence of signals from an aldehyde proton and three hydroxyl groups, one of which is involved in the formation of a strong intramolecular hydrogen bond (IHB) (15 m.d.), indicates the existence of a gossypol molecule in weakly polar solvents, mainly in the dialdehyde form³. The research was carried out according to the scheme described in.¹ The nature of the substituents in the molecule of gossypol derivatives largely depends on the protonation of the reactants. Gossypolacetic acid exhibits the greatest tendency to protonation. Gossypol acetic acid (GAA) – is a microcrystalline powder of light yellow color with a slightly greenish tint, insoluble in water, well soluble in ether, chloroform, alcohol, benzene, carbon tetrachloride. HAA –2,2-bis-(1,6,7-trioxy-3-methyl-5-isopropyl-8-aldehydonaphthyl) acetic acid (Fig. 3).

Molecular mass – 578.6.

Gross formula: $C_{30}H_{30}O_8 \cdot C_2H_4O_2$.

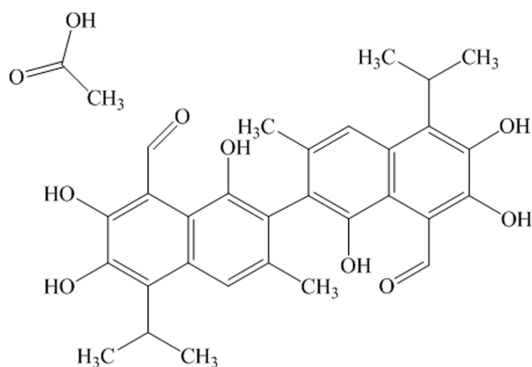


Figure 3. Structural formula of gossypol acetic acid (GAA).

The color changes under the influence of light. Liquidus temperature – from 1770 to 1800 °C (with decomposition). 0.002% solution in chloroform has a UV spectrum in the 310-430 nm range with a maximum absorption at 366 nm². The most studied derivatives of gossypol are Schiff bases based on it, some of which are used for quantitative methods of analysis and isolation of gossypol.² In works,¹⁴⁻¹⁵ for amines of gossypol, a benzoid structure was proposed, although this class of compounds is characterized by the phenomena of tautomerism, in which sigmatropic proton transfer leads to a benzoid-quinoid equilibrium, more or less shifted to one side or another.

Under the action of salts of two and multivalent metals on aqueous-acetone or aqueous-alcoholic solutions of gossypol, gossypolates of these metals are formed, which in most cases are very difficult to dissolve in water. On the contrary, in some organic solvents (for example, alcohols, ethers, ketones, benzene, toluene, chloroform, and CCl₄), these gossypolates are usually more or less soluble.

The reactions of the formation of gossypolates of heavy metals are still not well understood. In most cases, even the composition of the resulting products has not been precisely established. An exception in this respect is the formation of azo compounds of gossypolates of the monovalent silver metal. It was found that silver ions and azo compounds of gossypol interact with each other in molar ratios of 1:1, 1:2, 2:1, and 3:2. The physicochemical properties and structure of the complexes have been investigated by potentiometric method and mass spectroscopy (and ESI FT-IR, as well as PM5 Semi-empirical analyze).^{4,6-7,16-18}

Research shows that most gossypolates are yellow in various shades. Other colors have the following gossypolates: gossypolate of mercury II is green, Fe (III) is dark green (almost black), chromium, molybdenum, antimony III, tin, and iron II is dark brown with a red tint. Solutions of antimony, molybdenum, and tin gossypolates in some organic

solvents are colored in bright red colors of various shades; Fe (III) gossypolate solution – is in olive green color. Nickel gossypolate, colored yellow, when dissolved in some organic solvents, gives a violet solution.

Due to the presence of a functionally active group, under certain conditions, it forms complex compounds with colored ions and heavy metal ions. The formation of complex compounds is mainly carried out due to an undistributed pair of electrons in hydroxyl groups attached to hydrocarbon atoms in the 1,1'-position of the molecule, and aldehyde groups located in the 8,8'-positions.

When acidifying or shaking solutions of gossypolates of di- and multivalent cations in organic solvents with solutions of mineral acids, many of them are easily destroyed to form the corresponding salt of the cation and free gossypol.

Gossypolates of some heavy metals, for example, tin, molybdenum, and Fe (III), are more or less resistant to acid solutions. To study the spectrophotometric reaction of Fe (III) complexation with gossypol acetic acid, first of all, optimal conditions were selected.

The optical density of the alcoholic solution of the reagent and the ion association between the reagent and Fe (III) ($Fe-R_{reagent}$) was measured, respectively, at $\lambda_{reagent} = 430$ nm and $\lambda_{comp} = 460$ nm on a spectrophotometer relative to a blank solution.

The molar extinction coefficient at 460 nm is $\epsilon_{460}^{max} = 54000$.

One of the main conditions for the complexation reaction is the acidic condition. Thus, buffer solutions are used to keep pH constant, and to obtain reproducible results. For the optimal pH value, pH values were prepared with different values and different compositions of buffer mixtures. The optical density (A) of the complex increases accordingly with increasing pH of solutions.

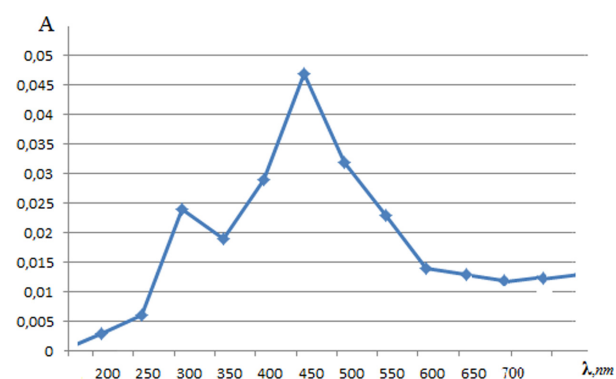


Figure 4. Dependence of the optical density of the complex compound ($Fe-R_{reagent}$) on the wavelength of the absorbed light, $\lambda_{max(comp)} = 460$ nm.

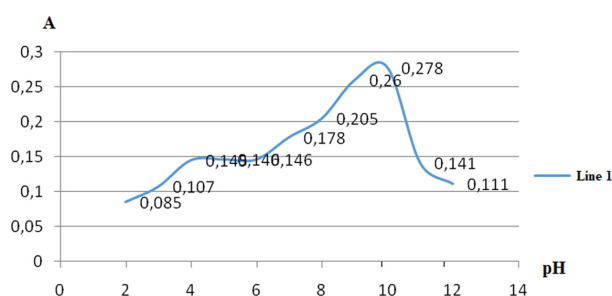


Figure 5. The graph of the dependence of the optical density of the complex compound ($Fe-R_{reagent}$) on the solution medium.

The maximum optical density of the complex is observed in a narrow range of pH of the solution (pH=10,0–10,25).

Proceeding from this, pH=10.11 was chosen as optimal, and from the experimental data, it can be seen that the maximum yield of the complex is observed when using a universal buffer solution, which was used in further studies (Fig. 5). Using optimal conditions, a calibration curve was drawn up, which is linear in the concentration range of 1.5–38.0 mcg/25 ml of metal (Fig. 6).

Deposition of the obtained values on the coordinate axes gives a number of points lying on one straight line, the slope of which, according to the theory, should be equal to the number of particles coordinated by the central ion.

Solutions of iron complexes ($Fe-R_{reagent}$) obey Beer's law in the range of 1.5–38.0 mcg/25 ml with an accuracy of 3–4%. On this basis, we have developed a new spectrophotometric determination of iron with the reagent gossypolacetic acid.

The molar absorption coefficient was calculated, and the sensitivity according to Sendel sensitivity was determined (Table 1).

Molar ratios are established by the method of molar ratios and by the saturation method with a variable concentration of one of the components (metal-reagent).

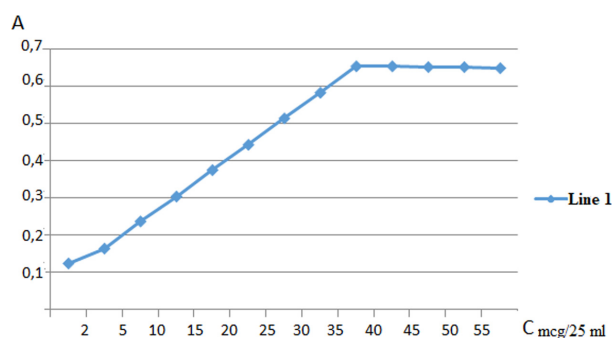


Figure 6. Submission of the iron complex with GAA to Beer's law.

Table 1. Some analytical characteristics of the Fe (III) complex with GAA

Analytical characteristics	$Fe-R_{reagent}$
1 Optimal volume of 0.05% reagent solution, ml	4
2 Optimal solution medium, pH	10,11
3 Stability of the complex in time, min.	180
4 Sendel sensitivity, mcg/sm ² in 25 ml	0,0058 mcg/sm ²
5 The absorption spectrum of the complex, λ , nm	460
6 Reagent absorption spectrum (in ethanol)	430
7 Molar absorption coefficient, ϵ_{460}^{max}	$5,4 \cdot 10^4$

Table 2. Results of verification of the method for the determination of Fe (III) in model solutions (n = 3, P = 0,95)

Iron entered, mg/l	Iron found, mg/l	S	S _r
	5,09		
5,00	5,08	0,185	0,036
	5,04		

Footnote: n – number of parallel definitions; P – confidential probability; S – standard deviation; S_r – relative standard deviation.

The accuracy and reproducibility of the method for the determination of Fe (III) ions with the reagent of gossypol acetic acid were checked by the “added - found” method on the basis of the equation of the calibration graph:

$$Y_i = a + bX_i = 0,0159 + 0,01441X_i$$

The results are shown in Table 2.

Thus, the proposed method for the determination of Fe (III) ions, high selectivity, fast productivity, and ease of analysis make it possible to use them in the practice of chemical analysis in the detection of Fe (III) ions in various objects.

As can be seen, the relative error in the determination of 5 mg/l is 0.036, which is acceptable for spectrophotometric methods of analysis. At the same time, high sensitivity, rapidity, non-deficiency of reagents (obtained from local raw materials), and ease of implementation make it possible to use the developed method in the practice of chemical analysis when detecting Fe (III) ions in various objects. On the basis of the developed spectrophotometric methods, an analysis of the composition of multicomponent model solutions containing Fe+3 ions was carried out. Heavy metals, such as chromium, manganese, cobalt, copper, and some others, can also be found in the composition of drinking water with iron. Therefore, it was necessary to elucidate the effect of foreign ions on the spectrophotometric determination of iron with the GAA reagent.

Experiments have shown that the determination of iron in the form of a complex is not displaced by cobalt ions (1:1 and 1:10), manganese (1:1), and chromium (1:1 and 1:10), but

manganese ions (1:10), copper (1:1 and 1:10). The interfering influence of foreign ions is eliminated by masking with pyridine and sodium fluoride, respectively.

CONCLUSIONS

Thus, we may come to the conclusion that a new spectrophotometric method for the determination of iron using gossypol derivatives has been developed. The optimal conditions for the determination of Fe (III) have been found, and the composition and stability constants of the iron complex with gossypolacetic acid have been determined. The developed technique was used to determine iron in model solutions. The influence of extraneous interfering ions was studied. A method for the spectrophotometric determination of Fe (III) ions was proposed, which was applied to the analysis of drinking water. The developed method for the determination of iron using GAA is characterized by high selectivity and reproducibility with S_r not exceeding 0.185, which indicates a metrologically sound recommendation of the proposed methods for the analysis of drinking water.

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REFERENCES

1. Абдурахманова, У. К.; Применение госсипола в аналитической химии // Материалы VIII Международной научно-практической конференции «Молодежь и наука: реальность и будущее». – Россия, **2015**, *1*, 32.
2. Маркман, А. Л.; Ржехин, В. П. Госсипол и его производные. – М.: Пищ. пром-ть, 1965; p 250.
3. James, A. *JAOCS*. **2006**, *83*, 269.
4. Przybylski, P.; Schroeder, G.; Brzezinski, B. *J. Mol. Struct.* **2004**, *699*, 65.
5. Przybylski, P.; Bejcar, G.; Schroeder, G.; Brzezinski, B. *Journal of Molecular Structure*. **2003**, *654*, 245.
6. Ramasvamy, H. N.; O’Connor, R. T. *J. Agr. Food Chem.* **1969**, *17*, 1406.
7. Ramasvamy, H. N.; O’Connor, R.T. *J. Amer. Oil Chem. Soc.* **1968**, *45*, 841.
8. Abdullaev, N. D.; Tyshchenko, A. A.; Nazarova, I. P.; Ul’chenko, N. T.; *et al. Chem. Nat. Compd.* **1990**, *26*, 129.
9. Brzezinski, B.; Olejnik, J.; Paszyc, S. *J. Mol. Struct.* **1990**, *239*, 23.
10. Baram, N. I.; Ismailov, A. I.; Kamaev, F. G.; Leontev, V. B. *Khim. Priir. Soedin.* **1976**, *2*, 249.
11. Brzezinski, B.; Olejnik, J.; Paszyc, S.; Aripov, T. F. *J. Mol. Struct.* **1990**, *220*, 261.
12. Brycki, B.; Brzezinski, B.; Marciniak, B.; Paszyc, S. *Spectrosc. Lett.* **1991**, *24*, 509.
13. Bronislaw, M.; Schroeder, G.; Kozubek, H.; Brzezinski, B. *J. Chem. Soc. Perkin Trans.* **1991**, *29*, 1359.
14. Alley, P. W.; Shirley, D. A. *J. Org. Chem.* **1959**, *24*, 1534.
15. Dechary, J. M.; Brown, L. E. *J. Amer. Oil Chem. Soc.* **1956**, *33*, 76.
16. Przybylski, P.; Brzezinski, B. *J. Mol. Struct.* **2003**, *654*, 167.
17. Przybylski, P.; Schroeder, G.; Brzezinski, B. *J. Mol. Struct.* **2003**, *658*, 115.
18. Wood, A. B.; Robinson, F. V.; Araujo Lago, R. C. *London. Chem. Ind.*, 1969; p 1738.
19. Булатов, М. И.; Калинин, И. П. Практическое руководство по фотоколориметрическим и спектрофотометрическим методам анализа, Л.: Химия, 1986; 317 стр.