# ISOLATION OF GOSSYPOL FROM HEXANE—ACETONE MISCELLAS

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Continuing investigations on the complex treatment of cotton seeds by direct extraction, we have studied the isolation of gossypol from the miscellas obtained in the extraction of the oil by a binary mixture of hexane and acetone in a ratio of 85:15 by volume [1].

Recently, gossypol has found ever-increasing independent use [2], and we were therefore faced with the task of maximizing its extraction from the oil in the native form. For this purpose we used 5% aqueous solutions of $NH_4OH$ [3], borax [4], and sodium carbonate, and a 2% solution of caustic soda [5]. The method was based on the interaction of gossypol with alkalis, but if highly concentrated solutions are used, irreversible changes take place in the gossypol as a result of which it loses its valuable properties. Miscellas of different concentrations were subjected to raffination.

TABLE 1

| Index (%) and number of the initial miscellas | | | Reagent | Index of the miscellas after raffination | | | |
|---|---|---|---|---|---|---|---|
| acid No., mg KOH | concentration | free gossypol | | acid No., mg KOH | free gossypol content, % | yield of gossypol, % of theor. | purity of the gossypol, % |
| 1) 3,8 | 5,45 | 0,45 | $NH_4OH$ | 0,5 | 0,08 | 68,0 | 78,0 |
| | | | Sodium carbonate | 0,3 | 0.08 | 12,0 | 18,0 |
| | | | Borax | 0,7 | 0,1 | 14,0 | 16,0 |
| | | | NaOH | 1,2 | 0,1 | 64,3 | 65,8 |
| 2) 7,7 | 18,2 | 1,7 | $NH_4OH$ | 2,3 | 0,3 | 55,0 | 74,0 |
| | | | Sodium carbonate | 2,1 | 0,1 | 39,0 | 18,0 |
| | | | Borax | 0,7 | 0,1 | 50,0 | 28,0 |
| | | | NaOH | 1,19 | 0,26 | 57,1 | 71,4 |
| 3) 10,3 | 23,05 | 1,58 | $NH_4OH$ | 3,0 | 0,2 | 60,0 | 78,0 |
| | | | Sodium carbonate | 6 3 | 0,12 | 47,0 | 28,0 |
| | | | Borax | 2,0 | 0,10 | 61,0 | 28,0 |
| | | | NaOH | 1,6 | 0,23 | 63,0 | 61,0 |
| 4) 7,7 | 25,5 | 2,07 | $NH_4OH$ | 4,7 | 0,15 | 74,0 | 62,0 |
| | | | Borax | 1,5 | 0,15 | 59,0 | 28,0 |
| | | | NaOH | 1,56 | 0,20 | 61,0 | 68,2 |

The amounts of the alkaline reagents were calculated on the basis of the acid numbers of the miscellas and their concentrations. The results of the extraction of gossypol by the reagents that we used are given in Table 1. As well as using the theoretically calculated amounts of alkalis, in some experiments we added an excess. As was found, in the preraffination of a miscella an excess of $NH_4OH$ does not affect the yield or purity of the gossypol. For example, when 100 $cm^3$ of miscella No. 3 was treated with 3 ml and 6 ml of 5% $NH_4OH$ the yields of gossypol proved to be 62 and 60%, respectively, and its purity in both cases was 78%. However, an increase in the excess of solutions of borax and sodium carbonate and amounts in excess of the theoretical figure led to the resinification of the product isolated.

The miscella darkened after its treatment with the $NH_4OH$ solution. Thus, while the initial miscella with a concentration of 17% had a color of 32 red units and 35 yellow units in a 13.5-cm layer, after preraffination with $NH_4OH$ solution its color had risen to 37 red units and sometimes even higher.

Table 1 shows that the highest yield of gossypol was obtained when the miscella was treated with $NH_4OH$ and NaOH solutions, and the use of borax and sodium carbonate lowered the yield appreciably.

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When a miscella was treated with a solution of caustic soda and, incidentally, with other alkalis, in addition to the gossypol the fatty acids reacted with the NaOH, as a result of which sodium soaps were formed as well as sodium gossypolates. To isolate the gossypol, the mass obtained was treated with 5% sulfuric acid. The gossypol and the fatty acids floated, and the aqueous lower layer was usually discarded. In the course of further work, it was found that part of the gossypol remained in it in the form of a bright yellow suspended precipitate which it was difficult to filter off. This is apparently the reason for the low yield of gossypol, which did not exceed 60-65% of the amount calculated theoretically. The addition of dry sodium chloride to the aqueous layer led to the coagulation of the particles and to the formation of a dark brown mass. The latter was defatted with petroleum ether, and the residue consisted of yellow crystals of gossypol which considerably increased its total yield.

TABLE 2

| Index | Number of the miscella | | | | |
|---|---|---|---|---|---|
| | 5 | 6 | 7 | 8 | 9 |
| Initial miscella | | | | | |
| Concentration, % | 11,8 | 11,8 | 17,2 | 17,2 | 17,2 |
| Amount of oil in the volume to be refined, g | 9,1 | 18,2 | 11,7 | 35,0 | 11,7 |
| Acid No., mg KOH | 7,4 | 7,4 | 10.1 | 10,1 | 10,1 |
| Concentration of free gossypol, % | 1,56 | 1,56 | 1,78 | 1,78 | 1,78 |
| Miscella after preraffination | | | | | |
| Acid No., mg KOH | 1,74 | 1,24 | 1,40 | 1,20 | 1,69 |
| Concentration of free gossypol, % | 0,20 | 0,008 | 0 | 0 | 0 |
| Amount of gossypol isolated from the upper layer, g | 0,08 | 0,18 | 0,103 | 0,306 | 1,118 |
| Gossypol from the lower layer, g | 0,04 | 0,075 | 0,081 | 0,250 | 0,768 |
| Total yield of gossypol, g | 0,12 | 0,255 | 0,184 | 0.556 | 1,886 |
| Yield of gossypol, % of theoretical | 84,5 | 89,8 | 88,2 | 89,3 | 90,3 |

Table 2 gives the results of the preraffination of a miscella with 2% NaOH and the total amount of gossypol isolated under these conditions. As can be seen from the Table, the treatment of the lower aqueous layer can increase the yield of gossypol by 25-40%. Its purity amounted to 65-75%, the neutralization number being 240-246 mg KOH. The color of the miscella after raffination fell from 37 to 9 red units in a 13.5-cm layer.

To eliminate traces of ammonium gossypolate and the excess of $NH_4OH$, A. L. Markman et al. recommend washing the miscella with water to neutrality [3]. However, we consider this operation to be superfluous, leading only to additional losses of fat and an additional consumption of water, since the miscella after its treatment with $NH_4OH$ solution, because of its increased acidity (see Table 2), required final raffination. However, in the case of the removal of free fatty acids from the prerefined miscella by distillation after the evaporation of the solvent from it a washing operation is necessary, but, unlike A. L. Markman et al., we recommend that it should be performed with NaCl solution in order to break the emulsion formed in the process and to accelerate separation into layers.

The next series of experiments was performed with the preliminary washing out of the acetone from the miscella before its preraffination with aqueous NaOH solution, since the acetone present in the hexane—acetone miscella and partially passing into the acid liquors may, when the ammonium salts of gossypol and of the fatty acids, are decomposed, retain part of the gossypol, lowering its yield.

| Index of the initial miscella, % | | Raffination of the miscella | |
|---|---|---|---|
| | | with the washing out of the acetone | without the washing out of the acetone |
| Concentration | 25,5 | — | — |
| Acid number, mg KOH | 7,5 | 3,8 | 5,0 |
| Free gossypol content | 2 07 | 0,07 | 0,15 |
| Yield of gossypol | — | 38,0 | 52,0 |
| Purity of the gossypol | — | 88,0 | 74,0 |

The washing out of the acetone before preraffination of the miscella with $NH_4OH$ solution considerably reduces the yields of gossypol, as has been reported by other authors in the refining of gasoline miscellas and gasoline—acetone miscellas [3].

Although the washing out of the acetone reduces the yield of gossypol, the question of the use of acetone and the impermissibility of its loss is a very important one. In this connection, we attempted to eliminate the acetone by the partial concentration of a miscella with an initial oil content of 11.8% by evaporating it on a water bath to the following concentrations:

| | | | | | |
|---|---|---|---|---|---|
| Concentration of the miscella, % | 20,5 | 23.4 | 32,5 | 36.8 | 39.6 |
| Acid No. of the miscella after preraffination, mg KOH | 3.4 | 2 45 | 3 04 | 2.31 | 2,23 |
| Concentration of free gossypol after preraffination, % | 0,08 | 0.11 | 0,13 | 0.15 | 0.12 |
| Yield of gossypol, % | 91,3 | 90,5 | 85.4 | 86.6 | 89,3 |

The miscellas obtained in this way were finally refined with caustic soda solution and, as the figures given above show, an increase in the concentration to 40% did not lower the yield of gossypol.

## EXPERIMENTAL

The miscellas were obtained by extracting cotton seed flake with hexane—acetone mixture (85:15) [1]. Preraffination with solutions of ammonia, sodium carbonate, and borax was performed by the method of A. L. Markman [3] and V. P. Rzhekhin [4].

In the extraction of gossypol with 2% NaOH, we used the method of preraffination [5] with the only difference that by adding a saturated solution of salt to the aqueous layer formed in the decomposition of the gossypolate by the acid we obtained additional amounts of gossypol.

The amount of free gossypol in the miscellas before and after raffination was determined by the p-anisidine method [6], and the purity of the gossypol by the reduction of Fehling's solution [7].

## SUMMARY

1. The influence of the nature of the alkali on the degree of extraction of gossypol from hexane—acetone miscellas by solutions of $NH_4OH$, borax, sodium carbonate, and caustic soda has been studied. It has been established that the maximum extraction of gossypol is achieved by using 2% caustic soda solution.

2. It is proposed to treat the aqueous layer formed in the decomposition of the sodium gossypolate with sodium chloride, which enables the yield of gossypol to be raised to 90%.

3. It has been shown that the concentration of the miscella in order to eliminate acetone does not lower the yield of gossypol on its isolation from caustic soda solution.

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