# A New method for globin and heme preparation from blood corpuscle concentrate

K. AUTIO

Technical Research Centre-Food Research Laboratory-ESPOO(FINLAND)

## Introduction

The large quantities of animal protein in slaughterhouse blood go to waste in many countries.Centrifugal fractioning of whole blood yields plasma and blood corpuscle concentrate, of which the dark color and strong flavor of the blood corpuscle concentrate provide major disincentives to its use in food products. Since the blood cell fraction contains about 80 % of the crude protein of whole blood, it would be valuable if a way could be found to separate hemoglobin into heme and globin fractions, not only so that the globin could be utilized as an ingredient in food but so that the heme could find application too. Heme iron, for example, might find use as a pharmaceutical product against anaemia.

Conventionally the separation of hemoglobin into heme and globin fractions has been achieved with organic solvents. Jope et al. (1949) presented a method in which globin was separated with acified acetone solution at -15 °C. In the method of Tybor et al. (1973) acetone was also used but in the presence of ascorbic acid: the hemoglobin was first oxidized to choleglobin and globin precipitated from this solution with acid acetone. Methyl ethyl ketone (Yonetani, 1967), ethanol (Lindroos, 1977), butanone (Teale, 1957; Yonetani, 1967) and acetic acid (Fisher, 1941) have also been used for separation of globin and heme. All these methods, however, have proved to be economically non-advantageous. Furthermore, the purity of the separation has not been good enough for both fractions to be utilized.

The heme can be separated off from the globin by adding hydrochloric acid to blood corpuscle concentrate. The hemine produced dissolves only sparingly at acid pH-value and can be separated by sentrifugation. Although the protein content of the supernatant is very high the brown color remains indicating that the precipitation of heme is not complete. Drepper et al. (1981) have recently developed a method based on hemine precipitation after partial enzymatic hydrolysis of hemoglobin in the acid pH range. Globin made by this method lacks functional properties. Also, off-flavors may be produced as a result of peptides formed under enzymatic hydrolysis. Sato et al.(1981) presented a chromatographic method in which heme and globin were separated using a CM cellulose column in the acid pH. However in their method 1 g of CMC is needed for 70 mg of hemoglobin. As such the method seems to be unsuitable for industrial scale.

A New separation method is described in the present paper. Low concentration of carboxymethyl cellulose (CMC) water-solution is added to the acid hemolized blood corpuscle concentrate. The CMC-hemine precipitate that forms is separated from the acid protein solution by centrifugation. The proteins are concentrated by isoelectric precipitation or by ultrafiltration and then spray-dried. The effect of pH and CMC concentration on the separation process is described.

## Materials and methods

Separation of blood: Pig blood cell concentrate was obtained from a slaughterhouse in Salo, Finland, where the blood was collected under hygienic conditions and separated in an Alfa-laval centrifugal separator. The separation of hemoglobin into heme and globin fractions has been described in more detail by Autio et al. (1983). The method was in principle as follows: The blood corpuscle concentrate was diluted with water to a protein concentration of 6-7 % (w/w) and the pH of the

suspension was adjusted to 1.2 - 2.0 by adding hydrochloric acid. Then 0.01 - 0.15 % (w/v) of CMC water solution was added to the acid suspension, or 0.01 - 0.15 % of CMC in acetate buffer pH 3.0 - 5.0 to diluted blood cell fraction. Two commercial CMC preparations were used: Hercofinn CMC 12 PDM 31 with a substitution degree of 1.1 and Finfix CMC with a substitution degree of 0.8. The protein/CMC (w/w) ratio varied from 5 to 200. The precipitated CMC-heme was centrifuged off in a Sorvall RC-5B centrifuge at 7000 r.p.m. The pH of the acid protein solution was adjusted to 7.0 with NaOH, which caused precipitation of the protein. Some of the water was decanted off and the suspension with a dry matter of 9 % was spray-dried on an Anhydro No 1 pilot scale spray-dryer. Air inlet temperature was 185 °C and air outlet temperature 80 °C. Atomization was by centrifugal disc. Alternatively the pH of the acid protein solution was adjusted to 3.0 and the solution was concentrated by ultrafiltration (Paterson Candy International, membrane type T4/A, nominal 85 % cutoff M.W. 10.000) to a dry matter content of 10 % and spray-dried.
Protein was estimated from Kjeldahl nitrogen values using the factor N x 6.25 (AOAC, 1970). Yields of globin preparations were calculated as the percentage of globin nitrogen of total nitrogen of the blood corpuscle concentrate.
Iron was estimated on a Perkin Elmer 603 atomic absorption spectrophotometer after wet oxidation.



## Results and discussion

Figures 1 and 2 show the effect of pH. At pH 4.0 and 5.0, when CMC has a negative charge and the protein a positive one, CMC reacts with protein and the protein recovery in the supernatant is at minimum. At low pH-value (1.5 - 2.0) when the CMC is fully protonated and the reaction with protein is eliminated, CMC reacts with heme; probably a co-ordinate bond is formed between the non-ionized carboxyl group and Fe atom of heme.

The protein recovery is best at low pH value. However, when the pH of

the system is lowered below 1.4, the ionic strength is increased, which may prevent co-ordination between Fe and carboxyl group (Table 1) and causes the Fe-content in the protein solution to increase. Another important factor influencing the separation is the concentration and degree of substitution of CMC. The concentration effect is described in Figure 3. The greater the substitution of CMC, the less CMC is needed. When the ratio of CMC and heme is correct almost all of the heme will react: as the concentration of CMC is increased more protein is precipitated and the protein preparation is freer of iron content. On the other hand, if the heme fraction is to be utilized as well, it will be important for the iron content in the heme fraction to be as high as possible; and this is achieved by low CMC concentration. Thus if both fractions are to be utilized a two-step process is needed: In the first step separation is performed with dilute CMC or simply by adjusting the pH to 2.0 or lower with HCl. The precipitated heme is separated out by sentrifugation. In the second step concentrated CMC is added to the protein solution, which at this point still has high iron content. In this way the separation method can easily be modified according to whether the heme or protein or both fractions are to be utilized.

With the two-step process a 3 % iron content in the heme fraction was achieved. It might be added that the iron of pure heme is poorly absorbed in intestine because it is polymerized, and since globin prevents the polymerization it is reasonable not to decrease the protein content in the heme fraction too low.

Table 1. The protein recoveries and protein and iron contents in spray-dried globin fractions when 0.03 % of CMC water-solution was used at pH-values 1.4 and 1.2

| pH | Protein recovery (%) | protein (%) | iron (%) |
|---|---|---|---|
| 1.4 | 92 | 88 | 0.042 |
| 1.2 | 94 | 90 | 0.066 |

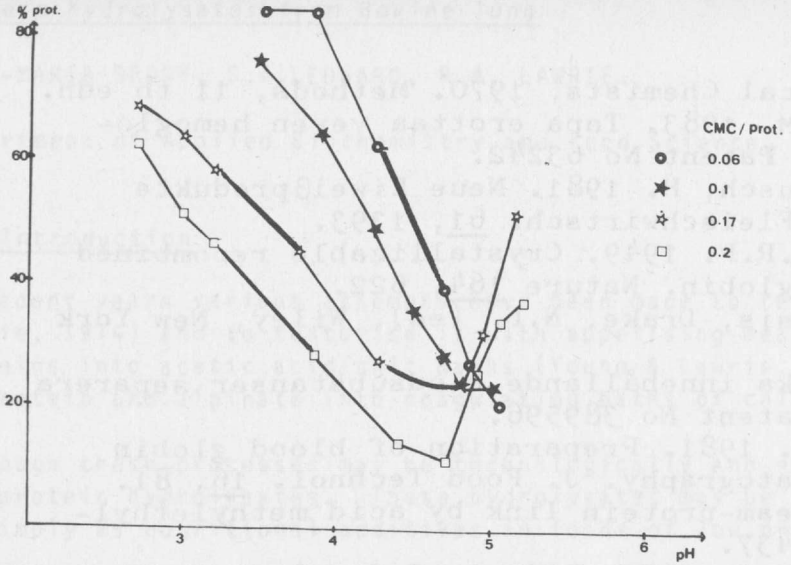


Figure 1. The effect of pH on protein recovery for different prot/CMC (w/w) ratios between pH 3.0 and 5.0 (substitution degree of CMC 0.8).

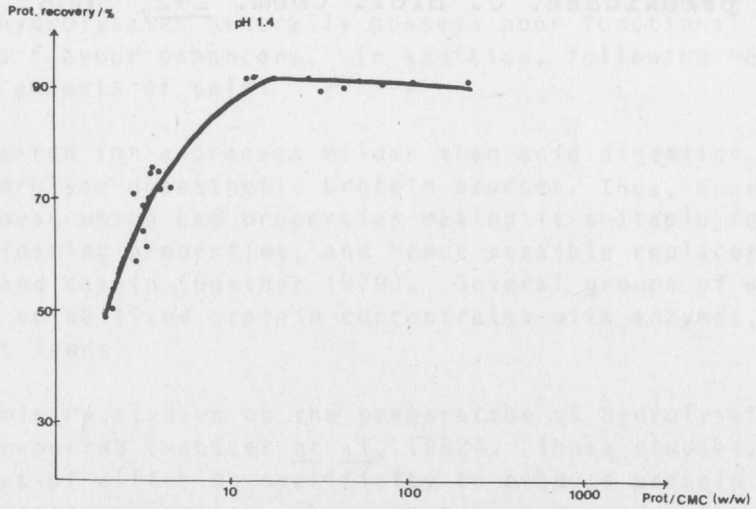


Figure 2. The effect of CMC concentration on protein re-very at pH 1.4 (substitution degree of CMC 1.1).

## References


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