Lists 1 and 2 are useful for coding new data. Lists 3–5 are used for rapid retrieval of data.

The system can be applied to practical problems by using either the cards and machine sorting or the printed lists. So far, the authors have used the printed lists for:

1. Selection of the liquid phase for separation of a mixture of two or more substances.

2. Tentative identification of unknown materials by comparison of the measured relative retention of the unknown with pertinent relative retentions in the lists. Two or more columns are used for a more positive identification[2].

3. Easy access to literature in which work of interest is described.

4. Comparison and correlation of data from different sources.

Several spaces on the CDC are unused in the present system. It is expected that some of these will later be assigned to data not now being stored in the system. The ratio of liquid phase to solid support and the specific retention volume, for example, are not coded at present; however, since data are being reported more precisely than in the past, it is planned to code this information.

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[1] C. F. SPENCER AND J. F. JOHNSON, *Anal. Chem.*, 30 (1958) 893.
[2] J. S. LEWIS, H. W. PATTON AND W. I. KAYE, *Anal. Chem.*, 28 (1956) 1370.

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Fractionation of sulphosalicylic acid filtrates on diethylaminoethylcellulose

In the course of a study on serum mucoproteins one of the aims of the authors was to investigate the high molecular substances that are not precipitable by sulphosalicylic acid, after the removal of the precipitant.

A method, formerly[1] developed for the separation of urine mucoproteins was used. It consists in the chromatographic separation of the substances on a column of

diethylaminoethylcellulose[2] and their gradient elution with phosphate buffers. The gradient was obtained with the aid of a closed mixer, which contained 0.01 *M* phosphate buffer at the beginning of the experiment. The same buffer, but with an ionic strength that had been increased to 0.6 by adding NaCl, was then added from a supply bottle. As soon as the ionic strength of the eluted solution had reached the value of 0.4, the addition of 0.4 *N* NaOH was started. By this means, a complex gradient of ionic strength and pH was attained. The eluted solution was collected in samples of about 5 ml, using an automatic fraction collector[3]. The samples were measured with a spectrophotometer and the height of their polarographic waves was determined, mixing 0.1 ml of the eluate with 1 ml of the BRDIČKA cobaltic solution[4].

The above-mentioned high-molecular substances were prepared during the so-called "BRDIČKA polarographic filtrate test"[4], by deproteinizing serum with sulphosalicylic acid. As the excess of sulphosalicylic acid interferes with the separation on an ion-exchange column when the final concentration is 0.42 *M*, this acid was removed by repeated dialysis and the dialysate was concentrated by freeze drying. Although BRDIČKA[4] and other authors state that substances causing the polarographic wave are not dialysable, a decrease in polarographic activity was always observed during dialysis. It was found that the most efficient dialysis and thus also the minimum decrease in activity could be achieved by short dialysis without using electric current.

The filtrate obtained from 200 ml of serum was subjected to dialysis in a cellophane sack for 48 hours and after freeze drying it was dissolved in 12 ml of water, re-dialysed in a disk dialyser according to SEEGERS[5] and again lyophilized. During this procedure the polarographic activity decreased to one half of the original value, while the amount of sulphosalicylic acid was reduced to 1/5000–1/10,000 of the original value. Thus, the sulphosalicylic acid constituted about 1/10 by weight of the dry-frozen substances.

In Fig. 1 the results of the separation are shown for the filtrates of normal and pathological sera, as well as for a sample of the urine mucoprotein prepared according to TAMM AND HORSFALL[6] and submitted to the action of sulphosalicylic acid and dialysis in the same way as the filtrates. The figure shows clearly the considerable similarity of the serum mucoproteins that pass into the filtrate and the urine mucoproteins obtained by alcoholic fractionation. In the experiments, carried out with urine mucoproteins, the peaks were called A – E and the zones of their occurrence were plotted on the volume scale. In zone C of the urine mucoproteins that had not been subjected to the action of sulphosalicylic acid several sub-fractions were found, the largest of which was called C_1. The main fraction found in the present experiment corresponds to this sub-fraction C_1. Another striking peak found in the zone of fraction C belongs to sulphosalicylic acid, either bound or free. This is confirmed by the spectra of samples from the peaks of the individual fractions (Fig. 2). The shape of these spectra as well as other properties, leads to the conclusion that fraction E consists of a dye of the urochrome type and fraction C_1 of a component with a rather high content of aromatic amino acids; on the other hand the other fractions have a typical mucoprotein shape.

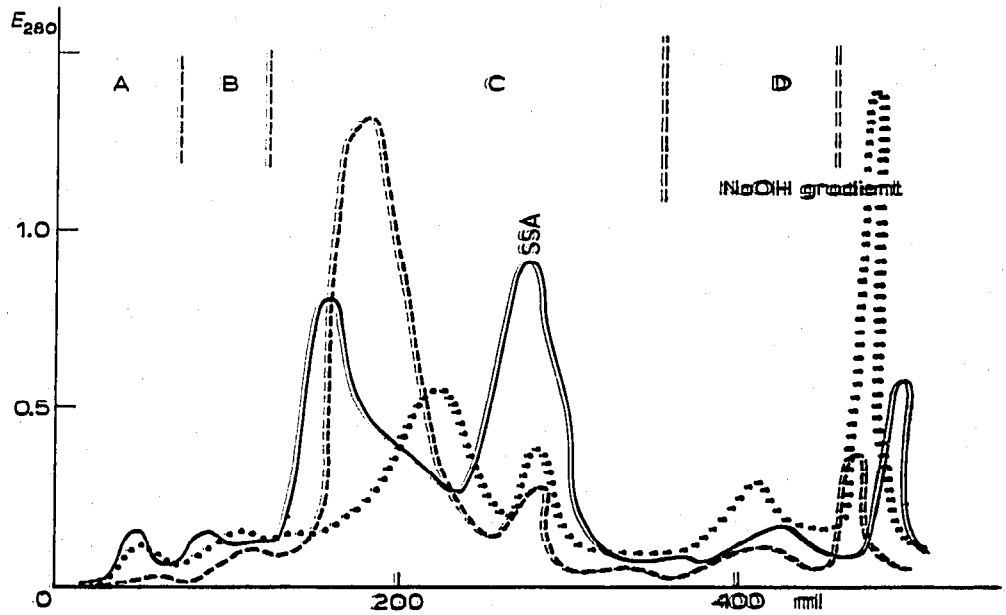

Fig. 1. Absorbances at 280 mμ. —— filtrate from normal serum; – – – – filtrate from pathological serum; ····· urine mucoprotein.

When the polarographic activity of the fractions was investigated (Fig. 3) it was found that all the protein components were active. Urochrome assumed, to be present in fraction E, and sulphosalicylic acid should not yield a catalytic wave with cobalt. The activities found, namely at the peak of sulphosalicylic acid, indicate that these substances are partially bound to proteins. From a comparison of Figs. 1 and 3 it follows that component B possesses the highest polarographic activity, related to the absorbance at 280 mμ. The activity of the fractions decreases in the sequence: A, C, D, E. From the individual areas in Fig. 3 the conclusion can be drawn that component C_1 participates maximally in the polarographic activity of the filtrate. It should, however, be kept in mind that after dialysis only one half of the original

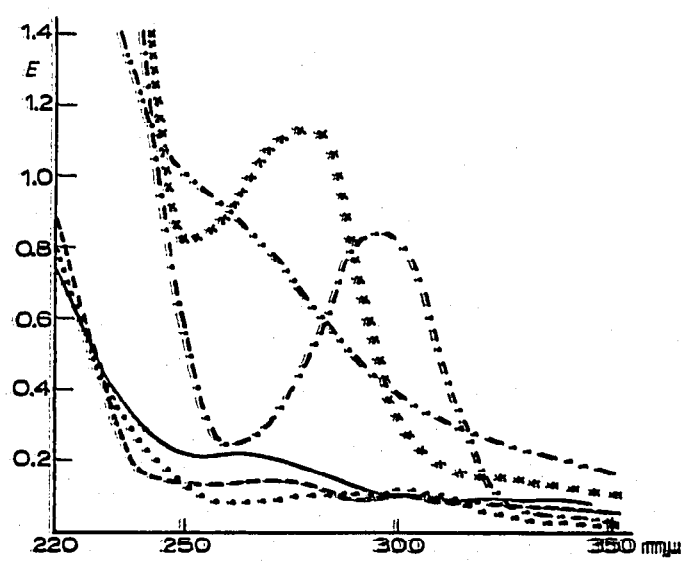

Fig. 2. Spectra of components according to Fig. 1. – – – – A; —— B; × × × C; ·····D; – · · – E; – · – · – sulphosalicylic acid.

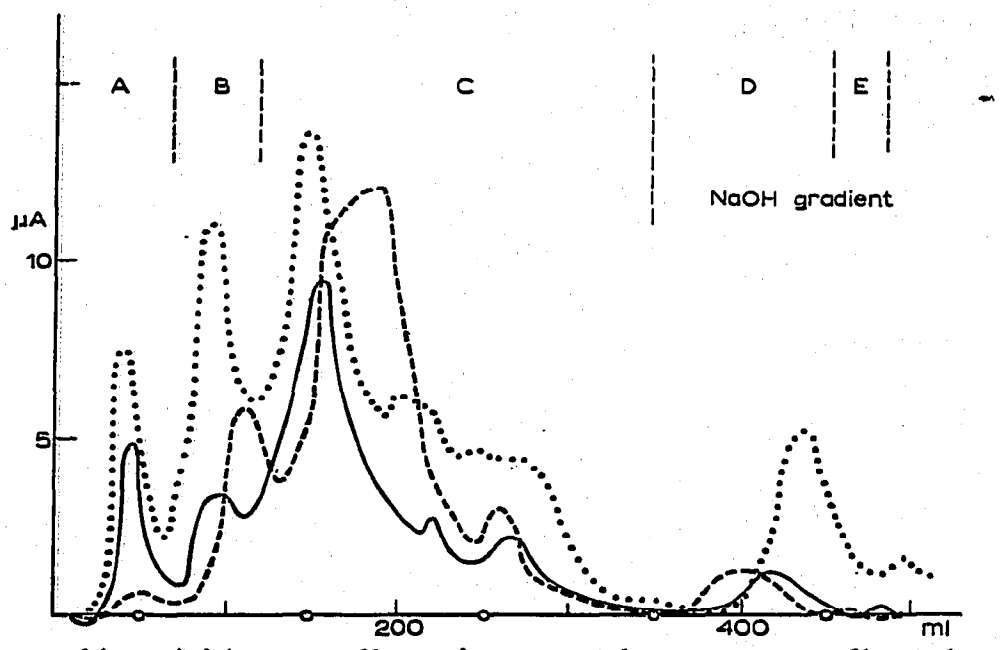

Fig. 3. Polarographic activities; —— filtrate from normal serum; – – – – filtrate from pathological serum; ····· urine mucoprotein.

polarographic activity remains in the filtrate and thus the activity of the original filtrate may be also influenced by other components, which were removed, either partially or completely.

During these experiments, attention was paid to the bond between sulphosalicylic acid and the protein. Attempts to remove all the sulphosalicylic acid from the filtrate by dialysis were unsuccessful, for this operation was accompanied by considerable losses of mucoprotein. A constant ratio between the content of sulphosalicylic acid and the mucoprotein could not be reached, even after a very long period of dialysis. These facts, as well as the presence of a comparatively small quantity of proteins in the fractions of the sulphosalicylic acid peak, support the assumption of a bond, even though it may be a weak one, between the proteins and sulphosalicylic acid. In view of the affinity of the mucoproteins to ions this phenomenon is not unexpected.

Further investigation of the components by analytical and physicochemical methods is being carried out.

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[1] Z. BRADA, *Experientia*, (in the press).
[2] H. A. SOBER, F. J. GUTTER, M. M. WYCKOFF AND E. A. PETERSON, *J. Am. Chem. Soc.*, 78 (1956) 756.
[3] A. KOČENT, Z. BRADA AND B. KEIL., *Chem. listy*, 51 (1957) 2376.
[4] R. BRDIČKA, *Research (London)*, 1 (1947) 25.
[5] W. H. SEEGERS, *Record of Chemical Progress*, Winter Issue 1952, publ. by Friends of the Kresge-Hooker Scientific Laboratory, p. 143–152.
[6] J. TAMM AND F. L. HORSFALL, *Proc. Soc. Exptl. Biol. Med.*, 74 (1950) 108.

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