der Substanz *XI* mit Essigsäureanhydrid bereitet. Aus 1,50 g Substanz *XI* und 15 ml Essigsäureanhydrid wurden nach dreistündigem Kochen, Verdünnen mit Wasser, Extraktion mit Hexan, Trocknen des Auszugs, Abdestillieren des Hexans und Destillation des Rückstandes 1,30 g (94,8%) Substanz *XII* vom Sdp. 262°C/749 Torr erhalten; n_D^{20} 1,5150, Smp. −9,6 bis −10,6°C. Für $C_{14}H_{22}$ (190,3) berechnet: 88,35% C, 11,65% H; gefunden: 88, 24% C, 11,78% H. Molekülmasse 190.

1-*Isopropyl-2-methyladamantan* (XIV). 1,2 g Substanz *XII* wurden in ätherischer Lösung in Gegenwart von 0,06 g PtO_2 bei Raumtemperatur hydriert. Die erhaltene Substanz siedete bei 263°C/753 Torr; Smp. −66,5 bis −65,0°C, n_D^{20} 1,5051. Für $C_{14}H_{24}$ (192,3) berechnet: 87,42% C, 12,58% H; gefunden: 87,64% C, 12,42% H. Molekülmasse 192. NMR-Spektrum τ: 8,14, 8,33, 8,54, 8,75 (insgesamt 14 H); 9,28 (Dublett $C\begin{smallmatrix}CH_3\\CH_3\end{smallmatrix}$ CH); 9,04 (Dublett CH_3).

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Übersetzt von M. Wichsová.

FORMATION OF IMMOBILIZED PROTEIN FILMS ON PVC MEMBRANE FILTERS

T.I.Přistoupil and M.Kramlová

Institute of Haematology and Blood Transfusion, Prague 2

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PVC membrane filters were shown recently[1,2] as suitable carriers for the formation of homogeneous protein layers spread and immobilized during a simple chromatographic development at pH 7·2. The areas covered by the protein films were linearly proportional to the amount of the given protein similarly as had been observed on nitrocellulose membranes especially at acid pH values[3,4]. With the PVC membranes a nearly linear relationship was also found[5] between the areas and the logarithms of molecular weight of equal amounts of standard proteins spread at pH 7·2. In the present work we investigated the behaviour of standard proteins at different pH values on PVC membranes of different mean pore size.

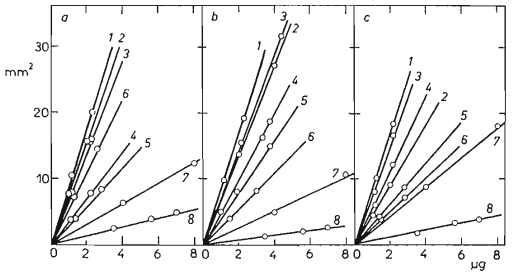

FIG. 1

Area *versus* Concentration Relationship of Proteins at Different pH Values

pH: *a* 3·7, *b* 7·2, *c* 9·06 *1* ribonuclease; isoelectric point (pI) 9·45; molecular weight (m.w.) 13000; 2 cytochrome-c; pI 10·0, m.w. 12300; *3* myoglobin; pI 6·99, m.w. 17500; *4* ovalbumin; pI 4·59–4·71, m.w. 44000; *5* serumalbumin; pI 4·9, m.w. 69000; *6* trypsin; pI 10·4–10·8, m.w. 24000; 7 gamma globulin; pI 6·6–7·2, m.w. 169000; *8* fibrinogen; pI 7·2, m.w. 341000; Proteins were spread on PVC membranes Sartorius SM 12801 in 0·1M acetate (pH 3·7), 0·1M phosphate (pH 7·2) and 0·1M borate (pH 9·06) buffers diluted 1 : 1 with 0·9% NaCl. The values of isoelectric points and molecular weights were taken from the literature[11–14]. Ordinate, area of the protein film in mm^2; abscissa, amount of applied protein in μg.

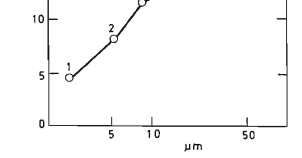

FIG. 2

Serumalbumin Spread on PVC Membrane Filters of Different Pore Size

Bovine serumalbumin (3·7 μg) was spread at pH 7·2 on PVC membranes Sartorius SM 12807 (1), 12806 (2), 12804 (3) and 12801 (4). The areas were plotted *versus* the mean pore size of the membranes on a semilogarithmic paper. Ordinate area of the protein film in mm^2; abscissa, mean pore size of the membranes in μm.

Material and Methods

PVC membranes Sartorius SM 12801, 12804, 12806 and 12807 with pore sizes 5—8 μm, 0·8 to 0·9 μm, 0·45—0·6 μm and 0·2—0·3 μm, resp., were used in strips 3—5 mm wide and 10—20 mm long. Bovine serum albumin, human gamma globulin and fibrinogen (prepared by Cohn's ethanolic fractionation), ovalbumin and horse myoglobin (prepared by salting-out techniques with ammonium sulphate), trypsin (a recrystallized sample of Lachema), horse heart cytochrome-c (Sigma) and crystalline bovine pancreatic ribonuclease (Reanal) were dissolved to about 0·4% solutions in 0·9% NaCl and diluted 1 : 1 by the addition of either 0·1M acetate buffer pH 3·7, 0·1M phosphate buffer pH 7·2 or 0·1M borate buffer pH 9·06. The same buffers diluted 1 : 1 with 0·9% NaCl were used to spread the proteins chromatographically. The exact concentration of the protein samples was determined separately by dry weight. Moistening of the PVC strips, quantitative application of the 1—4 μl samples to the starting edge of the strips, one-dimensional ascending development lasting 1—2 min on SM 12801, detection and measuring of the areas, were the same as described before[1,2,5]. The standard deviation of the mean of individual measurements was ±3% (*cf.*[3]).

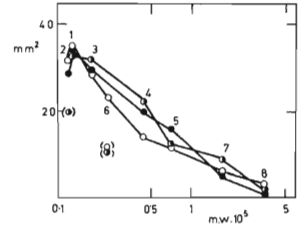

FIG. 3

Area *versus* Molecular Weight Relationship of Proteins at Different pH Values

PVC membranes SM 12801 and buffers pH 3·7 (○), 7·2 (●) and 9·06 (◑) were used to spread the standard protein samples (*cf.* Fig. 1). The calibration curves plotted on a semilogarithmic paper are valid for 4·0 μg of proteins. Dots in bracketts are reproducible but questionable, since the corresponding layers were not homogeneous.

RESULTS AND DISCUSSION

The calibration curves in Fig. 1 showed that the area -*vs.*- concentration relationship was different but characteristic for each individual protein and also for each pH tested. In most cases the areas were smaller at pH values near to the isoelectric point of the given protein, where the mutual electrostatic repulsion between molecules is the lowest. Further results are shown in Fig. 2 and Fig. 3.

As to the practical aspect of using the calibration curves (Fig. 1) as a basis for a rapid estimation of molecular weight or size of proteins, it seems to be preferable to work at pH values rather distant from the isoelectric points. In our experiments this postulate was fulfilled at pH 3·7, which is distinctly below the isoelectric points of all proteins tested. When plotting that "pH 3·7" calibration curve on a logarithmic paper a nearly linear relationship was obtained (Fig. 4) and the lines for 2·0 and 4·0 μg of proteins were parallel (*cf.*[1,2,14]).

It seems to us that protein molecules are neither fully uncoiled nor expanded as gaseous monolayers[6–9] at the interphase, since they do not spread spontaneously and they need chromato-

graphic development to form a homogeneous layer. Most probably, the coiled molecules form rather condensed liquid or solid films and are immobilized at the PVC surface by simultaneous short — range interactions with the numerous and tightly packed —CH_2—CHCl-groupings.

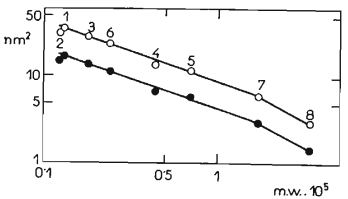

FIG. 4

Area *versus* Molecular Weight Relationship of Different Amounts of Proteins

Proteins were spread on PVC membranes SM 12801 at pH 3·7. The curves plotted on a logarithmic paper are valid for 2·0 (●) and 4·0 μg (○) of proteins.

The surface of PVC membranes filters seems to be chemically more homogeneous and therefore more feasible in these experiments than that of cellulose nitrate filters (*cf.*[4,10]) which contain also carboxylic and hydroxyl groups. Further investigation concerning the formation and behaviour of films of different protein samples and of other macromolecular substances on various types of membrane filters is under experimentation now.

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