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Wool cortical cells: a support for thin-layer chromatography

Unlike cellulose and its derivatives, which have found diverse application, wool keratin is a naturally occurring polymer with potential as a chromatographic support which has been largely neglected. This is notable in view of the well known uses of a variety of chemically related materials of both natural and synthetic origin. Thus, columns of methyl-esterified albumin adsorbed on Kieselguhr[1,2], various proteins deposited on cellulose[3], silk fibroin[4], and of poly-L-lysine on Kieselguhr[5], have found application in the fractionation of biological materials. Various synthetic polyamides[6,7] (nylon or perlon type) have been used in column and thin layer fractionation procedures: polyacrylamides have been developed for molecular sieve chromatography[8].

Wool in fibrous form has been shown to effect partial resolution of racemic mixtures[9] by a process of stereoselective adsorption. As fabric[10] or felt[11] discs packed into columns, it has been utilized in studies of dye migration. However, wool fibres, even when chopped, are not especially convenient or effective for use in chromatographic procedures, no doubt because of their relatively small specific surface.

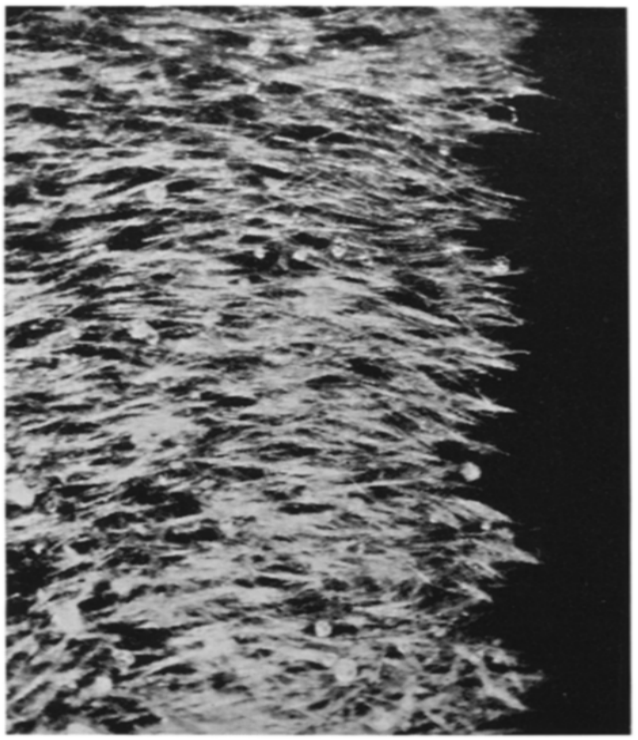
In view of interest in the use of proteins as chromatographic supports we wish to report the successful use of wool cortical cells[12] for this purpose. Cortical cells are readily isolated from fibres by enzymatic hydrolysis. In this work they were prepared by papain digestion of scoured 64's quality wool fibres in the presence of sodium hydrogen sulfite[13]. The cells were deposited on glass plates by doctoring a slurry* to form coherent films quite suitable for thin-layer chromatography. It was observed that layers formed by this means were characterized by the orientation of a large proportion of the component cells in the direction of application (see Fig. 1). These films also exhibited strong internal cohesion and rigid adherence to glass surfaces even in the absence of binder.

Cortical cell films were rather hydrophobic, nevertheless, satisfactory migration rates in the direction normal to the cell orientation have been obtained with both aqueous and non-aqueous irrigants in both the ascending and descending modes. Acid and alkaline chromogenic reagents have been used for detection purposes by spraying or by total immersion. The incorporation of a fluorescence indicator into the layer has permitted detection of substances having absorption in the ultraviolet region.

Initially, to demonstrate the viability of chromatography on thin keratin layers we wish to describe the migration of typical disperse dyes taken from the azo and anthraquinone classes. Replicate observations were made on plates prepared from three separate wool digests. All dyes migrated reproducibly as discrete coloured spots (Fig. 2) with pyridine–water (1:3, v/v) as irrigant in the ascending mode. The R_F values given in Table I are arithmetic means; all experimental observations are embraced by the limits quoted. The examples cited demonstrate that a wide range of R_F values is possible under suitable conditions.

Preliminary experiments have shown that keratin layers are well suited to the study of ionic dyes (including reactive dyes) and other textile auxiliaries. Clearly,

* We use the apparatus of C. Desaga, G.m.b.H., Haupstrasse 60, Heidelberg, Germany.



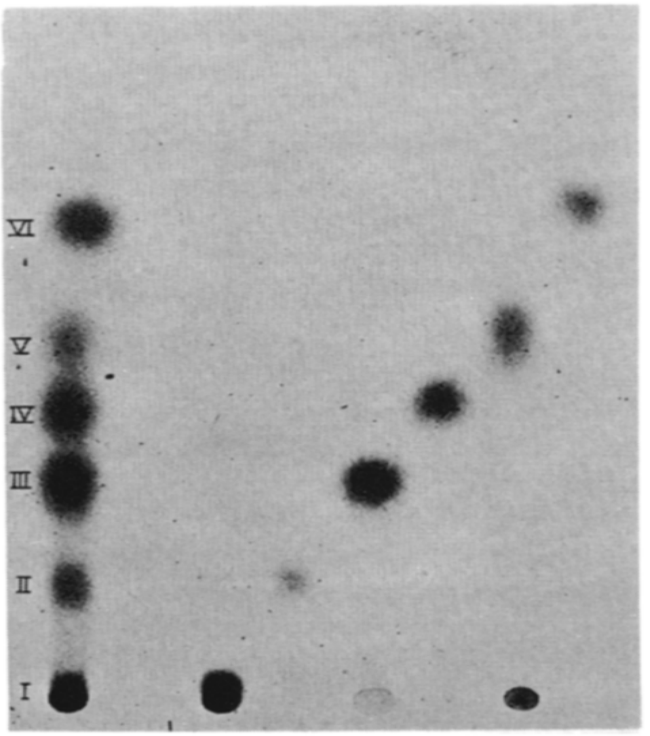


Fig. 1. Photomicrograph (125 ×) of edge of cortical cell layer formed on glass. Individual cells have dimensions of approximately 4 × 100 μ (ref. 12).

Fig. 2. Chromatography of azo dyes on a thin layer of wool cortical cells (see Table I).

TABLE I

C.I. Disperse[18] *dye No.*	*Fig. 2 key*	*Name*	R_F *value*
		Azobenzene	
Yellow 7	I	4-Phenylazo-4′-hydroxy-3′-methyl-	0.03 ± 0.01
Black 2	V	4,4′-Diamino-3′-methoxy-6′-methyl-	0.47 ± 0.02
		4-Nitroazobenzene	
Red 13	II	2-Chloro-4′-(N-ethyl-N-β-hydroxyethyl)amino-	0.18 ± 0.02
Orange 5	III	2,6-Dichloro-4′-(N-methyl-N-β-hydroxyethyl)-amino-	0.29 ± 0.02
Red 1	IV	4′-(N-Ethyl-N-β-hydroxyethyl)amino-	0.38 ± 0.02
Red 17	VI	4′-(N,N-Bis-β-hydroxyethyl)amino-2′-methyl-	0.61 ± 0.03
		Anthraquinone	
Red 15	—	1-Amino-4-hydroxy-	0.40 ± 0.02
Orange 11	—	1-Amino-2-methyl-	0.45 ± 0.03
Blue 14	—	1,4-Di-N-methylamino-	0.50 ± 0.03
Blue 1	—	1,4,5,8-Tetraamino-	0.53 ± 0.03
Violet 4	—	1-Amino-4-N-methylamino-	0.57 ± 0.02
Blue 23	—	1,4-Di-(N-β-hydroxyethyl)amino-	0.76 ± 0.03

however, they have a wider potential—particularly where the amphoteric nature of the substrate may be exploited or where advantage may be taken of interactions with the reactive amino, carboxyl and thiol groups[14,15] or with the hydrophobic side chains[16,17]. Studies on layer and liquid chromatography of other classes of compounds are in progress.

Commonwealth Scientific and Industrial Research Organization, P. R. BRADY
Division of Textile Industry, P.O. Box 21, Belmont, J. DELMENICO
Victoria 3216 (Australia) R. M. HOSKINSON

1 J. D. MANDELL AND A. D. HERSHEY, *Anal. Biochem.*, 1 (1960) 66.
2 T. YAMANE AND N. SUEOKA, *Proc. Natl. Acad. Sci. US*, 50 (1963) 1093.
3 T. I. TIKHONENKO, *Biokhimiya*, 27 (1962) 131.
4 T. Y. HUH AND C. W. HELLEINER, *Anal. Biochem.*, 19 (1967) 150.
5 S. R. AYAD AND J. BLAMIRE, *Biochem. Biophys. Res. Commun.*, 30 (1968) 207.
6 H. ENDRES AND H. HÖRMANN, *Angew. Chem., Intern. Ed. Engl.*, 2 (1963) 254.
7 J. DAVIDEK AND Z. PROCHAZKA, *Collection Czech. Chem. Commun.*, 26 (1961) 2947.
8 S. HJERTÉN, *Arch. Biochem. Biophys., Suppl. 1* (1962) 147.
9 K. HARADA, *Nature*, 218 (1968) 199.
10 J. LENOIR, *Bull. Soc. Chim. France*, 9 (1942) 475.
11 H. EGLI, *SVF Fachorgan Textilveredlung*, 11 (1956) 328.
12 P. ALEXANDER AND R. F. HUDSON, in C. EARLAND (Editor), *Wool, Its Chemistry and Physics*, Chapman and Hall, London, 1963, p. 4.
13 S. BLACKBURN, *Biochem. J.*, 47 (1950) 443.
14 D. H. SIMMONDS, *Australian J. Biol. Sci.*, 7 (1954) 98.
15 J. H. BRADBURY, G. V. CHAPMAN AND N. L. R. KING, *Australian J. Biol. Sci.*, 18 (1965) 353.
16 H. A. SCHERAGA, *J. Phys. Chem.*, 65 (1961) 1071.
17 H. ZOLLINGER, *J. Soc. Dyers Colourists*, 81 (1965) 345.
18 *Colour Index*, Society of Dyers and Colourists, Bradford, 1956.

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