factory for the two viruses studied. The method should be useful when it is desirable to use a single preparation of purified virus for a series of experimental studies and especially useful when freezing is undesirable[8]. For any particular virus one would probably have to determine the optimum pH and ionic strength of the storage medium before the method could be successfully used.

Résumé. On décrit une méthode d'accumulation des virus végétaux. Elle ne comprend pas la congélation ou la conservation chimique. La contamination microbienne est évitée par filtration à travers un microfiltre aseptique placé dans un réservoir également aseptique.

P. R. Desjardins and J. V. French

Department of Plant Pathology, University of California, Riverside (California 92502, USA),
5 November 1968.

[8] P. R. Desjardins and T. O. Diener, Phytopath. *57*, 809 (1967).

Methylated Cellophan Membranes for Possible Use in Multi-Membrane Electrodecantation

In multi-membrane electrodecantation[1], cellophan membranes spaced 1 mm apart are inserted in the separation chambers of the apparatus. This arrangement reduces the horizontal distance of migration of a protein before it meets a barrier. On this obstruction the protein concentrates and slides to the lower region of the vessel. The protein compound which is isoelectric at the pH of operation of the electrodecantator remains stationary and may be separated from the migrating fractions after completion of the experiment. If electrodecantation is conducted for long periods, concentration of the isoelectric component may occur in the lower regions of the separation cells. This may be due to one of two reasons. The pH of the buffer in which the protein mixture is contained may not be exactly isoelectric for the component to be purified, or, the buffer in the separation sections of the electrodecantator moves on account of electroendosmosis of the buffer through the cellophan membranes. At very low ionic strength this movement of the fluid is very noticeable. The electroendosmosis is due to the Helmholtz double layer effect in the pores of the cellophan. This migration of the electrolyte may be reduced by the addition of small amounts of Mg ions to the buffer. The divalent ions have a suppressing effect on the zeta potential in the double layer. Unfortunately divalent ions are insoluble on the alkaline side of neutrality and consequently will have no effect on the zeta potential.

As the major zeta potential in cellophan is due to dissociation of carboxyl groups which formed by oxidation during the manufacture of the membrane it follows that if these groups could be eliminated the zeta potential would be greatly diminished and the electroendosmosis in the membranes would be reduced to a minimum. An effective means of elimination of the carboxyl groups is to methylate the membranes prior to trimming them to the correct size for the electrodecantator.

The methylation is done by washing traces of glycerine from the surface of the cellophan followed by thorough dehydration in absolute alcohol. The remaining ethanol is removed with dry methanol. The membrane is then submerged in a mixture of dry methanol containing 4% by weight of dry HCl vapour. They are left in this mixture for 4 days at room temperature after which they are washed free of the methanol-HCl mixture. The methylation of the carboxyl groups seems to be complete after this treatment as additional methylation with diazomethane does not appear to have any further effect.

The effectiveness of removal of the charges is semi-quantitatively assessed by the electroendosmosis which the membranes show under standardized conditions.

The membranes are clamped between the flanges attached to 2 glass tubes and a potential gradient of 15 V/cm is applied across the membrane. The electrolyte solution was 0.014 *M* NaCl and the current density was 6.6 mA/cm². Reversible Ag/AgCl electrodes are used. The migration of the salt solution through the membrane was measured in capillaries attached to the electrode vessels. After passing the current for 1 h to allow for initial temperature equilibration of the apparatus readings were taken. The results are shown in the Table.

It is evident from the Table that the electroendosmosis shown by the treated membranes is much less than that shown by the untreated cellophan and that the removal of the carboxyl groups is complete after the first methylation. The residual electroendosmosis is probably due to normal Helmholtz double layer effect shown by any surface due to adsorption of ions. It would not be possible to eliminate this effect.

Electroendosmosis of cellophan medium 0.014 *M* NaCl potential gradient 15 V/CM, current density (6.6 mA/cm²)

Material	Rate of migration of 0.014 *M* NaCl in ml/min
Untreated cellophan	0.0125
Cellophan methylated with methanol HCl	0.00166
Cellophan methylated with methanol HCl followed by diagomethane treatment	0.0018

[1] A. Polson, Biochim. biophys. Acta *11*, 315 (1953).

The methylated membranes are brittle when dry, but when moist they have sufficient mechanical strength to render them useful in the form of an assembly pack in the electrodecantation apparatus. Care should be taken not to fold them as they crack easily at the position of the bend[2].

Zusammenfassung. Die Ladungen, eine Folge der Karboxylgruppen an Zellophanmembranen, können durch Methylierung entfernt werden. So behandelte Membranen zeigen eine beträchtliche Verminderung ihrer Elektroendosmosis und sind daher für Multimembranelektrodekantation sehr geeignet.

A. POLSON

C.S.I.R. and U.C.T. Virus Research Unit, University of Cape Town (Republic of South Africa), 3 December 1968.

[2] Acknowledgment. Grateful acknowledgment is made to Dr. B. W. RUSSELL for the electro-endosmotic measurements on the membranes.

Separation of Cardiotonic from Flavanoid Compounds of the Squill, *Urginea maritima* Baker

STOLL et al.[1] have identified most of the cardiotonic glycosides of the Squill; anthocyanins[2] and flavanoid compounds[3] have also been found. The ethyl-acetate extracts consist mostly of the cardiotonic and flavanoid compounds; however, a good method for their separation is not yet available. The method here described is simple and reliable enough to separate both groups of compounds.

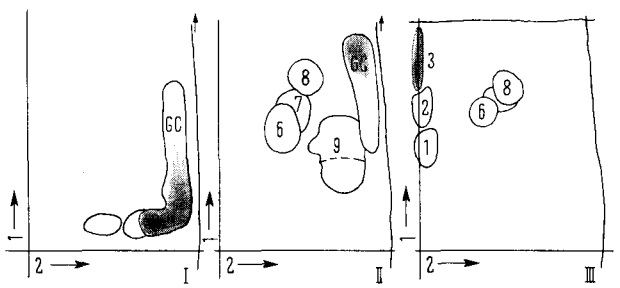
Materials and methods. Squill extracts. The ethyl-acetate extracts of the triploid, tetraploid and hexaploid Spanish bulbs, obtained by the method of STOLL et al.[1], were used.

Chromatography. The fractions of the extracts were checked by paper chromatography[4] for both cardiotonic and flavanoids. Thin layer chromatography (TLC) in polyamide (Woelm) developed with water–ethanol–methylethylketone–acetylacetone (65:1:5:15:5, by vol.)[5] for flavanoids and Silicagel G developed with water saturated methylethylketone for cardiotonics. The chromatograms were sprayed with 10% $SbCl_3$ in chloroform or 10% aminoethylester of diphenylboric acid in methanol[6].

Filtration through polyamide. 10 g of the dry ethyl-acetate extracts suspended in 1 l of distilled water were stirred for 15 min with 100 g of polyamide (Ultramid-Pulver BASF) free of monomers[7]; the slurry was poured into a funnel, with a sintered glass plate, the water was filtered out; then the polyamides were washed with 2 l of 10% aqueous methanol, filtered out and added to the water (I); 10 l of 50% methanol were passed with stirring (II) and finally 10 l of absolute methanol (III). With fraction II, where the separation was not complete, the process was repeated over the same polyamide used; washing with 10% methanol, the remaining cardiotonics were passed and added to fraction I. The polyamide washed with dimethylformamide did not retain any appreciable amount of these compounds (see Figure).

Results and discussion. Fraction I was shown to contain cardiotonics and some flavanopolyglycosides, that were isolated by preparative paper chromatography, after separating most of the cardiotonics by crystallization of the concentrated liquids; fraction II contained most of flavano-3-glycosides, very little dihydroquercetin and polyglycosides; fraction III contained all flavanoids with 3-OH free and some of the 3-glycosides. This sequence is in accordance with their Rf using aqueous solvents on paper. The isolation of the individual flavanoid compounds from these simple fractions was achieved by the ordinary chromatographic techniques and will be published elsewhere.

The precipitation with lead acetate or hydroxyde[1] has to be repeated more than 3 times to achieve any substantial separation, and some alteration occurred mainly on the 3-OH free compounds. We have worked some 25 runs of the extracts by column chromatography on cellulose powder, carboxymethylcellulose, Magnesol, polyamides etc.; the results were not satisfactory; the polyglycosides of both groups behaved on the chromatographic systems in a similar way, quercetin, dihydroquercetin and flavanomonoglycosides are difficult to



Whatman No. 1 chromatograms of the fractions sprayed with $SbCl_3$. Solvents: (1) *n*-butanol–acetic acid–water (4:1:5, by vol. upper layer); (2) 2% acetic acid. GC, cardiotonic glycosides; 8, dihydroquercetin; (3) condensation product; 1,2,3-OH free flavanoids; other spots, flavanoid-3-glycosides.

[1] A. STOLL, E. SUTER, W. KREIS, B. B. BUSSEMAKER and A. HOFMANN, Helv. chim. Acta *16*, 703 (1933).
[2] F. A. VEGA and C. MARTIN, Nature *197*, 382 (1963).
[3] F. A. VEGA and M. FERNANDEZ, Naturwissenschaften *51*, 483 (1964).
[4] J. B. HARBORNE, *Comparative Biochemistry of the Flavanoids* (Academic Press, London and New York 1967).
[5] E. STAHL, *Dünnschicht-Chromatographie* (Springer-Verlag, Berlin 1967), p. 669.
[6] R. NEU, Naturwissenschaften *44*, 181 (1957).
[7] H. ENDRES and H. HÖRMANN, Angew. Chem. *2*, 254 (1963).