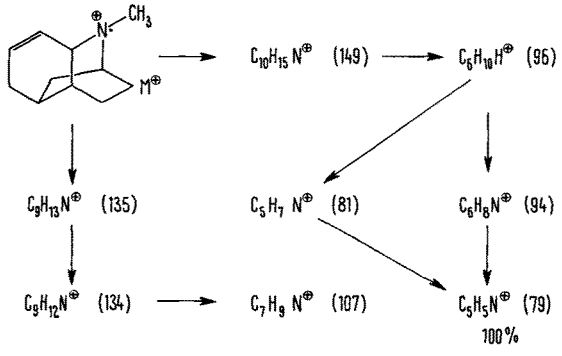
complexe-procède par une perte de CH_2-CH_2 (135) suivi de la perte d'un hydrogène radicalaire (134) et l'obtention du pic 107 et ensuite aromatisation interne avec un transfert d'hydrogène.

Une faible intensité du pic à $M^{\oplus}$-1 ne permet pas de le suggérer comme intermédiaire important. Dans une région des petites m/e seul pic important est *m/e* 42 CH_3-$N^{\oplus}$ = CH qui confirme la structure de la partie gauche de la molécule **1** (Schéma II).



Autres pics nonexpliqués (51,39) parvient vraisemblablement de la fragmentation de l'ion pyridinium[5].

Summary. The separation of a new alkaloid from cranberries leaves was carried out by means of specific solvents and thin layer chromatography. The determination of its molecular structure was made possible by infrared, ultraviolet, nuclear magnetic resonance, mass spectra and the degradations.

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Université de Moncton, Faculté des Sciences, Moncton (N.B., Canada), 7 juin 1971.

[5] H. BUDZIKIEWICZ, C. DJERASSI et D. H. WILLIAMS, *Interpretation of Mass Spectra of Organic Compounds* (Holden-Day Incorporation, San Francisco 1965), p. 253.

[6] Remerciements. Ce travail a été supporté par le Conseil National de Recherches du Canada. Nous tenons à remercier sincèrement Dr S. G. LEBLANC, Université de Moncton, pour sa contribution.

Drying of Agarose Gel Beads

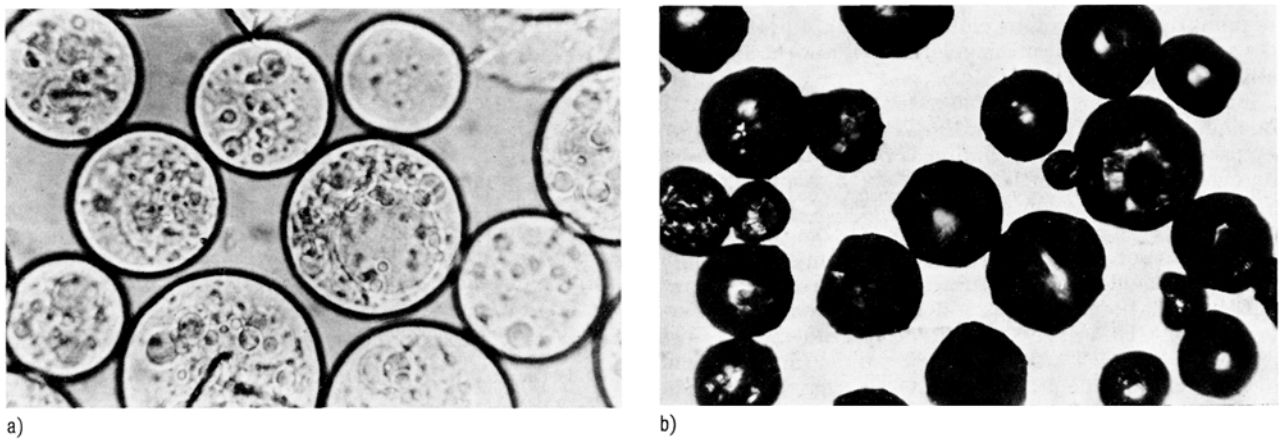
It is well-known that agarose gel beads (Sepharose®, Sagarose®) cannot be dried since they will shrink severely and irreversibly[1].

The gel is considered to be composed of randomly oriented and moderately hydrated fibers held together in a network by hydrogen bonding[2]. However agarose gels are not destroyed in an 8*M* urea solution[3], so we may assume that the gel structure relies not only on hydrogen bonds but also on some other forces, e.g. the hydrophobic interaction established between the hydrophobic patches of the anhydro-galactose (C_3 and C_6) that constitutes the agarose fibers.

If the hydrophobic bonds create contact points between the agarose fibers, orienting them into a network, we could take out the free-water from the network threads and meshes (pores) without damaging the hydrophobic bonds holding the gel structure. The only condition is to take out the water gradually and slowly with an organic solvent like acetone. Also in order to prevent network shrinkage during drying, we have conferred a better rigidity to the gel matrix by fastening the fibers by glyceric bridges with epichlorhydrin.

The drying of agarose beads with acetone was done according to the following technique: a water suspension of 6% agarose gel beads, prepared after HJERTEN[4], was mixed with an equal volume of acetone and occasionally stirred for 3 h. The supernatant was decanted and the procedure repeated 3 times. Finally the suspension was kept overnight, at room temperature, and washed once more on the filter with acetone. The agarose beads

[1] P. CUATRECASAS, J. biol.Chem. *245*, 3069 (1971).
[2] T. C. LAURENT, Biochim. biophys. Acta *136*, 199 (1967).
[3] M. K. JOUSTRA, in *Modern Separation Methods of Macromolecules and Particles.* (Ed. T. GERRITSEN, Wiley-Interscience, New York 1969), p. 195.
[4] S. HJERTEN, Biochim. biophys. Acta *79*, 393 (1964).



a) b)

Fig. 1. Microphotographs of agarose gel beads, a) dried and rehydrated; b) dried.

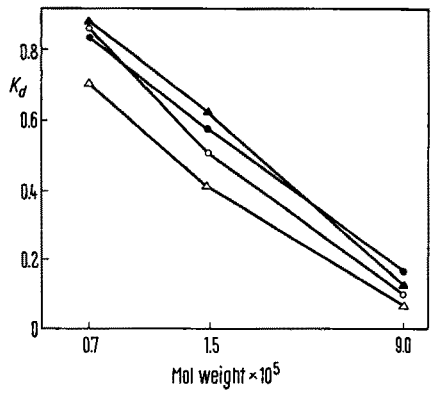


Fig. 2. The protein molecular weight selectivity curves of agarose gels. -●-●-●-, not dried; -▲-▲-▲-, dried and rehydrated; -○-○-○-, treated with epichlorhydrin (0.5 ml/100 ml packed agarose beads); -△-△-△-, treated with epichlorhydrin (2.0 ml/100 ml packed agarose beads).

were spread in a thin layer on a Petri dish and dried overnight at room temperature.

Before drying, the agarose beads can be treated with epichlorhydrin 0.5 or 2.0 ml to 100 ml packed agarose according to the procedure described previously[5]. The cross linked agarose beads were than dried according to the procedure mentioned above; the gel can be also dried at 60 °C. After the cross linking, the gel beads are not dissolved even in boiling water (0.5/100 is the smallest epichlorhydrin quantity that produces this effect). The water content of the air-dried beads was 16% for the non-treated gel and 13% for the gels treated with epichlorhydrin.

For the dried agarose gel beads rehydration, distilled water or 0.1 *M Tris*-HCl buffer pH 8.0 were used. The reimbibition was very quick; in a few minutes the beads reached the original shape (Figure 1). The total volume of the reimbibed gel bed was 8–10% smaller than the original gel bed, which shows that the beads did not exactly reach their initial size.

The molecular weight selectivity curves (K_d-log M plots) of the different agarose gel bead preparations are illustrated in Figure 2. A 95/1 column equilibrated with 0.1 *M Tris*-HCl, pH 8.0 was used. The flow rate was 12–15 ml/h. HSA (MW = 0.7×10^5) (N.B.Co); rabbit IgG obtained by chromatography (MW = 1.5×10^5) and bovine IgM (MW = 9.0×10^5) isolated from bovine γ-globulin, fr. II (Serva) were used for calibration. The void volume was determined with lyophylized *E. coli* cells (Seravac).

A relationship between the partition coefficient (K_d) of the above-mentioned proteins and the various treatments applied to agarose gel beads before drying, is shown in Figure 3. The results show that the mild drying with acetone does not modify the resolution power of agarose gel beads, the differences observed being within the error limits of the method used.

The epichlorhydrin treatments change the slope of the selectivity curves (Figure 2) and determine a lowering of the K_d values, an effect which is more pronounced for the agarose gel beads treated with 2.0/100 epichlorhydrin, especially for MW = 1.5×10^5 (Figure 3). After this treatment, the exclusion limit of the 6% agarose gel beads decreases from 2×10^6 to approx. 10^6, which means that the gel acquires the resolution power of an 8% agarose gel. As we used proteins with a MW $< 10^6$, it is not surprising that the agarose gels treated with epichlorhydrin have a somewhat better separation power than the original agarose gel (Figure 2).

The mild drying with acetone was also applied to agarose gel beads coupled with various proteins. No alteration in the antigenicity[5] of the conjugated proteins was observed. It seems that the agarose beads protect the proteins against denaturation during gel dehydration.

Our results show that agarose gel beads can be dried by gradual dehydration with acetone, the dry beads conserving their capacity for reimbibition and their resolution power. Moreover, the agarose beads protect against denaturation the protein with which they are conjugated. After epichlorhydrin treatment the gels acquire a good stability in boiling water and can be sterilized in the wet state by autoclaving. The glyceric bridges introduced in the agarose network lower its exclusion limit, a fact which could be utilized for the preparation of some agarose gels with separation limit under 10^6, starting from a 6% (or less) agarose gel and without resorting to higher agarose gel concentration (8–10%).

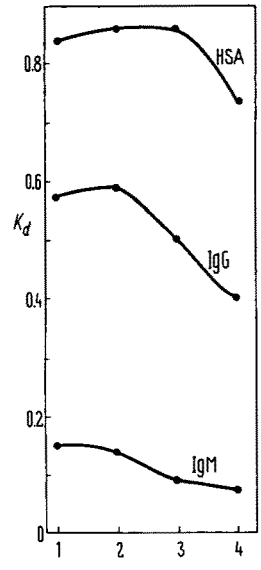


Fig. 3. Relationship between K_d values and the different treatments applied to agarose gel beads, 1. not dried; 2. dried and rehydrated; 3. treated with epichlorhydrin (0.5 ml/100 ml packed agarose beads); 4. treated with epichlorhydrin (2.0 ml/100 ml packed agarose beads).

Zusammenfassung. Granulierte und perlförmige Agarosegele können durch milde Entwässerung mittels Aceton getrocknet werden, ohne dass sie dabei ihr Quellvermögen und ihre Auftrennungsfähigkeit einbüssen.

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Bucuresti 63 (Romania),
14 May 1971.

[5] H. D. SCHELL and V. GHETIE, Revue roum. Biochim., Bucharest 8, 251 (1971).