Densities of Collagen Dehydrated by Some Organic Solvents

Unswelling of collagen gels in organic solvents results in considerable volume changes. In our previous work[1] we followed these volume changes, among other questions, and found the values given in the Table.

The effect of aliphatic alcohols indefinitely soluble in water increases with increasing number of carbon atoms. Unswelling in methanol gives rise to increase of gel volume in spite of the fact that dehydration takes place. The greatest volume changes occur during unswelling in acetone and dioxan.

The influence of acetone on collagen was studied by HEIDEMANN and RIESS[2]. They found that exhaustively dehydrated collagen exhibited a striking increase of density up to 2.0 g/cm³ and were able to derive some conclusions for structural changes of acetone-dehydrated collagen. In this work we tried to extend this observation to further solvents and to find out whether a correlation exists between densities of collagen preparations dehydrated by organic solvents and the volume changes of collagen gels unswelled in the same solvents.

We used insoluble collagen from bovine skin. Soluble collagen was removed by extraction with citrate buffer. Dehydration was carried out either by repeated extraction of insoluble collagen in the cold or by exhaustive extraction at a temperature of 30 °C. Densities of collagen preparations were determined by means of the apparatus used by Brunauer, Emmett and Teller for determination of adsorption isotherms similar to the work of HEIDEMANN and RIESS[3].

The densities of collagen preparations obtained by dehydration by solvents in the cold are given in the Table.

Density of collagen dried in the air or by lyophilization varies from 1.3–1.4 g/cm³, as has been shown by several authors[2,4–6]. Consequently, it is obvious that dehydration of collagen by ethanol, *n*-propanol, acetone and 1:4-dioxan under mild conditions causes only a little increase in density and their effect is about identical. On the other hand, by dehydration with methanol and *n*-butanol a considerable decrease in density occurs. Whereas the decrease in density of collagen dehydrated by methanol corresponds to the volume changes of collagen gels during unswelling in methanol, the density of collagen dehydrated by *n*-butanol is lower than could be expected from the unswelling power of *n*-butanol. It is possible that a certain effect may be attributed to water-collagen-solvent interaction.

The results obtained by exhaustive extraction were analogous except for the extraction by acetone and dioxan. Density of collagen dehydrated by acetone was 1.81–1.92 g/cm³, which is the value corresponding to the data given in the work cited above[2]. Density of collagen dehydrated by dioxan was 1.61 g/cm³, this fact indicating that structural changes evoked by 1:4-dioxan were less considerable.

Some of the collagen preparations were subjected to analysis by the X-ray diffraction method with the aim of determining the side chain distance. The preparations dehydrated both by cold extraction with propanol and by exhaustive extraction with the same solvent had the side chain distance of 11.60 Å. This value indicates that no structural changes in collagen occurred. The distance of sidechains of collagen dehydrated by butanol was 12.10 Å; this result confirms the data obtained by density measurements and indicates that a certain loosening of collagen structure takes place.

Considering the results given here, it is possible to suggest that a correlation exists between volume changes of collagen gels unswelled by organic solvents indefinitely soluble in water and the densities of collagen preparations dehydrated by an exhaustive extraction with the same solvents.

Collagen dehydrated by acetone and dioxan exhibits a tightening of structure, the influence of the latter not being so significant. Methanol and butanol exhibit an opposite effect on collagen. As shown by density measurement and the X-ray diffraction method, a loosening of structure takes place manifested by the enlarging of the distance between side chains[8].

Solvent	Volume[a]	Density[b]	*M* volume[c]	X-ray diffraction[d] d[a]	d[b]	d[c]	Å
Methanol	121	1.11–1.17	3689–3888				
Ethanol	71	1.41	3060				
n-Propanol	36	1.39–1.44	2998–3105				
n-Butanol	55	1.18–1.28	3372–3657				
Acetone	12	1.42–1.48	2916–3039				
1:4-Dioxan	13	1.43	3018				
n-Propanol[e]				1.15	2.80	4.85	11.60
n-Propanol[f]				1.15	2.80	4.85	11.60
n-Butanol[f]				1.10	2.20	4.90	12.10

[a] Volume of collagen gel, % of original volume, exposed to unswelling for 10 min. [b] Density of dehydrated collagen g/cm³. [c] Volumes of collagen molecules cm³ · 10¹⁶, calculated from the density of dehydrated collagen preparations and molecular weight of collagen 260,000[7]. [d] X-ray diffraction and side chain distance of collagen preparations. [e] Extraction in the cold. [f] Exhaustive extraction.

Zusammenfassung. Der Einfluss von organischen Lösungsmitteln auf die Dichteveränderungen des Kollagens wurde geprüft. Methanol und Butanol führt zur Strukturauflockerung, Aceton und Dioxan zur Strukturverdichtung.

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