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CONTRIBUTION TO THE COMPLEX ANALYSIS OF GELATIN

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Gelatin, the partially hydrolised product of collagen, is largely applied in various domains, especially in photographic and food industries and in pharmaceutical technics, which requires many-sided knowledge of its properties. Since in the hydrolytic process there is no unitary substance obtained, but fractions differing both in molecular mass (within 600 and 400,000) and structure and other physico-chemical properties, the gelatin samples practically impossible to reproduce. That imposes their complex characterization by means of different parameters described in literature (1—9). Such parameters are: the melting and the setting points, the viscosity in dependence on the concentration, the transmittance and the turbidity, the moisture, the pH value, the isoelectrical point, the colloid-protector action, content in different chemical reagents (bound sulphur, reducing compounds, haloids etc.), the complexing capacity and others.

In our investigations, we partly relied on some of these methods — that were modified according to the necessities —, simultaneously adding some new ones, elaborated by our research group in order to achieve the initial desideratum.

The methods were grouped so that the information — that had been obtained directly and through the possible correlations, respectively through their complementary interpretations — should ensure a more complete description of the gelatins proceeded from different sources.

The first objective to investigate is the fractionation of the samples, determination of the most probable molecular mass, characterization of the size and shape of molecules obtaining in this way important data regarding the structure or even the stereochemistry of component compounds. The fractionation and determination of the molecular mass were made by gelchromatographic method, working with a Sephadex-150 respectively a Sephadex-200 column, the separation domain of which corresponds to molecular masses of components of hydrolized collagen. The column was calibrated by means of some macromolecular compounds with known molecular mass (gamma-globulin, hemoglobin, alpha-chimotripsin, insulin etc.). The equation of regression line, concording with the theoretical Determann-equation (10):

$$\lg M = M_0 - (k_1 - k_2\, d)\, \frac{V_e}{V_{ex}}$$

can be written as:

$$\lg M = (5.935 \pm 0.036) - (0.660 \pm 0.082)\, \frac{V_e}{V_{ex}}$$

$$n = 4;\ r = -0.986;\ s_0 = \pm 0.12,$$

where V_e — elution volume; V_{ex} — external volume, k_1, k_2 and M_o — constants; d — density of gel. The calculated molecular mass generally varies between 100,000 and 200,000 (Table 1). It can be shown that the molecular mass is also correlated with some other physico-chemical parameters, namely with intrinsic viscosity — according to the Staudinger-equation (11):

Table 1

The most important physico-chemical parameters of some gelatin sorts

Nr. crt.	Gold number (mg)	Swelling degree (at 120', ml/g)	Isoelectrical point (pH)	Transmittance (%)	Physical retardance (degree)	Chloride ions (ppm)	LASER diffusion z/ϱ	Molecular mass dispersion (M.10⁻³)
1	2	3	4	5	6	7	8	9
1	0.005	7.6	4.98	90	5.3	30	1.93 0.19	147
2	0.005	10.4	4.96	67	4.3	170	2.16 0.14	173
3	0.005	9.9	4.83	80	5.0	170	2.16 0.16	170
4	0.005	13,0	4.94	76	4.3	14	2.62 0.21	193
5	0.005	11.8	4.84	68	2.9	3	2.95 0.13	164
6	0.005	5.8	4.82	84	2.9	130	2.00 0.31	155
7	0.010	16.8	4.91	79	5.8	300	1.80 0.23	132
8	0.005	13,6	4.53	81	4.0	120	1.94 0.19	91
9	0.005	9.4	4.66	81	4.0	340	2.07 0.21	155
10	0.010	8.3	4.52	79	5.8	330	1.88 0,20	110
11	0.010	10.6	4.81	79	4.0	500	2.12 0.13	107
12	0.010	6.8	4.94	90	4.4	44	2.10 0.10	125
13	0.010	8.6	4.94	92	4.6	150	2.06 0.11	136
14	0.010	9.0	4.95	89	4.6	70	1.95 0.10	226
15	0.010	9.0	4.95	32	6.6	330	2.47 0.15	207
16	0.010	8.9	4.97	32	8.4	—	2.95 0.12	147
17	0.010	11.5	4.99	68	6.7	710	2.50 0.14	112
18	0.010	10.4	4.95	42	8.7	390	2.41 0.12	125
19	0.005	6.4	4.92	53	5.8	300	2.14 0.08	117
20	0.005	5.6	4.93	50	6.7	190	3.00 0.04	121
21	0.005	9.9	4.90	51	5.4	100	2.81 0.09	121
22	0.010	10.5	4.86	53	5.2	190	2.00 0.16	182

Table 1, continued

1	2	3	4	5	6	7	8	9
23	0.005	—	—	99	4.6	—	—	125
24	0.010	—	—	88	5.4	—	—	136
25	0.005	7.1	4.99	52	4.9	30	2.37/0.09	—
26	0.0005	8.0	4.98	54	5.0	10	2.18/0.13	—
27	0.0005	5.4	4.87	50	5.2	100	2.08/0.07	—
28	0.005	5.5	4.88	52	6.7	80	2.05/0.05	—
29	0.010	9.1	4.85	76	4.7	10	2.04/0.11	—
30	0.005	8.3	4.88	88	4.7	80	1.85/0.14	—
31	0.005	13.8	4.91	89	4.5	260	1.88/0.14	—
32	0.005	7.7	4.85	79	5.2	710	2.56/0.08	—
33	0.010	9.0	4.90	58	5.2	200	2.24/0.06	—
34	0.010	7.8	4.98	99	5.2	50	2.13/0.06	—
35	0.010	7.3	4.88	88	7.1	50	2.17/0.09	—
36	0.010	7.5	4.82	90	6.8	180	2.18/0.10	—
37	0.010	7.0	4.97	61	5.2	70	2.28/0.05	—
38	0.001	9.6	5.10	64	6.1	185	1.73/0.06	—
39	0.005	9.9	5.13	32	6.3	570	3.58/0.09	—
40	0.005	8.7	5.05	63	7.1	150	2.22/0.12	—
41	0.010	8.3	5.04	36	5.4	30	2.38/0.14	—
42	0.010	10.3	5.10	38	9.4	410	3.14/0.11	—
43	0.010	11.9	5.00	95	7.2	180	1.71/0.18	—
44	0.005	7.3	4.93	94	5.2	70	1.78/0.16	—
45	0.005	7.3	4.91	83	5.8	2120	1.91/0.13	—
46	0.005	11.2	4.88	81	5.5	180	1.88/0.16	149
47	0.005	10.0	4.85	77	5.8	475	1.90/0.16	—
48	0.005	9.0	4.98	88	8.1	150	1.99/0.13	87

$$(\eta) = K\,M^{\alpha}$$

respectively with their suppressing capacity on the first degree polarographic maximum of the oxygen (12):

$$h = b\,M + a,$$

that makes possible the determination of specific constants of the investigated gelatins.

In order to characterize the shape of gelatin molecules, we used the light scattering (LASER) method, working with an indigenous installation.

There were recorded the scattering curves $I = f(\Theta)$ in horizontal polarized light at 4360 Å, from which there was calculated the dissymmetry coefficient for the angle $\Theta = 50°$ (Table 1):

$$Z = \frac{I(\Theta)}{I(180° - \Theta)}$$

The depolarization factor $\varrho = \frac{H}{V}$, which depends on the molecular anisotropy degree, was determined at 6328 Å and 90°, using for light source a He–Ne LASER.

Since the molecular structure is responsable for its behaviour, i.e. the physico-chemical properties, in the following we aimed at finding the most adequate methods for quantitative determination of the most important characters. Such structural properties are, for example, those which reflect the polarity of molecules (the balance of polar and apolar groups), as their adsorption capacity and hydration degree. The principal parameters from this viewpoint are: the gold number as the measure of protection action of liophyl colloid (gelatin), the action degree of physical retardance as the measure of physical maturation, the swelling degree in water or in several solutions, the surface-activity, measured for instance on the basis of their suppression activity on polarographic maxima of the oxygen, the electrical conductibility and the dielectric constant of gelatin solutions, as well as the isoelectrical point (Table 1).

Concerning the determination of the isoelectrical point, the importance of the knowledge of this parameter is justified by the fact, that in isoelectrical state most of the properties of macromolecular compounds have the maximum or minimum value (viscosity, electrophoretic mobility, swelling degree, aggregative stability, osmotic pressure, optical rotatory power etc.).

The method we propose for determination of this datum is based on the fact that the gelatin solutions as isostable liophyl colloid systems, have a minimum of stability in the isoelectrical state only in the case of depriving the macromolecules of their solvation shell at the corresponding pH. For this purpose we proposed to use aceton instead of ethanol, since the former has a larger dipole moment (2.85 D to 1.68 D) and — accordingly — a more remarkable variation of formation enthalpy of water solutions (—5.05 kcal/mole to —0.23 kcal/mole at the 1:1 mixing ratio). We also substituted the acetate-acetic acid buffer system with citrate-citric acid one, because the citrate ion has a larger electrical charge than the acetate one and — accordingly — produces a larger neutralization effect on electrical charge of macromolecules, respectively the citrate ion occupies a more favourable place in the liotrophic series. Indeed, by means of this new method there were obtained much more exact and reproducible results than those furnished by other methods known from literature. The minimum stability has been graphically determined from nephelometric measurements (Table 1). The constancy of isoelectrical point in different gelatin sorts is to be remarked.

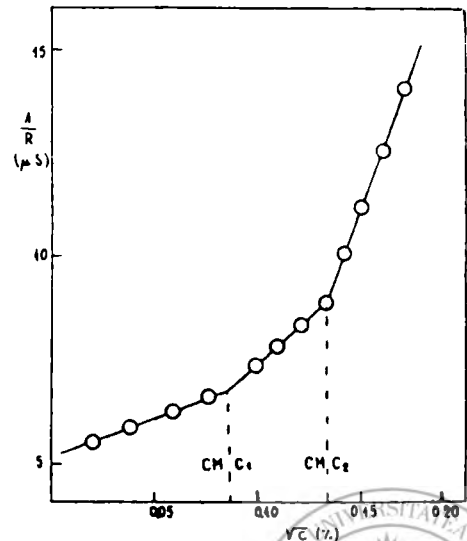

Fig. 1.

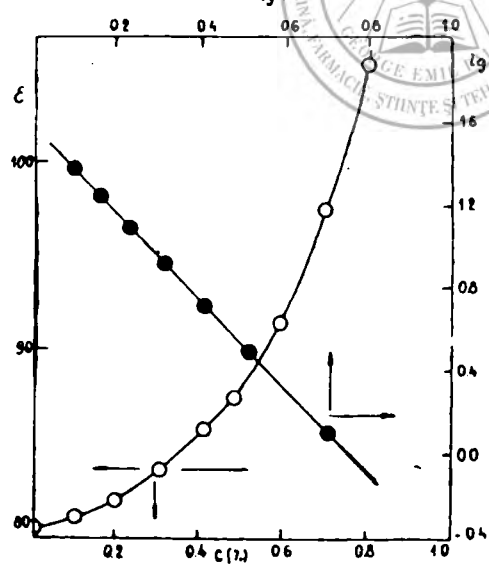

Fig. 2.

The conductometric and dielectrometric data make possible the determination of the critical micellar concentration, as well as the presence of ionic impurities. These values vary with the concentration of gelatin solutions according to some parabolas of different degrees (Fig. 1 and 2):

$$\frac{1}{R} = b\,C^{a} + \frac{1}{R_0} \qquad a = \frac{1}{2}$$

respectively

$$\varepsilon = b'\,C^{a'} + \varepsilon_0$$

These functions were linearized in the form (Table 2):

$$\lg\left[\frac{1}{R} - \frac{1}{R_0}\right] = \lg b + a \lg C$$

and

$$\lg\left[\varepsilon - \varepsilon_0\right] = \lg b' + a' \lg C$$

The larger the slope of these curves respectively straight lines, the larger the concentration of ionic impurities, respectively the gelatin is split into more little molecules by hydrolysing.

We consider that the comparative analysis of the obtained data by means of these independent methods permit a more profound characterization of the different gelatin sorts and the selection of the best characters, in accordance of the utilization domain.

Table 2

Conductometric and dielcometric parameters of several gelatin sorts

Nr.	$\frac{1}{R} = b \sqrt{c} + a$;						$\left\langle \frac{1}{R} \right\rangle = \mu S$		$\varepsilon = b'c^{a'} + \varepsilon_0$; $\varepsilon_0 = 80{,}4$	
	a_1	b_1	a_2	b_2	a_3	b_3	$CMC_1 \cdot 10^3$ %	$CMC_2 \cdot 10^3$ %	a'	b'
1	3.8	34.4	0.9	63.9	6.2	121.0	8.0	16.6	2.24	31.6
2	4.2	34.1	2.5	63.0	2.9	118.5	2.5	14.6	1.93	33.1
3	4.2	27.5	3.7	40.0	1.5	58.5	1.6	24.0	2.05	35.5
4	5.6	23.0	5.5	59.0	19.0	77.5	3.0	22.0	1.79	16.6
5	4.1	23.5	5.5	55.0	2.1	76.2	5.6	22.0	1.54	28.2
6	5.8	22.0	1.0	69.0	4.2	100.0	6.5	40.0	1.53	24.0
7	3.7	30.0	0.6	72.5	7.8	116.2	7.5	38.0	1.16	28.2
8	4.5	20.0	2.0	59.0	1.7	53.0	6.5	40.0	1.58	20.9
9	4.4	32.0	1.0	72.0	4.1	100.0	7.5	28.0	1.53	27.5
10	4.4	24.0	2.7	55.0	6.4	86.0	4.0	36.0	1,70	29.5
11	4.7	29.0	7.9	76.0	9.0	127.5	6.0	34.0	1.49	18.6
12	2.0	23.0	2.3	61.0	0.0	70.0	6.8	38.0	1,89	51.2
13	5.2	17.0	3.2	55.0	4.0	100.0	2.7	22.0	2.00	49.0
14	5.0	26.0	4.5	43.0	0.0	88.0	2.0	14.0	1.42	17.0
15	4.0	51.0	3.5	52.5	1.7	75.0	10.0	40.0	1,21	10.2
16	3.6	46.0	1.1	99.6	8.9	137.5	10.0	40.0	2.19	43.7
17	4.1	62.2	1.1	106.1	—16.6	185.0	8.6	41.0	2.06	52.5
18	3.4	73.0	—4.0	147.0	—11.2	190.0	10.0	40.0	1.78	42.7
19	3.8	27.3	1.1	79.5	—11.2	145.0	8.6	39.0	1.83	61.7
20	3.7	49.6	—2.5	115.0	—12.2	165.0	11.0	40.0	1.78	81.3
21	3.2	59.5	—5.0	162.4	—13.3	187.5	10.0	28.0	1.65	81.3

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