Centre de Recherches sur les Macromolécules, Strasbourg, France

Kurzmitteilung

Light-scattering of Aqueous Solutions

A New Clarification Technique

By G. Bernardi

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Clarification is a major problem in light-scattering. The difficulty seems to be related, to some extent at least, to the dielectric constant of the solvent; organic solvents which have low dielectric constants are much easier to clarify than aqueous solutions; among the latter, strong salt solutions are easier to clarify than weak salt solutions.

Despite the use of several different techniques[1], aqueous solutions often show dust particles which are observable by eye and exhibit a dissymmetry (defined as I_{45}/I_{135}, where I is the intensity of the light scattered at the angles indicated by the subscripts) higher than the theoretical unity value. The purpose of the present communication is to describe a novel clarification procedure.

The salt solution to be clarified is shaken for half an hour, or more, with half a volume of chloroform-isoamyl alcohol 5:1 (v/v)[2] at a rate great enough to cause a complete emulsification. The emulsions are then allowed to separate by gravity into a heavier organic layer and a lighter aqueous layer. The latter is transferred into centrifuge tubes while still strongly opalescent due to the presence of suspended droplets of the organic phase, and centrifuged two hours at 25,000 g. in a Phywe Pirouette centrifuge.

The top 25 ml. of the solution in each nylon tube (total volume 50 ml.) is transferred into the light-scattering cell with a clean pipette provided with a Propipette rubber bulb and a filter paper plug. During the suction process, the pipette tip is always kept 0.5–1 cm. beneath the liquid surface. The above precautions avoid contaminating the solution with dust from the surface and from the bottom. The pipette for removing the solution to be measured, is rinsed twice with the 25 ml. aliquots from two centrifuge tubes containing the same solution.

Table 1. Light-scattering of phosphate buffer

Θ	16	21	24	30	37.5	45	60	75	90	105	120	135	142.5
I′	1350	580	425	175	102	70	43	30	29	28	38	66	98
I″	7200 *) 6000	2200 *) 2000	1060 *) 1300	273	135	89	52	35	30	31	41	66	101

Θ Angles between the incident and the scattered beams.

I′, I″: Intensities of the light scattered at different angles Θ by 0.13 *M* NaCl + 0.01 *M* potassium phosphate, p_H = 6.8; I′: Buffer treated with chloroform-isoamyl alcohol; I″: Buffer not treated.

*) The two values listed refer to two buffers prepared separately with chloroform-isoamyl alcohol; the intensity of light scattered by benzene at 90° was 300.

Table 1 shows a comparison of typical data obtained in a low-angle light-scattering instrument[3] for a phosphate buffer as clarified either according to the above method or by the same procedure without emulsification. Essentially identical results were obtained when acetate buffers or 0.15 *M* NaCl solutions were employed. It appears that the dissymmetry of the solvent is about 1.06 when using the chloroform-isoamyl alcohol treatment as compared to 1.35 when this is not used. Furthermore, the scattered intensity at 16° of the "treated" solvent is quite reproducible and at least four times lower than the best values obtained with the "untreated" solvent.

An electrophoresis of the fine emulsion of chloroform-isoamyl alcohol in phosphate buffer p_H = 6.8, μ = 0.15, was carried out under microscopic observation and it was evident that the droplets migrated in the electric field in a direction which could be changed by reversing the polarity of the field. Therefore, a possible interpretation of the effectiveness of the clarification method described above would be an electrostatic attraction of the dust particles by the organic droplets.

The present method is being routinely used in our laboratory with several buffers commonly employed and with salt solutions of *e.g.* nucleic acids and polysaccharides, specially deoxyribonucleic acid. Results obtained with nucleic acid solutions have been reported elsewhere[3].

The present method might be questioned on two grounds: 1) Treatment with the organic mixture might introduce a third component and make difficult the interpretation of the results. 2) The components of the organic mixture might be selectively adsorbed onto the polymers.

A "third component" effect is not to be expected here since the organic mixture is practically insoluble in the salt solution. A selective adsorption

of chloroform or isoamyl alcohol onto the macromolecules is improbable; as a matter of fact it seems to be ruled out, at the least in the case of deoxyribonucleic acid, by the fact that this biopolymer does not change its optical melting curve, when in a solution saturated with chloroform-isoamyl alcohol.

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