UTILIZATION POSSIBILITIES OF LIGHT SCATTERING METHOD IN PHARMACY

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Abstract: Light scattering can serve as a method to put in evidence the presence of bacteria in a pharmaceutical emulsion. Using a control sample, a qualitative determination and a study of emulsion stability is possible. This method permits the analysis of drugs effect on osmotic transport through cell membrane, which is presented by effect of C vitamin on hemolysis. The action of some vegetable extracts upon water transport through erythrocyte cell membrane is presented with light scattering analysis on different concentration saponin solution.

Key words: light scattering, osmotic transport, emulsion purity control.

INTRODUCTION

The well known light scattering method for study of bacteria water suspension, can be extended for bacteriological purity control of pharmaceutical emulsion. With this method is possible to put in evidence the effect of drugs on osmotic transport at level of erythrocyte cell membrane, or in analysis of hemolytic effect of some vegetable extracts.

METHODS AND RESULTS

BACTERIOLOGICAL PURITY CONTROL OF PHARMACEUTICAL EMULSION

The presented experimental results were obtained by extension of well known light scattering method for the study of bacteria water suspension [3, 5, 6, 8] for case of pharmaceutical emulsions. The experimental technique is the same, but the theoretical aspect is different. In the case of pharmaceutical emulsions, by light scattering on existing micro-organisms take part the particles of emulsion components take part too [2, 4, 9]. The measured light intensity by different observation angles is given by the sum of two components, the presence of bacteria

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in emulsion is indicated by difference of measured intensities for analyzed probe and that measured on a sterile emulsion.

For registration of scattered light by different angles of observation, $I = f(\theta)$, an IFB model light diffusion installation was used, adapted for utilization of a He-Ne laser source. Emulsions were prepared with an emulgator formed from Tween and Span types non-ionic tensids, at an HLB value 11, used paraffin oil in proportion 20%. The dispersion degree determined by microscopy was $18 \cdot 10^9$ particles/cm^3.

The light scattering curves presented at Fig. 1 were registered for a sterile emulsion (curve "e"), and respectively the same emulsion infected with Escherichia coli germs in concentration $6 \cdot 10^7/cm^3$ (curve "e+b").

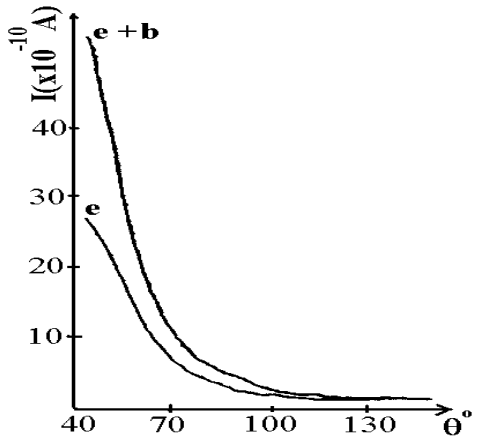

Fig. 1. Light scattering for a sterile (e) and an infected (e+b) emulsion.

Analyzing the scattered intensity by different angles, the presence of germs are signed at low angles, under 100^o, with maximal sensibility of method at $\theta = 40^o$.

By this angle of observation, besides bacteriological purity control, can be made quantitative determination of germs concentration at a given moment, or observation concerning stability in time of emulsion, us well on effect of utilized conserving agent.

EFFECT OF SOME DRUGS ON OSMOTIC TRANSPORT AT LEVEL OF ERYTROCYTE CELL MEMBRANE

The followed scope was to present a method that permit to make some observation regards, how different drugs action on osmotic resistance of erythrocyte membrane, respectively upon water transport through erythrocyte

membrane. This process was follow by valuation of hemolysis speed, determined through laser beam scattering method. The method is based on analysis of a controlled hemolysis, provoked artificially in a hypotonic erythrocyte suspension, process followed through light scattering curves. This curves describe the variation in time of diffused intensity by well chosen observation angle. The slope of this curves, registered automatically, measures the hemolysis velocity, a parameter that is inverse proportional with osmotic resistance of erythrocyte membrane. The effect of studied drugs can be evaluated through the value of hemolysis velocity, determined graphically, in comparison with a standard sample. Representing the hemolysis velocity in function of drug concentration, we obtain information regards action mode of respective medicine.

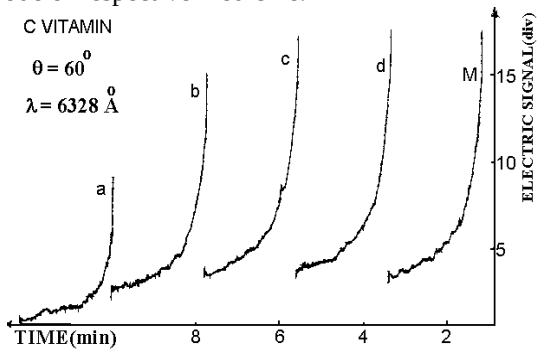

Fig. 2. Light scattering curves of some samples with C vitamin in different concentrations: a. $2.5 \cdot 10^{-4}$ M; b. $2.5 \cdot 10^{-5}$ M; c. $2.5 \cdot 10^{-6}$ M; d. $2.5 \cdot 10^{-7}$ M; M – witness.

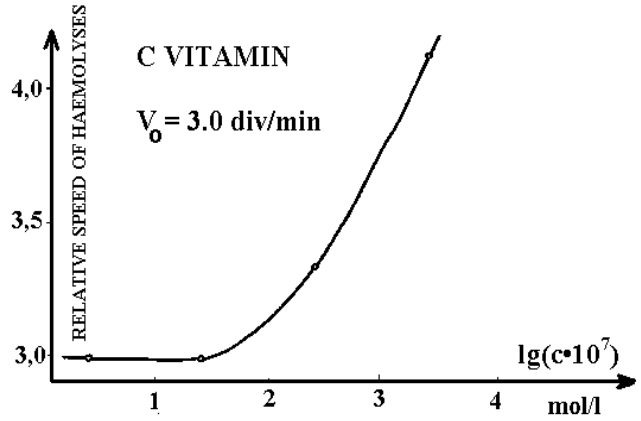

Fig. 3. The change of relative speed of hemolysis on C vitamin concentration.

On Fig. 2 and Fig. 3 is presented, for example, registrations obtained for C vitamin, and respectively the curve that describe variation of hemolysis velocity in function of concentration.

This curves show that in studied concentration interval, C vitamin has such a hemolytic effect that decrease the osmotic resistance of erythrocyte membrane. The effect is evidenced for concentrations over $2.5 \cdot 10^{-5}$ M, the hemolysis velocity increasing exponentially with concentration raising.

ANALYSIS OF VEGETABLE EXTRACTS

The afore-mentioned described method permits the study of action of some vegetable products upon water transport through erythrocyte membrane. Beside qualitative observations of extract effect upon osmotic resistance of erythrocyte membrane, the method permits a quantitative dosage of substance present in extract, responsible for observed effect [1, 7]. For exemplification is presented the case of some extracts in which the active substance is saponina. Fig. 4. presents the registration of scattered light obtained for a series of etalon probe, prepared with different concentration from pure solution of saponina.

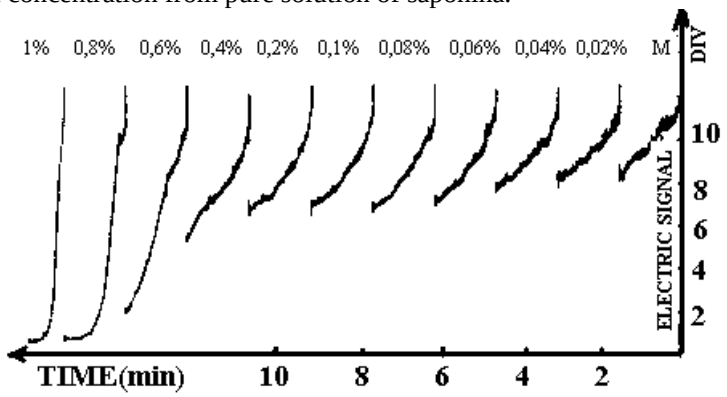

Fig. 4. Light scattering curves registered for standard samples with saponina in different concentrations.

The substance has a strength hemolytic effect, in special for concentrations higher than 0.1%. In studied concentration interval, the effect is increasing exponentially with concentration (Fig. 5). The presented curve may be utilize as "test curve" for dosing saponina from vegetal extracts prepared in same circumstances, with condition that this extract does not contain other substances which action upon osmotic resistance of erythrocyte membrane.

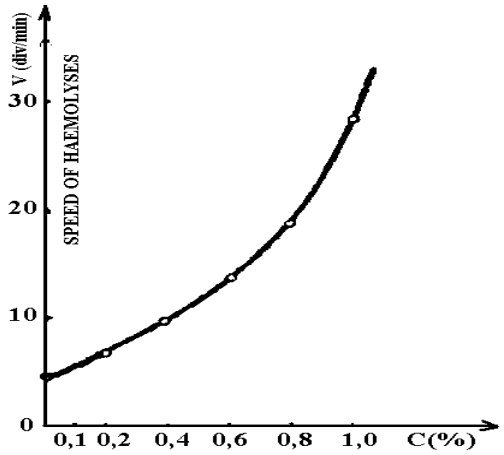

Fig. 5. The effect of saponina concentration on hemolysis speed.

CONCLUSIONS

The light scattering effect in biological substances with elaborated methods, has a various application in pharmacology practice and can be used with successes by biological purity control of pharmaceutical emulsions, by study of drags effects upon osmotic transport at level of cell membrane, or by analysis of some vegetal extracts.

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