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EMULSIFYING PROPERTIES OF PROTEINS AND POLYSACCHARIDES I. METHODS OF DETERMINATION OF EMULSIFYING CAPACITY AND EMULSION STABILITY

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ABSTRACT

A new method of determination of the emulsifying characteristics of protein and some other substances, based on a study of the dependence of the nonpolar emulsified phase quantity at the phase inversion point upon the emulsifier quantity, is proposed. Emulsifying characteristics of proteins under the conditions where the amount of oil in emulsion is lower than necessary for phase inversion may be compared by means of the diagrams plotted in the coordinates of "a separated phase fraction—initial nonpolar phase fraction" (emulsion stability diagrams). Some changes in the terms denoting emulsifying characteristics are proposed.

INTRODUCTION

Functional properties of proteins and some other nutritional substances are complex characteristics evaluated in model systems and describe the ability of the substances to fulfil certain structural functions in real nutritional systems (produced or ready for use) [1]. In this paper we shall primarily deal with the functional properties of proteins, among which emulsifying characteristics, namely, emulsifying capacity and emulsion stability, are of much importance, describing protein behaviour in a wide range of nutritional systems.

By emulsifying capacity (EC_a) is implied the ratio of an oil quantity emulsified under specified conditions at the phase inversion point to a protein quantity in the system. The concept of emulsifying capacity was originally introduced by Swift et al. [2]. Later a number of workers [3—6] investigated the influence of other factors, such as the equipment design, oil addition rate and blender speed, temperature, pH, ionic strength of the solution, on the EC_a value of different proteins. EC_a was shown to depend, to some extent, upon these parameters. This led the authors [3—6] to think that EC_a of proteins may serve as a comparative characteristic of their emulsifying properties, only when it is determined under the same conditions, for the

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same nonpolar phase, with the same system parameters and emulsifying regime. Other workers [7] concluded that EC_a should be determined under conditions optimized for all relevant parameters. Optimization of all parameters may, however, be very difficult.

Thus with existing methods EC_a may not be used as a standard characteristic for direct comparison of the emulsifying properties of different proteins or of results obtained by different authors [1].

MATERIALS AND METHODS

Determination of emulsifying capacity (or activity of emulsifier)

The phase inversion point was determined by a sharp fall of the electric conductivity during titration of 5 ml aqueous solution of protein or polysaccharide with a nonpolar phase. Emuslification was performed in a cylindrical vessel. For each nonpolar phase its addition rate (4.6 ml/min) and blender speed (3,400 ± 150 r.p.m.) were adjusted so that determined characteristics remained practically unaffected by them.

Evaluation of emulsion stability

The procedure was as follows. To a protein, polysaccharide or their complex solution, some amount of a nonpolar phase was added so that the total volume was 25 ml. The system was emulsified in a square-sectioned vessel for 3 min at the blender speed of 3,000 ± 150 r.p.m. The choice of time and speed was dictated by the above discussed considerations. Then emulsion was transferred into a measuring test tube, 25 ml in volume, thermostatted for 1 h at 25°C, then the volumes of separated aqueous and nonpolar phases were measured. By the end of thermostatting the phase separation was practically over. These data were used to plot a stability diagram (Fig. 3) laying off as abscissa the initial volume fraction of the nonpolar phase and as ordinate, on the left and on the right, respectively, volume fractions of the separated aqueous and nonpolar phases. In the case of full decay of the emulsion, the experimental points lie at the diagram diagonal.

Bovine serum albumin (BSA) and ovalbumin (OA) of the Oline plant of chemical reagents (USSR), sodium salt of dextran sulphate (DS), and beet pectin of the Krasnodar pectin plant (USSR) were used in the work.

Decane and cotton oil were used as a nonpolar phase.

RESULTS AND DISCUSSION

1. General approach to the determination of emulsifying capacity

Emulsifying capacity is an important characteristic. It is usually determined from the equation

$$EC_a = F/P \qquad (1)$$

where EC_a is apparent emulsifying capacity, F is maximum amount of emulsified oil, P is protein quantity in the system [2]. F is determined by phase inversion when a nonpolar phase is continuously added to an aqueous phase; that is, a protein solution or suspension of a definite concentration, the arbitrarily chosen protein concentration remaining constant for all proteins under comparison [8—12], whether the protein is fully or partially dissolved. In our opinion, this invalidates the method, inasmuch as F would depend on protein concentration, i.e., the latter would be determinant for the EC_a value, and in addition F dependence on P may vary from protein to protein. Moreover, EC_a value of a partially dissolved protein is not indicative of its emulsifying properties; as the stabilizing effect would, presumably, depend upon the properties of both the dissolved protein and its suspension, dissolved protein fraction and several other, generally uncontrolled factors (e.g., degree of suspension dispersity). It may be noted that the correlation between solubility and EC_a of proteins reported in [13, 14] may be accounted for by the fact that EC_a depends primarily upon dissolved protein quantity in suspensions under study.

Considering Eq. (1) again, it may be noted that if F is determined in a dynamic regime (i.e., when the fact of emulsion existence is established during the emulsification process), then

$$F_{P\,=\,0} = F_O \neq 0.$$

In other words,

$$F = F_P + F_O,$$

where F_P is nonpolar phase quantity at the phase inversion point additionally emulsified by the protein action. Fig. 1 shows a scheme of the expected F dependence upon P (or protein concentration, C_P). F_{max} reached at some $P = P^*$ (see Fig. 1) may be accounted for by the fact that generally F cannot increase unlimitedly with an increase of P in the system. In the case of a linear F growth with P (or C_P) at $P < P^*$ emulsifying capacity, EC, can be easily determined from the equation

$$EC_a = \frac{F}{P} = \frac{F_P}{P} + \frac{F_o}{P} = EC + \frac{F_o}{P} \qquad (P < P^*) \qquad (2)$$

where it is assumed that, similar to EC, F_o is constant. The assumption of EC independence of the emulsifier quantity is apparently based on the assumption that emulsion dispersity and the structure of the adsorption layer are invariable. It may be rewritten in a more practicable form:

$$F = EC \cdot P + F_o \qquad (P < P^*) \qquad (3)$$

According to eq. (3), EC is plotted as the slope of a linearly increasing part of the diagram, $F = f(P)$. It may be rewritten in a more general form for the

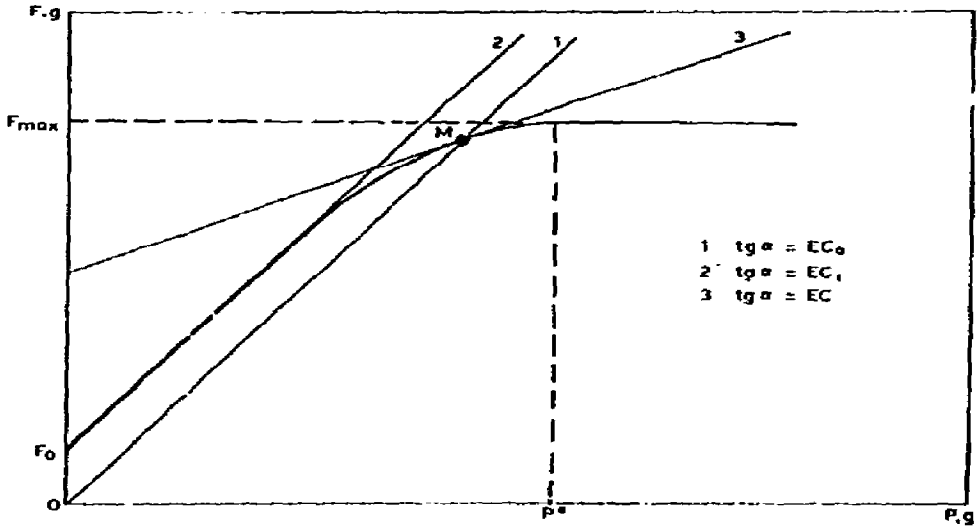

Fig. 1. The determination of emulsifying capacity (*EC*), saturation concentration (*P) values and emulsification limit (F_{max}).**

non-linear F dependence on P:

$$EC = \frac{dF}{dP} \qquad (P < P^*) \tag{4}$$

(the tangent slope at some point M, Fig. 1).
Since generally EC may depend upon the quantity (concentration) of the emulsifier also when $P < P^*$, it would be reasonable to introduce the concept of intrinsic emulsifying capacity, namely

$$EC_i = \lim_{P \to 0} \frac{dF}{dP} = \lim_{P \to 0} EC \qquad (P < P^*) \tag{5}$$

(see Fig. 1).

Experimentally obtained F dependence on P in coordinates specified above, for two proteins, pectin and BSA-DS complex, is shown in Fig. 2. We can see that at sufficiently low concentrations of emulsifier, EC, determined from eq. (4) is actually independent of the latter parameters. We can also see that for ovalbumin (cotton oil emulsion) the F—P dependence plot has the same shape as that in Fig. 1. For BSA, however, abnormal F—P dependence is displayed for reasons so far unknown. Perhaps the abnormality arises from emulsion dispersity and/or emulsifier concentration-dependent structural rearrangements in the adsorption layer. At any rate it proves that EC should not be determined for a single arbitrary concentration of the emulsifier but should rather be determined from eq. (5).

Thus emulsifying capacity (the intrinsic one included) under given condi-

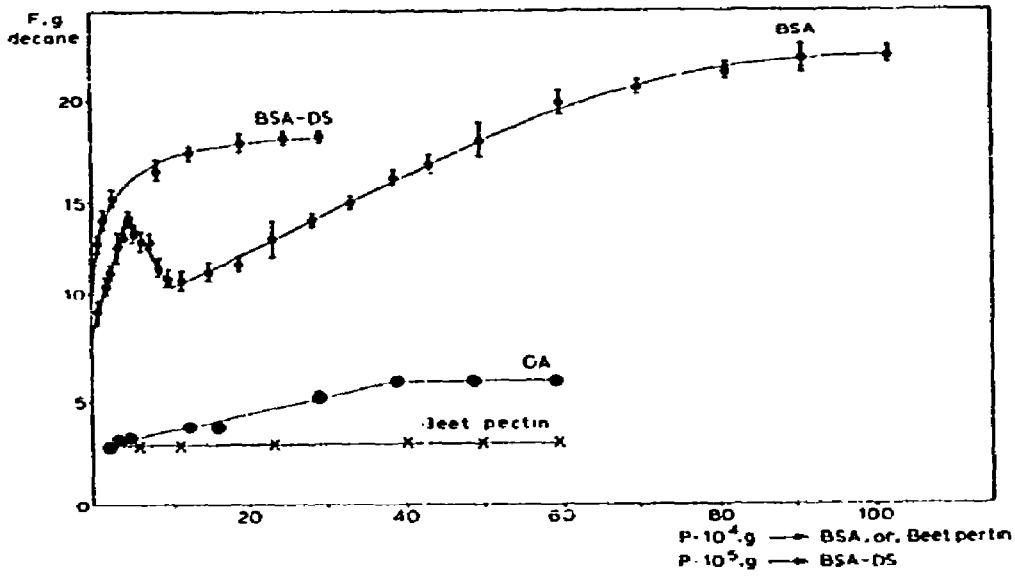

Fig. 2. Dependence of emulsified decane quantity at the phase inversion point (F) upon emulsifier quantity (P).

tions may be evaluated only on the basis of F (or EC_a) dependence upon the concentration, and only at $P < P^*$. For example, P^* value for BSA (decane emulsion) under the experimental conditions was 0.01 g. At higher quantities of the protein EC estimation has no significance. P^* and F_{max} values (we shall refer to them as "saturation concentration" and "emulsification limit," respectively) may also be regarded as emulsifying characteristics of the dissolved substance.

2. *"Emulsion stability diagram" method*

Characteristics of emulsion that contain a nonpolar phase in quantities smaller tha required for phase inversion, can be investigated as exemplified by "emulsion stability" (ES) and "emulsifying activity" (EA). ES is usually expressed as a fraction of oil separated from the emulsion during a given period of time at constant temperature and gravitational field [15—18].

Essentially, EA was evaluated in a similar way. (For example, Iasumatsu et al. [14].) Franzen and Kinzella calculated emulsifying activity as "height of emulsified layer divided by height of total contents in the tube after centrifugation at 1300 g for 5 min" [19].

Further, in this work, a method of evaluation of emulsion stability (ES) as a function of the initial nonpolar phase fraction (Q) at constant total volume, is used. In the diagram in Fig. 3 a nor polar phase volume fraction, p.c., is plotted as abscissa, while aqueous and nonpolar phase volume frac-

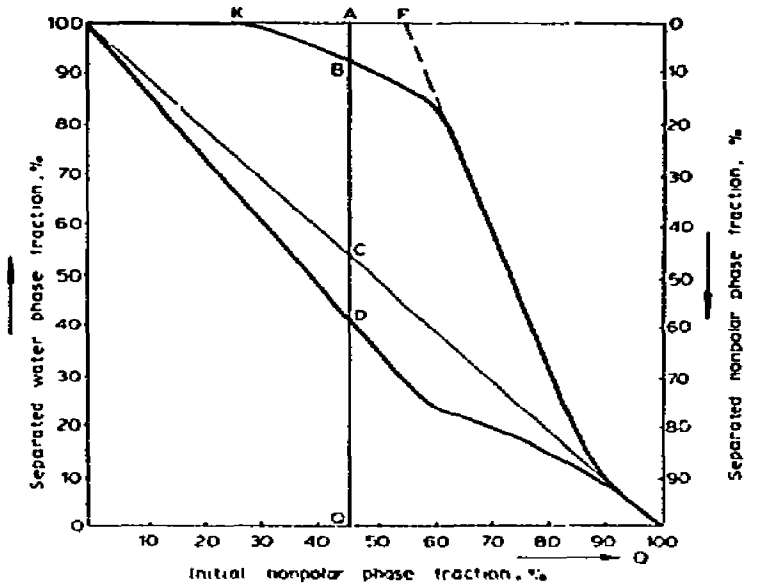

Fig. 3. Emulsion stability diagram.

tions, separated under given experimental conditions, are plotted as ordinate, at the left and at the right, respectively. Emulsions with various Q were transferred into measuring test tubes and thermostatted at 25°C till phase separation was practically complete. Then volumes of the separated aqueous and nonpolar phases were measured, thus two points for one vertical section of the diagram were obtained. Quite clearly, upon full emulsion decay there will be one point lying at the diagonal (Fig. 3). This figure gives an example of section AQ (for $Q = 46$), BC/AC gives ES, while CD/CQ describes flotation stability (FS) of emulsion of composition Q. The lines drawn across experimental points define free phases and emulsion regions. For example, for a decane water emulsion stabilized with BSA, $ES = 100\%$, until $Q = 30\%$. At $Q > 30\%$ partial decane separation occurs, at $Q = 55\%$ a sharp decrease of the emulsion quantity takes place. Diagrams like that depicted in Fig. 3 enable the following quantitative characteristics of emulsions to be obtained:

(a) Q value at which separation of a nonpolar phase occurs (point K in the diagram).

(b) Q value, above which emulsion stability decreases rapidly (point F in the diagram).

(c) Volume fraction of the nonpolar phase in a concentrated emulsion. This is determined by BC/BD.

(d) For each section of the diagram, ES and FS are determined as unseparated aqueous and nonpolar phase fractions of their total amount in the system (BC/AC and CD/CQ, respectively). For the diagram as a whole, a

relative area corresponding to the unbroken emulsion (S) may serve as a characteristic similar to ES. Likewise, an area corresponding to the unseparated aqueous phase (part of S area under the diagonal) may serve as a measure of flotation stability relevant to all emulsions described by a given diagram and being similar to FS.

3. Some proposals regarding the terminology

As mentioned above, the dynamic method of determination of emulsifying characteristics of nutritional substances, which we propose, led us to introduce special terms for P^* and F_{max}, namely "saturation concentration" and "emulsification limit." Furthermore, the term "emulsifying capacity" applied to dF/dP and $\lim_{P \to 0} (dF/dP)$ values does not reveal their significance. Two other terms: "emulsifier activity" for dF/dP and "intrinsic emulsifier activity" for $\lim_{P \to 0} (dF/dP)$ seem to us more pertinent.

To summarize, we propose the following terms and designations:

$\frac{dF}{dP} = A$, activity of emulsifier,

$\lim_{P \to 0} \frac{dF}{dP} = \lim_{P \to 0} A = A_i$, intrinsic activity of emulsifier,

P^*, saturation concentration

F and F_{max}, emulsification level and limit, respectively.

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