Short communication

A Simple Method of Determination of Partition Coefficient for Biologically Active Molecules

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Abstract. A simple method is presented for the determination of partition coefficient of an effector between water environment and biological material, based on concentration-dependent effects. The method allows the determination of partition coefficients for biological objects such as algae, bacteria and other microorganisms.

Key words: Partition coefficient — Oxygen evolution rate — Algae — Trimecaine — *Chlorella vulgaris*

The understanding of a number of biological processes requires the knowledge of partition coefficients (K_p) determining the distribution of an effector between biological material and water environment. Information on this partitioning can be obtained by calculating K_p.

Sedimentation or centrifugation of biological suspensions and measurements of the effector amounts in the supernatant are used for determination of K_p. To evaluate the effector concentration in the supernatant, various physico-chemical methods, including spectrophotometry, conductometry, polarography etc., can be used.

Indirect methods for the determination of K_p, requiring no phase separation procedures, can be divided in three groups.

i. The first group is based on studying changes of some physical or biochemical parameters of the biological material in dependence on effector concentration, using various amounts of biological material. Several authors have used this approach by following depression of phase transition temperature of phospholipid membranes (Kaminoh et al. 1988; Inoue et al. 1990; Gallová 1993) or changes of ultrasonic velocity in them (Babincová and Hianik 1994). Other authors estimated K_p by studying concentration-dependent properties of phospholipid membranes using molecular probes (Ondriaš et al. 1983; Lissi et al. 1989; Šeršeň et al. 1989). Another approach for the determination of the number of binding sites of an effector in chloroplasts was reported by Izawa and Good (1965) and Tischer and

Strotmann (1977) who exploited changes in IC_{50} values (i.e. effector concentrations in chloroplast suspensions inducing 50% inhibition of Hill reaction rate with respect to untreated controls) at various chloroplast concentrations.

ii. The second group of methods is based on the observation of spectral changes of an effector upon changing its concentration in the biological material (Welti et al. 1984; Balgavý et al. 1992).

iii. Also, the partition coefficient of an effector can be determined in the aqueous phase of biological suspensions, from the depression of its freezing temperature (Hill 1974; Vanderkooi et al. 1977).

In this report a simple method is presented for the determination of water/biological material K_p, using concentration-dependent inhibitory effect of trimecaine on oxygen evolution rate (OER) in the algal suspension of *Chlorella vulgaris*.

Trimecaine (2-diethylamino-2′, 4′, 6′-trimethylacetanilidinium chloride) was obtained from Slovakofarma (Hlohovec, Slovakia), and was used without further purification.

The OER of algae was measured by a Clark type electrode (SOPS 31 atp., Chemoproject Prague) in a chamber prepared according to Bartoš et al. (1975) at 24 °C. The composition of the applied algal medium is described in Sidóová et al. (1992). Irradiation (ca 100 W/m^2) of the algal suspensions was carried out with a 250 W halogen lamp through a water filter. The algal suspension was accomodated in the dark for 4 h prior to OER measurements.

The relation between chlorophyll (Chl) content and the volume of algal cells was determined by centrifugation. The concentration of Chl (Chl_a + Chl_b) was estimated spectrophotometricaly (SPECORD UV-VIS, Zeiss Jena, Germany) according to Inskeep and Bloom (1985) after extraction in N,N-dimethylformamide.

K_p calculations are based on the assumption that effector concentrations in algal cells having the same inhibitory effects on OER must be equal, independent of the amount of algae in the suspension.

The formalism for the calculation of K_p starts from the definition

$$K_p = \frac{C_a}{C_w} = \frac{N_a \cdot V_w}{N_w \cdot V_a} \tag{1}$$

where C_i are concentrations, and N_i the numbers of the effector molecules in algal cells or water, and V_i are volumes of algae and water. The indices i denote the algal (a) and aqueous (w) phase, respectively. Taking into account that $N_t = N_a + N_w$ (N_t is the total number of effector molecules in algal suspension) and using mathematical procedures, the N_a or (C_a) can be expressed as follows

$$N_a = \frac{N_w \; V_a \; K_p}{V_w} \tag{2}$$

$$N_a = N_t \frac{V_a \; K_p}{V_w + V a \; K_p} \tag{3}$$

$$C_a = C_t \frac{V_t \; K_p}{V_w + V_a \; K_p} \tag{4}$$

It is evident that the effector concentrations in algal cells must be equal (i e $C_{a1} = C_{a2}$) at two different amounts of algae showing the same inhibitory activity of the effector (e g at IC_{50}), and K_p may be calculated as follows

$$K_p = \frac{C_{t1} \; V_{t1} \; V_{w2} - C_{t2} \; V_{t2} \; V_{w1}}{C_{t2} \; V_{t2} \; V_{a1} - C_{t1} \; V_{t1} \; V_{a2}} \tag{5}$$

Provided that the total volumes V_{ti} in all events are constant ($V_t = 1$ ml) and $V_w \gg V_a$ (this condition is met in our experiments because the algal concentrations employed are lower than 65 mg Chl/l, which corresponds to $9\,1 \times 10^{-3}$ ml of algal volume), whereby $V_{w1} = V_{w2} = V_t$, K_p can be expressed as follows

$$K_p = \frac{(C_{t1} - C_{t2}) \; V_t}{C_{t2} \; V_{a1} - C_{t1} \; V_{a2}} \tag{6}$$

If $V_{a2} = 0$ and $C_{t2} = C_{t0}$, eq (6) can be modified to

$$K_p = \frac{(C_{t1} - C_{t0}) \; V_t}{C_{t0} \; V_{a1}} \tag{7}$$

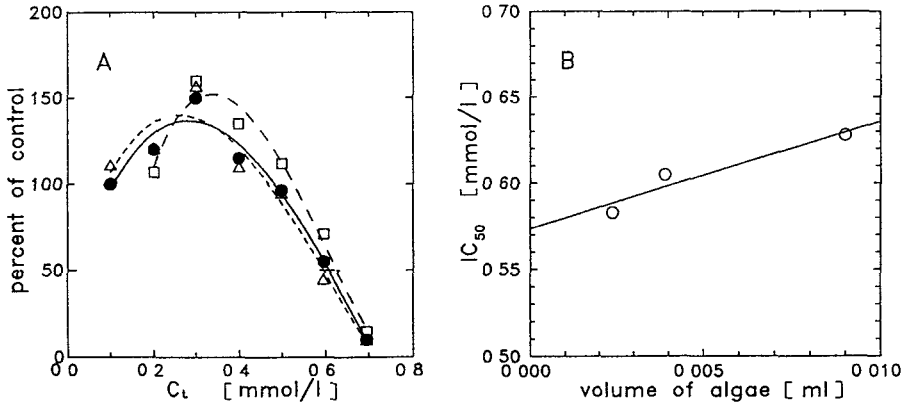

Figure 1. *A* The dependences of OER in algal suspension upon trimecaine concentration at various amounts of algae Squares, 64 1 mg Chl/l (long dashes), filled circles, 28 1 mg Chl/l (full line), and triangles, 17 1 mg Chl/l (short dashes) *B* The dependence of IC_{50} for trimecaine upon algal volume in suspension

After substitution of C_t by IC_{50} (the IC_{50} values were read from Fig. 1*A* at intersections of the horizontal dotted line with the experimental curves of the OER dependences upon effector concentrations), the K_p can be calculated by

$$K_p = \frac{V_t \cdot IC_{50}}{[IC_{50}]_0 \cdot V_{a1}} \tag{8}$$

where $[IC_{50}]_0$ is the itercept with the ordinate, and IC_{50}/V_{a1} is the slope of the dependence of IC_{50} on algal volume (Fig. 1*B*).

Using centrifugation it was found that the algal suspension containing 1 mg Chl represents algal volume of 0.14 ml. By applying this Chl content-algal volume relation the partition coefficient for trimecaine $K_p = 10.9 \pm 3.3$ was calculated, using the data presented in Fig. 1, according to formula (8). In parallel, the value of $K_p = 9.8 \pm 2.5$ was determined by the centrifugation method using changes of trimecaine absorbance at 263 nm in the supernatant. It is obvious that the K_p values obtained by both methods are in a good accordance.

The present work shows that measurements of a certain quantitative property which can be immediately evaluated as the function of effector concentration (e.g. oxygen evolution or CO_2 consumption in photosynthesis of algae, CO_2 evolution or oxygen consumption in other microorganisms) can in general be used to determine K_p for living biological objects such as algae, bacteria or other microorganisms. If, for some reason, the phase separation method cannot be used, the above method of K_p determination can be applied.

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