Discussion Letter

On the relation between surface area and partitioning of particulates in two-polymer aqueous phase systems

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Partitioning in dextran-poly(ethylene glycol) aqueous two-phase systems is an established method for the separation of biomaterials. Size and surface properties are generally regarded as parameters which contribute to the determination of the materials' partition coefficients, K. While molecular weight or surface area can be one of the determinants of the K value of biomaterials in the size range of macromolecules to very small particulates (e.g. some viruses), partitioning liposomes of identical surface properties and different but distinct sizes indicate that surface areas greater than about 0.2 μm^2 do not affect the K value obtained. Analysis of available partitioning data of much larger particulates (i.e. cells) reveals that surface properties per unit area outweigh surface area per se in determining the K value in non-charge-sensitive, charge-sensitive and biospecific affinity phase systems.

Liposome; Cell; Aqueous phase system; Partitioning; Surface area; Surface property

1. INTRODUCTION

Partitioning in dextran-poly(ethylene glycol) aqueous two-phase systems is an established, sensitive method for the fractionation of biomaterials and for obtaining information on their surface properties [1-3]. Depending on the concentrations of polymers and on the ionic composition and concentrations chosen the phase systems can have appreciably different physical properties [4]. Thus the incorporation of some salts (e.g. alkali phosphates) gives rise to a Donnan potential between the phases (top phase positive) which are deemed charge-sensitive; while with others (e.g. alkali halides) there is virtually no potential difference (non-charge-sensitive phases). Increasing the polymer concentrations increases the interfacial tension between the phases, makes the phases more dissimilar and causes the partitioning to become more one-sided [2,5]. Incorporating a polymer-ligand [e.g. a PEG-ligand such as the ester of poly(ethylene glycol) and palmitic acid, poly(ethylene glycol)-palmitate] which tends to partition into one of the phases (in the present case, the top, PEG-rich phase) can be used to specifically extract biomaterials which react with the ligand (affinity partitioning).

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The mechanism of partitioning is complicated [2,5] but, according to Albertsson's generalized application [2] of the Brønsted equation, the partition coefficient, K, which is C_T/C_B, depends on $e^{\lambda M/kT}$, where C_T and C_B are the partitioning material's concentration in the top and bottom phases, respectively, M is the molecular weight, k the Boltzmann constant and T the absolute temperature. λ is a factor which depends on properties other than molecular weight (i.e. interactions between the surface properties of the material and the two phases). Albertsson has further proposed that for particulates M should be replaced by A, the particle's surface area, in the above equation [2]. Thus size and surface properties determine the partitioning behavior of biomaterials and do so in a most sensitive manner since these parameters are *exponentially* related to the partition coefficient.

While the appropriateness of applying the Brønsted equation to partitioning phenomena has recently been called into question [6] the suggested formulation is also a Boltzmann-type expression (i.e. one in which an exponential relation exists between parameters contributing to the partitioning behavior and the partition coefficient) and includes a surface area term.

Albertsson has shown experimentally that a linear relation pertains between log K and the surface area of a number of proteins and virus particles in a dextran-methylcellulose system [7,8]. A variety of non-heme

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proteins (but not heme proteins), at their isoelectric points, were similarly found to have decreasing K values with increasing molecular weight in dextran-poly(ethylene glycol) phases [9]. For DNA, log K is proportional to molecular weight (and charged groups) in dextran-methylcellulose [10] as well as in dextran-poly(ethylene glycol) [11] systems.

Although size is regarded as a parameter which contributes to the determination of K values of partitioned biomaterials in general, the experimental data cited relating log K and surface area cover only macromolecular and viral sizes.

Unlike the partitioning of soluble materials which occurs between the two bulk phases the partitioning of particulates takes place between one of the phases and the interface [2,4,5]. That is because particulates have their lowest energy in the interface since the formation of an interface requires energy and the adsorption of particulates to the interface reduces its area. The partition coefficient of particulates, often called the partition ratio, is the ratio of the number of particulates in top phase divided by the number at interface + bottom phase [4].

The transition of partitioning of biomaterials from between two bulk phases to one phase and interface is size dependent. Brook et al. [5] have calculated that adsorption of a particle to the interface would first be expected to occur at a diameter of about 0.03 μm and adsorption would be strong with particles larger than this in phase systems having interfacial tensions of the order associated with a composition of 5% (w/w) dextran T500 (Pharmacia LKB, Uppsala, Sweden): 4% (w/w) poly(ethylene glycol) 8000 (Union Carbide, Long Beach, CA).

Ideally the involvement of size in contributing to or determining the K value of biomaterials is best probed with substances of identical surface properties where size is the only variable. We have here further examined the partitioning behavior of unilamellar liposome populations of identical composition and of different but distinct sizes [12].

Biological cells differ in both size and surface properties and it is thus not possible to conduct an experiment such as the one with liposomes. Yet it remains of interest to assess the relative contributions of different physical parameters to the K value obtained. Pertinent cell partitioning data in the literature, which have never been scrutinized specifically for information on the relationship of surface area to K, were also examined.

2. EXPERIMENTAL

2.1. *Small particulates*

Data in ref. 12, in which two sets of unilamellar liposome vesicles composed of either egg phosphatidylcholine (EPC)/egg phosphatidylglycerol (EPG) or EPC/egg phosphatidic acid (EPA) (each 6:4 mole ratio) and having measured diameters of approximately 0.05, 0.10, 0.20, 0.30 or 0.4–0.5 μm were prepared by repeated extrusion of multilamellar vesicles through polycarbonate filters and partitioned in dextran-poly(ethylene glycol) aqueous phase systems, were further analyzed (see Fig. 1 and below).

2.2. *Large particulates*

Data in [13–16] were examined (see below) for what they reveal about the involvement of size (or surface area) in contributing to the K value of cells (erythrocytes).

3. RESULTS AND DISCUSSION

It is apparent from Fig. 1 that there is a marked decrease in log K with increasing vesicle surface area with smaller vesicles (up to about 0.1 μm^2), as previously indicated [12]; while with larger vesicles virtually the same log K value is obtained irrespective of size.

When erythrocytes from different species, which range in volume from about 35 to 135 μm^3 [17,18], are partitioned in charge-sensitive phases at some distance from the critical point (i.e. at higher polymer concentrations) there is a reasonable correlation between log K and the cells' relative electrophoretic mobilities [13]. The electrophoretic mobility of cells depends on the charge per unit area and is not dependent on the size, shape or orientation of the particle being measured [19].

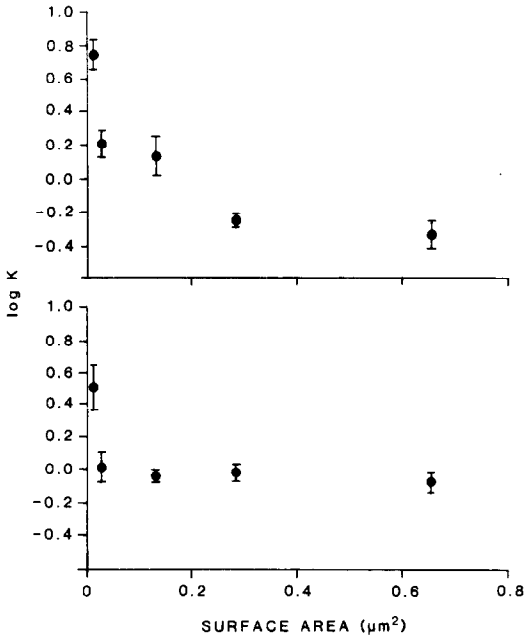

Fig. 1. Relationship between log partition coefficient (K) and surface area of unilamellar liposome populations. Two sets of vesicles were prepared each of identical surface properties and of different but distinct sizes [12]. Vesicles of EPC/EPG (6:4) (top) and EPC/EPA (6:4) (bottom), radioactively labeled for ready analysis of K, were partitioned in 5% dextran T500 (Pharmacia LKB, Sweden): 5% (w/w) poly(ethylene glycol), 6000 (BDH, UK) containing 0.11 M Na-phosphate buffer, pH 6.8, and sampled at 25 min. Given are log K values (defined as the ratio of number of vesicles in top phase/number of vesicles at interface + bottom phase) ± SEM for 4–5 determinations. (See text and [12] for details.)

When erythrocytes are partitioned in non-charge-sensitive phases nearer the critical point, an excellent correlation exists between the cells' log K value and their membrane *ratio* of poly/monounsaturated fatty acids [14], a parameter which does not pertain to total surface area.

When different species' red blood cells are partitioned in a dextran-poly(ethylene glycol) system (with NaCl) at some distance from the critical point and also containing poly(ethylene glycol)-palmitate, the partitioning depends on the cells' hydrophobic interaction with the palmitoyl ligand. A correlation between the cells' K values and the ratio described for the non-charge-sensitive phases above is again in evidence [16]; some correlation is also observed between the cells' K values and their membrane's relative percentages of phosphatidylcholine and sphingomyelin, being higher with the former and lower with the latter [13,14]. The latter parameter is again one that is not dependent on total cell surface area.

Microscope studies on the interaction between cells and phase droplets after mixing and during the partitioning process show that the relative avidity of red blood cells from different species for adsorption on the surface of droplets of one phase suspended in the other is species-specific but not cell-size dependent [20].

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