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Development of a dynamic delivery method for in vitro bioassays

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ABSTRACT

Measuring the biological activity of hydrophobic chemicals using in vitro assays is challenging because their aqueous solubility is low and the high density of bio-suspensions strongly decreases the bioavailability of hydrophobic pollutants. Dynamic dosing by partitioning from a stable polymer has a potential to overcome these limitations. Poly(dimethylsiloxane) (PDMS) was chosen due to its documented bio-compatibility and excellent partitioning properties. PDMS sheets were loaded with five polycyclic aromatic hydrocarbons (PAHs) and then immersed in model bio-suspensions composed of membrane vesicles ("chromatophores", composed of 30% lipids and 70% proteins) isolated from the photosynthetic bacterium *Rhodobacter sphaeroides* or phospholipid bilayer vesicles (liposomes) composed of palmitoyl-oleoyl phosphatidylcholine (POPC). Method development included the determination of partition coefficients between chromatophores or liposomes and water, desorption rate constants from PDMS to bio-suspensions, and diffusion resistances in both PDMS and bio-suspensions. The release of the PAHs from the PDMS into the bio-suspensions was measured and modeled as a combination of diffusion in pure water and diffusion in a completely mixed solvent composed of water and bio-suspensions. The mass transfer resistance for the release was lower in the PDMS than in the tested solutions, which demonstrates that PDMS can efficiently deliver PAHs even to dense biosuspensions. The contribution of aqueous diffusion to the mass transfer decreased with increasing hydrophobicity of the PAHs indicating that hydrophobic chemicals are efficiently transported with suspended biomaterial. The passive dosing system is versatile and offers a number of applications. Promising are tests with instantaneous response, where the time-dependent effect can be translated to concentration-effect curves but the system is also applicable for assuring constant dosing for longer-term testing.

1. Introduction

In vitro bioassays are promising tools for screening of biological activity of chemical pollutants but they often lack sensitivity as compared to in-vivo testing (e.g., Schirmer et al., 2008). Chemicals are typically administered in the assay medium by dissolving them directly into the solution or by adding them as dissolved in co-solvents. Depending on the chemical properties, it may become challenging to maintain constant exposure condition in the assay medium during the course of the experiment. For example, volatile compounds can be lost during the experiment and lipophilic chemicals can bind onto the plastic surfaces of the plate and into cellular matrices (Schirmer et al., 1997; Hestermann et al., 2000; Heringa et al., 2004; Gülden and Seibert, 2005; Schreiber et al., 2008). In addition, in many cell-based bioassays, the presence of large amounts of serum protein is necessary, which has a high sorptive capacity for hydrophobic chemicals and therefore decreases the bioavailable concentration in a given assay system (Heringa et al., 2003; de Bruyn and Gobas, 2007). Although solubility of hydrophobic chemicals may be enhanced by co-solvents (Bendels et al., 2006), the presence of co-solvents may interfere with biological activity by changing the conformation and mobility of proteins (Makhatadze and Privalov, 1995; Fadnavis et al., 2005). Therefore, it would be ideal to introduce chemicals into the assay medium without using co-solvents.

Partition controlled delivery (partitioning driven administration or passive dosing) is a new experimental technique without organic co-solvents (Mayer et al., 1999; Brown et al., 2001; Mayer and Holmstrup, 2008). Stable polymers such as poly(dimethylsiloxane) (PDMS), containing hydrophobic organic chemicals, can be placed in the assay medium and compounds are passively delivered from PDMS to the assay medium by thermodynamic partitioning. Because partitioning processes between solution and dissolved

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humic acids (e.g., Schlautmann and Morgan, 1993) or liposomes (e.g., Cócera et al., 2001) are known to be extremely fast, it is reasonable to assume that the diffusion in the aqueous boundary layer at the polymer–solution interface is the rate-limiting step of the overall dosing process. Using this passive dosing technique, different concentrations for a dose–response determination may be obtained by exposing the assay medium to loaded polymer for different times. With longer exposure time, more chemical will have desorbed into the assay medium, leading to a higher concentration. Time required for reaching equilibrium between the dosing phase and assay medium depends on physico-chemical properties of chemicals as well as the dimensions of the experimental set-up (Brown et al., 2001; Kramer et al., 2007; Kwon et al., 2007b; Jahnke et al., 2008).

Theoretical prediction of mass transfer is essential to enhance the applicability of this emerging method. In order to provide theoretical interpretation of the new dosing technique, we investigated how fast chemical substances migrate from PDMS to the assay medium. Five polycyclic aromatic hydrocarbons (PAHs) with various molecular size and hydrophobicity were chosen as model hydrophobic compounds. We used membrane vesicles of approximately 100 nm diameter as surrogates for any type of biomaterial in an in-vitro assay. Two types of membrane vesicles were used: liposomes composed of phosphatidylcholine, which are pure phospholipid bilayer vesicles, and isolated bacterial membrane vesicles called "chromatophores" composed of approximately 30% membrane lipids and 70% proteins (Escher et al., 1997).

Liposome–water and chromatophore–water partition coefficients were obtained using a PDMS depletion method (Ter Laak et al., 2005) and literature PDMS–water partition coefficients (K_{PDMSw}) (Kwon et al., 2007b). Apparent mass transfer resistances in the PDMS phase were directly measured and mass transfer resistance in the suspension was derived from these data and the measured overall resistance of desorption. Three simple kinetic models were evaluated based on the experimental data to explain time–concentration profile of test chemicals in the model assay media for a bioassay.

2. Theory

The two-compartment mass transfer model comprised of PDMS and the suspension of assay medium containing biomaterial is described as (Kwon et al., 2007b):

$$\frac{dC_{sus}}{dt} = k_d \frac{V_{PDMS}}{V_{sus}} C_{PDMS} - k_a \frac{V_{PDMS}}{V_{sus}} C_{sus} \quad (1)$$

$$\frac{dC_{PDMS}}{dt} = k_a C_{sus} - k_d C_{PDMS} \quad (2)$$

where C_{PDMS} (mol m^{-3}_{PDMS}) and C_{sus} (mol $m^{-3}_{suspension}$) are concentrations of a chemical in PDMS and the assay medium, V_{PDMS} and V_{sus} are the volumes of PDMS and the suspension (m^3), k_d is the desorption rate constant (s^{-1}) and k_a is the absorption rate constant (s^{-1} $m^{-3}_{suspension} m^{-3}_{PDMS}$). If the partition coefficient between PDMS and the assay medium, $K_{PDMSsus}$ ($m^{-3}_{suspension}$ m^{-3}_{PDMS}), has been independently measured, k_a should be equal to $K_{PDMSsus}$ k_d:

$$\frac{dC_{sus}}{dt} = k_d \frac{V_{PDMS}}{V_{sus}} (C_{PDMS} - K_{PDMSsus} C_{sus}) \quad (3)$$

$$\frac{dC_{PDMS}}{dt} = k_d (K_{PDMSsus} C_{sus} - C_{PDMS}) \quad (4)$$

If no chemical is initially present in the medium (i.e., $C_{sus}(t = 0) = 0$), the analytical solutions of Eqs. (3) and (4) are:

$$C_{sus} = \frac{C_{PDMS,0} \frac{V_{PDMC}}{V_{sus}}}{1 + K_{PDMSsus} \frac{V_{PDMS}}{V_{sus}}} \left[1 - \exp\left(-\left(K_{PDMSsus} \frac{V_{PDMS}}{V_{sus}} + 1\right)\right) k_d t\right] \quad (5)$$

$$C_{PDMS} = \frac{C_{PDMS,0}}{1 + K_{PDMSsus} \frac{V_{PDMS}}{V_{sus}}} \left[K_{PDMSsus} \frac{V_{PDMS}}{V_{sus}} + \exp\left(-\left(K_{PDMSsus} \frac{V_{PDMS}}{V_{sus}} + 1\right)\right) k_d t\right] \quad (6)$$

where $C_{PDMS,0}$ is the initial concentration in PDMS (mol m^{-3}_{PDMS}). Detailed derivation for Eqs. (5) and (6) are provided in our previous paper (Kwon et al., 2007b). The desorption rate constant, k_d, is the only parameter needed to be determined to predict time-dependent mass transfer because $K_{PDMSsus}$ can be easily determined in an independent experiment. The overall mass transfer resistance, $R_{overall}$ (m^{-1} s), can be defined as:

$$k_d = \frac{1}{R_{overall}} \frac{A}{V_{PDMS}} \quad (7)$$

where A is the interface area (m^2). Due to the additivity of mass transfer resistances in a simple film diffusion model, we may divide $R_{overall}$ into two terms, R_{PDMS} and R_{sus}, which are mass transfer resistances in PDMS and the assay medium, respectively. R_{PDMS}, can be experimentally determined by directly contacting two PDMS disks and measuring the rate of migration from one to the other (Mayer et al., 2005), while R_{sus} can be obtained by subtracting R_{PDMS} from $R_{overall}$ obtained using Eq. (7) with experimentally determined k_d.

$$R_{overall} = R_{sus} + R_{PDMS} \quad (8)$$

If we assume that diffusion occurs in a finite thickness of boundary layer, R_{PDMS} is

$$R_{PDMS} = \frac{\delta_{PDMS}}{D_{PDMS}} \quad (9)$$

where δ_{PDMS} is the thickness of diffusion boundary layer in PDMS (m) and D_{PDMS} is the diffusion coefficient in PDMS (m^2 s^{-1}). δ_{PDMS} can be regarded as one-half the thickness of a PDMS square if the concentration gradient is linear in PDMS (Ai, 1997; Salaün and Buffle, 2004).

R_{sus} can be characterized by two limiting cases. Since exchange of hydrophobic chemicals between water and small suspended particles (d < 100 nm) is thought to be instantaneous, one may assume that the solute–vesicle complexes are completely labile and the suspension behaves like a solvent mixture such as methanol/water mixture (model I). If the boundary layer can be characterized by mixed solvents, R_{sus} can be regarded as:

$$R_{sus} = \frac{\delta_{sus}}{D_{sus}} K_{PDMSsus} \quad (10)$$

where δ_{sus} is the boundary layer thickness of assay medium and D_{sus} is the effective diffusion coefficient of the solute in the particle suspension incorporating diffusivities of free and associated species to vesicles. Although the thickness of laminar boundary layer is known to be proportional to $D^{1/3}$ (Levich, 1962), it is assumed that δ_{sus} is the same for all selected PAHs because their diffusivities do not differ by a factor of 1.4.

Alternatively, one may assume that the aqueous boundary layer still limits overall mass transfer of hydrophobic chemicals even though the aqueous boundary layer is reduced as compared to pure water by adding bio-suspensions (model II). A solute molecule in PDMS must dissolve in water before it eventually is transferred into bio-suspensions in the bulk. Then, R_{sus} can be determined by the thickness of reduced aqueous boundary layer (δ), aqueous diffusion coefficient (D_w), and partition coefficient between PDMS and water (K_{PDMSw}):

$$R_{sus} = \frac{\delta}{D_w} K_{PDMSw} \quad (11)$$

Note that δ is a conceptual thickness not an actual aqueous boundary layer, therefore the subscript w was omitted to differentiate it