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Continuous-flow microfluidic method for octanol-water partition coefficient measurement

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ABSTRACT

To determine the octanol–water partition coefficient ($\log K_{ow}$ or $\log D$), a microfluidic method is developed to reduce time and coast of analyze. A double *Y* serpentine microfluidic chip is fabricated used soft lithography facilities. To generate a biphasic parallel flow, hydrophilic microchannels walls are generated by oxygen plasma treatment. Epifluorescence microscopy is used to measure the intensity of fluorescence molecules in microchannel. Using microfluidic parallel diluter this intensity show a strong linear correlation with molecule concentration in solution. The equilibrium between phases is reached in less than 5 s. To validate this microsystem, we have determined the $\log D$ of fluorescein natrium molecule. The obtained results compare well with previous measurements using traditional shake-flask or microfluidic drops flow methods, with a relative difference to the literature less than 3%. Rapid microfluidic partition coefficient determination is useful for studying liquid–liquid partition of organic pollutant.

1. Introduction

Determination of partition coefficient of organic species is a widespread approach in order to predict their transport and accumulation in biological [1] and environmental [2] media. Such parameter allows determining their lipophilicity, i.e.i.e. their tendency to be distributed between a non-polar lipid and aqueous phases. A traditional approach for the evaluation of lipophilicity is the determination of the partition of these species between two immiscible liquid phases, octanol and water [3–5]. The fate of pollutants and their partition in different compartments air–water–soil–biotic lipids obeys to a complex balance depending on various parameters among which: (i) their lipophilicity expressed by the partition coefficient of the solute between two immiscible liquid phases such as octanol and water K_{OW}, (ii) their aqueous solubility *S*, (iii) their adsorption coefficient or partition coefficient between soil and water K_{oc}, (iv) their Setschenow constant $K_S^{\text{salt,solute}}$ and finally, (v) the Henry's constant K_H which affects the transfer of compounds between water and air [2,6–8]. On the other hand physiological and pharmacokinetic processes of xenobiotics are also positively related to lipophylicity [9]. In pharmacokinetic context, the partition coefficient K_{OW} of the drug influences its main ADME properties (absorption, distribution, metabolism, and excretion) [10]. In addition, Sandermann et al. [1] has developed a relationship between K_{OW} and partition coefficient $K_{TG/PC}$ (TG: trioleylglycerol; PC; phosphatidylcholine) for more than 20 xenobiotics in order to predict their affinity to biotic layers. Because of the prime importance of K_{OW} in structure-property relationships of organic molecules, several methods for its experimental determination are proposed in literature including reversed-phase high-performance liquid chromatography (RP-HPLC) [4,11–13], titration techniques [14], microemulsion electrokinetic chromatography [15], solid-phase microextraction method [16], flow injection analysis [17] and finally, shake flask methods [2,18]. In addition, K_{OW} can be estimated using fragmental method and group contribution method [19]. However, the static method so-called "shake-flask" is the standardized method for K_{OW} in the case of octanof/water experimental determination. This method is known for its simplicity to express the partitioning process [20,21]. Nevertheless, this approach presents various drawbacks. Indeed, this static method necessitates a large amount of time to reach equilibrium and very often, several days are required in order to complete the experiment. Also, this experiment requires a large amount of solvent and analyte. Moreover, for compounds

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having a high vapor pressure, the static method would lead to inaccurate values of K_{OW} (by excess) due to the volatility during the sampling (from the aqueous phase). Recently, literature has shown an increased interest in the miniaturization of this partition coefficient determination method in order to have a fast, portable, solventless and sustainable device [22,23]. The main advantage of miniaturization is in the reduction of time to carry out these experiments. Indeed, the molecular transport of analyte between two immiscible phases is often controlled by diffusion and it is well known that by decreasing the characteristic length of diffusion, time required to complete the experiment is also reduced [24]. Therefore, the time scale is imposed by the short diffusion length of the analyte across two phases and large specific interfacial area [22]. In this paper, we investigated a microfluidic system where the partition of analyte (fluorescein disodium salt) occurs on-chip between two moving immiscible continuous phases of octanol and water. The originality of using this type of flow is that the two phases are spatially separated. This propriety allows the integration of electrochemical detection that is not possible in droplet flow system [22,23]. For this approach, we used a PDMS/glass microsystem fabricated using soft lithography methods [25]. In order to validate this continuous-flow method, we determined the partition coefficient of fluorescein.

2. Experimental

2.1. Materials

Sylgard 184 Silicone, a two-part poly(dimethylsiloxane) (PDMS) elastomer, was purchased from Samaro, France. Fluorescein natrium molecules were used as received from Aldrich. Solutions of these analytes were freshly prepared with demineralized and distilled water before operating the microfluidic system. Etertec photoresists were purchased from CTS.

2.2. Microchip design

We designed two configurations of microfluidic channels with Y-junctions: (i) straight microchannel shaving widths of 1 mm (200 μm thick), 500 μm (100 μm thick) and 250 μm (100 μm thick) and (ii) a microfluidic contactor system featuring two Y-junctions connected through a serpentine microchannel having a length of 350 mm, a width of 200 μm and a thickness of 50 μm. The first configuration was used to investigate the behavior the two immiscible phases when injected in the microfluidic channels. Indeed, it is well known in biphasic microfluidic that the oil/water interface behavior depends on the ratio of the flow rates of the aqueous and the organic phases and also on the surface properties of the microchannel walls. Therefore, using this geometry, the distance of contact between the two fluids is not sufficient to reach equilibrium of analyte between two phases. For this reason, we replace the straight line shape between the double *Y* inlets and outlets by a serpentine channel shape (Fig. 1). In this final configuration, we used parameters in order to achieve an interface where the two phases flowed in laminar parallel streams in the main channel of the system.

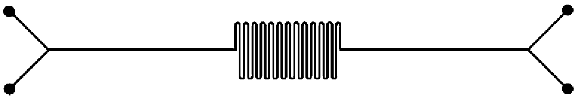

Fig. 1. Schematic representation of the microfluidic channel used for the determination of the partition coefficient between octanol and water.

2.3. Microfabrication

The microfluidic systems were fabricated using soft lithography method as already described elsewhere [25]. Briefly, we first generated a mold using fast and low coast dry film resist photolithography [26]. Molds were prepared from a 50 μm thick dry film photoresist (Etertec) which was laminated on a glass substrate. After irradiation of the film through a photomask (recommended dose ranged from 30 to 76 mJ cm^{-2}), non-irradiated areas were dissolved in a solution containing potassium or sodium carbonate at a concentration of 1% in mass. These two types of substrates, consisting of dry film photoresist on glass or Si, were used as negative molds and were replicated in poly(dimethylsiloxane). After peeling off from the molds, PDMS slab was sealed to a glass slide using oxygen plasma (3 min, 200 mTorr). Native PDMS is hydrophobic (contact angle of water around 110°), but present hydrophilic properties after treatment with O_2 plasma (contact angle of water around 30°). In the same way, water contact angles on glass vary from 60° to 20° after O_2 plasma treatment. We observed that this hyrophylicity of PDMS promoted parallel flow between the two phases. In case of non-oxidized PDMS surface we obtained a droplet of water into octanol flow [22].

2.4. Experimental set-up

The actuation of liquids in microfluidic channels was provided by a syringe pump. Teflon tubing was connected between syringes (containing water and octanol phases) and the inlets of the PDMS microchannels. Behavior of liquid phased in the microfluidic systems was observed employing an inverted epifluorescence microscope using bright field and fluorescence (I3 filter characterized by an excitation band pass between 450 and 490 nm and an emission filter high pass wavelength above 515 nm) modes. Intensity measurements were carried out using a CCD camera. Fluorescein presents seven prototypic forms (Dianion, Monoanion I, Monoanion II, Quinoid, Lactone, Zwitterion and Cation) which make its spectral properties depend on the pH of the medium [27]. Here, we worked at pH7 in order to obtain fluorescein analytes mainly under its dianionic form which is reported to have a strong visible absorption band and intense florescence emission [28].

3. Theoretical basis

Any system containing a molecular species partitioned between two immiscible phases is a closed system (heat and work are exchanged with the outside). However, each phase is considered as an open system: the transport of solute is carried out from one phase to another. During a partition experiment, the transfer of a solute occurs spontaneously from the phase having the highest chemical potential μ to the other phase till chemical potentials in the two phases are equals. Therefore, in the case of an octanol/water experiment, an equilibrium relationship can be considered at the interface Eq. (1).

$$\mu_i^{\mathrm{oct}(T,C_i^{\mathrm{oct}})} = \mu_i^{w(T,C_i^{w})} \tag{1}$$

where $\mu_i^{\varphi(T,C_i^{\varphi})}$ is the chemical potential of solute *i* in the phase φ (oct for octanol and w for water) at the temperature *T* and at solute *i* concentration C_i^{oct} it is in octanol (oct) and C_i^{w} for water (*w*).

The chemical potential of solute in octanol phase is given by the following equation (assuming that the solutions are ideals)

$$\mu_i^{\mathrm{oct}} = \mu_i^0 + \mathrm{RT} \times \ln C_i^{\mathrm{oct}} \tag{2}$$

where μ_i° is the standard chemical potential of solute *i*.