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Zone fluidics for measurement of octanol–water partition coefficient of drugs

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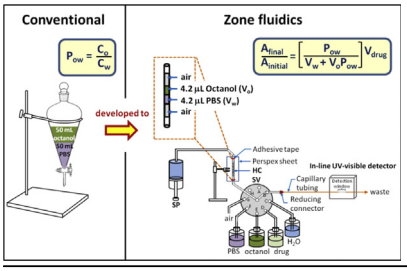
HIGHLIGHTS

- An automatedzone-microfluidic system is presented for screening of octanol–water partition coefficient (P_{ow}).
- Requires onlyone-phase measurement.
- Vertically aligned holding column improves eliminates zone breakage.

GRAPHICAL ABSTRACT



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This paper is dedicated to Prof. Kate Grudpan on the anniversary of his 60th birthday.

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ABSTRACT

A novel zone fluidics (ZF) system for the determination of the octanol–water partition coefficient (P_{ow}) of drugs was developed. The ZF system consisted of a syringe pump with a selection valve, a holding column, a silica capillaryflow-cell and anin-line spectrophotometer. Exact microliter volumes of solvents (octanol and phosphate buffer saline) and a solution of the drug, sandwiched between air segments, were sequentially loaded into the vertically aligned holding column. Distribution of the drug between the aqueous and octanol phases occurred by the oscillation movement of the syringe pump piston. Phase separation occurred due to the difference in densities. The liquid zones were then pushed into the detection flow cell. In this method, absorbance measurements in only one of the phase (octanol or aqueous) were employed, which together with the volumes of the solvents and pure drug sample, allowed the calculation of the P_{ow}. The developed system was applied to the determination of the P_{ow} of some common drugs. The log (P_{ow}) values agreed well with a batch method ($R^2 = 0.999$) and literature ($R^2 = 0.997$). Standard deviations for intra- and inter-day analyses were both less than 0.1log unit. This ZF system provides a robust and automated method for screening of P_{ow} values in the drug discovery process.

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1. Introduction

Drug discovery is a process by which new compounds are evaluated as possible drug candidate. The compound will be tested in the laboratory and in various clinical trials for efficacy and safety. Once a compound has successfully passed these tests, the results are sent to the country's Food and Drug Administration (FDA) for approval prior to introduction into the market [1,2].

In the early stages of the laboratory tests, the lipophilicity of the compound is measured. Lipophilicity is one of the physicochemical parameters used to describe the behavior of a drug in the body, such as absorption, distribution, metabolism and excretion (ADME). It reflects the capability of a drug to permeate cell membrane *via* transcellular pathway or theso-called passive mechanism [3]. Lipophilicity is generally reported in terms of the logarithm of the octanol–water partition coefficient (log P_{ow}). P_{ow} is traditionally determined by measuring the ratio of the concentration of drug partitioned in octanol (C_o) to its concentration in water (C_w) at equilibrium: $P_{ow} = C_o/C_w$. The standard method for determining P_{ow} recommended by the Organization for Economic Cooperation and Development (OECD), is the shake-flask method [4]. In this method the drug sample is added into an immiscible mixture of octanol and water. The mixture is shaken until equilibration is attained. The concentrations of the drug in the two liquid phases are found by measuring the absorbance (or other physical parameters) and comparing them to the respective calibration graphs. However, constructing calibration graph for each phase istime-consuming, which makes the traditionalshake-flask method not a popular method of choice.

Separation techniques such as liquid chromatography [5–7] and electrophoresis [7,8] are often used for indirect estimation of the log P_{ow} value. This technique requires a calibration plot of the capacity factor k' against the log P_{ow} of standard compounds. The log P_{ow} of a drug can thus be found from the measured capacity factor. However, this technique may give rise to incorrect values if the molecular structure of the standard compounds used in constructing the calibration plot is not closely related to the drug. Moreover, these chromatographic techniques require expensive instruments with low sample throughput [9]. This hinders the technique to be used in the stage of drug screening, where many drugs may need to be screened.

Recently many attempts have been developed for measuring the log P_{ow}; for example, parallel analysis on96-well plate format [10,11], frozen water phase method [12], water plug aspiration/injection method [13], use of a dialysis tubing with ultrasonic agitation [14] and use of magneticnano-absorbent method [15]. Although those methods have their particular advantages, they are not used on a regular basis.

In our opinion theshake-flask method is still an appropriate procedure for measuring the log P_{ow} values of drug candidates at the early stage of drug discovery. This is because the method is a direct and simple measurement. The major disadvantages of the conventionalshake-flask method are the volumes of solvent and sample required (tens of milliliters) and consequently a lengthy time to reach equilibrium (10 min or longer). Intensive labor and skill of the scientist are also required to achieve reliable results.

Flow techniques [16] have been applied to help resolving the drawbacks discussed previously. The flow system is amendable to miniaturization and automation. Continuous flow extraction has been developed for determination of log P_{ow} [17,18]. Organic and aqueous phases were continuously introduced into the flow system. Sandwich of the solvent segments was formed sequentially. The compound was partitioned between adjacent segments while flowing along the channel. However, in order to control the dispersion only between two adjacent immiscible segments, segmented flow technique [19] must be employed. With this technique, air bubbles are introduced at the ends of a pair of liquid segments. Dispersion is therefore limited to only this zone. Extraction based on the segmented flow technique requires only a short time to reach equilibration. This is because small volumes of solution are used, leading to large surface contact area to volume ratio. Moreover when liquid zones are maintained between air zones, a Taylor flow pattern is generated. This flow pattern constantly renews the boundary between the two immiscible phases leading to very efficient mass transfer of a compound between the liquid phases [20]. The segmented flow extraction for measuring P_{ow} has been presented [21]. It was reported that equilibrium time was complete within 4 min. However, in that work, extraction was carried out in tubing which was aligned horizontally. After strong shaking the extraction zone was often broken into many discrete segments, leading to irreproducible results.

In this work a zone fluidics system [22] in combination with segmented flow, was developed for determination of P_{ow} of drug. Volumes of the extraction solvents and drug solution were precisely metered in the microliter range. The system consisted of a syringe pump with a selection valve and a vertically aligned holding column fitted on the valve. Solvent and drug solutions were held in the holding column. The solution zones were shaken for distribution of the drug between the octanol and aqueous phases. Since the organic and aqueous phases have different densities, phase separation readily occurred. Measurement of the absorbance of only one of the liquid phase was used in this work. There was no need to constructabsorbance-concentration calibration curves for each liquid phase.

2. Experimental

2.1. Solvent and sample preparation

1-Octanol and phosphate buffer saline (PBS) are widely used as the distribution solvents for study of the lipophilicity of drugs [12,13,15,17,21]. This is because octanol, with a polar head andnon-polar tail, has a molecular structure similar to a phospholipid molecule of cell membrane. PBS with pH at 7.4 simulates the liquid in biological system.

In this work,1-octanol was obtained from Fluka (Switzerland). The PBS was prepared by dissolving 1.74 g of Na_2HPO_4 (Fluka, Switzerland), 0.93 g of NaH_2PO_4 (Fluka, Switzerland) and 8.5 g of NaCl (Merck, Germany) in 1.0 L of deionized water [23]. The pH was adjusted to 7.4 with HCl or NaOH. Equal volumes of the two solvents were saturated with each other by mixing on a shaker (IKA model HS250, Germany) for 24 h. The mixture was then allowed to stand for complete phase separation prior to use. Throughout this work the solvent used in the experiment are always the saturated liquid phases.

Acetaminophen, aniline, caffeine and ibuprofen were selected as representatives of lipophobic drugs, while imidazole and riboflavin were selected as examples of lipophilic drugs. The compounds were all of analytical grade. Stock lipophobic and lipophilic drugs were prepared in1-octanol and PBS, respectively, by dissolving appropriate amount in the solvent.

2.2. Calculation of P_{ow} from absorbance measurements of a single liquid phase

Using a lipophobic drug as an example, a stock solution of the pure drug was first prepared by dissolving a suitable amount in octanol and its absorbance measured at the wavelength of maximum absorbance (the absorbance should be $\leq$1.00 AU). This absorbance was designated as the initial absorbance ($A_{initial}$). For a given volume of drug sample (V_{drug}), applying Beer's law the