Characterization of physicochemical properties of substances using chromatographic separation methods

Natalija Nakov*, Jelena Acevska, Katerina Brezovska, Zoran Kavrakovski, Rumenka Petkovska, Aneta Dimitrovska

Faculty of Pharmacy, Ss. Cyril and Methodius University, Mother Theresa 47, 1000 Skopje, R. North Macedonia

Introduction

Convenient and high throughput methods for characterization of physicochemical properties of substances are highly demanded in modern pharmaceutical research and industry. It is well recognized that the lipophilicity (logP) is correlated to the pharmacokinetic properties of the active pharmaceutical ingredient (API), making this physicochemical property essential for drug candidate screening. The dissociation constant (pKa) is one of the key properties during the drug formulation stage, since pH-solubility, lipophilicity and salt formation are pKa dependent. An accurate estimation of the pKa value for the API and its degradation products/impurities is also important for the analytical method development, providing ruggedness and faster method optimization (Kerns & Di, 2004).

Numerous computational and experimental methods have been exploited for determination of logP and pKa value. The shake-flask method as a gold standard for assessing logP, and potentiometry and spectrophotometry as traditional methods for pKa determination, are time consuming, sensitive to impurities and require larger quantity of pure substance having good water solubility (Reijenga et al., 2013).

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) emerge as a method of choice for characterization of pKa and logP due to several reasons: require small amount of sample, capacity to deal with impure and complex samples, and good level of throughput.

pKa assessment based on RP-HPLC

The RP-HPLC determination of pKa is based on different chromatographic retention time (Rt) of the neutral and ionic form of the substance. The isocratic RP-HPLC approach includes series of isocratic experiments at different pH value of the mobile phase, using at least three different concentration of the organic modifier (Manderscheid & Eichinger, 2003). Several years ago, pH gradient mode was developed to speed up the throughput. This approach was proposed for rapid determination of pKa value in complex mixtures (Wiczling et al., 2006).

The apparent pKa value could be determined from the plot retention factor (k’) versus pH value at a particular organic content concentration using nonlinear regression analysis software (Nakov et al., 2020). The aqueous pKa (at “zero organic solvent”) can be estimated using several mathematical models such as the Yasuda-Shedlovsky linear extrapolation method (Wiczling et al., 2006), or different empirical equations (Angelov et al., 2008; Kazakevich & Lobrutto, 2007). Evasion of the complex aqueous pKa calculation could be obtained
using the solely aqueous RP-HPLC approach (Volna et al., 2017).

**Lipophilicity assessment based on RP-HPLC**

According to solvophobic theory, the $R_t$ in RP-HPLC is governed by lipophilicity, meaning that the $R_t$ is directly related to the compound’s dynamic distribution between the stationary and mobile phase. The lipophilic index is derived from the $R_t$ that is converted to the $\log k'$. Isocratic $k'$ represents a relative scale of lipophilicity. However, the extrapolated $\log k'_{w}$ (at “pure water”) is considered as more representative lipophilic indeces. The $\log k'$ and $\log k'_{w}$ values could be directly correlated to octanol-water $\log P / \log D$ via Collander equation (Liang & Lian, 2015).

In isocratic approach for $\log P$ determination, at least four isocratic mobile-phase rations are necessary to ensure reliability for linear fitting between the $\log k'$ and organic modifier content. The gradient elution approach speeds up the experimental work, but the mathematical calculation are far more complex (Wiczling et al., 2006. Valko, 2016). The gradient $R_t$s are measured and converted to Chromatographic Hydrophobicity Index (CHI) values. The CHI value is the percentage of organic modifier required to achieve equal distribution of the analyte between the mobile and stationary phase. The CHI value is well correlated to $\log P$, allowing estimation of $\log P$ from a single gradient experiment.

In recent years, the use of Immobilized Artificial Membrane (IAM), provide an alternative way to measure lipophilicity and cell membrane permeation (Giaginis & Tsantili-Kakoulidou, 2008). The IAM columns permit the use of solely aqueous mobile phase, leading to directly measurement of $\log k'_{w}$.

**Concluding remarks**

The greater use of the chromatographic principles for the characterization of physicochemical properties demonstrates their utility and predictivity, making these methodologies well recognized by the pharmaceutical industry. The future perspective is directed towards development of more reliable theoretical approaches and high throughput LC methods for characterization of physicochemical properties of substances using biomimetic stationary phases.

**References**

Angelov, T., Vlasenko, A., Tashkov, W., 2008. HPLC determination of pKa of parabens and investigation on their lipophilicity parameters. J. Liq. Chromatogr. Relat. Technol. 31, 188-197.

Giaginis, C., Tsantili-Kakoulidou, A., 2008. Current state of the art in HPLC methodology for lipophilicity assessment of basic drugs - A review. J. Liq. Chromatogr. Rel. Technol. 31, 79-96.

Kazakevich, Y., Lobrutto, R., 2007. HPLC for pharmaceutical scientists, fourth ed. John Willey & Sons, Inc.: Hoboken, New Jersey.

Kerns, E.H., Di, L., 2004. Physicochemical profiling: overview of the screens. Drug Discov. Today Technol. 1, 343-348.

Liang, C., Lian, H., 2015. Recent advances in lipophilicity measurement by RP-HPLC. Trends in Anal. Chem. 68, 28-36.

Manderscheid, M., Eichinger, T., 2003. Determination of pKa values by Liquid Chromatography. J. Chrom. Sci. 41, 323-326.

Nakov, N., Brezovska, K., Karcev, V., Acevska, J., Dimitrovski, A. 2020. Chromatographic and surfactant based potentiometric determination of aqueous dissociation constant of mupirocin. Curr. Anal. Chem. 16, 158-165.

Reijenga, A., Van Hoof, A., Van Loon, A., Teunissen, B., 2013. Development of methods for determination of pKa values. Anal. Chem. Insights 8, 53-71.

Valko, K., 2016. Lipophilicity and biomimetic properties measured by HPLC to support drug discovery. J. Pharm. Biomed. Anal. 130, 35-54.

Volna, T., Motyka, K., Hlavác, J., 2017. RP-HPLC determination of dissociation constant using solely aqueous mobile phase. J. Pharm. Biomed. Anal. 134, 143-148.

Wiczling, P., Kawczak, P., Nasal, A., Kaliszan, R., 2006. Simultaneous determination of pKa and lipophilicity by gradient RP HPLC. Anal. Chem. 78, 239-249.