Characterization of physicochemical properties of substances using chromatographic separation methods

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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

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using the solely aqueous RP-HPLC approach (Volna et al., 2017).

Lipophilicity assessment based on RP-HPLC

According to solvophobic theory, the Rt in RP-HPLC is governed by lipophilicity, meaning that the Rt is directly related to the compound's dymanic distribution between the stationary and mobile phase. The lipophilic index is derived from the Rt 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 $logP\ /\ logD$ via Collander equation (Liang & Lian, 2015).

In isocratic approach for $logP$ 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 Rts 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 $logP$, allowing estimation of $logP$ 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 bacis 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., Dimitrovska, 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., Hlaváč, 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.