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Tadpole toxicity prediction using chromatographic systems

Alejandro Fernández-Pumarega[a], Susana Amézqueta[a], Elisabet Fuguet[a,b,*], Martí Rosés[a]

[a] Departament de Química Analítica and Institut de Biomedicina (IBUB), Universitat de Barcelona, Martí i Franquès 1-11, 08028 Barcelona, Spain
[b] Serra Húnter Programme, Generalitat de Catalunya, Spain

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ABSTRACT

Toxicity has been emulated in tadpole species through chromatographic systems. The parameter studied to evaluate the non-specific toxicity of a compound is the narcosis concentration (C_{nar}), which is defined as the concentration needed for the immobilization of the organism. Because experimental investigation with animals is lengthy, costly, technically difficult, and ethically questionable, there is a great interest in developing surrogate physicochemical systems able to emulate biological systems to obtain the same information in a faster, more economic, and easier manner.

In order to see which chromatographic systems would be able to emulate tadpole narcosis, both, tadpole narcosis data and data in several chromatographic and electrophoretic systems, were fitted to a linear solvation energy relationship (LSER) model. Thus, by comparison of the models it was possible to see which of the chromatographic systems were more similar to the biological one. The physicochemical systems that best emulate tadpole narcosis were an HPLC system based on an immobilized artificial membrane (IAM) column, and two micellar electrokinetic chromatography (MEKC) systems based on sodium taurocholate (STC) and a mixture of sodium dodecylsulphate (SDS) and Brij 35 as surfactants. A system based on a RP18 HPLC column also was selected for comparison because it is a common column in most analytical laboratories.

To establish the models, a set of compounds with known C_{nar} values were analyzed in the chromatographic, and electrophoretic selected systems and, then, the retention factor (k) was correlated to the concentration of narcosis. Statistics showed that the system based on STC micelles was the best to emulate toxicity in tadpoles. The robustness and predictive ability of the developed models were validated.

1. Introduction

Aquatic wildlife is exposed to different chemical pollutants coming from industrial or municipal wastes or from urban or agricultural runoff. Tadpoles, amphibian anuran larvae, are useful models for studying environmental hazards due to their trophic importance, environmental sensitivity, research tractability and impeding extinction [1]. However, the experimental investigation through animal tests is usually a lengthy, costly, and technically difficult process, even ethically questionable. For these reasons, surrogate physicochemical systems capable of estimating toxicity in a faster, more economic and easier manner have become of potential interest. Liquid chromatography and capillary electrophoresis systems offer clear advantages over other physicochemical systems (based on liquid–liquid partition, for example) due to the high level of automation of the techniques, low cost, and low consumption of reagents, among others. Undoubtedly, implementation of physicochemical testing in common practices of toxicity estimation will contribute to more sustainable development of chemicals.

Several authors have proposed linear solvation energy relationship (LSER) equations that can be used to predict the narcotic or the lethal effect of chemical compounds [2–8]. These equations take into account several molecular descriptors related to structural, physicochemical, topological, geometrical, fragment, electrostatic, quantum chemical and thermodynamic parameters of the compounds.

Abraham et al. [2] proposed an equation (Eq. (1)) to estimate the narcotic concentration of compounds to tadpoles through the solvation parameter model (SPM). This model assumes that narcotic and lethal effects are related to the partition of a solute between two phases, and this partition depends on five molecular descriptors:

$$\log\left(\frac{1}{C_{nar}}\right) = 0.582 + 0.770E - 0.696S + 0.243A - 2.592B + 3.343V; \quad (1)$$

* Corresponding author at: Departament de Química Analítica, Universitat de Barcelona, c/ Martí i Franquès 1-11, 08028 Barcelona, Spain.
E-mail address: elifuguetj@ub.edu (E. Fuguet).

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$n = 114,\ \mathrm{SD} = 0.337,\ F = 217$

E represents the excess molar refraction, S is the solute dipolarity/polarizability, A and B are the solute's effective hydrogen-bond acidity and hydrogen-bond basicity, respectively, and V is McGowan's solute volume. The coefficients of the equation are characteristic of the system and reflect its complementary properties to the corresponding solute property. In this case, the most important descriptors are the solute hydrogen bond basicity, that contributes negatively; and the solute volume, which contributes positively. For any partition system, the coefficients of this correlation equation can be obtained by multiple linear regression analysis between the property to be correlated (which must be an equilibrium constant or some other free energy related property) and molecular descriptors for the set of compounds.

Bowen et al. [7] described a more general correlation that should be applicable to different species of tadpoles (Eq. (2)). To this end, they introduced a flag descriptor for each tadpole species different from *Rana temporaria* (I_{pip} for *Rana pipiens*, I_{jap} for *Rana japonica*, I_{xen} for *Rana xenopus laevis*, I_{brev} for *Rana brevipoda porosa* and I_{not} for non-identified tadpole species):

$$\log\left(\frac{1}{C_{\mathrm{nar}}}\right) = 0.543 + 0.736E - 0.367S + 0.049A - 2.852B + 3.043I_{\mathrm{pip}} + 0.150I_{\mathrm{jap}} + 0.524I_{\mathrm{xen}} + 0.146I_{\mathrm{brev}} + 0.473I_{\mathrm{not}} \quad (2)$$

$n = 240,\ \mathrm{SD} = 0.322,\ F = 331$

Similarity of two different systems (i.e. a biological and a physicochemical one) can be measured when they both are characterized through the same mathematical model. In the same way that the tadpole narcosis, several physicochemical systems ruled also by the passive transport of solutes between two phases have been successfully described by means of the Abraham solvation parameter model [9]. When two systems are similar in terms of model coefficients, a good correlation is established between their solvation properties. Then, the biological property of a new chemical compound can be predicted just by determining the surrogate physicochemical property. The main advantage of this procedure over QSAR studies is that it is not necessary to know the molecular descriptor values of the new compound to estimate the biological property.

Several tools are available to compare the coefficients of different systems characterized by the SPM, in order to choose the physicochemical systems that can best emulate tadpole toxicity. Some comparison approaches, like the measurement of the d distance [10] are based on evaluating the difference between the coefficients of the respective equations. Other approaches are based on the estimation of the precision of the correlation between the biological property and the physicochemical one [11]. Finally, some common procedures such as principal component analysis (PCA) of the coefficients of the compared systems [12], or dendrogram plots [13] can also be applied. These methodologies provide different information about the similarity of the compared systems. The parameter d is the euclidian distance between the SPM normalized coefficients, and provides information about how similar are the compared correlations. Instead, the prediction of the variance of the correlation between chromatographic and biological data is a measure of the precision with which a chromatographic system can emulate the biological one. This precision is a combination of the precision of biological data, the precision of chromatographic data, and finally the dissimilarity between the compared systems (which is related to the parameter d). The PCA reduces the chemical space dimensions by combination of the most significant variables, so the closest the compared systems are in the new dimensions, the more similar they are. Finally, the dendrogram clusters the different compared systems according to their similarities. Whereas d and the variance prediction provide direct comparison between pairs of systems (the biological and a physicochemical one), PCA and dendrogram compare the biological system to a set of selected physicochemical ones, so the final results can be affected by the nature of the physicochemical systems selected for comparison.

The aim of this work is to emulate nonspecific toxicity of neutral organic compounds to tadpoles (mainly *R. temporaria* and *R. japonica* tadpoles) through direct measurements in specific chromatographic and electrophoretic systems, basically high-performance liquid chromatography (HPLC) and micellar electrokinetic chromatography (MEKC) systems. Thus, the toxicity of test compounds could be directly estimated from the chromatographic retention factor of a simple linear correlation.

2. Materials and methods

2.1. Equipment

HPLC measurements were done using a 10 A series chromatograph from Shimadzu (Kyoto, Japan) equipped with a quaternary pump and a diode array detector and fitted with either an IAM.DD 2 immobilized artificial membrane column (10 cm × 4.6 mm i.d., 12 μm particle size) (Regis Technologies, Morton Grove, IL, US) or a XBridge C18 column (15 cm × 4.6 mm i.d., 5 μm particle size) (Waters, Milford, MA, US).

MEKC measurements were done using the CE capillary electrophoresis system from Agilent Technologies (Santa Clara, CA, US) equipped with a diode array detector. The fused-silica separation capillary (40 cm effective length, 50 μm i.d.) was obtained from Composite Metal Services Ltd (Shipley, UK).

2.2. Reagents

Methanol (HPLC-grade), hydrochloric acid (25% in water), sodium hydroxide (>99%), sodium dihydrogenphosphate monohydrate (>99%), disodium hydrogenphosphate (>99%) and sodium dodecyl sulfate (>99%) were from Merck. Acetonitrile (HPLC grade) was from VWR International (West Chester, PA, US). Taurocholic acid sodium salt monohydrate (98%) was from Acros Organics (Geel, Belgium) and Brij 35 was from Scharlab (Barcelona, Spain). Dodecanophenone (98%) was from Sigma–Aldrich. Water was purified by a Milli-Q plus system from Millipore (Bedford, MA, US), with a resistivity of 18.2 MΩ cm.

Tested substances were reagent grade or better and obtained from several manufacturers (Merck (Darmstadt, Germany), Sigma–Aldrich (St. Louis, MO, US), Carlo Erba (Milano, Italy), Baker (Centre Valley, PA, US), Panreac (Castellar del Vallès, Spain), Thermo Fisher Scientific (Waltham, MA, US), Scharlab (Sentmenat, Spain)).

2.3. Analysis by HPLC

In both cases (IAM and C18 columns) target compounds were analyzed with a binary system consisting of aqueous buffer (pH 7.0, 60%) and acetonitrile (40%) at 1 mL min^{-1}. The aqueous buffer was prepared dissolving 5 mM NaH_2PO_4–5 mM Na_2HPO_4 in HPLC-grade water and neutralizing with hydrochloric acid or sodium hydroxide up to the desired pH. The injection volume was 10 μL and the column temperature 25 °C. After a preliminary scan, detection wavelengths were set at 200, 214, 240 and 254 nm depending on the compound absorption profile. All measurements were taken in triplicate.

Solutes and the non-retained compound (potassium bromide) were dissolved in acetonitrile:water (2:3) at 100 mg L^{-1}. In the case