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Effects of structural modification of the daunosamine moiety of anthracycline antibiotics on pK_a values determined by capillary zone electrophoresis

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ABSTRACT

The thermodynamic acid dissociation constants (pK_{a1} and pK_{a2}) of 16 anthracycline antibiotics, including doxorubicin (DOX) and daunorubicin (DAU), their epimers, epidoxorubicin (EDOX) and epidaunorubicin (EDAU), as well as novel anthracycline derivatives containing piperidine (FPIP), morpholine (FMOR) and hexamethylenoimine (FHEX) rings in the formamidine group of the daunosamine moiety were determined by analysis of the dependence between measured electrophoretic mobility and the pH of the buffer using the capillary zone electrophoresis method. The results obtained confirmed the ampholytic character of anthracyclines with at least two ionization states. The determined values were in the range of 8.36-9.28 and 9.38-11.48 for pK_{a1} and pK_{a2} arising from ionization of amino and phenolic groups, respectively. Structural modifications in the daunosamine moiety of the studied anthracyclines affected their pharmacological properties, such as antiproliferative activity.

1. Introduction

It is always challenging to produce molecular derivatives with favorable physicochemical properties, enhanced therapeutic efficacy and lower toxicity in comparison with their parent compound. In recent years, a new approach based on chemical modification of reference molecules has arisen and allowed for rapid design and synthesis of innovative drugs. Physicochemical properties, such as lipophilicity, solubility and especially acid-base character, are dependent on the ionization state of the molecule and are particularly important because they affect molecule biological activity, metabolism and toxicity, protein–protein interactions, membrane transport, cellular uptake or extrusion, tissue distribution and binding to target receptors. The acid-base properties of ionogenic compounds are characterized by an acid dissociation constant (pK_a) that is often considered the most relevant parameter [1].

The pK_a defines the extent of molecular ionization in different pH environments, and from a biological point of view, is rather significant in the case of weak acids and weak bases [2]. The physicochemical properties of the nonionized and ionized forms of a molecule are different, and usually only nonionized form exhibits affinity to metabolizing enzymes, binding proteins, protein conveyors and, in the case of cytotoxic agents, penetration into tumor cells [3].

There are several methods currently employed for pK_a determination, like potentiometric titration, spectrophotometry and conductometry [4–6]. Potentiometric titration in aqueous solutions is seen as the “gold standard” method but it has certain drawbacks, including being time consuming, requiring large amounts of very pure and stable

Abbreviations: CAPS, *N*-cyclohexyl-3-aminopropanesulfonic acid; DAU, daunorubicin; DOX, doxorubicin; EDAU, epidaunorubicin; EDOX, epidoxorubicin; DAU-FMOR, morpholine derivative of DAU; DAU-FPIP, piperidine derivative of DAU; DAU-FHEX, hexamethylenoimine derivative of DAU; DOX-FMOR, morpholine derivative of DOX; DOX-FPIP, piperidine derivative of DOX; DOX-FHEX, hexamethylenoimine derivative of DOX; EDOX-FMOR, morpholine derivative of EDOX; EDOX-FPIP, piperidine derivative of EDOX; EDOX-FHEX, hexamethylenoimine derivative of EDOX; EDAU-FMOR, morpholine derivative of EDAU; EDAU-FPIP, piperidine derivative of EDAU; EDAU-FHEX, hexamethylenoimine derivative of EDAU; NM, neutral marker; SLC, the solute carrier; QSAR, quantitative structure–activity relationship

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substances and suffering from low sensitivity [7,8]. Techniques based on computer simulations are very popular in contemporary times, though data does not always correlate with the experimental results [9]. An alternative technique to potentiometric titration is capillary zone electrophoresis (CZE), which offers quick, highly reproducible and low-cost measurements that may be adapted to the high-throughput scale [4,10–12].

Generally, there are few approaches that allow the application of CZE for pKa determination [10]. A frequently used method is founded upon measuring the differences in the effective electrophoretic mobility of an ionizable compound in a series of buffers with different pH and constant ionic strength [13]. An analyte exhibits maximum electrophoretic mobility in full ionization, intermediate mobility when it is half-ionized and lack of mobility with the nonionized form. Molecular ionization depends on buffer pH, and its mobility is proportional to charge, making electrophoresis independent of the molecule's structural diversity [14]. With the CZE method, a small amount of a sample at a low concentration is required, and the sample does not need to be absolutely pure, while analyses are fast and accurate [15–17]. Therefore, this technique permits pK_a determination of different types of compounds dissolved in water or organic solvents. The measurements can be performed across a wide pH range (2–12), however it should be noted that analyses under extremely acidic conditions (pH < 2) necessitate large amounts of time and are less reproducible because of the slow electroosmotic flow (EOF) stemming from the suppressed dissociation of silanol groups on the inner wall of the fused silica capillaries [18,19]. The use of additional pressure fosters an increase in the migration velocity of ions as well as of the electroneutral/EOF flow markers, but decreases analyte separation efficiency [20,21].

DOX, and DAU as well as their epimers, EDOX and EDAU, belong to the first generation of anthracycline antibiotics, and three are currently used in cancer therapy. The most important side effect of standard anthracyclines is life-threatening cardiotoxicity. It is believed that anthracycline-induced cardiotoxicity may be closely linked to their toxic effects on coronary endothelial cells, leading to impaired endothelial function, and may be a risk factor for the development of cardiovascular events in cancer patients [22–24].

Modification of the basic structure of parent drugs is common in searching for novel derivatives with favorable therapeutic properties, reduced cardiotoxicity, and decreased resistance of tumor cells to cytotoxic mechanisms [25–27]. In the present context, it was apparent that special attention was paid to the derivatives that possesses at the C-3′ position of the daunosamine moiety a formamidine (−N=CH−N−) instead of an amine ($-NH_2$) group with attached additional morpholine, piperidine or hexamethyleneimine substituents. It was previously demonstrated that novel derivatives of anthracycline antibiotics possess desired biological properties, *e.g.,* the ability to overcome drug resistance and lower cardiotoxicity [28,29].

In the light of these data, the aim of this study was to assess the pK_a values of the standard anthracyclines along with their novel formamidine derivatives using a simple, quick and reproducible CZE method in bare fused silica capillaries with photodiode array detection.

2. Materials and methods

2.1. Chemicals

DOX, DAU, EDOX, EDAU, and their 12 novel formamidino derivatives with FHEX (DOX-FHEX, DAU-FHEX, EDOX-FHEX, EDAU-FHEX), FMOR (DAU-FMOR, DOX-FMOR, EDOX-FMOR, EDAU-FMOR) and FPIP (DOX-FPIP, DAU-FPIP, EDOX-FPIP, EDAU-FPIP) rings were synthesized at the Institute of Biotechnology and Antibiotics (Warsaw, Poland) [30]. The chemical structures of the studied compounds are presented in Table 1.

Acetic acid, acetone, bisTRIS, *N*-cyclohexyl-3-aminopropanesulfonic acid (CAPS), 10 M hydrochloric acid, 1 M sodium hydroxide and TRIS were purchased from Sigma-Aldrich (Steinheim, Germany). Boric acid and phosphoric acid were obtained from J.T. Baker (Deventer, Holland). All chemicals utilized in this study were of analytical grade purity. Deionized water was obtained from a Millipore Direct-Q 3UV system (Millipore, Molsheim, France).

2.2. Background electrolytes

The freeware computer program, PeakMaster 5.3, was used to calculate the compositions of the background electrolytes. A set of 12 buffer solutions were prepared in pH ranging from 5 to 11.5 at a constant ionic strength of 0.05 M as shown at Supplementary material. The pKa values of the buffer constituents were included in the program database, except borate and CAPS, which came from Mala et al. [31] and Goldberg et al. [32], respectively. The pH of the buffers was measured using the pHmeter, 827 pHLab, with a combined glass electrode, type LL Primatrode, and a negative temperature coefficient (NTC) temperature sensor (Metrohm, Switzerland). All buffers were filtered through a 0.22 µm pore size filter (Macherey-Nagel, Duren, Germany).

2.3. Sample solutions

The stock solutions (2 mM) of the studied anthracyclines were prepared by precisely weighing the appropriate amount of each compound and dissolving it in MilliQ water. The working solutions were then generated by mixing 10 µL of stock solution of the analyzed substance with 1 µL of 1.35 M acetone and further diluting it to 200 µL with MilliQ water. The final concentration of each compound was 100 µM. For calculation of effective electrophoretic mobility (m_{eff}) of the studied compounds acetone, was used as an EOF marker.

2.4. Instrumentation and operating conditions

A P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA, USA) with 32 Karat software (version 8.0, Beckman Coulter, Fullerton, CA, USA) was employed in the work presented here. PeakFit v.4.12 (SPPS, Chicago, USA) was used for the electropherogram treatment. Analytes were detected with a UV–vis spectrophotometric photodiode array detector set in the range of 190–400 nm (Beckman Coulter, Fullerton, CA, USA). The absorbance of the analyzed anthracyclines and EOF marker was monitored at absorbances of 233, 254 and 291 nm. Separation of the analytes took place in an uncoated fused-silica capillary (50.2 cm in total length and 40 cm in effective length with 50 µm I.D. and 375 µm O.D.) at 25 °C. Before each injection, the capillary was purged for 2 min with 0.1 M NaOH, followed by the same with MilliQ water, and finally with running buffer solution. The samples were injected hydrodynamically under a pressure of 3.447 kPa (0.5 psi) for 5 s with a 6 nL injection volume. The separation voltage was selected based on Ohm's plot created for all buffers utilized by applying an increasing voltage into capillaries filled with the background electrolyte. The separation voltage chosen together with the range of electric currents are found at Supplementary material.

3. Calculations

As mentioned earlier, determination of pK_a by CZE is related to m_{eff} of a molecule in different pH environments. For amphoteric compounds, the effective mobility, m_{eff}, is defined as the sum of products "$m_i\, x\, X_i$", where m_i is the actual ionic mobility of the ionic component of the analyte with charge number, i, and X_i is the molar fraction of the ionic component with charge number, i, related to all charged and non-charged forms of the particular analyte, *i.e.* $X_i = c_i/\Sigma c_i$, where $i = -1$, 0 and $+1$ for the analyzed compounds. The m_{eff} value can be expressed as a function of the pK_a values of each species and pH of each buffer. The effective mobility of a compound with one acidic and one basic