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Health Concerns of Bisphenols and the Determination of their Acid-Dissociation Constants by Capillary Electrophoresis

UREAP Report
Summer 2020

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Date of Project: May-September 2020

Date of Submission: October , 2020

In a study by Lehmler H et al (2018) analyzed urinary bisphenols levels in 1808 adults and 868 children participating in the national health and nutrition examination survey in 2013-2014 in order to investigate demographic and lifestyle factors associated with urinary levels of bisphenols. Specifically, Bisphenol F (BPF), Bisphenol S (BPS) and Bisphenol A (BPA) were studied as BPF and BPS are replacing BPA in the manufacturing of products containing polycarbonates and epoxy resins.

BPA is a high-volume industrial chemical with a global consumption of about 7.7 million metric tons in 2015. It us used for the manufacturing of poly carbonates and epoxy resins. Due to growing human health concerns the use of BPA based plastics in food and beverage applications is a concern. This comes from its potential toxicity in humans, causing for manufacturers to attempt to replace use of BPA with structurally similar chemicals such as BPS and BPF.

This experiment used ANOVA to compare results from the survey. Differences in urinary concentrations of BPA, BPF, and BPS (log transformed) were compared among various categorical variables. Linear regression analyses were done by including all variables simultaneously to mimic the independent effects of each variable. Also, urinary creatinine levels were adjusted in the model to account for urine dilution.

From the survey, BPA, BPS and BPF were detected in 95.7%, 89.4% and 66.5% of randomly selected samples from the survey respectively. Furthermore, medial levels of BPA in the U.S for adults were measured at 1.24 ug/L, while BPF and BPS levels were only 0.35 and 0.36 ug/L respectively. In children, median BPA levels were measured at 1.25 ug/L with BPF and BPS levels being recorded at 0.32 and 0.29 ug/L respectively. The limits of detection for BPA, BPF, and BPS were 0.2, 0.2 and 0.1 ug/L respectively. Urinary levels showed correlations with gender, race, family income, physical activity, smoking and alcohol intake that depended on the specific bisphenol. These results indicate that exposure of the general U.S. population to BPA substitutes is almost ubiquitous.

In a study conducted by Kinch C et al (2015), zebrafish were used to link Bisphenol A (BPA) mechanistically to disease etiology. BPA is a ubiquitous endocrine disruptor that is present in many household items. Studies have shown it has roles in causing obesity as well as cancer, and more relevant to the current experiment, childhood neurological disorders like anxiety and hyperactivity.

Many other effects of BPA exposure have been noted, in humans and rodent models, BPA has been associated with an increased risk of developing social, psychiatric, and behavioral challenges later in life. BPA is a compound used in the production of diverse consumer products which makes it particularly dangerous to adults as everyday exposure is common. Furthermore, the fetal brain is especially vulnerable due to its immature xenobiotic metabolizing system and the blood-brain barrier

Results showed that by treatment of embryonic zebrafish with very low doses of BPA and Bisphenol S (BPS) a common analogue, resulted in 180% and 240% increases in neuronal birth (neurogenesis) within the hypothalamus. Also, restricted BPA/BPS exposure specifically during the neurogenic window caused hyperactive behaviors in zebrafish larvae. While human results are still scarce, high maternal urinary BPA during gestation has been linked to hyperactivity and other behavioral disturbances in the child.

The findings of this experiment provided mechanistic support that the neurogenic period may be a window of vulnerability and opens up other areas of how endocrine disruptors might prevent early brain development. Furthermore, BPA may not be the only problem and the results suggest the removal of all bisphenols from consumer merchandise.

In another experiment, Ullah A et al (2018) evaluated the toxicity of Bisphenol A (BPA) and its analogs, BPB, BPF and BPS. The effects of chronic exposure to low bisphenol doses in male rats would have an effect on spermatogenesis with outcomes on oxidative stress and the reproductive system. This is in questions because BPA is an estrogen-mimic endocrine disrupting chemical, commonly used in the making of polycarbonate plastics and epoxy resins with toxic effects for male reproductivity. Because of this, manufacturers have begun to move towards its analogs as substitutes in products, although now, analogs such as BPB, BPF and BPS are proving to be toxic as well.

In this study, male rats (22 days old) were exposed to water containing 0.1% ethanol as a control group and different concentrations of BPA or its analogs in doses of 5, 25 and 50 ug/L in the drinking water for 48 weeks. This formed 13 groups, each group with its own bisphenol (BPA, BPB, BPF or BPS) at the for mentioned concentrations plus the control group. Bisphenol solutions in the water bottles were replaced daily with fresh solutions. After the experimental period, animals were weighed, euthanized and had blood collected from the heart. Reproductive organs were also dissected and weighed and used for testing.

Results of this study showed a large change in the gonadosomatic index (GSI) and relative reproductive organ weights. Oxidative stress in the testis was significantly elevated while sperm motility, Daily sperm production (DSP) and the number of sperm in epididymis were reduced. Plasma testosterone, LG and FSH concentrations were reduced and estradiol levels were high in the group receiving 50ug/L doses. These results suggest that exposure to BPA and its analogues for chronic duration can induce structural changes in testicular tissue and endocrine alterations in the male reproductive system. Furthermore, exposure for extended periods of time to BPA and its analogues are capable of suppressing gonadotropins secretions from pituitary, exhibiting estrogenic and antiandrogenic effects in mammals.

In this study, Moreman J et al (2017) analyzed the toxicity and teratogenic effects of the bisphenols BPA, BPS, BPF and BPAF in zebrafish embryo-larvae and an assessment on their estrogenic mechanism in an estrogen responsive transgenic fish. This experiment targeted the heart, liver, somite muscles, fins, and corpuscles of the Stannius. The effects of development and toxicity were studied in the wild type strain while estrogenic potency ad tissue targets for estrogenic responses were analyzed in the transgenic zebrafish.

BPA is a chemical incorporated into plastics and resins which contains estrogenic activity and is associated with adverse health effects in humans and wildlife. Because of this, similarly structured BPA analogues are widely used although their potential toxicity and estrogenic activity in vivo so less known. They are also endocrine disrupting chemicals (EDC’s) which possess structural similarities with endogenous hormones that can alter hormone biosynthesis, biodegrading or excretions and consistent exposure can alter biological homeostatic. This experiment shows that there are differences in the effects and potencies between the different bisphenol analogues. Furthermore, this study shows that BPA alternative induce similar toxic and estrogenic effects as BPA, and that BPAF is actually more potent then BPA.

The results found that the toxicity was most prominent in BPAF, then BPA, followed by BPF and lastly BPS. At concentrations between 1.0 and 200 mg/L (higher than environmental levels) developmental deformities for larval exposures such as cardiac edema, spinal malformation, and craniofacial deformities were observed. The rank order for estrogen city was BPAF, followed by BPA and BPF, with BPS showing the lowest levels. The bioconcentration factors were 4.5, 17.8, 5.3 and 0.067 for exposure concentrations of 1.0, 1.0, 0.1, and 50 mg/L for BPA, BPF, BPAF and BPS respectively. Final results show that the bisphenols tested are toxic to fish although concentrations in the environment are lower than what was used in the lab.

Bisphenol A (BPA) is a well-known endocrine disrupting chemical with estrogenic activity was studied by Ullah et at (2019) in this experiment. Widespread exposure of individuals to BPA is suspected to affect a variety of physiological functions including reproduction, development and metabolism. It is also used in many plastic consumer products such as food container, water pipes, paper products, electronics, toys and medical equipment. Humans can be exposed to it via dietary or non-dietary pathways. While BPB, BPF, and BPS have been used as an alternative to BPA, data from many agencies that monitor the environment have shown that these chemicals are going to become a serious threat to both human and animal life.

This study observed rat models and the mechanism in which Bisphenol A (BPA) and three of its analogues Bisphenol B (BPB), Bisphenol F (BPF) and Bisphenol S (BPS) cause generation of reactive oxygen species (ROS), sperm DNA damage and oxidative stress. Furthermore, the selective bisphenols will be compared on the toxic effects they present to antioxidant enzymes of sperm.

In the in vitro section of this experiment, sperm were incubated with different concentrations (1, 10, and 100 ug/L) of BPA and its analogues BPB, BPF, and BPS for 2 hours. Then, the species were observed for increases in DNA fragmentation, formation of ROS, and affected levels of superoxide dismutase at higher concentration groups. In the in vivo part of this experiment, the rats were exposed to different concentrations 95, 25, and 50 mg/kg/day) of BPA, BPB, BPF, and BPS for 28 days.

It was observed that in the higher dose (50 mg/kg/day) groups treated with BPA, BPB, BPF, and BPS, there was DNA damage although the motility of the sperm was not affected. Results of the in vivo and in vitro studies showed that the selected bisphenols exhibited altered DSP, affected sperm quality and DNA damage. Furthermore, evidence of genotoxic potential and oxidative stress-inducing ability was present, which may be due to the generation of ROS and LPO in sperm.

Bisphenol A (BPA) was studied by Zhang Y et al (2018) and is known to have disrupting activity on the thyroid hormone signaling. Due to concerns regarding the potential toxicities of alternatives, this study aims to investigate whether Bisphenol S (BPS) and Bisphenol F (BPF), two leading analogues to BPA also present interference with the thyroid hormone signaling pathway.

BPA is one of the highest volume chemicals used in the production of diverse consumer products across the world. And its use has resulted in ubiquitous existence in the environment and organisms. Studies have measured BPA concentrations in picomolar and nanomolar levels in the water, and levels have even reached micromolar levels in human blood and urine samples. Given the widespread human exposure and possible adverse effects, BPA has been banned or is planning to be banned in many products creating the need of a new substance to help create these products.

In this experiment, a series of *in vitro* and *in vivo* assays were conducted. The results from the fluorescence competitive binding assay showed that BPS and BPF, like BPA, bound to thyroid hormone receptors (TRa and TRb), although with less binding potencies that BPA. Furthermore, the molecular docking data also showed their binding potencies to the thyroid hormone receptors. Also, in the coactivator recruitment assay, BPS and BPF recruited coactivator to TRB although not with TRa, also with weaker potencies than BPA.

In the in vivo assay, the three bisphenols like T3 induced thyroid hormone response gene transcription in tadpole, although the presence of T3 altered T3 induced gene transcription in a biphasic concentration response manner. These results demonstrated that BPS and BPF, have the potential to interfere with the thyroid hormone signaling pathway like BPA. In conclusion, they found that generally, bisphenols could act as thyroid hormone signaling agonists in the absence of T3; however, in the presence of T3, they would agonize or antagonize thyroid hormone actions or display biphasic concertation-response under certain conditions, highlighting the potential risks of using BPS and BPF as alternatives to BPA.

The experiment by P.M. Nowak et al (2018) used capillary electrophoresis to determine the acid-base dissociation/deprotonation constant (pK_a) of several cathinone's (2-methylmethcathinone, 3-methylmethcathinone, 4-methylmethcathinone, a-pyrrolidinovalerophenone, methylenedioxypyrovalerone, and ephedrine as well as 1-phenylethylamine. This study shows an effective method for enhancing the reliability of parameters and estimating the magnitude of errors that result from insufficient dissipation of Joule heating. Lack of sufficient temperature control is a source of error in the determination of pK_a values. This method relies on the relationship between electrophoretic mobility and actual temperature, and performing pK_a determination with corrected mobility values.

The method proposed for minimizing the impact of Joule heating on the accuracy of pK_a values determined is based on the estimation of actual temperature reached at various temperatures of coolant, using the current values and then, plotting the obtained electrophoretic mobilities against these values. Then, after function fitting the corrected electrophoretic mobility values corresponding to the temperature of interest can be observed by interpolation. Comparing the data obtained from trials without any correction to ramping and Joule heating effects with data with correction methods, the magnitude of the systematic errors attributed to both effects can be estimated. The ramping effect can be measured by comparing the non-corrected and ramping-corrected trials while comparing the ramping-corrected and fully-corrected trials shows the temperature rise effect.

Results showed that the average pK_a error caused by Joule heating noted at 25°C was relatively small, only 0.04-0.05 pH units. Also, it was demonstrated that the thermal dependencies of electrophoretic mobility, modelled during the correction procedure may be useful to determine the optimal temperature for providing maximum separation efficiency.

The study conducted by Foulon C et al (2007) showed the use of capillary electrophoresis to determine the acidity constants of benzoxa-, benzothia-, and benzoselena-zolinone derivatives. This study also compared the results acquired to the results of determining acidity constants by potentiometry as well as spectrophotometry experiments. This experiment utilized heteroatom or 6-benzoyl substituted derivatives to induce changes in acidity constants to determine pK_a values. Acidity constants were determined simultaneously for two compounds characterized by different electrophoretic mobility and pK_a in the presence of a neutral marker.

The knowledge of a substance's acidity constant is a key parameter for understanding its chemical interactions between the compound of interest and its pharmacological target. Relationships between the acidity constant and structure can prove useful in drug design and explaining the biopharmaceutical properties of substances like the ones used in this experiment. Many biologically active molecules are fully or partially ionized at physiological pH and it has often been shown that the presence of ionizable groups is necessary for biological activity and solubility. This is the importance of accurate determination of pK_a values for the discovery of new molecules.

The results of this study showed that the acid-base dissociation constants of ten weak acids containing benzoxa-, benzothia-, and benzoselena-zolinone rings were determined. The use of capillary electrophoresis allowed for convenient, quick determination of the pK_a values of these compounds. The results obtained were in good agreement with values obtained using bot the potentiometric and spectrometric methods.

Beach D et al (2018) used a CE-MS/MS technique that is well suited for analyzing polar marine toxins and offers very high-resolution separation. This method was developed with a custom-built interface for sensitive multiclass analysis of paralytic shellfish toxins, tetrodotoxins, and domoic acid in seafood. A highly acidic background electrolyte (BGE) (5 M formic acid) was developed to maximize protonation of analytes and to allow a high degree of sample stacking to improve the limits of detection in this experiment. The experiment used very low pH BGE where the EOF of the CE was almost entirely suppressed.

Recently, the CE-MS/MS method for the analysis of neurotoxin B-N-methylamino-L-alanine (BMMA) was successful at separating the analyte from potentially interfering matrix components including several isomers by the same group, which helped create the method for this experiment. This includes the utility of CE-MS/MS as a multiclass analytical method by using a highly acidic BGE.

This method was applied to a wide range of regulated toxin analogues which exhibited a high degree of selectivity between toxin isomers and matrix interference. And minimal ionization suppression was observed when the response from neat and mussel-matrix-matched standards was corrected with multiple internal standards. The method was then transferred to a commercial CE-MS/MS system to demonstrate its widespread applicability for use in both R and D and routine regulatory settings. Accuracy of this method was evaluated through quantitative analysis of mussel matrix (certified) reference materials and spiked samples.

The analysis of shellfish matrix reference materials and spiked samples demonstrated good accuracy and precision. The limits of detection in mussel tissue were 0.0052 mg/kg for tetrodotoxins, 0.169 mg/kg for domoic acid and between 0.0018 and 0.120 mg/kg for paralytic shellfish toxins in which all showed good linearity.

In a study by Zrnčić M et al (2015), the determination of the thermodynamic acid dissociation constant (pK_a) of 22 common pharmaceuticals by capillary electrophoresis in aqueous media was done. The species of interest belong to different pharmacological groups such as macrolides, fluoroquinolones, sulfonamides, B-lactams, tetracyclines, and other miscellaneous pharmaceuticals. The electrophoretic mobilities of the investigated analytes were monitored in a pH range of 2.00 to 10.82. Obtained data were plotted on effective mobility vs pH graphs. And mobility measurements were done in triplicate.

The acid dissociation constant is an important factor in pharmaceuticals as it can allow for better prediction of there fate and behavior in an environment. Furthermore, the acid-base property of a pharmaceutical substance is one of several crucial properties that can be used to estimate the adsorption, distribution, metabolism and excretion of compounds in biological systems. And, the retention and electrophoretic behavior of ionizable compounds strongly depends on the pK_a of the compound and thee pH of the mobile phase. Also, the knowledge of an analytes pK_a is useful in the optimization of machines like capillary electrophoresis for the separation of compounds.

This study utilized sodium dihydrogen phosphate and disodium hydrogen phosphate the determination of the thermodynamic pK_a values was done by extrapolations to zero ionic strength. And experimentally obtained acid dissociation constants were interpreted using structural formulae of investigated analytes and the moieties corresponding to specific pK_a were identified.

While the pK_a values for many of the pharmaceuticals are not well known, the results of this experiment were in good agreement with the analytes that have been determined and published. They were also compared to predicted values using ChemSpider and/or ACD Labs computational programs. Most r^2 values obtained were higher than 0.99. Also, the %RSD values for the effective mobilities calculated were less than 1.5% for all compounds.

This experiment done by MORI M et al (2001) focused on the application of capillary electrophoresis (CE) using sulfated B-cyclodextrin (SCD) for the separation of various sizes of alkylphenols as well as bisphenol A and S. In this study the application of CE using SCD was examined to separate a mixture of BPA, BPS and six alkylphenols with varied chain lengths from ethyl to heptyl phenol. This system was also applied to the separation of 4-octylphenol (OP) and 4-nonylphenol (NP) isomers

The addition of sulfated B-cyclodextrin was crucial to the study as in trials without SCD, all the phenols migrated with the same velocity as the electroosmotic flow. This is because most of the compounds used are electrically neutral, making them difficult to separate by only running a buffer. Upon addition of SCD, separation of alkylphenols was achieved based on the capabilities of each analyte to bind to do hydrophobic cavity of B-cyclodextrin. This method is known as micellar electro kinetic capillary electrophoresis (MEKC) using sodium dodecyl sulfate (SDS) which separates neutral compounds on the bases of their distribution into the micelles. Furthermore, CD is used as an additive with SDS to improve separating in MEKC.

Binding constants between analyte phenols and SCD were determined from Benesi-Hildebrand plots obtained by CE and UV-visible measurements and showed that the greater the hydrophobicity of phenols the largest the binding constants. This system of using SCD was also effective for the separation of 4-octylphenol and 4-nonyl phenol isomers having longer alkyl chains. The binding constants between SCD and analyte phenols were also estimated and the effect of organic solvents on the separation of alkylphenols was examined.

Results showed that using CE and SCD together would prove to be a good method for the separation of alkylphenols and bisphenols, specifically highly hydrophobic alkylphenols. Also, the migration order in the running buffer was correlated with the length of the alkyl chain in the phenols. Addition of an organic solvent cause a decrease in resolution between alkylphenols but improved separation between BPA and BPS. This also provided complete separation of OP and NP's.

In a study by Konášová R et al (2015), the acid dissociation constants (pK_a) of six widely used triazole fungicides (cyproconazole, epoxiconazole, flusilazole, tebuconazole, penconazole, and propiconazole) were determined by pressure assisted capillary electrophoresis (pCE). The pK_a values were determined from the dependence of effective electrophoretic mobility of the triazole fungicides on $p[H^+]$ of the background electrolyte (BGE) by non-linear regression analysis. Due to BGE's being strongly acidic, and there for having an increased ionic strength, $p[H^+]$ was chosen over pH for this experiment. Before pK_a calculations were done, the measured effective electrophoretic mobilities were corrected to a reference temperature of 25°C and constant ionic strength of 25 mM.

The acid dissociation constant of a weak base is important to determine as it provides information about a form of the compound (neutral or protonated) in a solution. The protonation of a weak base can increase its solubility in water and may change its adsorption in the soil. Furthermore, the ionized form has different abilities to enter into organisms as compared to the neutral form. The knowledge of pK_a of weak electrolytes is important in analytical chemistry as it provides information on how to optimize experimental conditions for separation and analysis. Specifically, the motivation to determine the pK_a of triazole fungicides were to better estimate potential risk to the environment by these compounds, and to fill in missing or ambiguous values in literature to be used in future experiments.

CE was found to reduce the time on the CE analysis of the strongly acidic BGE's and increased the throughput of pK_a determination by CE. Also, attachment of the substituents with different structures in the position B to the triazole ring resulted in the decreased electron density of the ring and decreased basicity of the above triazole fungicides as compared to the unbound molecule.

Results showed that the pK_a values were found to be in the range of 1.05 – 1.97 which are confirmed by values calculated by SPARC online pK_a calculator. It was also determined that the analyzed triazole fungicides are very weak bases that remain in the non-protonated form in the soil at natural environmental pH.

In 2018, Qian C et al studied the effects of adding external pressure to analytes in the process of capillary electrophoresis (CE) which usually results in an increased band broadening, especially if the pressure induced flow is significant. This negatively effects the resolution in pressure assisted capillary electrophoresis (PACE). Although, frontal analysis (FA) can potentially benefit from using an external pressure while avoiding the negative effects in other modes of CE.

In CE, charged molecules with different electrophoretic mobility are separated in the presence of an electric field and species are driven by an electroosmotic flow (EOF). This produces a flat shaped flow profile of the EOF driven flow. On the other hand, pressure driven laminar flow in an open tube has a parabolic flow profile. This parabolic profile has a negative effect on the separation efficiency making it uncommon that a significant large pressure is applied during a CE process. The addition of pressure in the capillary can have many effects. In PACE the pressure is applied on the same direction with the analyte migration. This can be used for accelerating the migration of low-mobility molecules.

Frontal analysis, when combined with CE, requires a much larger amount of sample than traditional CE methods. In CE-FA, only the front of the plateaus, instead of the peaks, are measured in the electropherograms, and the concentrations of ligands are determined with a pre-made calibration curve. As a result, CE-FA is not dependent on baseline resolution of the analytes, thus more tolerant to band broadening caused by the application of an external hydrodynamic pressure.

In this experiment, the possible impact from the external pressure was simulated by COMSOL Multiphysics. Results showed that under a typical CE-FE set up, the detected concentrations of analyte will not be significantly affected by and external pressure lower than 5 psi. Furthermore, based on experimental results, it was concluded that PACE-FA can reduce the time of binding analysis while maintaining accuracy of the measurements.

Qian C et al studied a popular method for determining binding affinities of molecular interactions in 2017 known as capillary electrophoresis frontal analysis (CE-FA). Consequently, the current data processing method requires specific mobilities of the binding pair in order to obtain accurate binding constants.

CE based methods can be roughly categorized into two groups, there is affinity CE and vacancy affinity CE. In these methods the binding constant is deterred by the shift in mobility of ligand molecules while in the Hummel-Dreyer method, vacancy peak method and CE frontal analysis, the binding constant is determined by concentration change of related species due to the binding interaction. Although it is not common yet, the method of CE with frontal analysis can be a better choice for high-throughput determination of binding constants because of the simplicity in procedure and its ability to determine binding stoichiometries.

The purpose of this study is to show that significant errors can result when the mobilities of interacting pieces do not meet the requirements. This lowers the applicability of CE-FA in many real-world applications. In this experiment, an electrophoretic mobility-based correction method is developed based on the flux of each species. The equations and effectiveness of this method were evaluated via a simulation program and a pair of model compounds.

In this experiment, ibuprofen and hydroxypropyl-B-cyclodextrin are used to demonstrate the differences in the obtained binding constant by CE-FA when different calculations methods are used. The results are compared with those obtained by affinity capillary electrophoresis (ACE). The results suggest that CE-FA, with the mobility-based correction method can be generally applicable. Specifically, for the binding pairs that did not fulfill the prerequisites of the current CE-FE data processing method; the new equation derived in this work has proved effective in correcting the systematic error caused by mobility difference of various species. This mobility-based correction method has also shown no restrictions to the type of molecules involved in the interaction.

In this experiment by TAKAYANAGI T et al (2016), the acid-base equilibrium of hexamethylenetetramine (hexamine) was analyzed with its effective electrophoretic mobility by capillary zone electrophoresis (CZE). Hexamine, is degradable in weak acidic aqueous solutions and produces ammonia and formaldehyde. CZE is an alternative method for the analysis of acid-base equilibrium in homogenous solutions including conventional potentiometric and spectrophotometric titrations. In CZE, the equilibrium reactions are analyzed in an aqueous solution without any organic co-solvents and without any side reaction, such as a partition to the stationary phase.

In CZE, the acid-dissociation constants (pk_a) values are determined though measurements of the effective electrophoretic mobility of the equilibrium species of interest. Because this is still a separation technique, other substances and impurities are allowed to coexist and are resolved.

To determine the pK_a value, N-ethyl quinolinium ion was used as an internal standard of the electrophoretic mobility. The CZE signal of hexamine became closer to the EOF with increasing pH of the separation buffer suggesting hexamine came to be neutral form acidic to neutral pH conditions. Changes in the effective electrophoretic mobility of hexamine were plotted against the pH of the separation buffer where the effective electrophoretic mobility of hexamine was standardized with that of the N-ethyl quinolinium ion.

In this study, the effective electrophoretic mobility of hexamine was measured in the range of pH 2.8 to pH 6.9. The acid-base dissociation equilibrium of the protonated hexamine was analyzed based on mobility change. The monoprotic acid-base equilibrium of hexamine was confirmed through comparisons of its electrophoretic mobility with the N-ethyl quinolinium ion and with the monocationic N-ethyl derivative of hexamine. This was also compared to the slope analysis of the dissociation equilibrium. The results of this experiment determined the acid dissociation constant to be pK_a = 4.93 +- 0.01 at an ionic strength of 0.020 mol dm^{-3-}.

In this study by Takatangi T et al (2015), the acid dissociation constant of pravastatin was determined under degraded conditions. It was determined with residual pravastatin through its effective electrophoretic mobility in order to study the ability of CZE analysis. Pravastatin was degraded in an acidic solution of pH = 2.0 for 5 hours. The degraded solution was subjected to the measurement of the effective electrophoretic mobility by capillary zone electrophoresis. The results of this experiment agree with some reported values.

Pravastatin, is a cholesterol-lowering compound that completely inhibits the microsomal enzyme 3-hydroxy-3-methylglutaryl-co enzyme A (HMG-CoA) reductase. Pravastatin is degradable under acidic pH conditions, and its lactonized and/or C-6-epimerized forms are supposed to be generated. The acid dissociation constant (K_a) has been determined by capillary zone electrophoresis (CZE) in pressure-assisted mode using the effective electrophoretic mobility with changing pH of the separation buffer.

CZE analysis is convenient using the effective electrophoretic mobility of an analyte with changing pH of the separation buffer. CZE includes the electrophoretic separation, therefore like normal capillary electrophoresis, is a separation method that will resolve impurities or degraded species from the analytes of interest. In this experiment, the degraded species are neutral and therefor migrated at the EOF time. CZE separation was utilized to determine the pK_a by electrophoretic ally separating the degraded species.

The results of this experiment found that the K_a value was successfully determined by the CZE analysis or residual pravastatin. The pK_a value determined was 4.46 +/- 0.03 (I = 0.01 – 0.02; error: standard error) which agreed with one obtained freshly prepared solution and reported values. The signal height of the electropherogram of pravastatin was decreased by the degradation and the degraded species were detected by and MEKC format.

The ionization constant (pK_a) and limiting ionic mobility of 3-chlorotyrosine (CT) and 3-nitroyrosine (NT) were determine by capillary one electrophoresis (CZE) by Ren H et al (2013). Furthermore, the diameter of the hydrated ion was calculated as well. CT and NT are oxidation products of reactive oxidative species (ROS) and other radicals under inflammatory conditions. Chlorination of tyrosine decreases the ionization constant of the phenolic group which results in an altered protein conformation and function similar to the way that nitration of tyrosine can alter enzymatic activity or protein function.

The determination of the ionization constant is important as pK_a plays an important role in biochemical activity and electrophoretic behavior along with other physiochemical parameters. And CZE is a useful tool for the determination of these values. In this study, it is assumed that a cationic capillary, with revered and detectable EOF, should be more suitable for cations at low pH due to electrostatic repulsion; this was based on the conclusion that electrostatic interactions can bring variations in pK_a values.

When attempting to determine the limiting ionic mobilities and pK_a values by CZE, the team conducting this experiment found that great caution should be taken, especially when monitoring experimental conditions or determining the parameters used in the calculations. They also suggested that the charge of both the analyte and the capillary inner surface should be taken into account when calculating pK_a and ionic mobility, and that electrostatic interaction should be avoided. Furthermore, some formulas in this paper are helpful to use if the diameter of a compound is unknown.

This experiment was done over a wide pH range, measurements were carried out in a polyionic liquid (PIL) modified capillary at a low pH (1.80-4.00) and a bare fused capillary at an upper pH of 3.00-11.00. The electrostatic interaction between analytes and the inner wall were suppressed. The parameters were calculated form a simple theoretical model. Results were compared and agreed with predictions from the database of Peakmaster software.

Nowak P et al (2016) conducted an experiment in which the acid dissociation constant (pK_a) of 20 structurally diverse coumarin derivatives were determined by capillary electrophoresis; for many of these compounds the pK_a values were determined for the first time. Coumarins acidity strongly depends on chemical structure making them very diverse and a good modal for theoretical investigation of structure0acidity relationships.

Notably, in this experimental an amine permanently coated capillary was used instead of the bare silica or other coated capillaries for the determination of pK_a as it ensured good precision and shorter migration times. Also, the traditional methodology relying on measurements in a broad pH range and fitting of a sigmoidal function was compared to an alternative simplified method that requires less measurements. This reported for the first time where only two electrophoretic mobility values suffice for pK_a estimation. In this method, the first value corresponds to the partially ionized form and its measured experimentally while the second one corresponds to the totally ionized form and is either measured experimentally by the two-valve method, or directly from the molecular mass (one-valve method). The reliability of this method has been confirmed by the analytical predictions, comprising resolutions, migration times and order, and peaks overlapping.

For the determination of pK_a in this study, the traditional method utilized the relations between the effective electrophoretic mobility and pH. In the case of the new approach, the mobility values related to partial ionization and total ionization were used while experimentally measuring electrophoretic mobility.

The values obtained in this experiment varied between 4.16 – 9.10 pH units. This suggests some interesting structure-acidity relationships. Furthermore, it was shown, despite a limited measurements number, that the alternative approach may be consistent with traditional methodology, yielding the relatively low pK_a deviation. In conclusion, the use of an amine capillary companied with the simplified calculation method is a fast and reliable method for pK_a determination.

In this experiment, Tůmová T et al (2016) determined the thermodynamic acidity constants (pK_a), and actual ionic mobilities of polycationic antimicrobial peptides (AMPs) by capillary electrophoresis. Bacterial resistance to conventional antibiotics is a major reason for intensive research into new therapeutics. And the determination of physiochemical properties of potent drug candidates is important for physical modeling of pharmacokinetics and can reduce time taken in the early stages of drug development especially when solubility, permeability, acid-base properties, lipophilicity and protein binding data is already known.

In this study, the effective electrophoretic mobilities of AMPs were measured with a series of background electrolytes within a pH range of 2.00-12.25 at a constant ionic strength of 25 mM at ambient temperature. Furthermore, a polybrene coated fused silica capillary was used in order to suppress sorption of cationic AMPs to the capillary wall. The Haarhoff-Van der Linde peak fitting function was used to calculate correct migration times of some AMPs peaks that had been altered by electromigration dispersion. Furthermore, the measured effective mobilities were corrected to 25°C. Also, mixed acidity constants and ionic mobilities of the AMPs were determined by the nonlinear regression analysis of pH dependence of their effective mobilities, and the mixed acidity constants were recalculated to thermodynamic pK_a using the Debye-Huckel theory.

The results of this experiment found that the thermodynamic pK_a of imidazolium group of histidine residues was in the range of 3.72 – 4.98. And the pK_a of the a-NH_3^+ group was in the range of 6.14 – 6.93. And finally, that the pK_a of the ε-NH_3^+ group of lysine was in the range of 7.26 – 9.84 and varied depending on the particular amino acid sequence of the AMPs.

The actual ionic mobilities of AMPs with positive charges from one to six elementary units achieved values of $9.8 – 36.5 \times 10^{-9}$ $m^2V^{-1}s^{-1}$; also, the actual ionic mobilities of peptides from the first to the third protonation degree are growing proportionally to the increasing effective charge. This is untrue for tetra to hexavalent AMPs though as the growth of their effective and ionic mobilities is much lower, which maybe be due to the condensation of counter ions.

Matyjaszczyk K et al (2017) determined the thermodynamic acid dissociation constants (pK_a) of 16 anthracycline antibiotics by the analysis of the dependence between measured electrophoretic mobility and the pH of the buffer using the capillary zone electrophoresis (CZE) method. pK_a determination in anthracyclines is important as it can lead to optimization of their efficacy/toxicity ratio. Furthermore, it can also influence drugs uptake into tumor cells, as well as their penetration into the cardiomyocyte and vascular endothelium.

CZE is a well-known approach for pK_a determination. Often, it is done by measuring the differences in the effective electrophoretic mobility of an ionizable compound in a series of buffers with different pH and constant ionic strength. The analytes will show maximum electrophoretic mobility while fully ionized and lack mobility when not ionized allowing for the separation of species.

In this study, 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 the species of interest. Modifications in the daunosamine structure of anthracycline caused a significant change in the pK_a determined in this experiment. Two pK_a values were determined for each molecule according to the assumption of two ionized states of each analyte. The greatest differences between the set of pK_a values obtained was observed for EDOX-FPIP and DAU-FPIP while the parent drugs showed the greatest similarities.

The results from this experiment confirmed the ampholytic character of anthracyclines with at least two ionization states. The pK_a values were determined to be in the range of 8.36 – 9.28 and 9.38 – 11.48 for pK_{a1} and pK_{a2} respectively. These values come from the ionization of amino (pK_{a1}) and phenolic groups (pK_{a2}). Structural modifications in the daunosamine moiety of the studied anthracyclines affected their pharmacological properties, such as antiproliferative activity.

In this study a Malý M et al (2020) created new method for determining the data of a weak electrolyte by nonlinear regression of effective electrophoretic mobility versus buffer composition dependence when measured in a set of background electrolytes (BGEs) with various pH. This method differs from traditional approaches as the nonlinear regression is performed on limiting mobility data calculated by Peak Master's correction engine, instead of the raw experimental mobility data. This removes the requirement to perform all measurements at a constant ionic strength. Corrections for experimental data for zero ionic strength were done via the extended Debye Huckel model and Onsager-Fuoss law.

This study was done to devise the computer program AnglerFish that performs the necessary calculations in a user-friendly fashion. This includes calculating all thermodynamic pK_a values and limiting electrophoretic mobilities for arbitrarily charged substances with any number of ionic forms in just one run. It is also equipped with the buffer composition of the set of BGEs and experimentally measured effective mobilities of the inspected weak electrolyte.

Thermodynamic acidity constants (also known as acid or acid-base dissociation constants or ionization constants) and limiting ionic mobilities are fundamental physiochemical characteristics of a weak electrolyte, that is, a weak acid or weak base or ampholyte. And acidity dissociation constant and electrophoretic mobilities are fundamental physiochemical constants that characterize and ionic constituent. Both constants determine the effective mobility of the analytes in the electromigration movement in capillary electrophoresis and therefore have a direct impact on the quality of separation in these methods. And the ability to accurately quantify these constants is essential to multiple fields in chemistry.

Finally, in an experiment conducted by Tanikami Y et al in 2020, the acid dissociation constants (pK_a) of nine kinds of flavin (FL) analogues as molecular catalyst candidates were determined by capillary zone electrophoresis (CZE). The effective electrophoretic mobility of the flavin analogue of interest has been measured with the residual substance. The pK_a values of the flavin analogues were analyzed through the changes in effective electrophoretic mobility with varying pH of the separation buffer. In some cases, specifically analogues possessing carboxylic acid moiety, a pressure-assisted CZE was utilized for the estimation of pK_a values; this method also aided determinations of values at weakly acidic pH conditions where the EOF is slow.

Acid dissociation constants have traditionally been determined by potentiometric and spectrophotometric titrations. Although, the homogeneous titrations are not applicable to such substances as these are not pure or degradable. The spectrum shift is indispensable for the pK_a determination by the spectrophotometric titration. The advantage of CZE for determining the pK_a of the selected analytes is that its method is based on the change in the effective electrophoretic mobility with protonation/deprotonation reactions under varying pH (pH 7 in this study). Furthermore, CZE analysis is applicable to substances that contain impurities or are degradable.

As a result of this study, the acid dissociation constants were determined by CZE for a series of molecular catalysts candidates of FL analogues. Even though some of the FL analogues were degradable under alkaline conditions and/or light exposure, the pK_a values were successfully determined. This was done by analyzing changes in electrophoretic mobility by the protonation or deprotonation in CZE. CZE was also useful for the separation of the target analyte from the surrounding matrix.

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