Quantitative analysis of active pharmaceutical ingredients (APIs) using a potentiometric electronic tongue in a SIA flow system

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Abstract

An advanced potentiometric electronic tongue and Sequential Injection Analysis (SIA) measurement system was applied for the quantitative analysis of mixtures containing three active pharmaceutical ingredients (APIs): acetaminophen, ascorbic acid and acetylsalicylic acid, in the presence of various amounts of caffeine as interferent. The flow-through sensor array was composed of miniaturized classical ion-selective electrodes based on plasticized PVC membranes containing only ion exchangers. Partial Least Squares (PLS) analysis of the steady-state sensor array responses, measured in API mixtures prepared by the SIA system permitted a correct quantitative analysis of acetylsalicylic acid and ascorbic acid. Further optimization using multiway PLS fed by dynamic responses without additional feature extraction did not improve significantly the resolution of acetaminophen. Lastly, the chemometric treatment, involving the extraction of dynamic components of the transient response employing the Wavelet transform, the removal of less-significant coefficients by means of Causal Index pruning and training of an Artificial Neural Network (ANN) with the selected coefficients, allowed the simultaneous determination of all the three studied APIs, while counterbalancing any interference due to caffeine.

Keywords: pharmaceutical analysis; active pharmaceutical ingredients (APIs); ion-selective electrodes; automated electronic tongue; sequential injection analysis

1. Introduction

Multidrug pharmaceutical preparations containing paracetamol and non-steroidal anti-inflammatory drugs (NSAID) such as acetylsalicylic acid or ibuprofen are commonly used for the flue treatment and flu-like illnesses. Frequently, they are combined with antihistamines e.g. diphenhydramine and pheniramine maleate and other active pharmaceutical ingredients (APIs) as caffeine, codeine, derivatives of pyrazolones, barbiturates. Paracetamol and NSAIDs are applied for treatment of mild to moderate pain and fever, while the antihistamines prevent many of the symptoms of an allergic reaction like nasal congestion. Addition of codeine or caffeine improves the pharmacological efficiency of paracematol and NSAIDs. Moreover, the multidrug formulations are less harmful to the main metabolic organs, because smaller amount of each component are introduced in comparison with monocomponent drug [1,2].

Since analgesic drugs as acetylsalicylic acid, ibuprofen or paracetamol are generally available over the counter (OTC) they are daily used by millions of people for household relief of pain and thus accidental and deliberate overdose are common. Paracetamol is a tolerable drug in standard dose, while NSAIDs often cause gastro-toxicity which is depended on dose and product. High risk of gastrointestinal bleeding is especially associated with taking even low-dose of aspirin, whereas ibuprofen seems to carry lower risk of gastro-toxicity. Regarding the extended use of such formulations, the development of analytical methods for the quality assessment of these products is needed [3,4].

The analysis of pharmaceuticals involves pre-clinical and early phase clinical studies of a candidate for a new drug as well as routine analysis during production. Preclinical studies include the characterisation of a drug, identification of the effective dose and range and the tissues in which side effects may occur. During clinical trials, high-performance liquid chromatography (HPLC), UV-Vis spectrophotometry and potentiometric titration are useful to characterize drugs in 0 (preclinical) and I phase of clinical trials [5]. Moreover, several methods of API analysis in pure form, mixtures and commercial products have been reported. HPLC is the most common technique used for targeted NSAID analysis, especially coupled with MS detection employing different ionization systems [6,7]. As an example, HPLC with optical detection was applied for the determination of active pharmaceutical ingredients: paracetamol, acetylsalicylic acid, caffeine in pharmaceutical preparations [1,2,8,9]. Conventional and derivative spectrophotometry, often combined with chemometric procedures, was

also proposed for the multi- component analysis of drugs [10-14]. Voltammetric measurements at modified or unmodified electrodes enabled the resolution of various pharmaceutical compounds [15-18].

On the other hand, portable and compact electrochemical sensors, providing fast measurements with high selectivity and sensitivity, could be competitive to classical instrumental techniques dedicated for the analysis of pharmaceuticals. Among them, potentiometric sensors – ion-selective electrodes (ISEs) – are the most promising for such purpose [19,20]. ISEs based on polymer membranes doped with the ion-pair complex of tetraoctylammonium cation were developed for the analysis of ibuprofen [21,22]. Similar approach was proposed for the design of ion-selective electrodes sensitive towards: naproxen [23], ketoprofen [24] and caffeine [25]. It is worth to note, that the use of potentiometric sensors provided measurements in flow injection mode (see a review on the application of Flow Injection Analysis (FIA) and Sequential Injection Analysis (SIA) for pharmaceutical analysis in [26]).

Recently, electronic tongue (e-tongue) systems composed of sensor arrays and pattern recognition tools were tested in pharmaceutical applications [27-29]. Since the majority of commonly used active pharmaceutical ingredients have a bitter taste, various methods for taste masking were developed and the e-tongue devices were exploited for the evaluation of the efficiency of such methods. An array of classical or miniaturized ISEs was proposed for the assessment of taste masking effect of selected pharmaceuticals modified with co-spray excipients (microencapsulation method) [29,30]. Potentiometric multisensor systems were employed for the quantification of the bitter taste of diverse active pharmaceutical ingredients [31,32]. Finally, the performances of commercial e-tongues dedicated to the analysis of pharmaceutical formulations were also compared [27,33,34].

In this work, an automated method for the quantitative analysis of selected active pharmaceutical ingredients (acetaminophen, ascorbic acid, and acetylsalicylic acid) involving an array of miniaturized potentiometric electrodes based on well-known lipophilic salts (ion-exchangers) was reported. Calibration and measurement with the electrode array was implemented with the aid of sequential injection analysis (SIA) system. Among different measurement conditions and data treatment, incorporation of dynamic components of the transient response is reported as the means to improve modeling capability and resolution.

2. Experimental

2.1. Chemicals and membrane materials

The active pharmaceutical ingredients (APIs): acetaminophen pK_a=9.50 (paracetamol, PARA), ascorbic acid pK_a=4.17 (ASC), acetylsalicylic acid pK_a=3.50 (ASA) were purchased from Sigma-Aldrich, whereas hydrochloric acid, lithium acetate, tris(hydroxymethyl)aminomethane (TRIS) and caffeine pK_a=0.70 (KOF) of analytical grade were purchased from Fluka. The solutions of APIs (0.04 mol/L acetaminophen, 0.014 mol/L ascorbic acid, 0.016 mol/L acetylsalicylic acid), caffeine (2.6 mmol/L), lithium acetate (1 mmol/L), TRIS buffer solution (1 mmol/L) were prepared with deionised water. The pH of solutions was adjusted by the addition of hydrochloric acid or sodium hydroxide solution. High-molecular-weight poly(vinyl chloride) (PVC), plasticizers: o-nitrophenyl octyl ether (o-NPOE), bis(2-ethylhexyl) sebacate (DOS), lipophilic salts: potassium tetrakis [3,5-bis(trifluoromethyl)phenyl] borate (KTFPB), tridodecylmethylammonium chloride (TDMAC), tributylhexadecylphosphonium bromide (TBHDPB) and ionic liquid 1-decyl-3-methylimidazolium chloride (IL) were obtained from Fluka. Freshly distilled tetrahydrofuran (Fluka) was used as a solvent for the membrane components.

2.2. Sensor array

The flow-through sensor array consisted of 8 miniaturized electrodes based on PVC membranes (plasticized using DOS or o-NPOE), containing an appropriate lipophilic salt, exhibiting generic anion (TDMAC, TBHDPB, IL) or cation (KTFBB) response. Two electrode specimens were prepared for each membrane composition. The method of membranes preparation and electrodes conditioning were the same as for the standard ISEs. The membranes contained: 1-3.5 wt% lipophilic salt, 64-66 wt% plasticizer, 32-33 wt% high-molecular-weight PVC (see Table 1). The membrane components (200 mg in total) were dissolved in 2 mL of THF. A detailed architecture of the miniaturized ion-selective electrodes compatible with a single flow-through module was presented in [35], whereas the design of the modular flow-cell system is a subject of a polish patent application [36]. NaCl solution (0.01 mol/L) was used as an internal filling. The constructed sensors were preconditioned overnight in a dilute solution of internal electrolyte for at least 24 hours.

2.3. Instrumentation and EMF measurements

All measurements were carried out in flow-through mode with cells of the following type: Ag, AgCl; KCl 3 mol/L | CH_3COOLi 1 mol/L | sample solution ‖ membrane ‖ internal filling solution; AgCl, Ag.

Potentiometric multiplexer (EMF 16 Interface, Lawson Labs Inc., Malvern, USA) was used for the characterization of the sensors. The calibration curves of the electrodes were examined by measuring the EMFs while increasing the concentration of the APIs in steps of 0.5 log c in the range $10^{-5.5}$ – $10^{-1.5}$ mol/L ($10^{-5.5}$ – 10^{-2} mol/L for acetylsalicylic acid). Repeatable sensor performances were recorded during at least 4 weeks.

For the quantitative analysis of APIs mixtures, the flow-through sensor array was connected to the Sequential Injection Analysis (SIA) system providing the automated operation and generation of API samples mixtures, thanks to the precise dosing and mixing of volumes of stock solutions. The SIA system was formed by two differentiated parts: the fluidic system and the measurement system [37,38], which were wholly controlled by a PC using a virtual instrument developed in LabView, where the other active elements were commanded through RS-232 communication lines.

The fluidic system consisted of an automatic microburette (Crison 2030 microburette, Crison, Spain) equipped with a 5-mL syringe (Hamilton, Switzerland), a holding coil (5m×1mm i.d. PTFE tubing, Bioblock, France), a 8-way Hamilton MVP valve (Hamilton, Switzerland) and a 7mL Perspex mixing cell (home built) with a magnetic stirrer. The burette was connected to the multiport valve through the holding coil placed in between, and fed through a carrier solution reservoir. In this way, connection between the common port to the other ones (i.e. sample, standard stock solutions, mixing chamber or sensor array port) was achieved by an electrical rotation of the valve; all the elements being connected together using low pressure liquid chromatography connectors.

The measurement system comprised the sensor array, a reference electrode (miniaturised silver/silver chloride electrode with a double junction) and an 8-channel signal conditioning circuit connected to the data acquisition analog inputs (National Instruments NI6221 Multifunction DAQ, TX, USA).

2.4. Data analysis

Data analysis was performed in MatLab (The MathWorks, Inc., Natick, USA), Solo (Eigenvector Research, Inc., Wenatchee, USA) and Origin (Microcal Software, Inc, Northampton, USA) software. Chemical images of samples were processed using Partial Least Squares (PLS) analysis in the case of models involving steady-state signals, and 3-way PLS in the case of models based on dynamic responses of the sensors. Analogously, ANN models were also built employing the dynamic responses, but in this case requiring a preprocessing step aimed to the reduction of signals complexity and dimensionality [39]. This was achieved by means of Discrete Wavelet Transform (DWT) as feature extraction tool and Causal Index (CI) pruning of the inputs to remove less-significant extracted coefficients that barely contribute to the final model.

3. Results and discussion

The studied active pharmaceutical ingredients remain in neutral or anionic form in solution, according to their pK_a values and pH conditions. Therefore, anion-sensitive electrodes based on various ion-exchangers were applied for the quantitative analysis of selected APIs. The electrodes constructed by incorporating tetraalkylammonium and phosphonium salts in the PVC/o-NPOE membranes exhibited almost theoretical values of the response slopes for acetylsalicylic acid (in the range 10^{-4} – 10^{-2} mol/L), whereas significantly worse response sensitivities (<20 mV/decade) were measured for the weak acids: acetaminophen and ascorbic acid, even in alkaline conditions. Comparable, poor sensitivity was observed in the case of potentiometric sensors based on ionic liquid (1-decyl-3-methylimidazolium chloride) towards all APIs, recorded in different pH conditions.

Additional cation-sensitive electrodes based on KTFPB in PVC/DOS membranes were introduced to the sensor array, since the studied mixtures of APIs contained also caffeine – a component commonly added to the pharmaceutical formulations with anti-inflammatory drugs. However, due to the very low value of pK_a, the neutral form of caffeine was predominant in solution under the studied pH range [25] and thus the electrodes showed rather small and non-linear response to this component.

3.1. Quantitative analysis of APIs – PLS and 3-way PLS

The measurements of the sensor array signals were conducted in 80 sample mixtures containing 3 APIs in different concentration ranges (1-20 mmol/L for acetaminophen, 0.3-7 mmol/L for ascorbic acid and 1.4-8 mmol/L for acetylsalicylic acid), prepared automatically by the Sequential Injection Analysis system (Figure 1) [36,37]. The given concentration ranges were matched to the level of such APIs in commercial drugs after their dissolution in water. Caffeine, added to each mixture (0.13-1.3 mmol/L), was considered as an interferent (see Figure 1). The experiments were performed in solutions at various pH in order to alter the structure of APIs and sense individual components in particular pH conditions. Therefore, four carriers of different pH: HCl solution (pH=1.8), lithium acetate solution (pH=5.0) and TRIS buffer solution (pH=8.0 and pH=10.5) were used. In these solutions anionic species of acetaminophen were present only at pH=10.5 (existed in small amounts at pH=8.0), whereas acetylsalicylic acid and ascorbic acid remained in appreciable amount in neutral form only at pH=1.8.

Data matrices involving the steady-state signals of the sensors after step introduction of the samples were formed. Target matrices contained direct information on 3 APIs concentrations (three-column calibration) or on 1 API concentration (single-column calibration). Various variants of train data were considered (but in each case, 4:1 data division was used to form the train and the test set, respectively):

- 4 PLS models based on data from single measurements in the given pH,
- 6 PLS models based on the combination of signals recorded in solutions of 2 values of pH,
- 4 PLS models based on the combination of signals recorded in solutions of 3 values of pH,
- 1 PLS model based on the combination of all signals recorded in all 4 values of pH.

The PLS models were built with a number of Latent Variables (1 to 8 for PLS and 20 for 3-way PLS) enabling the minimization of the RMSE value. Their performance was characterized after performing the linear fitting of the obtained data (PLS predicted concentrations of API) versus the expected data (real concentrations of API). The values of the slope (“a”), intercept (“b”) and determination coefficient (“R^2”), were calculated for the train and test samples, assuming that in the perfect case they should reach values of 1, 0 and 1, respectively. It was found, that the data

dimensionality i.e. the combination of data from the experiments performed in various pH conditions influenced the classification results. The best results were obtained for the fusion of signals recorded in solutions of pH: 1.8, 8.0, 10.5 (see Table 2). However, the concentration of only 2 APIs i.e. acetylsalicylic acid and ascorbic acid was properly predicted. Even though acetaminophen should be in the anionic form in the solution of highest pH value, the responses of the electrodes to this API did not provide satisfactory result. Therefore, single-column calibration was attempted i.e. separate PLS-1 models were created for each API, based on the fusion of signals recorded in the same 3 values of pH. The same train and test data matrices were used; however the target matrix was limited to one column – concentration of single API. Unfortunately, the prediction of API concentration remained unsatisfactory, especially for acetaminophen (Table 2).

In the next step, dynamic responses of the sensor array were applied in the train data for multiway processing to enhance the amount of information gained by PLS model. Whole responses (1800 data points per sensor) and the initial 30s of sensors responses (first 300 data points per sensor) recorded in various combinations of pH were used in multiway models. Better results were obtained in the case of 3-way PLS analysis, but only for the train subset. Still, the results obtained for the test subsets were not satisfactory, which was caused by poor prediction abilities of the 3-way PLS models (see Table 3). However, quite better results were noticed when whole dynamic response was considered (1800 points).

3.2. Quantitative analysis of APIs – DWT-ANN

As an alternative approach, the usage of ANNs was also considered. Compared with PLS, ANNs are more flexible modelling methodologies, since both linear and non-linear functions (or its combination) can be used in the processing units, thus being especially suited when sensor responses show non-linear behaviors [39,40]. Moreover, allowing for more complex relationships between a high-dimensional descriptor space and the given retention data, which might lead to better predictive power of the resulting ANN model compared with other linear methods, although if linearity exists, a proper behavior will be obtained also with the latter.

In this way, same approaches as previously described were also followed, i.e. the usage of the steady state signal and building of models based on single- and multi-pH values, with the same data division for the train and test subsets. However, none

of those approaches provided enough satisfactory results. The next step was then the usage of the dynamic profile, as previously done with 3-way PLS. Nevertheless, this was not as straightforward as in the previous case, as in here, before building the ANN model, a preprocessing step devised for reducing the input dimensionality was required.

For the dynamic treatment, a two-step feature extraction process was thus followed to achieve the reduction of signals complexity and dimensionality. Firstly, the response profile of each sensor was compressed employing DWT, and afterwards ANNs were used as feature selection tool for variable selection [39]. In this way, the dynamic potentiometric response (from 2.1s to 10.0s, comprising a total of 80 values; see Figure 2) was compressed employing Daubechies wavelet mother function and a second decomposition level down to 25 coefficients, without any loss of relevant information. The obtained coefficients were then fed to an ANN model, which upon its training, was used for the removal of the less-significant coefficients by Causal Index (CI) pruning of the inputs [39,40]. This process was then iteratively repeated until selection of an optimal subset of coefficients that allows doing the prediction task as well as possible, with as few variables as possible. Lastly, the architecture of the ANN model was optimized, as usual, by systematically fine-tuning its topology (i.e. training algorithm, number of hidden layers, number of neurons, transfer functions, etc.) to achieve the correct quantification of the desired compounds.

Subsequently, after pruning and optimization of the ANN model, comparison graphs of predicted vs. expected scores, both for the train and test subsets, were built (Figure 3) and the linear fitted regression parameters were calculated to easily check the performance of the model (Table 4). As can be seen, acetylsalicylic acid (ASA) can be perfectly modelled in all the cases with low difficulty, while counterbalancing the changing concentrations of caffeine. On the other side, acetaminophen (PARA) and ascorbic acid (ASC) modelling was also plausible, although obtained correlation seem to be worst. Nevertheless, in all cases the obtained regression parameters were close to the ideal ones; i.e. values of intercept close to 0, and slope and R^2 close to 1.

4. Conclusions

HPLC systems with UV detection are commonly applied for the routine analysis of non-steroidal anti-inflammatory drugs (e.g. acetylsalicylic acid) in pharmaceutical pain relievers. However, sophisticated instrumentation and skilled personnel are essential to provide the proper chromatographic results. Therefore, a simpler analytical approach based on sensor arrays systems would be beneficial due to the automation and shortening of the analysis.

In this work, a flow-through sensor array of miniaturized potentiometric electrodes containing only an ion-exchanger in polymeric membranes coupled with Sequential Injection Analysis system was proposed for the quantitative analysis of selected active pharmaceutical ingredients. Simple chemometric data processing involving Partial Least Squares (PLS) analysis of the steady-state responses of the sensors as well as multiway PLS fed by dynamic sensors responses enabled the determination of acetylsalicylic acid and ascorbic acid in mixtures, while correcting any interference derived from the presence of acetaminophen and caffeine. However, the extraction of dynamic components of the transient response employing the Wavelet transform, the removal of the less significant inputs by means of Causal Index pruning and training of an Artificial Neural Network with the selected coefficients allowed the simultaneous determination of the 3 APIs counterbalancing any interference caused by caffeine.

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410 Table 1. Components used for the preparation of potentiometric electrodes.

411

Electrode type	Lipophilic salt	Plasticizer	Polymer	Internal filling/ Conditioning solution
1	3.5 wt% TDMAC	64.0 wt% o-NPOE	32.5 wt% PVC	0.01/0.001 mol/L NaCl
2	2.0 wt% TBHDPB	65.5 wt% o-NPOE	32.5 wt% PVC	
3	2.0 wt% IL	65.5 wt% o-NPOE	32.5 wt% PVC	
4	1.0 wt% KTFPB	66.0 wt% DOS	33.0 wt% PVC	

412

413 Table 2. Parameters of linear fitting of real and PLS-predicted concentration of APIs.

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		Three-column calibration			Single-column calibration		
		acetaminophen	ascorbic acid	acetylsalicylic acid	acetaminophen	ascorbic acid	acetylsalicylic acid
TRAIN	a	0.72±0.06	0.84±0.05	0.98±0.02	0.22±0.05	0.68±0.06	0.94±0.03
	b	0.00±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00
	R^2	0.72	0.84	0.98	0.22	0.68	0.94
TEST	a	0.52±0.28	0.60±0.20	0.96±0.03	0.11±0.08	0.58±0.19	0.94±0.04
	b	0.01±0.00	0.00±0.00	0.00±0.00	0.01±0.00	0.00±0.00	0.00±0.00
	R^2	0.19	0.41	0.98	0.12	0.40	0.98

415

416 Table 3. Parameters of linear fitting of real and 3-way PLS-predicted concentration of
417 APIs.
418

		Calibration based on 1800 data points			Calibration based on 300 data points		
		acetaminophen	ascorbic acid	acetylsalicylic acid	acetaminophen	ascorbic acid	acetylsalicylic acid
TRAIN	a	0.86±0.04	0.75±0.08	0.98±0.08	0.69±0.06	0.65±0.06	0.98±0.01
	b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	R^2	0.96	0.92	0.99	0.91	0.89	0.99
TEST	a	0.31±0.18	0.80±0,19	0.97±0.05	0.60±0.12	0.41±0.10	0.97±0.03
	b	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
	R^2	0.00	0.45	0.96	0.62	0.49	0.98

419

420 Table 4. Parameters of linear fitting of real and DWT-CI-ANN-predicted concentration of
421 APIs.
422

		acetaminophen	ascorbic acid	acetylsalicylic acid
TRAIN	a	0.96±0.02	0.96±0.01	0.98±0.01
	b	0.37±0.17	0.13±0.04	0.084±0.057
	R^2	0.99	1.00	1.00
TEST	a	0.98±0.27	1.00±0.35	1.03±0.09
	b	-0.42±3.00	0.13±1.23	-0.11±0.39
	R^2	0.82	0.73	0.98

423

Figure captions:

Figure 1. Random distribution of concentrations of the four species for the (●) train and (★) test samples prepared automatically by the SIA system. Acetaminophen (PARA), ascorbic acid (ASC) and acetylsalicylic acid (ASA) are plotted in x,y,z coordinates respectively, whereas caffeine (KOF) is plotted as a color-scale in the scatter plot.

Figure 2. Exemplary dynamic responses of the electrode based on TDMAC (PVC/o-NPOE membrane) recorded in arbitrary mixture of APIs (1.4 mmol/L acetaminophen, 0.91 mmol/L ascorbic acid, 6.6 mmol/L acetylsalicylic acid and 0.68 mmol/L caffeine) in 4 carriers at various pH values

Figure 3. Model performances characterized after linear fitting of the real concentrations of APIs to the predicted data by the DWT-CI-ANN model. Train set (●, solid line), test set (○, dotted line) and theoretical diagonal line (dashed line).

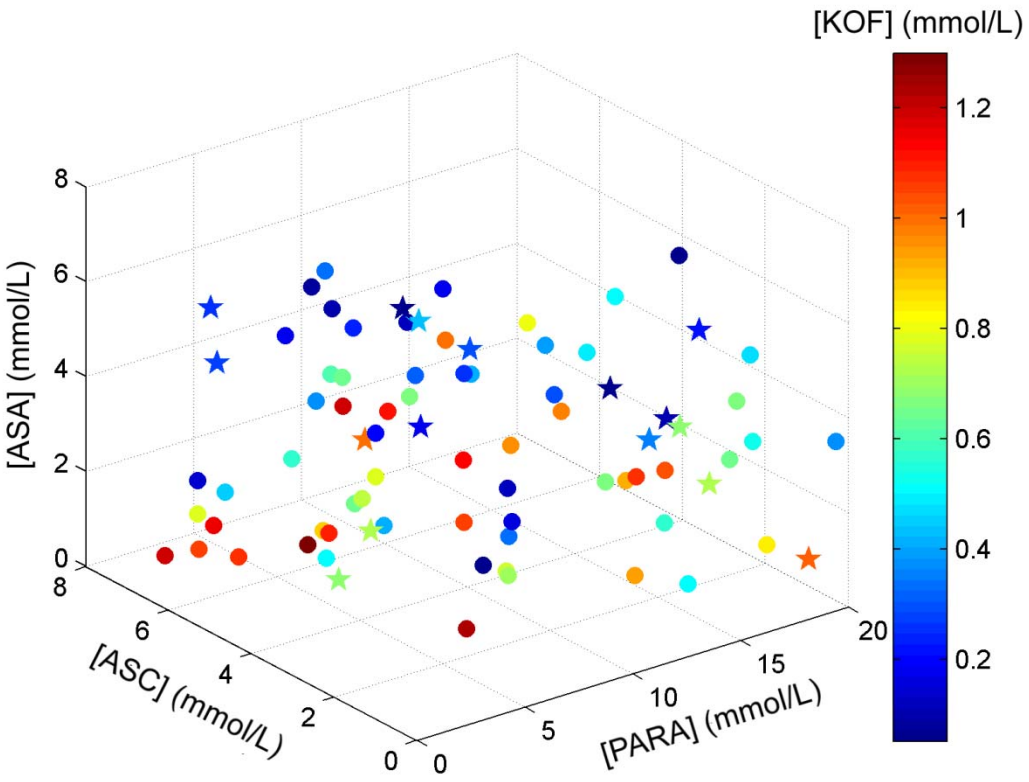
[KOF] (mmol/L)
1.2
1
0.8
0.6
0.4
0.2
[ASA] (mmol/L)
8
6
4
2
0
[ASC] (mmol/L)
8
6
4
2
0
[PARA] (mmol/L)
0
5
10
15
20

Figure 1.

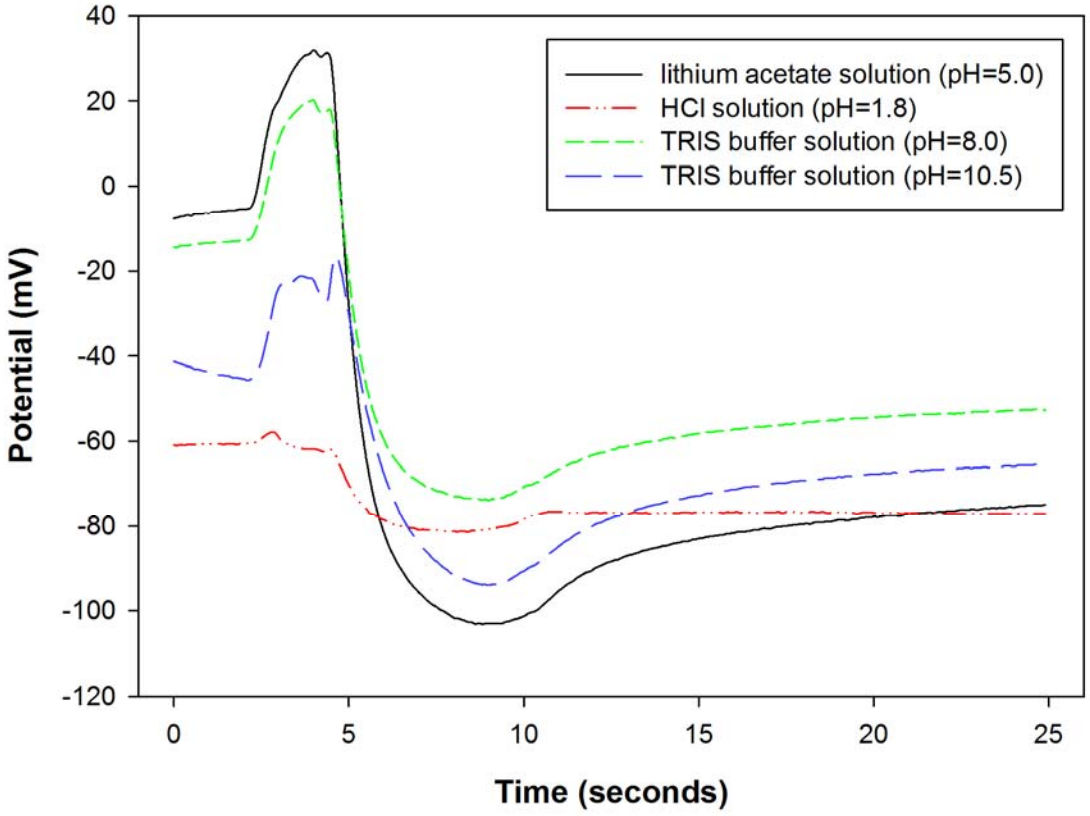
lithium acetate solution (pH=5.0)
HCl solution (pH=1.8)
TRIS buffer solution (pH=8.0)
TRIS buffer solution (pH=10.5)
Potential (mV)
Time (seconds)
40
20
0
-20
-40
-60
-80
-100
-120
0
5
10
15
20
25

Figure 2.

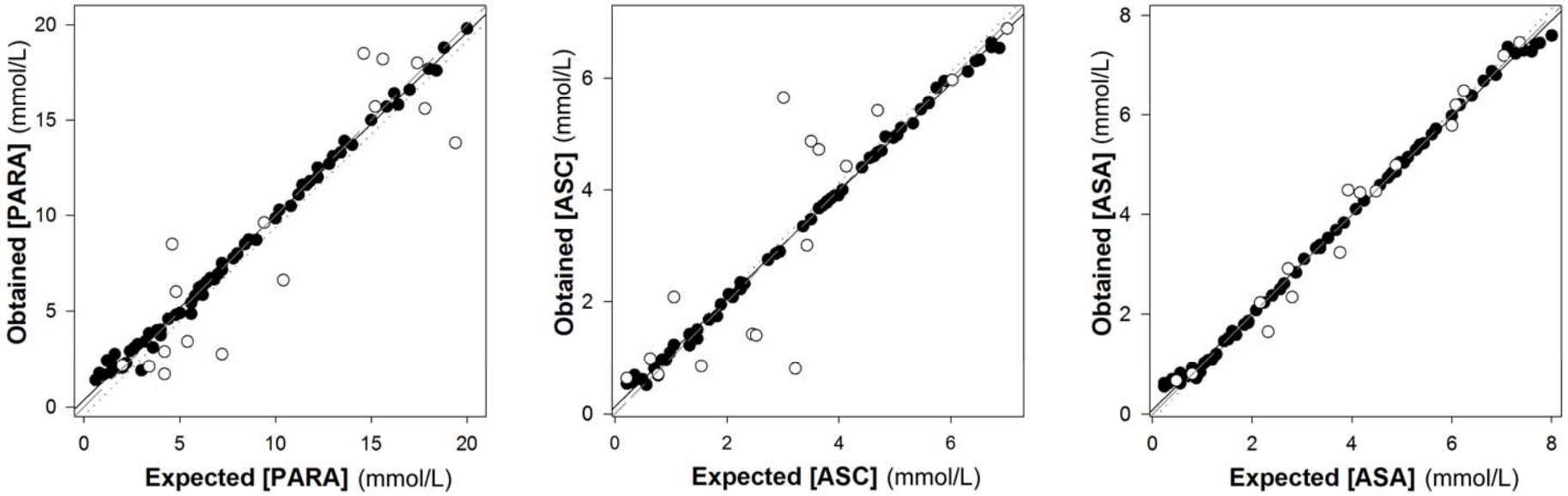
Obtained [PARA] (mmol/L)
Expected [PARA] (mmol/L)
Obtained [ASC] (mmol/L)
Expected [ASC] (mmol/L)
Obtained [ASA] (mmol/L)
Expected [ASA] (mmol/L)

Figure 3