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A comparative study on two electronic tongues for pharmaceutical formulation development

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ABSTRACT

Electronic tongues are sensor array systems which are able to determine single substances as well as complex mixtures of various substances. They are increasingly used for taste assessment of pharmaceutical formulations. Two systems are available on the market, the AlphaMOS electronic tongue Astree2 and the Insent taste sensing system TS-5000Z. Both systems measure based on potentiometry but sensor technologies are different. Therefore, these electronic tongue systems were compared to each other with respect to general aspects like software handling, sensors, and measurement procedure, but also on the basis of analytical experiments in order to figure out the applicability and limitations for use in the pharmaceutical field. By investigation of substances with different ionic character, like sodium saccharin, acetaminophen, ibuprofen, quinine, and caffeine, it was shown for both systems that ionic substances are easier to detect than neutral ones. Further, the performance qualification could only be done for the TS-5000Z, whereas the validation step, a correlation to human taste assessment, was passed by both systems. The results were even more reproducible than those from the panel. Taste masking by complexation of ibuprofen and quinine hydrochloride by maltodextrin, could be evaluated by both systems. Data from the Astree2 system have to be normalized in order to compare inter-day results, while the Insent taste sensing system refers each measurement to a standard solution and therefore reaches better inter-day results. Both systems offer the opportunity to be used for the development of taste-masked pharmaceutical formulations.

1. Introduction

Electronic tongues are sensor array based robotic systems which can be used for the investigation of single substances as well as complex mixtures [1]. They measure aqueous solutions non-specifically, but data were shown to have good reproducibility with low detection limits and high sensitivity [2]. These characteristics make those systems unique in the field of analytical systems and therefore their application to various fields of interest is continuously growing [3,4]. Most research has been done in the area of food, where for example different qualities of wine, tea, beer have been compared to each other [5]. The use of those systems in the area of pharmaceutical development has also attracted more interest [6]. This fact can be explained by the increasing importance of developing palatable medications in order to be superior to competitors and, more importantly, to improve acceptance by children. The new EU legislation [7], which demands the development of medicinal products appropriate for children, leads to the general question how to reliably assess the taste of active pharmaceutical ingredients (APIs) as well as finished drug products without raising ethical concerns. Therefore, electronic tongues offer a promising tool for objective investigation of possible taste masked formulations. For example, an optimization of matched placebo and active formulations by electronic tongue measurements could be performed before starting clinical studies. From a taste perspective, the compliance to the study would then be likely to be high and no ethical permission would have been needed for this preliminary formulation optimization.

Electronic taste sensing systems are based on different underlying techniques, as for example, electrochemical measurements, like potentiometry, amperometry, and voltammetry, or impedance spectroscopy [8,9]. However, potentiometry is the technique which is mostly used for pharmaceutical applications [1]. To date, two electronic tongues are available on the market, the Alpha MOS electronic tongue Astree2 and the Insent taste sensing system TS-5000Z. Both systems are potentiometric systems. They can be equipped with different sensors, whereas each lipid membrane sensor for the TS-5000Z is claimed to be associated with a specific taste stimulus, e.g. sour, salty, umami, bitter, sweet and the nociceptive sensation astringency. However, this only means that

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one sensor is most sensitive to this specific sensation, but also detects other substances. A detailed description about the development of these sensors and the entire taste sensing system has been published by Kobayashi et al. [10]. Sensors for the Alpha MOS electronic tongue Astree2 are based on chemically modified field effect transistor technology (ChemFET) which is similar to the ion selective FET technology, but sensors are coated with specific materials. These sensors are not assigned to a specific taste sensation and their way of measurement is described to be cross-selective. This means, that each sensor of the set detects substances of the sample, but with different intensities. Samples for both systems should be liquid and particle-free. Therefore tablets or other solid dosage forms need to be dissolved before investigation or dissolution profiles should be recorded [1].

The implementation of both electronic tongue systems for pharmaceutical purposes has been shown in various research papers in the recent years. For example, different antibiotic formulations were evaluated [11–13] and fast dissolving films containing a bitter tasting drug substance could be investigated [14] using the Insent taste sensing system. In addition the Insent taste sensing system was combined with a disintegration testing apparatus (Ph.Eur.) for taste assessment of orally disintegrating tablets containing propiverine hydrochloride [15]. The Alpha MOS electronic tongue has been shown to be potentially useful for the bitterness comparison of original and generic products [16] as well as for development of taste masked formulations [17–22]. Krause [23] investigated sodium benzoate pellets using both systems, the Insent taste sensing system and the Alpha MOS electronic tongue. A good correlation between dissolution profiles and detection via UV spectroscopy as well as human taste assessment and electronic tongue data could be achieved for both systems. In addition, measuring principle, system preparation, sample measurement, and the way of data analysis were compared in a non-experimental way.

Nevertheless, a systematic approach by comparing the applicability to different APIs has not been performed yet. Further, solid dosage forms were used by Krause [23] whereas liquid multicomponent mixtures were not investigated. Those multicomponent formulations reveal special challenges as other analytical techniques could fail due do misleading interferences or the missing ability to simultaneously detect more than one substance.

Therefore, the aim of this study was to test both electronic tongues under the same conditions with respect to their applicability to drug substances and liquid taste masked drug products. The ability to qualify the systems, their validity in comparison to human taste assessment, as well as their use for formulation development according to a rational approach, described earlier [24], should be investigated. Based on this, systematic experimental results with both systems should be shown and special characteristics elucidated in order to provide guidance using the electronic tongues in the pharmaceutical field.

2. Experimental

2.1. Chemicals and reagents

2.1.1. Electronic tongues

Potassium chloride (analytical grade) was acquired from Grüssing GmbH (Filsum, Germany). Tartaric acid (Ph.Eur.) was purchased from Sigma–Aldrich Laborchemikalien GmbH (Seelze, Germany). Absolute ethanol (purity 99.8%) was purchased from VWR International (Leuven, Belgium). Hydrochloric acid (1 mol/l) and potassium hydroxide solution (0.1 mol/l) were obtained from Merck KGaA (Darmstadt, Germany). The inner solution for sensors and reference electrodes of the taste sensing system TS-5000Z consisting of 3.33 mol/l potassium chloride in saturated silver chloride solution was provided by Insent Inc. (Atsugi-shi, Japan).

Sodium-L-glutamate (analytical grade) was purchased from Sigma–Aldrich Chemie Gmbh (Steinheim, Germany), sodium chloride (Ph.Eur.) from VWR International (Leuven, Belgium). Water was demineralized by reverse osmosis. Distilled water was obtained by in-lab distillation of demineralized water.

2.1.2. Drug substances and drug formulations

Acetaminophen (Ph.Eur.) was obtained from Rhodia Deutschland GmbH (Freiburg, Germany). Caffeine (Ph.Eur.), caffeine citrate (Ph.Eur.), quinine hydrochloride (Ph.Eur.) and sodium saccharin (Ph.Eur.) were purchased from Caesar & Loretz GmbH (Hilden, Germany). Ibuprofen (Ph.Eur.) and ibuprofen lysinate (Ph.Eur.) were donated by BASF SE (Ludwigshafen, Germany). Maltodextrin (Glucidex IT 17 L exp.; research grade) were generously provided by Roquette Frères (Lestrem, France).

2.2. Taste sensing system TS-5000Z

2.2.1. Sensors

Sensors and reference electrodes were purchased from TecLabS Europe OHG (Essen, Germany). The TS-5000Z (Fig. 1) was equipped with seven lipid membrane sensors indicating different taste qualities and three corresponding reference electrodes. There are three sensors indicating bitterness (Table 1), bitterness sensor 1 (SB2AC0), bitterness sensor 2 (SB2AN0), and bitterness sensor 3 (SB2C00). The other sensors represent the gustatory stimuli umami (SB2AAE), saltiness (SB2CT0), sourness (SB2CA0), and astringency (SB2AE1). In addition, a sweetness sensor is available detecting sugars and sugar alcohols, but as it is not commercially available yet, it was not included in the present sensor set. Furthermore a so called “aftertaste” can be measured for bitterness, umami, and astringency. 0.2 ml inner solution (see Section2.1.1) was filled into each sensor prior to the beginning of experiments. The reference electrode was completely filled up with inner solution. All sensors were preconditioned in standard solution for one day before the measurement.

2.2.2. Preparation of standard and washing solutions

Two washing solutions for negatively and positively charged sensors respectively were made by diluting absolute ethanol to ethanol 30% with distilled water and adding 100 mmol/l hydrochloric acid for the negatively charged sensors or 100 mmol/l potassium chloride and 10 mmol/l potassium hydroxide for the positively charged sensors. A standard solution serving as cleaning and reference solution was prepared by dissolving 30 mmol/l potassium chloride and 0.3 mmol/l tartaric acid in distilled water.

2.2.3. Electronic tongue system and measurement setup

All measurements were performed by the taste sensing system TS-5000Z (Insent Inc., Atsugi-shi, Japan). A sensor check was conducted routinely before every measurement in order to assure that sensors were working in the correct mV range. Each sample was measured four times, whereas one measurement cycle consisted of measuring a reference solution (V_r), afterwards the sample solution (V_s), a short ($2\times$ 3 s) cleaning procedure and measurement of the aftertaste ($V_{r'}$) followed by a cleaning procedure for 330 s. The so called “aftertaste” was measured by determining the change of membrane potential caused by adsorption of the substance to the lipid membrane after the short cleaning procedure. Both, sensor output for taste, also called relative value (R), and sensor output for “aftertaste”, also called CPA value (change of membrane potential caused by adsorption) were calculated in relation to the preliminary

Fig. 1. Taste sensing system TS-5000Z (Insent Inc., Atsugi-Shi, Japan).

Table 1
Sensors of the Insent taste sensing system TS-5000Z.

Sensor type	Sensor name	Corresponding taste sensation	Aftertaste
SB2AAE	Umami sensor	Umami	X
SB2CT0	Saltiness sensor	Saltiness	
SB2CA0	Sourness sensor	Sourness	
SB2AE1	Astringency sensor	Astringency	X
SB2AC0	Bitterness sensor 1	Bitterness of cationic substances	X
SB2AN0	Bitterness sensor 2	Bitterness of cationic and neutral substances	X
SB2C00	Bitterness sensor 3	Bitterness of anionic substances	X
Reference electrode	–	–	

determined sensor response to the reference solution (V_r).

$$R = V_s - V_r \quad (1)$$

$$\mathrm{CPA} = V_{r'} - V_r \quad (2)$$

The whole measurement procedure was performed for all samples and repeated afterwards up to four times. For further data treatment the first run was discarded as recommended by the supplier in order to enable conditioning of the sensors.

2.3. Astree2 electronic tongue

2.3.1. Sensors

The Astree2 electronic tongue (Alpha MOS, Toulouse, France) (Fig. 2) can be equipped with a seven-sensor probe of potentiometric working ChemFET sensors. In addition, an Ag/AgCl reference electrode and a stirrer are included. In this study, the sensor set for pharmaceutical applications was used, whereas sensor BA was exchanged by sensor GA or JB from another sensor set for some measurements due to stability reasons (Table 2). According to the manufac-

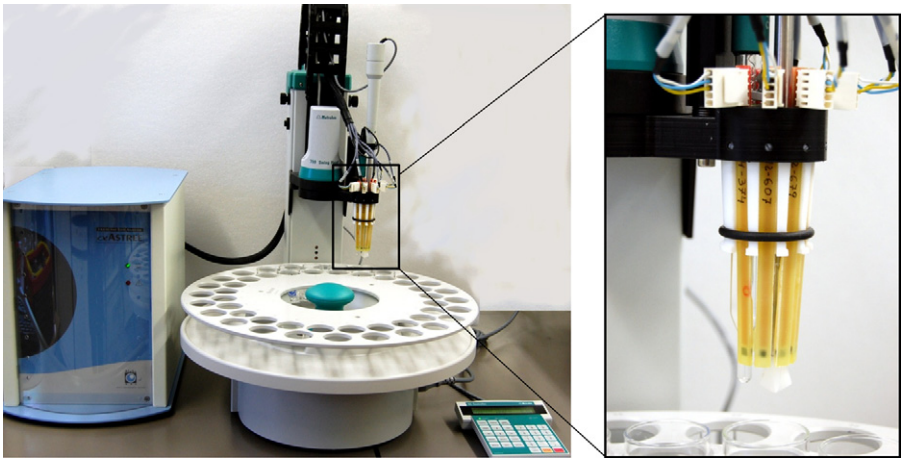

Fig. 2. Astree2 electronic tongue (AlphaMOS, Toulouse, France).

Table 2
Sensors of the Astree2 electronic tongue.

Sensor	Taste
ZZ	
AB	
BA/GA/JB	Cross-selective
BB	
CA	
DA	
JE	

turer, the new pharma set does no longer include sensor BA.

2.3.2. Preparation of cleaning, conditioning, calibration, and diagnostic solutions

An aqueous solution of 0.01 mol/l hydrochloric acid for a "conditioning" and a "calibration" step was prepared by diluting hydrochloric acid 1 mol/l with demineralized water. "Diagnostic" solutions were prepared by dissolving 0.18713 g sodium-L-glutamate (0.01 mol/l), and 0.05844 g sodium chloride (0.01 mol/l) in 100 ml demineralized water respectively and diluting hydrochloric acid 1 mol/l to 0.01 mol/l. For cleaning the sensors between sample measurements, an aqueous solution (dem. water) with the lowest concentration of the drug substance to be investigated was used. It has to be noted that supplier does not recommend the use of any other diagnostic solutions than the certified ones purchased from the company.

2.3.3. Electronic tongue system and measurement setup

The Astree2 electronic tongue was used with an auto sampler with 48 sample positions. The sample volume per beaker was 25 ml. Three tests, to confirm that the electronic tongue is working according to the suppliers requirements, were performed at the beginning of every week of measurement or after storage of the sensors in dry state for more than two days. In the "Conditioning" phase, which serves to rehydrate the solid state sensors, sensors are immersed in 0.01 mol/l of hydrochloric acid in triplicate and the dispersion in-between the measurements as well as the stability of the sensor signal are evaluated. In the "Calibration" step sensors are immersed in 0.01 mol/l of hydrochloric acid again, but in addition to meeting the dispersion and the stability criteria, sensor values are adjusted to a specific target value. In the "diagnostic" step three samples representing salty (sodium chloride 0.01 mol/l), sour (hydrochloric acid 0.01 mol/l), and umami (mono sodium glutamate 0.01 mol/l) taste need to be identified and distinguished. This means that a discrimination index (DI) of at least 94 needs to be obtained after conducting a principal component analysis with all sensors. The discrimination index is determined by calculating the ratio between the sum of areas of sample groups and the area of the whole PCA map and can be between 0 and 100. Resulting, a high DI is obtained, when sample groups are small (high reproducibility) and not overlapping (good to differentiate). Before the beginning of each measurement a short run with three different concentrations of the API is performed for conditioning of the sensors. For this, the measurement time is set to 300 s and sensors are immersed in one cleaning beaker after each sample for 10 s. 4 replicates of the whole setup are performed.

For measurement, sensors are dipped in the sample solution for 120 s and in three beakers with cleaning solution for 10 s each afterwards. After completing the measurement of every sample, the whole procedure is repeated up to 8 times. For data evaluation, the last three runs were used.

2.4. Concentration series of single substances

Different concentrations of substances with different ionic character were chosen according to their solubility, dissolved in demineralized water, and measured by both electronic tongues. *Anionic substances*: Sodium saccharin (8 concentrations between 1 mmol/l and 500 mmol/l), ibuprofen lysinate (10 concentrations between 0.013 mmol/l and 30 mmol/l). *Neutral substances*: Ibuprofen (10 concentrations between 0.013 mmol/l and 0.13 mmol/l), acetaminophen (10 concentrations between 0.13 mmol/l and 66 mmol/l), caffeine (10 concentrations between 0.05 mmol/l and 90 mmol/l). *Cationic substances*: Caffeine citrate (10 concentrations between 0.03 mmol/l and 45 mmol/l), quinine hydrochloride (9 concentrations between 0.02 mmol/l and 5 mmol/l).

2.5. Validation–correlation with human taste panel

0.12 mmol/l, 0.25 mmol/l and 0.49 mmol/l ml quinine anhydrous were dissolved in demineralized water and measured by the electronic tongues. For correlation with human data it was referred to the studies performed by Turner [25]. The number of healthy human volunteers able to assess the three quinine hydrochloride solutions mentioned before in the correct order was 19. According to the measurement protocol, each assessor evaluated and recorded the initial bitterness intensity of the three blinded quinine hydrochloride samples on a 100 mm visual analogue scale in triplicate and in a randomized order. 0 mm on this not further subdivided scale represented "no bitter taste" whereas 100 mm represented "extremely strong bitter taste".

2.6. Formulation development

Sample solutions were prepared by dissolving 1.2 mmol/l, 2.4 mmol/l, 4.9 mmol/l, 9.7 mmol/l and 19.4 mmol/l of maltodextrin (MG = 11751 g/mol) in demineralized water. For formulations 5 mmol/l of quinine hydrochloride (MG = 396.9 g/mol) were added to the solutions containing 4.9 mmol/l, 9.7 mmol/l, and 19.4 mmol/l maltodextrin resulting in molar ratios of 1:0.96, 1:1.92 and 1:3.85. For ibuprofen formulations, 9.7 mmol/l of ibuprofen (MG = 206.3) were added to maltodextrin solutions with 1.2 mmol/l, 2.4 mmol/l, and 4.9 mmol/l, and 9.7 mmol/l respectively, resulting in molar ratios of 1:0.125, 1:0.25, 1:0.5 and 1:1. For reference values, 1 mmol/l and 5 mmol/l of quinine hydrochloride and 0.013 mmol/l and 0.13 mmol/l of ibuprofen in demineralized water were used. All samples were shaken for 24 h and, after filtration of the ibuprofen formulations, investigated by the electronic tongue systems as well as by UV-spectroscopy at 221 nm and 264 nm for ibuprofen formulations (Spekol®, Analytik Jena AG, Jena, Germany).

2.7. Evaluation of results

The results were expressed as sensor response values obtained either by direct measurement of mV values and multiplication by a certain gain factor (Astree2 electronic tongue) or relative measurement of mV values of the sample solution to a standard solution (TS-5000Z). The last 20 s of 120 s measurement were used in case of the Astree2 electronic tongue whereas the whole 30 s of measurement were used for the TS-5000Z. Either sensor signal results alone or combined by multivariate data analysis were evaluated. For the multivariate data analysis raw data was pretreated by mean centering and scaling to unit variance. Data processing, graphical illustration, and statistical interpretation of the results were carried out using Excel 2007 (Microsoft, Redmond, US) and SIMCA-P+ v11.5 (Umetrics AB, Umeå, Sweden).

Table 3
Comparison of general aspects: software, sensors and measurement.

	Insent TS-5000Z	Astree2
Software		
Measurement	Comparable	
Data analysis	More display tools	More scientific tools
Software validation	No special validation tools	CFR Part 11 compliant
Language	English, Japanese	English, German, French, Japanese, Korean, Chinese
Sensors		
Handling	Demounting; special storage	No demounting; storage in air
Lifetime[a]	Up to one year	One to three months
Limitations in terms of sensor damage	Only pH 2–8, no alcoholic solutions > 40%[b]	Not observed
Performance qualification	Passed according to ICH guideline Q2 [2]	Not passed according to ICH guideline Q2
Measurement		
Principle	One sensor indicating one taste sensation, cross selective	Cross selective
Preparation	Sensor check (30 min)	"Conditioning", "calibration", "diagnostic" (5–24 h)
Sample volume	2× 40 ml	25ml
Duration of each analysis	Exchange of sensors → duration ↑ 2× 7h for all sensors	Several short cycles 6h

[a] Observed for both sensor sets under the same extent of usage and measurements for data obtained in this study
[b] Quoted by the manufacturer

3. Results and discussion

3.1. General–theoretical comparison

Due to the different underlying sensor technologies of both taste sensing systems, sensor handling and preparation, as well as measurement results are different from each other. Table 3 shows the main differences of both electronic tongues with respect to provided software, sensors, and measurement setup.

Both systems provide a software for managing the system, creating measurement sequences, as well as evaluation of the raw data after measurement and statistical analysis by a multivariate data analysis tool offering, for instance, principal component analysis and partial least square regression. In addition, raw data can be exported and used by external software as it was done in the present study. One main difference is that the software for the Astree2 electronic tongue is directly available on the computer which is connected to the electronic tongue. Further, the electronic tongue unit can be equipped with up to four auto sampler systems. The Insent taste sensing system TS-5000Z is driven via a server which is connected to a terminal computer for data analysis and measurement sequence creation. This offers the opportunity to connect several electronic tongues to one server, but always requires a second computer system. Regarding the creation of measurement sequences and system control both software systems are comparable. With respect to multivariate data analysis the Astree2 software is higher sophisticated, as important statistical values are specified and more options for analysis are provided. In addition, the Alpha MOS system offers validated software, which is compliant to the Code of Federal Regulations (CFR) 21 part 11 announced by the Food and Drug Administration (FDA). This part deals with the guidelines on electronic records and electronic signatures in the United States. The software system can therefore be regarded as fit to be used in industrial environment. Nevertheless, both software systems can be used getting a first impression of the results and also allow to integrate external sensory data about odor or texture as well as analytical data such as GC/MS. Further, data from both systems can be easily exported to have the ability of independent statistical treatment.

The ChemFET sensors of the Alpha MOS system are easy to handle as they are attached to the sensor head once and can be stored in a beaker with demineralized water for short-time storage (1 day) or in air for long-time storage. Their lifetime, however, was only one to three months for the samples investigated in this study. The lipid membrane sensors of the Insent taste sensing system TS-5000Z require more maintenance since they need to be mounted before every measurement and demounted afterwards in order to store every sensor separately in potassium chloride solution. With careful treatment, their lifetime was one year or even longer for the measurements presented in this study. The cost for one set of sensors is approximately the same for both electronic tongue systems. Of course, depending on the type of sensor materials, limitations with respect to compatibility to sample solutions and sensor damage can occur. As most sensor coatings are of organic nature, sensors are fragile towards organic solvents and high concentrations of lipids or surfactants. They should therefore preferably be used in aqueous systems. However, other experiments showed that small concentrations of surfactants for example did not damage the sensor membranes. If it is necessary to use agents increasing the solubility of APIs, it is recommendable to start with small concentrations and to directly check sensor performance after extensive cleaning. In addition, according to the manufacturer, the Insent taste sensing systems should only be exposed to pH values between 2 and 8 and alcohol concentrations may not exceed 40%. Other limitations for the Alpha MOS system have not been observed so far.

Tables 1 and 2 show that sensors of the TS-5000Z are assigned to a taste stimulus offering the possibility of univariate data analysis. However, a partial cross-selectivity of these sensors has been observed as well and will be discussed later. The Astree2 sensors measure in a cross-selective way meaning that a taste specific interpretation is not aimed for or possible with this sensor set. Both systems are able to offer the stage of operational qualification (OQ) before starting measurements. It is recommended to perform a so called "sensor check" for the Insent taste sensing system TS-5000Z prior to every measurement in order to check whether sensors are measuring stable in the correct voltage range while investigating a potassium chloride/tartaric acid standard solution. This sensor check lasts between 10 and 30 min. In addition, a maintenance measurement of solutions representing different taste qualities should be performed monthly. Here, the specified mV range is narrower. At the beginning of every week of the measurement period or after storage of the sensors in dry state, three tests, so called "conditioning", "calibration", and "diagnostic", should be performed with the Astree2 electronic tongue. The conditioning step serves for rehydration of the sensors after storage in air. In the second step, sensors are calibrated to certain mV values, whereas three different sample solutions representing salty, sour, and umami need to be distinguished in the diagnostic step. This procedure can last from 5 h up to one day depending on how often the system is used. However, like the measurement procedures of both systems, this procedure is automated in the software and can be performed at night.

The sample volume for the Insent taste sensing system is 2× 40 ml, whereas 25 ml are needed for the Astree2 electronic tongue. With the default settings of the Insent taste sensing system TS-5000Z, a maximum number of 10 samples can be investigated in one measurement procedure. The Astree2 electronic tongue has 48 possible positions on the sample table leading to a maximum number of 12 samples when using 3 cleaning beakers per sample, like in the present study. Cleaning procedures of both systems were evaluated to be effective for substances used in this study and no carry over effect could be observed. In general, the measurement time with the Alpha MOS system is shorter (approximately 6 h) as there is no check of sensor values included between the sample solutions compared to the Insent taste sensing system (approximately 2× 7 h) and the cleaning procedure is shorter. In addition, all 7 sensors can be used at the same time, whereas sensors of the Insent taste sensing system need to be exchanged and the measurement restarted, when all 8 sensors available are intended to be used, due to interaction between some sensors.

3.2. Performance qualification (PQ)

The performance qualification based on an adapted protocol to ICH guideline Q2 of the Insent taste sensing system SA402B has been shown in our previous study [2]. Quinine hydrochloride was used as a bitter tasting model drug and all important topics required according to the guideline were tested. If parts of the guideline were not applicable to the working principle of an electronic tongue, an alternative approach was introduced. As sensors of the SA402B and the TS-5000Z are identical and only the robot system is different, results could be repeated with the TS-5000Z without any problems.

Measurement results of the different concentrations of quinine hydrochloride investigated by the Astree2 system (Fig. 3) make obvious why the same approach could not be used for the Astree2 electronic tongue. As a log concentration dependent sensor response was only obtained for sensor CA as well as for sensor JE in a small range compared to the Insent taste sensing system, the detection limit, the quantitation limit as well as linearity could not be determined according to ICH guideline Q2. The non-specificity of sensors can be confirmed as their measurement principle is based on this prerequisite. Like for the Insent taste sensing system, sensors are sensitive to changes in analytical conditions, as for example temperature and preliminary investigations, and therefore reproducibility can be affected. Issues of reproducibility will be further discussed later. Concluding, the performance qualification, which is based on the log-linear relationship between concentration and sensor response as well as reproducibility of this relationship, could not be done for the Astree2 electronic tongue using quinine hydrochloride as bitter tasting model drug. Nevertheless, by investigating another API, for which sensors are more sensitive, might allow applying our proposed approach for the performance qualification and may lead to more satisfactory results.

3.3. Concentration series of single substances

According to a rational approach, drug substances and excipients should be investigated individually first, in order chose the sensors which are sensitive to these substances for formulation evaluation later on [18]. To find out feasibilities of the electronic tongue systems to detect different APIs, substances were categorized into anionic, cationic and neutral by structure and salt form. Different concentrations were investigated for each substance (Table 4). To compare sensor responses, molar amounts of substances were used. Fig. 3 clearly shows that detection of ionic substances can be done more easily by both systems compared to neutral substances. As the measurement principle is potentiometric increased conductivity by ionic substances leads to better detection. Nevertheless, detection might be increased, but also interaction with the sensor membranes plays an important role. This can be seen as the Astree2 electronic tongue shows difficulties to detect quinine hydrochloride dependent on the concentration, the same for ibuprofen lysinate. For these substances, no clear log linear relationship was obtained. Only few sensors show ranges of linearity, which are quite small and not in congruence with the whole shape of the concentration curve. As the ingredients of the thin layer on the semi conductor part of the sensor are not disclosed by the manufacturer, interactions between a specific substance and electronic tongue sensors are hardly predictable. Therefore an explanation about sensor - API interaction cannot be drawn and a case by case investigation is still necessary. Sensors of the Insent taste sensing system, for example, are labeled according to their intended specificity for a taste stimulus. But, as can be seen in Fig. 3, also the sensor labeled for sourness, detects bitter tasting quinine hydrochloride. In addition, the three sensors for bitterness should reflect the variability in bitter substrates and corresponding receptors in reality. But, these sensors are only different from each other in detecting molecules with different ionic structures, rather than representing up to 40 known different subtypes of bitterness receptors on the human tongue. These results obtained from measurement with both electronic tongues emphasize again, how important a preliminary calibration is in order to put sensor responses to formulations in the right context later on. This is the first time different substances have been systematically compared to each other by electronic tongue measurement based on calibration curves. And, in order to assure that the unpleasant tasting drug substance will be detected in the formulation later on, this calibration step is a major prerequisite and has often not been considered in previous studies [1]. Therefore, results of API characterization or formulation development shown for substances which cannot be detected according to different concentrations or taste intensities, respectively, might be questionable at all.

Table 4
Feasibility of detecting log linear relationships between sensor responses and different concentrations of single substances.

Substance	Insent TS-5000Z	Astree2
Sodium saccharin (anionic)	√	√
Ibuprofen lysinate (anionic)	√	(√)
Ibuprofen (neutral)	(√)	(√)
Acetaminophen (neutral)	√	×
Caffeine (neutral)	×	(√)
Caffeine citrate (cationic)	√	√
Quinine hydrochloride (cationic)	√	(√)

√ = log linear relationship observed; (√) = log linear relationship observed, but either with a small slope or only for few sensors; × = no log linear relationship observed.

The measurement of so called “aftertaste values” is an additional option offered by the Insent taste sensing system. Aftertaste sensor responses towards the substances are shown in Fig. 4. As described by the supplier, aftertaste values are only available for taste impressions mediated by G-protein coupled receptors in reality, like bitterness, umami, astringency, and sweetness. However, there are no studies giving evidence that these aftertaste values are comparable to human taste. They are likely not to be as aftertaste sensation is very complex and not only depended on substance–receptor interaction. Nevertheless, these values are recorded for all sensors and should therefore also be included in the first evaluation of raw data. In general, ranges of mV values are much smaller compared to the sensor responses and standard deviations are high for most sensors due to the additional washing procedure. However, depending on the substance, these values might have an important impact for evaluation of the results as they are a measure for high drug-polymer affinity. Aftertaste values of sensor

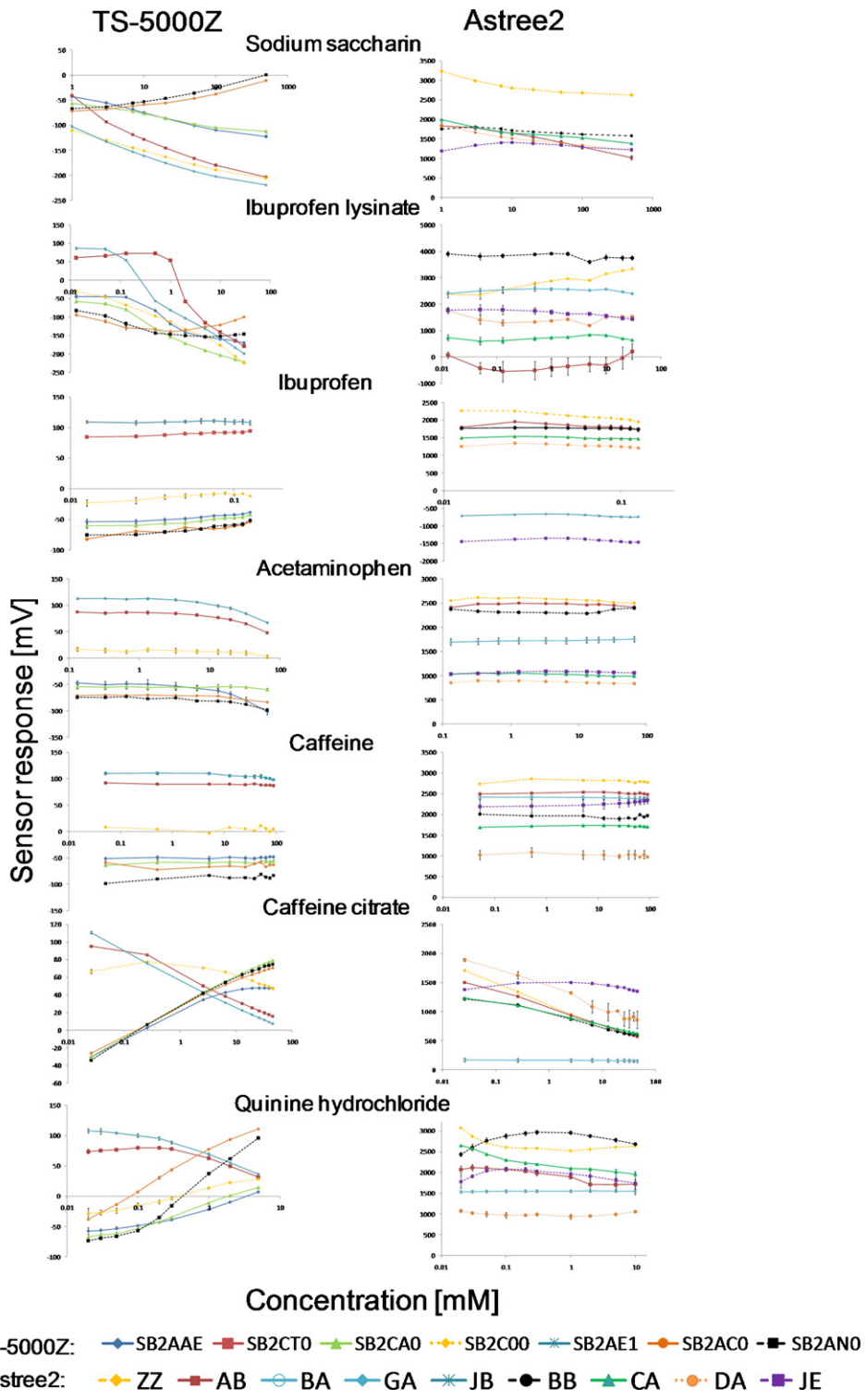

Fig. 3. Concentration series of anionic, neutral and cationc substances ($n = 3$, $\bar{x} \pm s$).

SB2C00, for example, offer additional information for ibuprofen measurement, compared to sensor responses. These values should be therefore always considered, especially, when information obtained from the sensor responses are not satisfactory enough. In principle, the same protocol could be performed with the Astree2 system, but not recommended as the comparability to a real aftertaste is not proven according to the supplier.

3.4. Validation–correlation with human taste panel

For the rating of the three different concentrations of quinine hydrochloride by human taste panel, ranges of bitterness scores were 1–100 mm for 0.12 mmol/l, 2–100 mm for 0.25 mmol/l, and 4–100 mm for 0.49 mmol/l. This variability in data can be explained by the missing ability of human sense of taste to exactly quantify taste as well as by the inter-individual differences which are

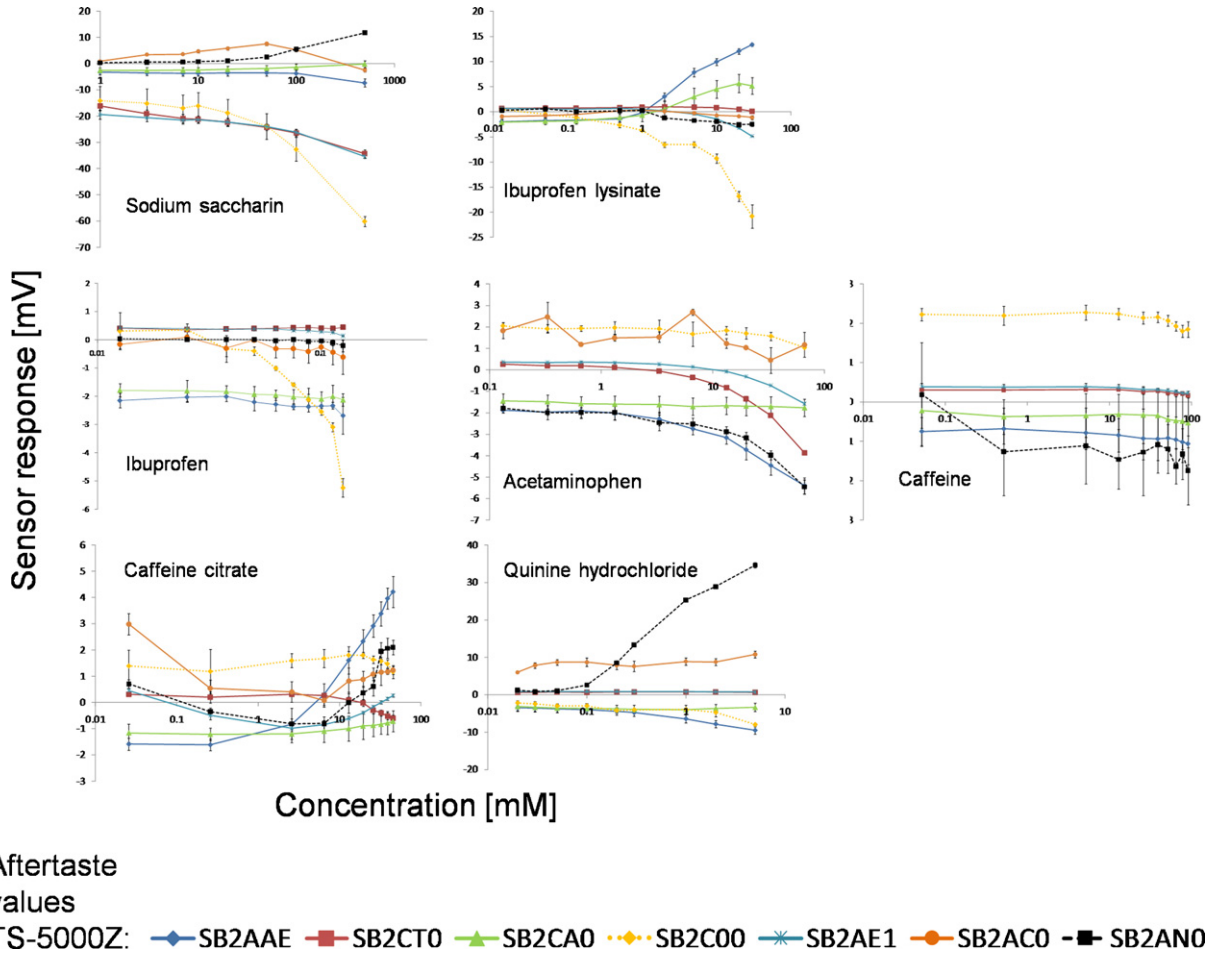

Fig. 4. Aftertaste values for anionic, neutral and cationic substances (TS-5000Z; $n = 3$, $\bar{x} \pm s$).

based on genetic variance as well as habituation effects. However, focusing on the evaluation by one individual, a differentiation of the samples as well as a ranking in the correct order could be done. In order to correlate these results with sensor responses obtained from the electronic tongues, a partial least square regression was performed using all sensors available (Fig. 5a and b). A log-concentration dependency of sensor responses of the TS-5000Z to quinine anhydrous has been observed in the same way like for quinine hydrochloride but with different absolute mV values (Fig. 3). Therefore, the correlation to human taste assessment is pretty good. A R^2 of 0.993 together with a root mean square error of estimation (RMSEE) of 1.13 mm was obtained. The RMSEE is a measure for the remaining error of prediction and therefore showing the accuracy of prediction. This means that a prediction of the human bitterness score by the TS-5000Z could be done with a remaining error of 1.13 mm with respect to the 100 mm scale the bitterness was rated on. From the correlation with data from the Astree electronic tongue a R^2 of 0.990 and a RMSEE of 1.52 mm were obtained. The univariate concentration dependency of individual sensor responses was, similar to responses to quinine hydrochloride, and therefore worse compared to the results of the Insent electronic tongue. However, the correlation by PLS correlation is quite acceptable. This can be explained by the fact that sensors of the Astree2 electronic tongue could distinguish between the samples and therefore a correlation to human data could be established. Concluding, data from both systems could be correlated with the rating of quinine hydrochloride solutions by a human taste panel. The prediction by the electronic tongue systems was even more precise due to the higher reproducibility. This over-precision is important for analytical reliability in vitro, but has to be considered for in vitro/in vivo correlation. In addition, this example of correlation does only show feasibilities of the two electronic tongues in direct comparison for one specific substance. Of course, further

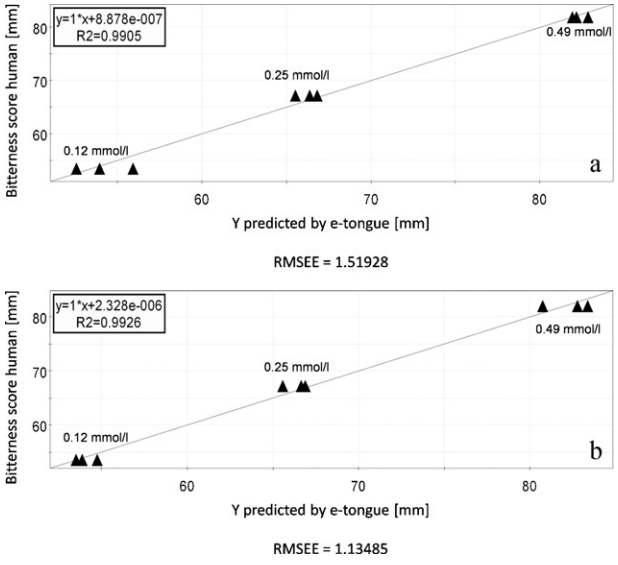

Fig. 5. Correlation of e-tongue data ($n = 3$) and human taste assessment of quinine anhydrous ($n = 19$; $\bar{x}$): (a) Astree2 electronic tongue (sensors ZZ, AB, GA, BB, CA, DA, JE); (b) Insent taste sensing system TS-5000Z (all sensors + aftertaste).

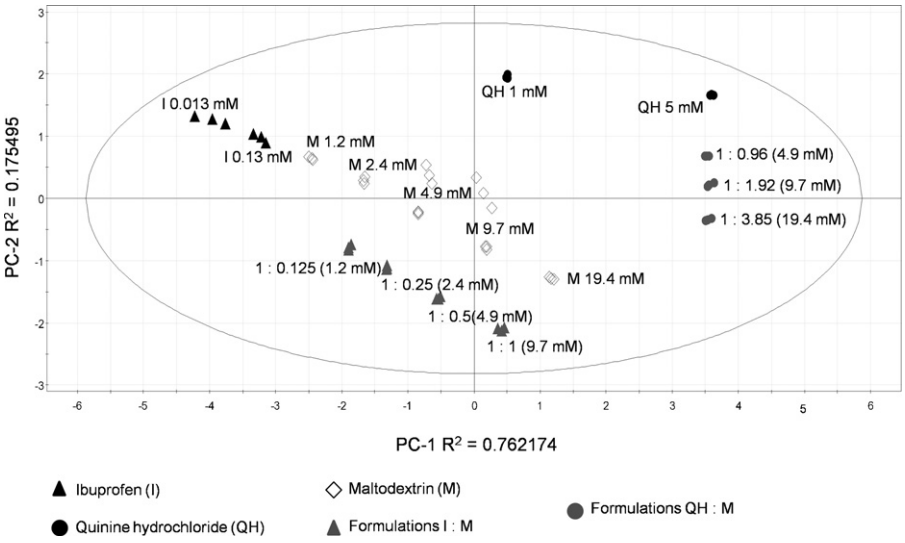

Fig. 6. Principle component analysis (PCA) showing the investigation of binary formulations by the TS-5000Z (sensors umami, saltiness, sourness, bitterness 1–3, astringency).

validation would be needed to make statements about the general comparability of electronic tongue measurements to human taste sensation.

3.5. Formulation development

In order to verify the applicability for formulation development according to a rational approach [24], a screening with maltodextrin and quinine hydrochloride or ibuprofen as unpleasant tasting drugs was performed. Maltodextrin was assumed to have taste masking properties due to its helical structure and thereby high complexing capacities. To improve comparability to the previous study, the same molar ratios of drug to complexing agent were chosen. All sensors of the TS-5000Z were chosen for data evaluation after measurement of the single substances showing a concentration dependent sensor behavior. Aftertaste values were not included due to missing reproducibility. For evaluation of the results from the Astree2 electronic tongue, all sensors were used except the GA as it did not contribute to differentiation of the samples. Conducting a principal component analysis (PCA) with these sensors (Figs. 6 and 7), mainly the same conclusion can be drawn from both data sets. A taste masking by complexation of bitter tasting quinine hydrochloride was not achieved, as complex formulations are closely located to the pure quinine hydrochloride drug substance on the PCA map. This missing taste making effect has been analogically described before for the interaction of β-cyclodextrin and quinine hydrochloride [24,25]. In contrast, an interaction between the ibuprofen and the maltodextrin has obviously occurred, as the different complex formulations were detected similarly to the maltodextrin by both electronic tongue systems. This interaction could be proven by UV-spectroscopy showing increased solubility of ibuprofen. Nevertheless a taste masking at the same time could be excluded as only the increased amount of ibuprofen was detected by the sensors, rather than a reduced amount leading to taste masking of ibuprofen. This could be shown by comparison to a preliminary calibration with ibuprofen and investigation of the ibuprofen-maltodextrin complex at saturation solubility of ibuprofen. In addition, other reference techniques, like ^{1}H NMR or FT-IR would allow to further characterize this complex. But, as the major objective was focused on the electronic tongue performance, complex characterization was not further deepened.

The main difference between the data sets from both electronic tongues lies in the weaker reproducibility of data from the Astree2 electronic tongue. This can be explained by the different measurement procedures. For the Insent taste sensing system,

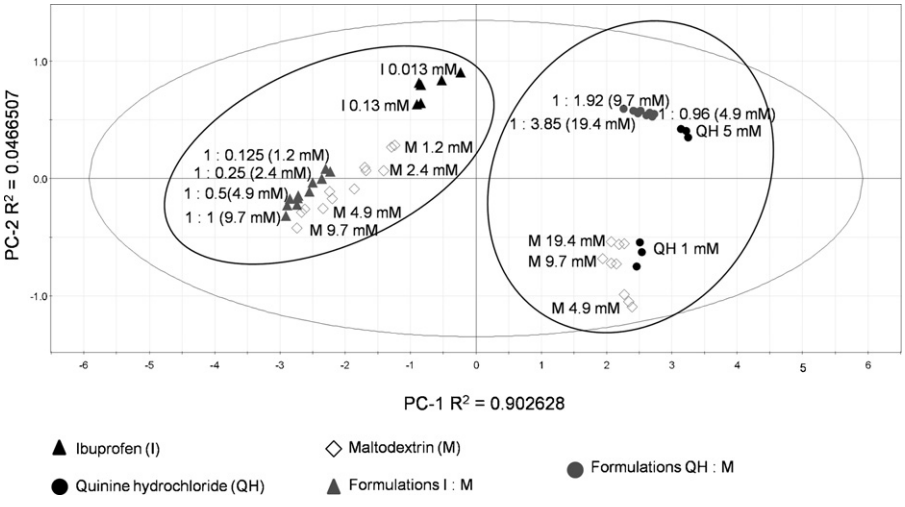

Fig. 7. Principle component analysis (PCA) showing the investigation of binary formulations by the Astree2 (sensors ZZ, AB, BB, CA, DA, JE).

the measurement of the standard solution and calculation of the relative value leads to more reproducible data. The principle component analysis of the Insent data shows that maltodextrin samples (4.9 mmol/l and 9.7 mmol/l) investigated in duplicate on different days can be measured in a reproducible manner as they are located close to each other. In contrast, the PCA map of the Astree data shows two groups consisting each of samples which were measured within one measurement run. Therefore, it becomes obvious that a normalization of these results to an external standard would be essential to obtain comparable results from the Astree2 electronic tongue, whereas data from the TS-5000Z usually not require additional normalization due to the sampling protocol. As described earlier [2], it is therefore recommendable to always use an external standard in order to have the opportunity of data normalization. In order to avoid the use of such an external standard it is necessary to investigate all samples, which should be compared, within one measurement for the Astree2 electronic tongue.

4. Conclusions

The comparison of the two systems contributed to demonstrate to what extent they are suitable to be used for pharmaceutical analysis and formulation development. As identically sample solutions could be investigated at the same time and under the same analytical conditions, inter-day/-laboratory/-analyst issues influencing the comparability could be excluded. In the context of previous studies, this is the first time that both electronic tongues could be systematically tested under the same conditions. Whereas much research dealing with the Astree2 electronic tongue is based on case studies so far [1], research with the Insent taste sensing system deals with the systematic development of sensors and measurement setups over years. The present study, further demonstrates the importance of this systematic approach in order to obtain reliable data.

Generally, both systems offer the same advantages like working well when comparing complex drug-loaded to drug-free formulations due to non-specificity, as well as offering acceptable correlation to human taste assessment. And both systems are also facing challenges as it is almost impossible to give absolute statements regarding the taste of samples based on electronic tongue data. Neither have taste-specific sensors, but sensors with different affinities to molecular substructures. However, the concentration–response correlations of the Insent sensors offer additional opportunities as univariate data evaluation and calibration. Further, data from both systems, especially those from the Astree2 electronic tongue, can be affected by changes in analytical conditions and reproducibility of data can therefore worsen. This is why an external standard for data normalization should always be available and used if necessary. Taking these findings into account, in our study, the Insent taste sensing system was shown to provide more reliable (in vitro/in vivo correlation) and precise (reproducibility and repeatability) data due to the different sampling protocol and sensors' sensitivity for the substances investigated in this paper. The Insent system shows longer analysis cycle times mainly determined by the more extensive cleaning procedure of the sensors. Concluding, electronic tongues are analytical systems able to support and facilitate the development of taste masked formulations. Nevertheless, more experiences with respect to validation by human taste panel should be gained in the future.

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