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Rational development of taste masked oral liquids guided by an electronic tongue

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ABSTRACT

Human taste testing is often associated with ethical concerns, organizational and validation issues. Electrochemical sensor array systems, so called electronic tongues, offer an alternative to assess the taste of multi-component liquid formulations. Therefore, it should be investigated how an electronic tongue can be implemented in the rational development of taste masked formulations.

Taste masking of bitter tasting quinine hydrochloride (QH) in a liquid formulation was carried out by screening sweetening agents (sucrose, glucose, fructose, mannitol, sucralose, sodium saccharin, acesulfame potassium, and monoammonium glycyrrhizinate), strong and weak cation ion exchange (IE) resins (Amberlite™ IRP69, Amberlite™ IRP88, and Indion 234), and soluble complexing agents (α-, β-, hydroxypropyl-β-, sulfobutyl ether-β- and γ-cyclodextrin and maltodextrin).

Amberlite™ IRP88 showed the best binding capacity for quinine (1.9 g quinine/1 g IE). The addition of sulfobutyl ether-β-cyclodextrin (SBE-β-CD) could significantly reduce the bitter taste of QH (79% reduction of free QH). The SBE-β-CD formulation was further improved by adding sodium saccharin as secondary taste masking agent. It could also be shown that presence of strawberry flavor and the preservative domiphen bromide does not affect evaluation of taste masking efficiency. The introduced stepwise approach was shown to be applicable to rationally develop novel taste masked formulations.

1. Introduction

The taste of a pharmaceutical formulation has major influence on the adherence of a patient to the medication. Particularly, patients suffering from chronic diseases are affected and compliance issues may arise. Children, whose sense of taste is not finally developed yet, might refuse taking unpleasant tasting medicine (Cram et al., 2009). Children are more sensitive to bitter tasting substances compared to adults as, from an evolutionary point of view, bitter taste of substances is often associated with toxic attributes (Mennella and Beauchamp, 2008). This sensitivity decreases during development and also due to adaptation to such substances. Infants further prefer sweet substances which is innate and presumably evolved to attract species to energy sources.

In fact, many active pharmaceutical ingredients have an unpleasant taste, like bitterness, saltiness, or sourness or cause an irritating mouth feeling, like astringency, metallic or spicy taste. For these reasons, taste masking and taste testing have become important topics for the development of a pharmaceutical formulation (Daniels, 2005a,b). Taste masking can be carried out using various techniques depending on the type of active pharmaceutical ingredient and the type of formulation (Ayenew et al., 2009; Wagh and Ghadlinge, 2009): solid dosage forms can be processed by introducing barriers like polymer coating of tablets or lipid extrusion for example. Liquid formulations can be modified by adding substances covering the taste such as sugars, sweeteners and sweetness enhancers. It can also be taken advantage of poorly soluble APIs or formation of less soluble salts to prepare suspensions. Further, complexation by complexing agents like cyclodextrins or ion exchange resins are commonly used methods. Recent investigations were performed dealing with so called taste suppressants (Sato et al., 2009; Lyall et al., 2010), which directly modify the interaction of API and taste receptor. In addition, viscosity enhancement, pH modification, and microencapsulation of the API can improve the taste of a formulation.

In order to determine whether the available masking technique is effective, different approaches are available. Human taste panels, animal models or analytical techniques are commonly used (Cram et al., 2009). But, determination of taste masking efficiency by human taste panels reveals challenges with respect to possible toxicity of the drug. This is especially true for new chemical entities, which often have unknown toxicity status. Further, taste assessment by human beings is affected by differently developed senses of taste and individual preference and intraindividual variations. Even if a trained and calibrated panel is used, evaluation of taste is susceptible to physical and physiological conditions. In children both

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limitations play a major role. Ethical concerns inhibit taste studies in children and in addition younger children have difficulties to give valid statements (Davies and Tuleu, 2008). Animal models might be valid, if the medication is intended for veterinary use, but they are hardly representative for human taste sensation. Therefore, analytical techniques were used in the past as for example dissolution testing and detection of the free amount of drug via UV spectroscopy (Kayumba et al., 2007; Hamashita et al., 2008). With this approach only single substances can be detected. To investigate multi-component mixtures, such as oral liquid formulations, these methods can hardly be applied as synergistic or suppression effects are difficult to show.

Electronic sensor array systems, so called electronic tongues, offer an alternative to characterize the taste masking efficiency for multi-component formulations. These systems have attracted increasing attention over the last years. They were initially implemented in the food sector, but are also used for pharmaceutical purposes (Toko, 1998; Ciosek and Wróblewski, 2007; Kobayashi et al., 2010). Studies were based on comparative investigations of different sorts of wine, beer or tea, for example, or on comparison of different pure active pharmaceutical ingredients or taste masked drug formulations. An investigation of pharmaceutical excipients and a rational stepwise development of a taste masked formulation using electronic tongue sensor responses have not been described yet.

Therefore, the development of a pleasant tasting formulation, containing quinine hydrochloride, as a model drug, based on a screening of different taste masking techniques by guidance of an electronic tongue should be evaluated in this study. According to the WHO guidelines quinine hydrochloride is used for the therapy of severe malaria, predominately for infants. But, the bitterness value of quinine hydrochloride is 200 000 meaning that 1 g of the substance diluted in 200 l of water still has a bitter taste (European Pharmacopoeia, 2010). Some investigations have been carried out before in order to minimize the bitter taste of quinine hydrochloride in a liquid formulation. For example, the inclusion of quinine by different cyclodextrins was studied and it was found that a large excess of β-cyclodextrin is needed to reduce the bitter taste of quinine (Turner, 2009). Saunders and Srivastava (1950) showed the successful interaction of quinine with different types of ion exchange resins and monitored the decrease of quinine of time by binding to the resin. Further, pleasant tasting suspensions have been developed after binding quinine sulphate to the cross linked polyacrylic copolymers Tulsion 339 and Tulsion 335 or to copolymers of acrylic acid and methacrylic acid, Indion 204 and Indion 234 (Rao et al., 2004). Other attempts were made by adding substances covering the taste to quinine hydrochloride such as sucrose (Stevens, 1996; Nakamura et al., 2002). However, assessment of successful taste masking was carried out using UV spectroscopy or human taste panels. Nakamura et al. (2002) evaluated the addition of sucrose and aspartame using an electronic taste sensing system. Nevertheless, a rational screening of various different sweetening agents alone, in mixtures and in combination with other taste masking substances in order to evaluate the optimal taste masking technique for an unpleasant tasting drug by means of an electronic tongue has not been performed yet.

These approaches should be verified by electronic tongue measurements. Further, taste masked quinine hydrochloride formulations using new additional taste masking excipients should be developed. For electronic tongue measurements the taste sensing system TS-5000Z (Insent Inc., Atsugi-Chi, Japan) was used, equipped with eight sensors representing the six different taste stimuli and gustatory impressions bitterness, sweetness, sourness, saltiness, umami, and astringency. The system was qualified according to ICH guideline Q2 before in order to show the fitness for purpose (Woertz et al., 2010).

By screening of different sweeteners, ion exchange resins and further complexing agents the stepwise development of a taste masked formulation should be achieved. The influence of these excipients on sensor responses should be evaluated and it should be demonstrated how and to what extent an electronic tongue could help to simplify and rationalize development of taste masked formulations in order to reduce human taste tests in the future.

2. Materials and methods

2.1. Sweetening agents and sweetness enhancer

Fructose (Ph.Eur. grade), sodium saccharin (Ph.Eur. grade), sucrose (Ph.Eur. grade), and quinine hydrochloride (Ph.Eur. grade) were purchased from Caesar & Loretz (Hilden, Germany). Glucose (analytical grade) and mannitol (analytical grade) were obtained from Roquette Frères (Lestrem, France). Sucralose (analytical grade) was acquired from Tate & Lyle Sucralose (Mc Intosh, USA), and acesulfame potassium (analytical grade) was purchased from Nutrinova (Frankfurt, Germany). Monoammonium glycyrrhizinate (Mafco Magnasweet 100; research grade) was provided by Mafco (Camden, USA).

Different concentrations of sweeteners were prepared in aqueous solution (demineralized water) equimolar to 7.5%, 3%, 1.5% sucrose or with respect to sweetness potency of 10% or 13% sucrose and mixed with 1 mM quinine hydrochloride in 100 ml demineralized water (Table 1).

2.2. Ion exchange resins

Two weak cation exchangers, Amberlite™ IRP88 (analytical grade; Rohm and Haas, Philadelphia, USA) and Indion 234 (analytical grade; Ion Exchange, Mumbai, India), as well as one strong cation exchanger, Amberlite™ IRP69 (analytical grade; Rohm and Haas, Philadelphia, USA), were investigated.

Table 1
Amounts of sweetening agents with respect to equimolar ratios (*E*) or sweetening potencies (*P*) of sucrose.

Sweetening agent	Sweetening potency	Concentrations calculated by considering sweetening potency (*P*) of sucrose [%]		Concentrations calculated equimolar (*E*) to sucrose [%]		
	1[a]	13[a]	10[a]	7.5[a]	3[a]	1.5[a]
Mannitol	0.6	–	–	11.67 (*P*)	5.83 (*P*)	2.5 (*P*)
Glucose	0.7	18.57	14.29	10	5	2.14
Fructose	1.3	10	7.69	5.38	2.69	1.15
Acesulfame potassium	200	0.065	0.05	4.12	2.06	0.88
Monoammonium glycyrrhizinate	252	0.052	0.04	0.028 (*P*)	0.014 (*P*)	0.006 (*P*)
Sucralose	600	0.022	0.017	8.13	4.07	1.74
Sodium saccharin	500	0.026	0.02	4.2	2.1	0.9

[a] Sucrose (reference).

Table 2
Principle component analysis (PCA) distances of excipients and excipient combinations for taste masking to water, quinine HCl (1 mM and 5 mM) and placebo (mean ± sd; $n = 3$; PCA created with sensors: bitterness 1, bitterness 2, sourness, umami; $R^2_{(PC\text{-}1)} = 0.85$; $R^2_{(PC\text{-}2)} = 0.14$).

No.	Excipients and excipient combinations	Distance to demineralized water	Distance to quinine HCl, 5 mM	Distance to quinine HCl, 1 mM	Distance to placebo = SBE-β-CD
1	Sulfobutyl ether-β-cyclodextrin (SBE-β-CD)	2.87 ± 0.18	2.75 ± 0.08	1.15 ± 0.07	–
2	Sodium saccharin (SSa)	0.21 ± 0.11	5.70 ± 0.13	3.88 ± 0.10	2.97 ± 0.08
3	Strawberry (S)	1.81 ± 0.16	3.86 ± 0.11	2.13 ± 0.09	1.24 ± 0.03
4	Domiphenbromide (Dom)	5.81 ± 0.18	3.31 ± 0.17	2.81 ± 0.11	3.70 ± 0.16
5	Quinine HCl (QH), 5 mM	5.60 ± 0.26	–	2.02 ± 0.03	2.75 ± 0.08
6	QH (5mM) + SBE-β-CD	3.74 ± 0.19	2.18 ± 0.01	0.38 ± 0.03	1.16 ± 0.02
7	QH (5mM) + SSa	4.77 ± 0.28	0.95 ± 0.03	1.11 ± 0.05	1.97 ± 0.11
8	QH (1mM) + SSa	3.06 ± 0.24	2.85 ± 0.01	0.83 ± 0.03	0.99 ± 0.06
9	QH (5mM) + Dom	6.86 ± 0.29	1.63 ± 0.06	3.13 ± 0.07	4.04 ± 0.14
10	QH(5mM) + S	5.51 ± 0.26	0.12 ± 0.01	1.97 ± 0.04	2.68 ± 0.08
11	SBE-β-CD + QH (5mM) + Dom	3.76 ± 0.19	2.19 ± 0	0.41 ± 0.04	1.2 ± 0.03
12	SBE-β-CD + QH (5mM) + SSa	3.36 ± 0.19	2.59 ± 0	0.62 ± 0.03	1.04 ± 0.02
13	SBE-β-CD + QH (5mM) + SSa + S	3.35 ± 0.20	2.59 ± 0	0.61 ± 0.03	1.03 ± 0.02
14	SBE-β-CD + QH (5mM) + SSa + S + Dom	3.40 ± 0.19	2.57 ± 0	0.60 ± 0.03	1.08 ± 0.02

250 mg of ion exchanger were added to an aqueous solution containing 1000 mg of quinine hydrochloride in demineralized water. Solutions were stirred for 20 h and 2 ml samples were drawn after 0, 5, 10, 15, 20, 30, 45, 60, 90, 120, and 1200 min.

2.3. Complexing agents

α-Cyclodextrin (Cavamax W6®; analytical grade) and γ-cyclodextrin (Cavamax W8®; analytical grade) were obtained from ISP (Cologne, Germany), β-cyclodextrin (Kleptose®; Ph.Eur. grade), hydroxypropyl β-cyclodextrin (Kleptose® HPB Oral Grade; Ph.Eur. grade), and maltodextrin (Glucidex IT 17 L exp.; research grade) were generously provided by Roquette Frères (Lestrem, France). Sulfobutyl ether-β-cyclodextrin (Captisol®; research grade) was purchased from CyDex Pharmaceuticals (Lenexa, Kansas, USA).

Different amounts of complexing agents were dissolved in 100 ml demineralized water and 200 mg (5 mM) or 40 mg (1 mM) of quinine hydrochloride were added. Samples were shaken for 24 h and subsequently analyzed. Molar ratios of 1:0.96, 1:1.92, 1:3.85, 1:5.77 (quinine hydrochloride:complexing agent) were prepared. Further, a calibration was established by dissolving 0.1 mM, 0.2 mM, 0.5 mM, 1 mM and 10 mM of quinine hydrochloride in demineralized water.

In order to characterize the formation of the complex [*DCD*] of quinine [*D*] and cyclodextrin [*CD*], binding constants were calculated according to Eq. (1) assuming a 1:1 inclusion complex.

$$K_c = \frac{[DCD]}{[D][CD]} \quad (1)$$

2.4. Formulation enhancement

Two drops of strawberry flavor (analytical grade; Nordmann, Rassmann, Hamburg, Germany), 0.01% (w/v) domiphen bromide (research grade; Sigma-Aldrich, Steinheim, Germany), 0.05% (w/v) sodium saccharin, 29 mM sulfobutyl ether-β-cyclodextrin, and 5 mM or 1 mM quinine hydrochloride were dissolved in different combinations in demineralized water in order to evaluate the influence on sensor signals (Table 2).

2.5. Electronic tongue measurements

2.5.1. Sensors

Sensors and reference electrodes (Table 3) were purchased from TecLabS Europe OHG (Essen, Germany). 0.2 ml inner solution (see Section 2.5.2) 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.

2.5.2. Preparation of standard, washing and sample solutions

Potassium chloride (analytical grade) was acquired from Grüssing (Filsum, Germany). Tartaric acid (Ph.Eur. grade) was purchased from Sigma-Aldrich Laborchemikalien (Seelze, Germany). Water was demineralized by reverse osmosis. Distilled water was obtained by in-lab distillation of demineralized water. 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 acquired from Merck (Darmstadt, Germany). The inner solution for sensors and reference

Table 3
Sensors for the taste sensing system TS-5000Z with corresponding taste sensations.

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
SB2GLt1	Sweetness sensor	Sweetness	X
Reference electrode	–	–	

electrodes consisting of 3.33 mol/l potassium chloride in saturated silver chloride solution was provided by Insent (Atsugi-chi, Japan).

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

2.5.3. Electronic tongue system and measurement setup

All measurements were performed by the taste sensing system TS-5000Z (Insent, Atsugi-chi, Japan). This electronic tongue can be equipped with up to eight lipid membrane sensors providing different taste qualities and four corresponding reference electrodes (Table 3). The underlying measurement principle is potentiometric and sensor responses are obtained as mV values consequently.

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 five times with the sweetness sensor and four times with the remaining sensors. One measurement cycle consisted of measuring a reference solution (V_r), afterwards the sample solution (V_s), a short (2×3 s) cleaning procedure and measurement of the aftertaste ($V_{r'}$). The 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 determined sensor response to the reference solution (V_r).

$$R = V_s - V_r \quad (2)$$

$$\mathrm{CPA} = V_{r'} - V_r \quad (3)$$

The whole measurement procedure was performed for all samples and repeated afterwards up to five times. For further data treatment the two first runs were discarded for sweetness measurements and the first run was discarded for measurements with the remaining sensors as recommended by the supplier in order to enable conditioning of the sensors. The method was validated as described in a previous analytical paper (Woertz et al., 2010).

2.5.4. Evaluation of results

The results were recorded as raw data [in mV] of the relative measurement of the sample to the reference. Either sensor signal results were evaluated alone or multivariate data analysis was performed. 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). To determine the distance between two samples (p, q) after multivariate data analysis Euclidean distances were calculated including all the variables (n) used for the model.

$$d(p, q) = \sqrt{\sum_{i=1}^{n} (p_i - q_i)^2} \quad (4)$$

2.6. Fourier transformation infrared spectroscopy (FT-IR)

The inclusion complexes of quinine hydrochloride with sulfobutyl ether-β-cyclodextrin, and β-cyclodextrin respectively, in demineralized water were characterized by in situ Fourier transformation infrared spectroscopy using the React IR (Mettler Toledo, Giessen, Germany) with a flow through cell. Measurements were performed at room temperature. An average of 256 spectra was evaluated in the range of 650–4000 cm^{-1} with a measurement time of 1 min and a spectral resolution of 4 cm^{-1}.

The spectrometer was purged with nitrogen in order to minimize effects of CO_2 and water from atmosphere. The reactor was cooled with liquid nitrogen.

2.7. UV spectroscopy

The reduction of quinine hydrochloride amount by binding to the solid ion exchange resin particles was determined by UV spectroscopy at 330 nm using a Spekol® (Analytik Jena AG, Jena, Germany). 2 ml samples were diluted to 100 ml and measured at room temperature.

3. Results

3.1. Sweeteners

Sensor signal patterns of formulations containing different sweeteners are shown in Fig. 1. For principal component analysis (PCA) sensors bitterness 1, bitterness 2, bitterness 3, and sweetness as well as the aftertaste of the four sensors were used. The principal component 1 (PC-1) represents ~49% of the information whereas PC-2 carries ~25% of the information. Pure sweetener solutions can be found on the left side of the map (Fig. 1a), on the right side (Fig. 1b) a 1 mM sample of quinine hydrochloride is represented. In between a standard consisting of 300 mM sucrose dissolved in standard solution is located in order to confirm reproducibility of measurements. Formulations containing 1 mM quinine hydrochloride and different sweeteners are located on the right part of the plot, but differing along the PC-2 indicating differences between the sweeteners.

3.2. Ion exchange resins

UV- and electronic tongue measurements with bitterness sensors 1 and 2 showed that the amount of free quinine hydrochloride in the loading solution decreased remarkably in the first 30 min and became stable to a certain value after that (Fig. 2). In equilibrium 1.9 g of quinine hydrochloride was bound to 1 g of Amberlite™ IRP 88, 1.2 g was bound to 1 g of INDION 234 and 0.36 g was bound to 1 g of Amberlite™ IRP 69. The root mean square error of estimation (RMSEE) serves as a measure describing the quality of the correlation by showing the remaining error of the prediction. It has to be regarded with respect to the investigated range. For example, the RMSEE of 0.009 describes that the decrease of quinine hydrochloride in solution could be predicted by the electronic tongue with an error of 9 mg within the concentration range of starting concentration and equilibrium.

3.3. Soluble complexing agents

Focusing on the principal component analysis (PCA) map built with the sensors bitterness 1, bitterness 2, umami and sourness, ~97% of the information is contributed by principal component 1 (PC-1) and ~1% of the information is shown by PC-2 (Fig. 3). Therefore the major part of information could be explained by the two components. Tasteless solutions of pure complexing agents in water with corresponding concentrations to formulations with quinine hydrochloride are located on the left side of the plot representing pleasant tasting placebo formulations. 5 mM samples of quinine hydrochloride are located on the right side of the PCA map known to have a bitter taste. 1 mM quinine hydrochloride

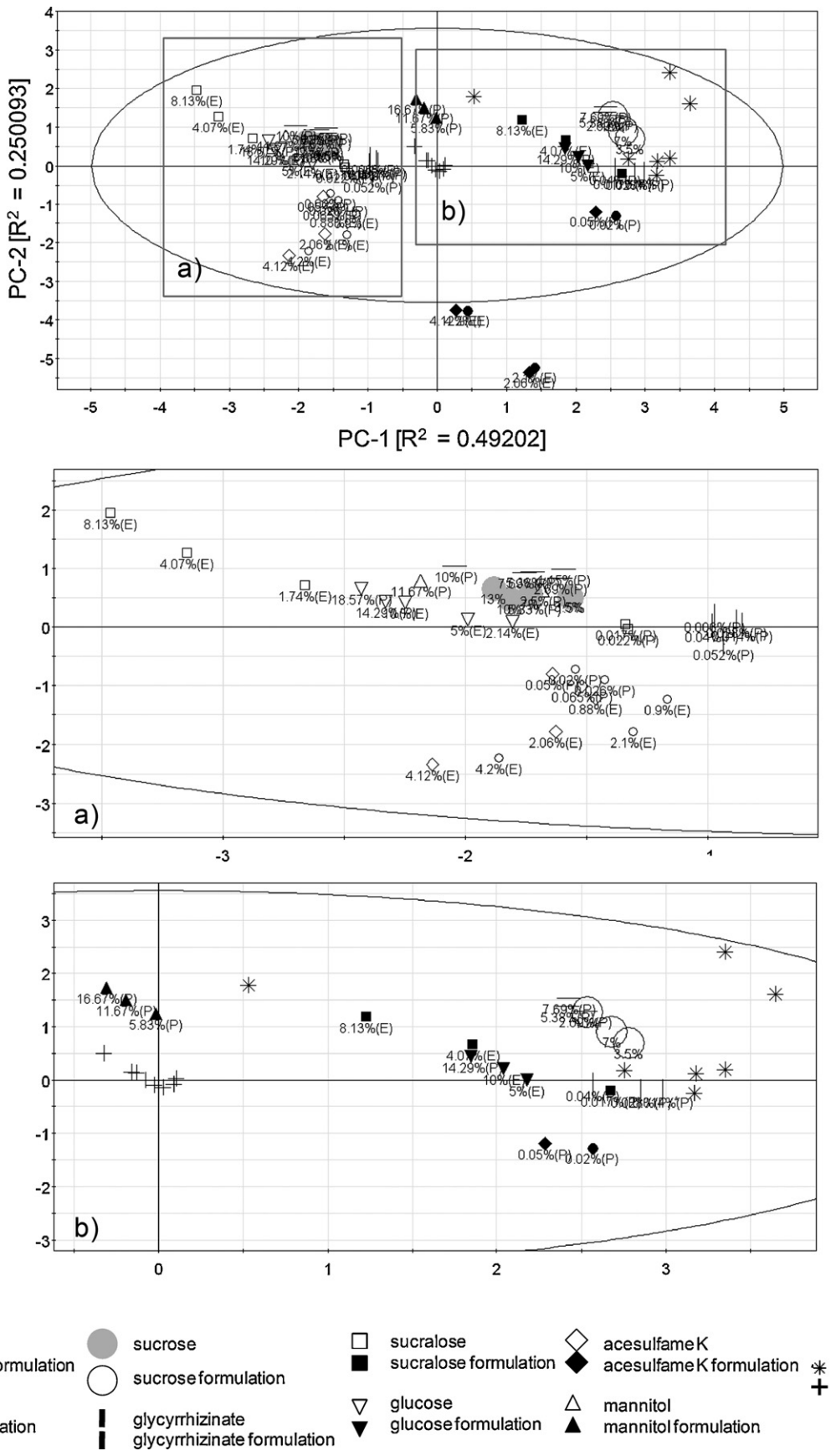

Fig. 1. Principal component (PC) analysis showing the influence of different sweetening agents with concentrations according to sweetening potency (*P*) or equimolar amounts (*E*) to sucrose created with the following sensors: bitterness 1 (+aftertaste), bitterness 2 (+aftertaste), bitterness 3 (+aftertaste), sweetness (+aftertaste).

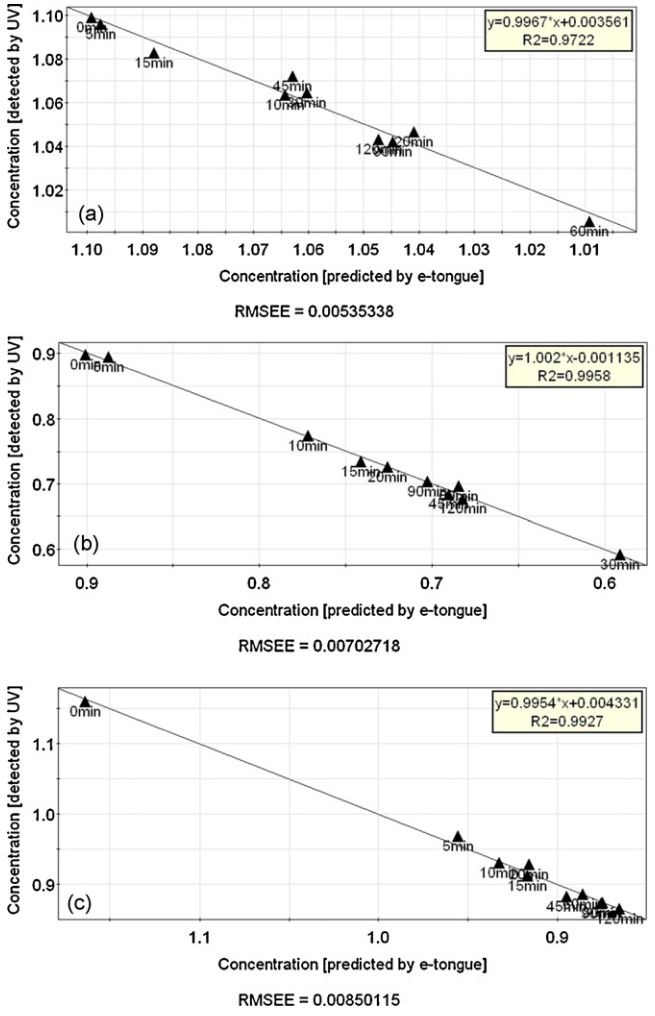

Fig. 2. Partial least square regression of the decrease of quinine hydrochloride through binding to solid ion exchange resins. (a) Amberlite IRP69, (b) Indion 234, and (c) Amberlite IRP88 over time evaluated via electronic tongue measurement (sensors bitterness 1 and bitterness 2) and UV spectroscopy as reference.

samples can be seen in the center of the map, showing reproducibility of the measurements. Also on the right side of the plot, formulations containing quinine hydrochloride in combination with different concentrations of α-cyclodextrin or β-cyclodextrin, hydroxypropyl-β-cyclodextrin, γ-cyclodextrin or maltodextrin can be seen. Small differences between the different molar ratios exist reflected by a shift of the sensor signal patterns off pure quinine hydrochloride 5 mM. A large shift of sensor signals towards the direction of quinine hydrochloride 1 mM can only be observed for the formulations containing sulfobutyl ether-β-cyclodextrin, whereas the largest distance was obtained for the highest concentration of cyclodextrin.

The FT-IR spectrum of quinine hydrochloride (Fig. 4) shows two peaks, the first at 1621 cm^{-1} belonging to the deformation of the ammonium group ($-NH_3^+$) located in the aliphatic ring, the second at 1512 cm^{-1} belonging to the aromatic system of the quinoline ring (Hesse et al., 2002). Cyclodextrin spectra show typical peaks at 1162 cm^{-1} belonging to C–O vibrations, in addition, the sulfobutyl ether-β-cyclodextrin reveals another peak at 1260 cm^{-1} coming from $-CH_2$ vibrations. The β-cyclodextrin, serving as a reference, was used in a 1:2 ratio here due to its limited water solubility. The quinine band typical for the $-NH_3^+$ vibrations disappears by binding to the sulfobutyl ether-β-cyclodextrin. The same can be observed after complexation by β-cyclodextrin. But, in addition, the $-CH_2$ vibration band of the sulfobutyl ether-β-cyclodextrin disappears.

Table 4
Free amount of quinine HCl [mM] and binding constants [M^{-1}] detected by bitterness sensors 1 and 2 (calculated based on partial least square regression; RMSEE = 0.0393).

Quinine HCl [1 mM]:sulfobutyl ether-β-cyclodextrin	Free amount of quinine HCl [mM] mean ± sd (n = 3)	Binding constant [M^{-1}]
1:0.96	0.44 ± 0.003	584
1:1.92	0.31 ± 0.002	360
1:3.85	0.25 ± 0.003	195
1:5.77	0.23 ± 0.009	134
1:10	0.21 ± 0.004	79

Table 4 shows binding constants and the free amount of quinine hydrochloride depending on the amount of sulfobutyl ether-β-cyclodextrin. An increase of quinine hydrochloride complexation can be seen up to the addition of 5.77 mM sulfobutyl ether-β-cyclodextrin. This was determined by calculating the ratio between the difference of free drug amount [Δ drug] and difference of added cyclodextrin amount [Δ CD]. The threshold indicating saturation was set to [Δ drug]/[Δ CD] < 0.01. Therefore, the addition of 10 mM of sulfobutyl ether-β-cyclodextrin does not change the complexation rate appreciably any more.

3.4. Formulation enhancement

Distances of excipients alone and in combination to demineralized water, quinine hydrochloride 5 mM and 1 mM as well as to sulfobutyl ether-β-cyclodextrin solution serving as pleasant tasting placebo are shown in Table 2. The principal component analysis was performed with sensors bitterness 1, bitterness 2, sourness, and umami. The major part of the information was contributed by PC-1 (85%) and the remaining part by PC-2 (14%).

After mixing quinine hydrochloride 5 mM with sulfobutyl ether-β-cyclodextrin the distance between quinine 5 mM and the quinine–cyclodextrin complex increases (2.18). Further the distance is shifted from 2.18 to 2.59 when sodium saccharin is added. Domiphen bromide (5.81), quinine hydrochloride (5.6) and strawberry flavor (1.81) can be differentiated from demineralized water according to their distance values. By addition of domiphen bromide and strawberry flavor to the quinine–cyclodextrin–sodium saccharin formulations (nos. 13, 14) distances remain constant with distance values ~3.4 to water, ~2.6 to quinine HCl 5 mM, ~0.6 to quinine HCl 1 mM, and ~1.05 to placebo.

4. Discussion

4.1. Sweeteners

In order to evaluate the comparability of the electronic tongue data to human taste, sweeteners were used according to the sweetening potency of the 10% or 13% sucrose standard (Schiffman and Gatlin, 1993). If the sensors were operating in the same way as human receptors, different concentrations with the same resulting physiological sweetness would lead to the same sensor responses. But, as the measurement principle is electrochemical and experiments from performance qualification (Woertz et al., 2010) had shown that sensors responses are depending on molar amounts as well as ionic structure of the substances, also equimolar amounts to sucrose were investigated. Due to its limited solubility, only three concentrations of mannitol according to sweetening potency of sucrose 7.5%, 3%, and 1.5% could be investigated. Amounts of monoammonium glycyrrhizinate were also only used according to

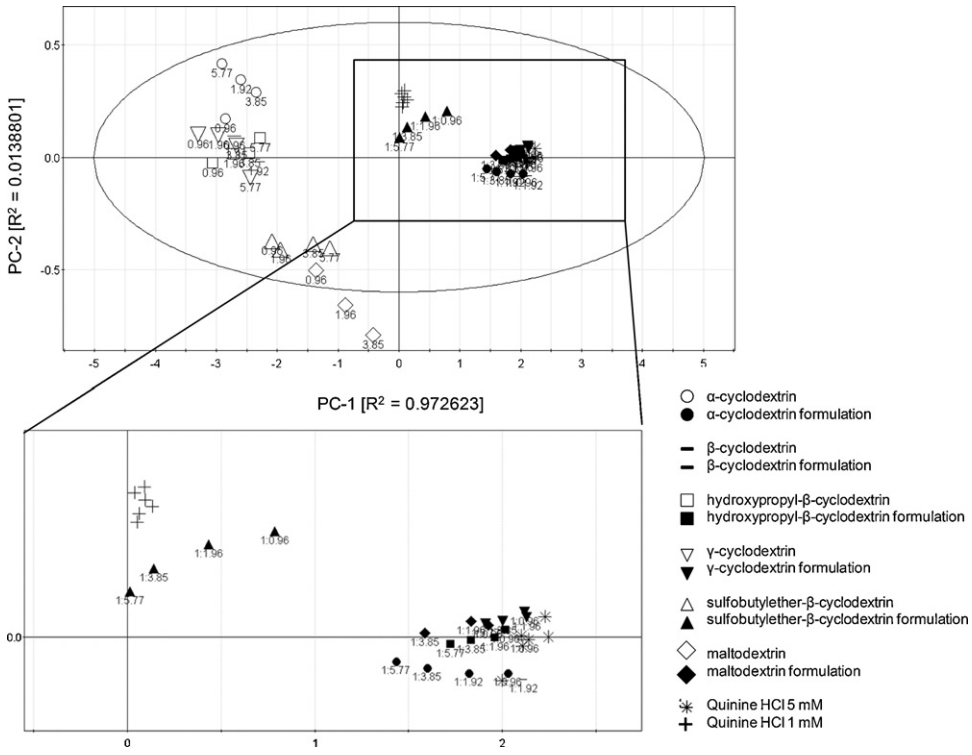

Fig. 3. Principal component (PC) analysis representing the inflence of complexing agents on taste properties of quinine hydrochloride built with the following sensors: bitterness 1, bitterness 2, sourness, umami.

sweetening potency as equimolar amounts exceed physiological concentrations by far.

Sensors included in the multivariate data analysis were chosen with respect to their ability to distinguish between the different substances. As evaluated in the performance qualification of the taste sensing system sensor bitterness 1 and bitterness 2 are most sensitive for quinine hydrochloride (Woertz et al., 2010). In addition the reduction of bitterness by the addition of sweeteners can be detected by bitterness sensor 3 and the sweetness sensors as evaluated in forgoing calibrations of the single substances. Information contributed by the different components shown by the PCA map (Fig. 1) is homogeneously distributed meaning that both components need to be included in the interpretation. This fact can be explained by the variability in chemical structure of the sugars, sugar alcohols, and sweeteners investigated. The scattering of quinine samples can further be explained by the inclusion of the sweetness sensor for multivariate data analysis. As this sensor is gaining a lot of information for sweet taste, bitter samples, like quinine hydrochloride, are difficult to characterize in a reproducible manner. For this reason, quinine samples with lower concentrations than 1 mM were not included in the analysis.

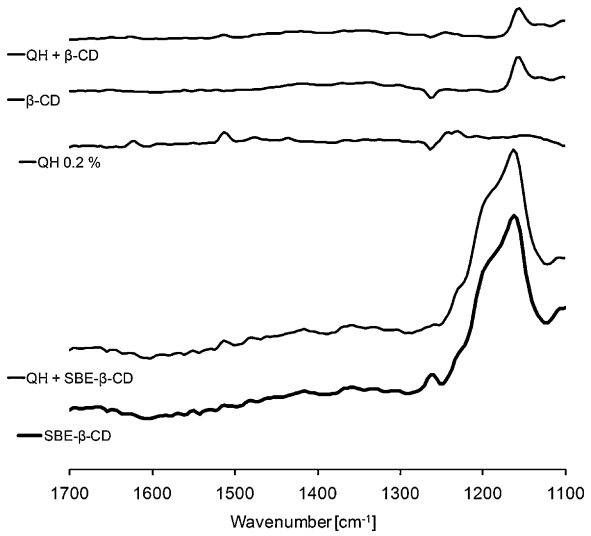

Fig. 4. FT-IR spectra of sulfobutyl ether-β-cyclodextrin (SBE-β-CD), quinine hydrochloride 0.2%:sulfobutyl ether-β-cyclodextrin complex (1:5.77) (QH + SBE-β-CD), quinine hydrochloride 0.2%:β-cyclodextrin complex (1:2) (QH + β-CD), β-cyclodextrin (β-CD), quinine hydrochloride 0.2% (QH 0.2%).

Firstly, excipients were rather detected according to molar amounts than according to their sweetening potency. This can be seen as samples concentrated according to sweetness potency of sucrose (*P*) do not have the same sensor signal patterns compared to sucrose with corresponding sweetness values. Sensor responses to artificial sweeteners, like sodium saccharin and acesulfame potassium, are quite different mainly due to their ionic structure. Secondly, the addition of sweeteners alone did not influence the sensor signal patterns towards quinine hydrochloride meaning that the bitter taste of quinine could not be masked. The only shift of the sensor signal patterns off the bitter tasting quinine sample on the right side was caused by the equimolar amounts of sodium saccharin and acesulfame potassium to 10% and 13% sucrose. These concentrations are physiologically neither relevant for taste improvement nor regarding acceptable daily intake.

4.2. Ion exchange resins

Sensors bitterness 1 and bitterness 2 were again used for data evaluation as they are most sensitive for quinine hydrochloride (Woertz et al., 2010). Therefore, they were chosen here to determine the binding capacity of the ion exchange resin. Both sensors were able to detect the decrease of quinine hydrochloride in the liquid phase in the same way as UV spectroscopy did with RMSEE values <0.009. Hence, these types of sensors can be implemented

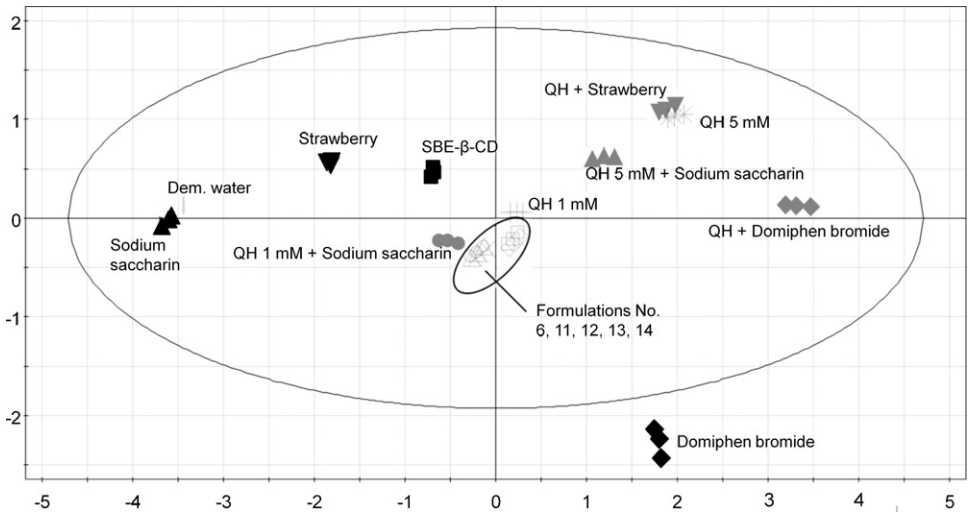

Fig. 5. Principle component analysis (PCA) of excipients and excipient combinations for taste masking created with sensors: bitterness 1, bitterness 2, sourness, umami; $R^2_{(PC\text{-}1)} = 0.85$; $R^2_{(PC\text{-}2)} = 0.14$.

for monitoring the binding process. Further, they can be used to determine the free amount of quinine hydrochloride within a finalized ion exchange resin formulation. The wider range of linearity and the lower limit of detection are the main advantages of the electronic tongue compared to UV spectroscopy. The decrease of quinine hydrochloride over time did not change remarkably after 2 h when the loading process was obviously finalized. As UV spectroscopy served as reliable reference method here and a good correlation could be established, comparison to lower concentrated quinine hydrochloride reference samples measured by the electronic tongue was not shown. Within the screening of the three different ion exchangers, the weak cation exchanger Amberlite™ IRP88 was found to have the best binding capacity for quinine hydrochloride. The velocity of binding shows the affinity of quinine to the ion exchange resin (Fig. 2) in accordance with the binding capacity. Whereas half of the quinine hydrochloride amount is bound to Amberlite™ IRP69 after 1 h, INDION 234 is loaded after ~15 min, and Amberlite™ IRP88 in less than 5 min. Further, the amount of quinine HCl decreases constantly when bound to Amberlite™ IRP88, whereas fluctuating concentrations of free quinine HCl were observed for the other ion exchange resins.

This can be explained by the different chemical structures of the ion exchange resins. The strong cation exchanger has less binding capacity as the ratio of specific weight of one molecule to the number of binding sites is smaller compared to the weak cation exchangers. Therefore, a smaller amount of the quinine could be bound. The difference between the two weak cation exchangers can again be explained by structural differences. INDION 234 consists of acrylic acid, whereas Amberlite™ IRP88 contains methacrylic acid. The additional methyl group presumably leads to a higher affinity for the guest molecule.

4.3. Soluble complexing agents

The previous calibration of different concentrations of quinine hydrochloride and complexing agents independently showed that bitterness sensors 1 and 2, the umami sensor, and the sourness sen-

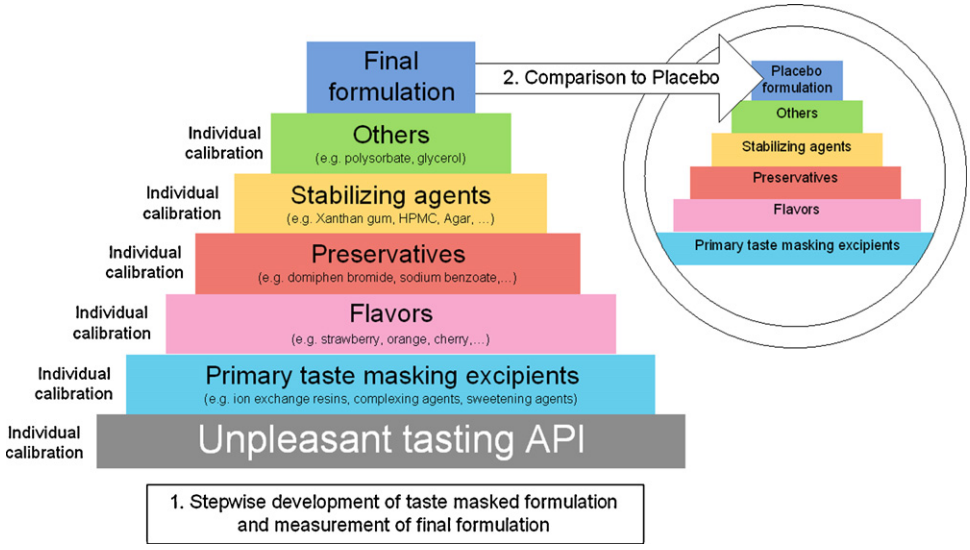

Fig. 6. Stepwise rational development of a liquid taste masked formulation guided by an electronic tongue.

sor were able to distinguish the substances from each other and a concentration dependent sensor response was obtained. Again, sensors bitterness 1 and bitterness 2 were most sensitive to quinine hydrochloride and in addition, complexing agents could be detected best by sensors umami and sourness. Therefore these sensors were chosen to evaluate the results of these screening measurements. Data from principal component analysis (Fig. 3) shows that all excipients except sulfobutyl ether-β-cyclodextrin did not change the sensor signal patterns to quinine hydrochloride appreciably. It was known from literature that quinine hydrochloride and β-cyclodextrin partly form inclusion complexes, but taste masking could not be achieved. Turner (2009) found by implementation of a human taste panel that a 14fold excess of β-cyclodextrin is needed in order to achieve improved taste properties of a quinine formulation. A molecular modeling study demonstrated (Fan et al., 2006) that the smaller part of the quinine molecule, the aliphatic ring, is complexed by β-cyclodextrin, whereas the larger part, the quinoline ring, is located outside the cavity and still available for receptor interaction. This fact was confirmed by our electronic tongue measurements and shows formation of incomplete inclusion complexes and therefore insufficient taste masking effects can be reliably detected by the electronic tongue. According to the electronic tongue studies, the α-cyclodextrin cavity could not serve for complexation of the whole molecule and also taste masking by γ-cyclodextrin could not be achieved. The new approach of using a maltodextrin, which is a modified starch, was not successful, too. It was assumed that the helical structure of amylose molecules could expand and form an inclusion complex similar to cyclodextrins, but sensor responses did not differ from sensor responses to pure quinine. The only exception was offered by the sulfobutyl ether-β-cyclodextrin, which led to, upon addition to quinine hydrochloride, a shift of the sensor signals towards the less bitter tasting 1 mM sample of quinine hydrochloride. As the cavity size is not significantly different compared to the conventional β-cyclodextrin, the sulfobutyl ether-substituent is obviously influencing the quinine-sensor membrane interaction differently.

FT-IR spectra (Fig. 4) confirm this hypothesis. The absence of the $-CH_2$ vibration band of the sulfobutyl ether-β-cyclodextrin indicates that an ionic interaction of the $-SO_3^{2-}$ group of the cyclodextrin and the $-NH_3^+$ group of the quinine occurred.

Therefore it is assumed that the ionic interaction of quinine hydrochloride and sulfobutyl ether-β-cyclodextrin plays a major role in the taste masking process of quinine. In order to further evaluate this, Table 4 shows the amount of detected quinine hydrochloride after complex formation and calculated binding constants. 56–79% of quinine hydrochloride was bound after adding sulfobutyl ether-β-cyclodextrin with different molar ratios. Binding constants calculated based on the assumption of 1:1 complex formation decrease with increasing cyclodextrin amounts. This is because the further addition of cyclodextrin does not lead to an increased complexation by the same factor. Consequently, the addition of a 10fold excess of sulfobutyl ether-β-cyclodextrin has only a small influence on sensor signal patterns compared to the 1:5.77 ratio.

This method of quantification of the free quinine hydrochloride amount offers an alternative as detection by UV-spectroscopic studies was not feasible. Spectra of the complexes were identical to the spectrum of pure quinine hydrochloride 1 mM (data not shown).

Concluding, an excipient reducing the bitter taste of quinine hydrochloride was found serving as basis for the further development of a final liquid quinine hydrochloride formulation. In addition different complexing agents could be evaluated and consistency to literature and to FT-IR spectra could be shown.

4.4. Formulation enhancement

In order to finalize the liquid quinine hydrochloride formulation, the sulfobutyl ether-β-cyclodextrin formulation was chosen and further improved. As sweeteners alone did not have an influence on the bitter taste of quinine hydrochloride, it was assumed that they could act as a secondary taste masking agent. Therefore, sodium saccharin was used. In addition, the influence of other substances, which are commonly used for liquid formulations, on sensor responses should be investigated. Domiphen bromide was chosen as a preservative and strawberry flavor was added.

The PCA map (Fig. 5) and calculation of distances (Table 2) showed again that, upon the addition of sulfobutyl ether-β-cyclodextrin to quinine hydrochloride, sensor signal patterns change significantly. The additional increase of distance shows that the formulation could be further improved by adding sodium saccharin. Moreover, the distance to pleasant tasting placebo solution becomes smaller. Sodium saccharin was successfully used as a secondary taste masking agent here, while the application as a primary taste masking agent to quinine did not change sensor signals remarkably. Obviously, masking potency of the artificial sweetener is dependent on the initial bitterness of the drug. Whereas domiphen bromide can be detected in water and quinine hydrochloride solution, and strawberry flavor can be differentiated from demineralized water, they do not change the sensor signal patterns appreciably any more when added to the taste improved formulation. This can be seen by constant distances between these formulations (nos. 12–14 in Fig. 3) and water, quinine hydrochloride and placebo.

Hence, taste improvement can be reliably detected without disturbing effects of other excipients which do not have taste masking effects.

On the basis of these results, a schematic, stepwise approach could be developed serving as a general protocol for rational formulation development of taste masked formulations (Fig. 6).

Measurements showed that it is mandatory to investigate all the excipients used for a formulation independently (individual calibration) first in order to determine the influence on the sensor responses. Therefore, at least five different concentrations of the API to be taste masked as well as all other substances used for the formulation need to be investigated. On the basis of the concentration sensor response relationship measurements, sensor responses towards the multi-component mixture can be interpreted and the sensors for multivariate analysis can be chosen. As described, flavors alone in water can be detected and distinguished by the sensors, whereas they hardly influence the sensor response to a complex formulation. The addition of a primary taste masking agent to the unpleasant API is the first step towards a taste masked formulation. The success of taste masking can be screened by the sensor array measurements. Subsequently, successfully taste masked formulations can be elaborated and further improved towards a final formulation. The final formulation can be compared to a corresponding placebo formulation. Therefore, the aim is to obtain a similar sensor signal pattern of formulation and pleasant tasting placebo. The rational approach and the harmlessness of taste testing a placebo formulation are the main advantages of this stepwise strategy guided by electronic tongue measurements.

This offers the perspective to create a database based on electronic tongue sensor responses and containing information about excipients used in oral liquid formulations in the future. Of course, these systems are hardly able to represent the human sense of taste as a whole, which is influenced by additional factors as olfactory effects and individual preference. Therefore, additional data about the correlation between human taste assessment and electronic tongue prediction would help to evaluate and

support electronic tongue data. Nevertheless, formulation development could be rationalized and simplified which makes electronic tongues promising tools to reduce human taste assessment tests.

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