Development and Critical Evaluation of Membrane Electrodes as Electronic Tongue Sensors for Pharmaceutical Applications

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List of abbreviations

ANN	Artificial neural network
ANOVA	Analysis of variance
API	Active pharmaceutical ingredient
AS	Artificial saliva
ASIC	Acid-sensing ion channel
ATP	Adenosine triphosphate
BA	Benzyl acetate
BATA	Brief-access taste aversion
BP	Bis(2-ethylhexyl) phosphate
CDO	Cyclodextrin oligomer
ChemFET	Chemically modified field-effect transistor technology
CPA	Change in membrane potential due to adsorption
DF	Drug formulation
DIN	Deutsches Institut fuer Normung
EDQM	European Directorate for the Quality of Medicines
EMA	European Medicines Agency
ENaC	Epithelial sodium channel
E-Tongue	Electronic tongue
EU	European Union
FDA	Food and Drug Administration
GPCR	G-protein coupled receptor
HCL	Hydrochloride
HCN	Hyperpolarization-activated cyclic-nucleotide gated channel
Hep	Heptakis(2,6-di-O-methyl)-ß-cyclodextrin
HHUD	Heinrich-Heine-University Duesseldorf
HP	Hydroxypropyl-ß-cyclodextrin
HPLC	High-performance liquid chromatography

HPßCD	Hydroxypropyl-ß-cyclodextrin
IbNa	Ibuprofen sodium dihydrate
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IPM	Isopropylmyristate
ISE	Ion selective electrode
ISFET	Ion-selective field-effect transistor
IUPAC	International Union of Pure and Applied Chemistry
K2P	Potassium channel
MOSFET	Metal oxide semiconductor field-effect transistor
NCE	New chemical entity
NPOE	2-nitro-phenyl octyl ether
OA	Oleic acid
OTC	Over the counter
P	Placebo
PARC	Pattern recognition
PC	Principal component
PCA	Principal component analysis
PIP	Pediatric investigation plan
PLS	Partial least square
PQ	Performance qualification
PVC	Polyvinyl chloride
QH	Quinine hydrochloride
Qu	Quinine hydrochloride
rNTS	Rostral nucleus of the solitary tract
RSD	Relative standard deviation
SAD	Single ascending dose
SBE	Sulfobutylethylether-ß-cyclodextrin
SLS	Sodium lauryl sulfate

ßCD	ß-cyclodextrin
TC	Trioctylmethyl ammonium chloride
THF	Tetrahydrofuran
TRC	Taste receptor cell
Tri	Triacetyl-ß-cyclodetxtrin
TRP	Transient receptor potential
VAS	Visual analogue scale

1 Introduction

1.1 The significant relevance of taste

1.1.1 The matter of taste for oral pharmaceuticals

1.1.1.1 Taste as a critical parameter for oral drug formulations

Oral pharmaceuticals are the most preferred dosage forms and include a great variety of different drug formulations that all have to pass the oral cavity during the administration of the drug product. These different formulations can be distinguished in solid and liquid peroral dosage forms and further in intraoral formulations that are intended to release the drug compound within the oral cavity. Regardless of the residence time of the different drug formulations in the mouth, they are all under suspicion to induce a bad taste when interacting with the taste buds on the human tongue. Many active pharmaceutical ingredients (APIs) and also some excipients are supposed to generate a certain taste as they are all interacting with the human taste buds [1]. Among numerous other compounds, a lot of APIs like quinine hydrochloride, caffeine citrate, paracetamol, sumatriptan succinate or azelastine hydrochloride are reported to be bitter tasting [2]. The chemical mechanism of provoking the bitter taste has not yet been completely understood. To gain further knowledge on the chemical features of bitter tasting substances, the database BitterDB was initiated. Here, summarized information about up to 687 bitter compounds (status Dec 2016), including information on molecular structure, bitterness thresholds, physico-chemical properties, related substances and targeted receptors are collected [3].

Independent from the reason for the unpleasant taste of a drug compound or a drug formulation, which might not only be associated with bitterness but also with an astringent or metallic mouth feeling, the acceptance and the compliance of a medicinal product is negatively influenced when it is unpalatable [4]. Especially children often refuse to take an oral drug product due to a bad taste or even due to poor experiences with non-proper pediatric drug formulations. As a result, the therapeutic success is reduced to a minimum. Since many children are not able to swallow common tablets and capsules [5], liquid oral formulations and orodispersible drugs are preferably used. These drug products have the drawback of a high residence time in the oral cavity besides the advantages of a high dose

flexibility and easy administration [6]. Even though adults might tolerate bitter medicines as they are trained to associate a good therapeutic effect with an unpleasant taste, children and also mentally ill people or the elderly who cannot comprehend the necessity of the drug administration might not be convinced to take an unpleasantly tasting drug formulation. This is why taste has become a critical parameter of oral pharmaceuticals and is emphasized by competent authorities for the development of pediatric drug formulations [7]. Content of such regulations are the development of child-appropriate formulations that offers a high quality and efficiency of the drug products.

1.1.1.2 Taste-masking strategies

To overcome the problem of bad tasting drug formulations, the effect of unpalatable compounds has to be reduced or even inhibited by technological approaches that are able to mask or alter the taste of the drug product. In general, there are two basic strategies to formulate a good or at least neutrally tasting drug product. On the one hand, excipients can be used to cover or alter the taste of the API. On the other hand, excipients which are able to reduce the interaction of the API with the human taste buds can be applied. Furthermore, masked API particles could be mixed with excipients of a strong pleasant taste, such as sweeteners or flavors, which are supposed to evoke a palatable taste sensation. The use of these excipients is dealt as one of the simplest approaches for masking the bad taste of an API and can be applied for solid and liquid drugs [8]. However, the addition of sweeteners and flavors with no other modification of the API might not always be successful, as highly bitter tastants and water soluble drugs cannot be covered. As a result, other apparently unpleasant taste sensations beside bitterness are activated [9, 10]. The inhibition of interaction between the bitter tastant and the human taste buds could be achieved by various technological modifications: One of the most common methods is the application of a water-soluble polymer layer (e.g., polyvinyl alcohol, polyvinyl pyrrolidone, hydroxypropyl cellulose, Eudragit E100 [11]). The water-soluble film remains intact within the oral cavity for a certain time that allows swallowing of the coated drug formulation without any interactions of the bitter API with the human tongue while the drug release is negligible affected [11, 12]. The film coating can either be applied to the finished drug product, such as tablets or capsules or on intermediates like granules, pellets and also powders as done by microencapsulation of small particles [13-16]. Furthermore, covering of the API could be done via incorporation in a poorly soluble

or lipophilic matrix. This is frequently done with the technique of hot-melt extrusion for instance [17-19]. A further modification of the drug is the formation of molecule complexes of the API together with ion exchange resins or cyclodextrins [20-27]. These complexes are able to reduce the solubility of the API and therefore decrease the contact with the taste buds. Furthermore, the concentration of API that is present on the taste buds can be decreased by ascending the size of the molecule complexes [19]. Additionally, the solubility of the API could be reduced by adjusting the pH value of the dissolving formulation and thereby inhibiting the interaction between the API molecules and the taste buds [8]. Moreover, the diffusion of the API to the taste buds in a liquid formulation can be decreased by increasing the viscosity of the formulation [28] or using a lipophilic vehicle that coat the taste buds or cause a higher viscosity of the formulation to reduce the release of the API [8].

The development and assessment of a suitable taste-masked drug formulation is only possible if the success or failure can be qualitatively described. The evaluation of taste, however, is a highly complex issue as it demands for adequate knowledge on human taste sensation. Furthermore, the taste of an object is accompanied by other sensory impressions such as visual appearance, texture and olfactory properties that are responsible for the evaluation of the palatability. Nevertheless, to assess the taste of drug formulations and to gain more detailed comprehension on the acceptance or aversion of a product, certain knowledge on the physiology of the human taste perception is of major importance.

1.1.2 Human taste sensation

Besides vision, audition, olfaction and somatosensation, gustation is one of the five basic human senses. Taste is generated by binding of chemical compounds (tastants) to taste receptors in the taste buds on the human tongue activating different signal transduction pathways [29]. The five basic tastes sweet, sour, bitter, umami and salty can be associated to different groups of foods with different functions for the human body and are detected by specific receptor groups (Figure 1). From an evolutionary point of view, gustation is not only the discrimination of different flavors but the ability of our organisms to discriminate nutritive foods from toxins and indigestible materials as described exemplarily by Chandrashekar et al. [30] and Chaudari et al. [31]: carbohydrates are dedicated to sweetness and imply a source for energy whereas at the same time salts were

essential for the water balance and blood circulation; umami, which mostly represent the taste of the amino acid L-glutamate, indicates the presence of proteins; sourness and bitterness taste sensations naturally cause aversive reactions as a mechanism of protection. Sourness is often associated with spoiled food and could furthermore negatively influence the acid-base balance of the body. Bitterness as the organism's safety mechanism to the ingestion of poisons leads further to the aversion of the particular materials. However, our modern living and dietary conditions trained the human taste sensation to accept bitterness and sourness in food like coffee or wine. However, overcoming of such aversive reactions to bitterness is still rarely observed in children as they are more sensitive to bitterness to protect them from toxins [32]. Beside the basic taste sensations, additional taste qualities like fatty or metallic are discussed [31]. In this context, possible receptors for the taste of fat were recently identified (Figure 1). Even though their function is not completely clarified, fat is already dealt as the sixth taste quality [33, 34].

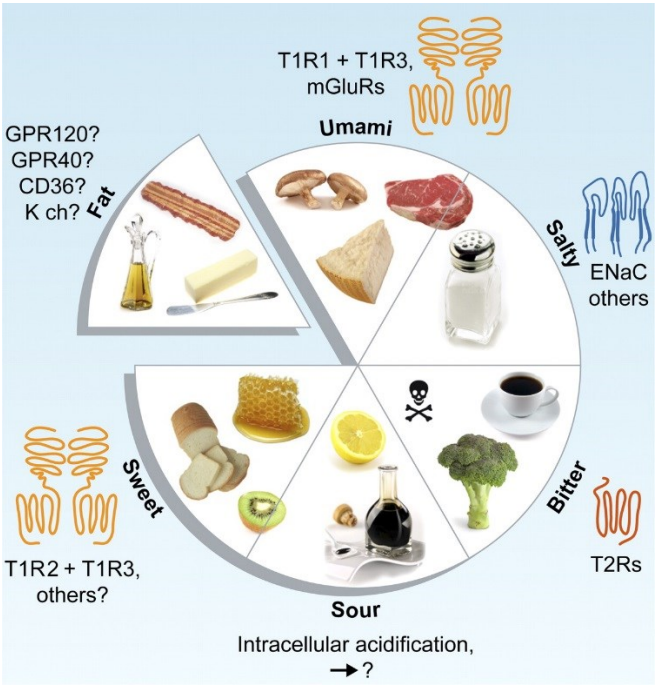

Figure 1: Taste qualities and receptors [31]

The taste buds on the human tongue can be defined as onion-shaped clusters of 50 – 150 taste receptor cells (TRCs) that are responsible for the signaling pathway of gustation [30, 33]. A human tongue possesses around 5000 taste buds that are not only located on the

surface of the tongue but also on the palate and epiglottis [35]. The taste buds are distributed over the tongue in three types of gustatory papillae: fungiforms, foliates and circumvallates (Figure 2A). Taste substances that are dissolved in the human saliva can be presented to the taste buds and bind to the apical sides of the TRCs [33]. The basal sides of the TRCs are connected to gustatory neurons and other nerves that are connected to the rostral nucleus of the solitary tract (rNTS) [33].

Recent studies reported the molecular characterization of TRCs and enabled the identification of TRCs in non-oral tissues like the gastrointestinal, bronchial and other tissues [3]. Receptor cells for bitterness in the intestine were suspected to play a role in digestive processes [36] whereas bitter receptors in the bronchial tissue were published to respond to irritants in the airways [37] even though their function is not completely elucidated [38, 39].

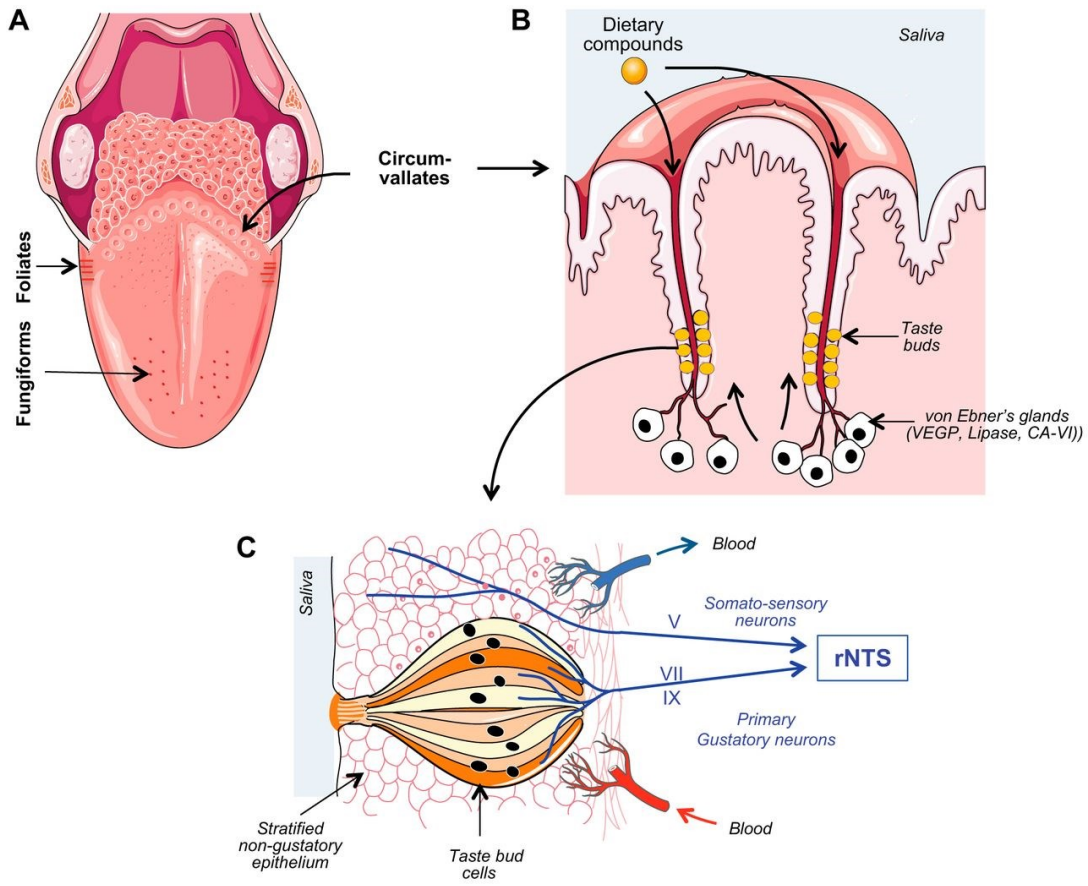

Figure 2: Physiology of the tongue with gustatory papillae (A), section of circumvallate papillae (B) and a schematic illustration of a taste bud (C) [33].

Human taste receptor cells (TRCs) can be divided into two groups: G-protein-coupled receptors (GPCRs) for umami, sweet and bitter substances and ion channels for sour and salty compounds [40]. The taste receptors are activated by the corresponding tastants as substrates causing either direct or second messenger mediated depolarization of the TRC (Figure 3). The apically located ion channels are activated by sour and salty substances such as acids (citric acid, tartaric acid, acetic acid, hydrochloride acid) and cations (Na^+, K^+, NH_4^+, Ca^{2+}) and causes direct entry of Na^+ and H^+ for membrane depolarization [30]. Several receptors that respond to sour taste quality have been proposed in the past like hyperpolarization-activated cyclic-nucleotide gated (HCN) channels , acid-sensing ion channels (ASICs) or potassium (K2P) channels [30]. A genetic and functional verified candidate is the PKD1L3-PKD2L1 channel a transient receptor potential (TRP) family member [30, 40]. Saltiness is transduced by the epithelial sodium channel (ENaC) or an amiloride-sensitive candidate (vanilloid receptor 1), a non-selective cation channel [40] (see also figure 1).

The signal cascade of GPCRs is initiated after binding of a tastant (umami, sweet or bitter) to the receptor and activation of the G-proteins gustducin or $G\alpha_{i2}$ [30]. Afterwards, a phospholipase C is stimulated and 1,4,5-inositol triphosphate triggers the release of intracellular Ca^{2+}, which further leads to activation of TrpM5 [37]. This cation channel enables the entry of Na^+ and K^+ and causes depolarization of the membrane, action potentials and the release of ATP as neurotransmitter for the taste perception [37].

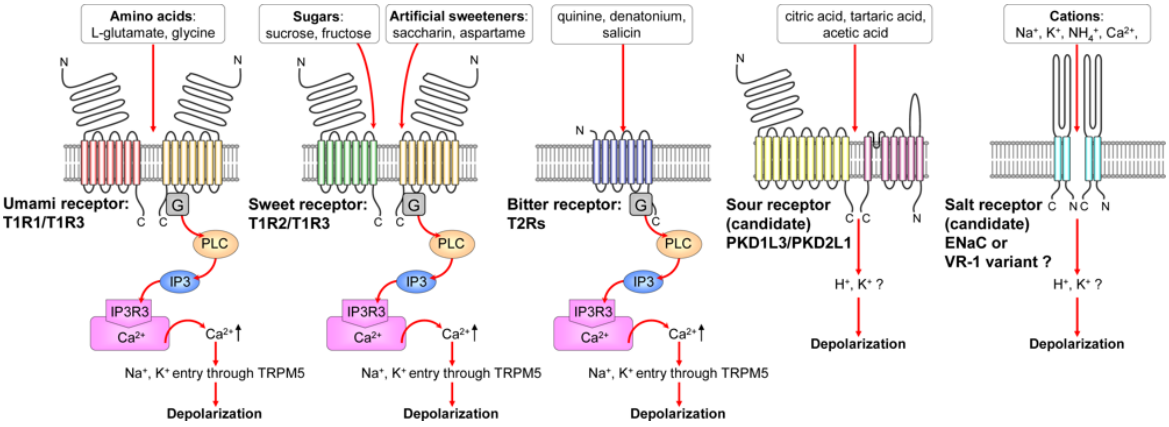

Figure 3: Types and structure of taste receptors and their signaling pathways [40]

Different types of GPCRs that are involved in taste transduction were identified such as T1R1, T1R2, T1R3 and several T2R subtypes that respond to different taste qualities (Figure 1). The taste quality umami is detected by receptors T1R1 and T1R3 after binding to amino acids such as L-glutamate. Sweetness receptors are T1R2 and T1R3, binding to different types of sugars (e. g. sucrose, fructose), artificial sweeteners (e. g. saccharin, acesulfame-K), sweet proteins (e. g. monellin, thaumatin) or D-amino acids. GPCRs of the T2R types are responsible for bitterness taste sensation. These receptors are numerous and comprises of up to 80 different types whose roles are still not completely clarified [41]. Currently, 25 different bitterness receptors are identified [42]. Some of these members are identified such as T2R4 responding to denatonium [30]. Other bitter receptors are still not characterized such as the receptors responding to noxious or toxic compounds such as quinine or atropine [30].

Due to the high variances in the human taste receptor expression, differences in the taste perception between the individuals might occur leading to more or less sensitive taste senses. Furthermore, living conditions (smoking, medication), experiences and preferences (desensitization of receptors) influence the human taste perception. To complete the complex context of taste sensation, gustation is often confused with the perception of smell or the perception of temperature and texture. Flavors are mostly volatile substances and detected by olfactory receptors rather than gustatory [31]. Certainly, taste and smell are spatially very close and are combined for the assessment of nutrition. Moreover, the ingestion of some chemicals like capsaicin or menthol can activate somatosensory nerve fibers that interact with the nerve fibers in the taste buds leading to cooling or heating effects on the tongue [31].

This high complexity of taste together with large inter-individual differences in the taste perception and preferences demonstrates the challenging part of developing a palatable oral drug formulation for a wide patient group and point out the huge need for suitable methods to assess the taste objectively.

1.1.3 The taste assessment as analytical tool for the characterization of oral pharmaceuticals

The assessment of a sufficiently taste-masked dosage form or the taste of an oral formulation is much more complex as the simple evaluation of the bitterness threshold of

a chemical compound and its classification by dilution according to the European Pharmacopoeia [43]. The need for a proper method to assess and interpret the taste of a drug product is urgent. At the moment, no generally accepted method to justify the choice for one or another drug formulation candidate with proper taste-masking characteristics can be applied, because a standardized method proposed by the same regulatory offices that demand taste-masking properties for specific drug products is not defined, so far.

The taste assessment of pharmaceuticals can roughly be divided into in-vivo and in-vitro methods. In-vivo taste evaluation can be performed either by human panelists or in animal studies mostly done with rats as test objects. In-vitro methods include the simple analysis of released API amount by drug dissolution studies, in-vitro assay methods using regulatory proteins expressed in taste receptor cells or sensor arrays used as electronic tongues. Due to a multitude of aspects during the development of a new pharmaceutical formulation, the application of different methods for the taste assessment might be necessary for each certain phase of development. For instance, due to unsolved toxicological questions it is preferred to use an in-vitro taste assessment in preclinical development of new chemical entities (NCEs), whereas during production and quality control human taste panels might be applicable [44]. This diversity of requirements on the taste assessment over the whole drug development process might be one reason for the lack of standardized taste assessment methods.

1.1.3.1 Human taste panels

In-vivo taste evaluation performed with human panelists in adults can be done either with healthy volunteers or with patient groups. While healthy volunteers are trained for the correct assessment of taste in a more objective way, patients represent an individual, untrained group of panelists. Their results are, however, essential for the evaluation of new therapeutics as the administration of therapeutic active formulations to healthy volunteers is ethically critical and restricted, even more if children are involved. Certainly, children are a critical target group especially for apparently taste-masked pharmaceuticals, in particular because they show differences in the taste perception compared to adults. Due to this fact, taste studies with children seem to be essential [44].

Even though DIN standard protocols are defined for taste assessment studies in general, none are published for the evaluation of taste-masked pharmaceuticals [45]. This is why

numerous different study designs were chosen for the various purposes of the according studies as presented by Pein et al. [45]. However, some general considerations about the performance of human taste studies are likewise applicable for the assessment of the taste-masking properties of a drug product. The selection of the volunteers requires the consideration of numerous characteristics such as gender, age, health, behavior (e. g. smoking, dental hygiene), pregnancy or medications [44]. The evaluation principles for human taste studies can be distinguished by their purpose and include simple expression of preferences between the tested formulations as it is often done with children using the facial hedonic scale [44]. With this visual analogue scale (VAS), the discrimination of the test formulation is done by correlation with pictures expressing affection and aversion (Fig. 4). Children above 5 years of age might also be able to express scorings in taste from 1 to 5 verbally [44]. This ranking of test formulations can also be done by adults according to one targeted characteristic like the scaling of bitterness or flavor intensity or the simple discrimination of two tested formulations. A further possibility for the evaluation of taste is the descriptive test method that is used to investigate the whole descriptive range of a sample [44]. Thereby, not only differences between samples are defined but characteristics such as the whole range of taste, texture or odor can be determined. Moreover, the intensity of a defined taste quality can be assessed by the volunteers [44].

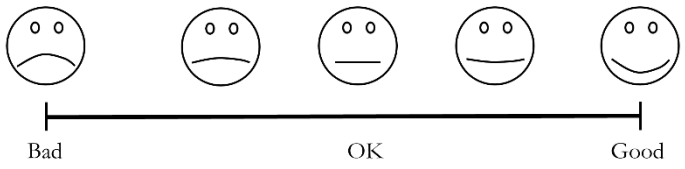

Figure 4: Example for a visual analogue scale (VAS) incorporating 5-point facial hedonic scale designed according to Mulla et al. [46].

Although human beings are the most suitable test objects for the assessment of taste characteristics, there are several reasons, why taste-studies are avoided as far as possible. Besides the obvious ethical concerns, human taste studies in particular with untrained patients show a poor reproducibility due to the high variances of taste preferences. The preparation, performance and evaluation of the obtained data request many resources like

personnel, time and money. Moreover, the data evaluation is highly complex and need sophisticated statistical methods.

1.1.3.2 Animal studies

Animal preferences tests are more and more frequently used to assess aversive and concentration dependent properties of tastants using either rats, mice, cats or dogs [47]. Among others, the rodent brief-access taste aversion (BATA) model is one method that showed promising results comparable to human taste panels using only a few animals (e. g. 10) [48]. Commonly rats are used as model animals that are exposed to different samples in a test area called “lickometer” [48]. Within this area, the different samples are presented to the animals for a short period of between 5 and 10 seconds. During that time, the water-deprived animals have the possibility to lick at different presented samples. The number of licks for each sample is recorded and is then correlated to the taste preference of the animals. Samples with a low number of licks are supposed to evoke an aversive behavior of the animals. For these particular samples, concentration dependent results can be obtained. This method can be used to screen NCEs in order to determine promising candidates with regard to the taste of the novel molecules [48]. Data evaluation offers promising candidates with the lowest aversion rate and are concluded to show a favored taste. Furthermore, the lickometer can be used to assess the taste of APIs with reduced efforts and costs compared to human taste panels. Data evaluation is again of high complexity and requires suitable statistical methods. The results of the experiments are displayed as “lick-ratio” curves representing a function of the sample concentration with a defined number of animals [48]. The interpretation of the data requires expert-knowledge to avoid misinterpretation. As the data does not follow a normal distribution, typical statistical models are not applicable. However, with trained personnel and animals, this method offers a frequently used alternative to human taste panels.

1.1.3.3 Drug dissolution studies

Under the assumption, that the unpleasant taste of a drug formulation can explicitly be associated with the contained API, the evaluation of the bitter taste can be correlated to the drug release rate. This approach enables simple drug dissolution tests to function for the taste assessment of oral pharmaceuticals. This approach is particularly applicable if the

route of administration is peroral and the drug is not supposed to remain in the oral cavity for a longer time.

The simplest way for the evaluation of the taste-masking efficiency of a drug formulation is the utilization of common drug dissolution tests as proposed by the European or US Pharmacopoeia. The drug release within the first minutes after administration of a peroral drug are of great interest as during this time an interaction of the drug with the taste buds is possible. In consideration of the conditions within the oral cavity, most drug dissolution studies that aim for an evaluation of taste-masked drug formulations are performed under modified conditions. If the drug release in the mouth is of interest, the dissolution method should be adjusted to more biorelevant approaches. This includes the use of artificial saliva as dissolution medium with regard to its composition, pH, viscosity, buffer capacity and surface tension [49] as well as a reduced volume of 1 – 2 mL [50] and a modified dissolution apparatus. UV spectroscopy is typically used for the analysis of the released drug amount. The implementation of an UV probe enables real-time evaluation of the drug release, which is beneficial in the first minutes of drug release [51]. However, with introduction of more biorelevant dissolution media that exhibit a high turbidity such as some artificial saliva types, application of UV spectroscopy might be not applicable since UV analysis is restricted to a clear test medium [45].

The obtained data of drug dissolution can be correlated to in-vivo data of human taste panels in order to define the taste-masking efficiency. Human bitterness thresholds can be related to the released drug amount or human taste studies can be performed with similar API concentrations as obtained by the in-vitro test [50]. Certainly, evaluation of the taste-masking efficacy via UV spectroscopy is limited to the presumption that an increase of API concentration leads to an increase in bad taste. Due to the fact that merely the API concentration is investigated, bad taste generated by excipients of the drug formulation is not taken into consideration [45].

1.1.3.4 Electronic tongues

One frequently used alternative for in-vivo taste assessment methods are electronic tongues as instrumental approach intended to mimic the human taste sensation with high reproducibility. The application of electronic tongues for the taste assessment of liquid samples are suitable for the characterization of oral pharmaceuticals without ethic

concerns. In general, electronic tongues are sensor arrays consisting of several different nonspecific sensors capable to respond to substances dissolved in multicomponent solutions [52]. The aim of electronic tongues is the imitation of the interactions between a tastant and its corresponding taste receptor on the human tongue. The interaction between the sensor and the liquid sample causes a concentration dependent response of the sensor that can be detected by an operational unit (Fig. 5). Thereby, each sensor of an array is supposed to give a sensor response when it is immersed in an aqueous solution. If the dissolved sample molecules come into contact with the sensor, the sensor will respond with a change in the sensor signal caused by the interaction of the sample molecules with the specific sensor [40]. This change in the sensor signal will be further recorded and evaluated using mathematical methods. The interaction of all sensors of one array with the sample solution results in a specific sensor response pattern, which is representative for the particular sample composition and can be interpreted as a taste pattern.

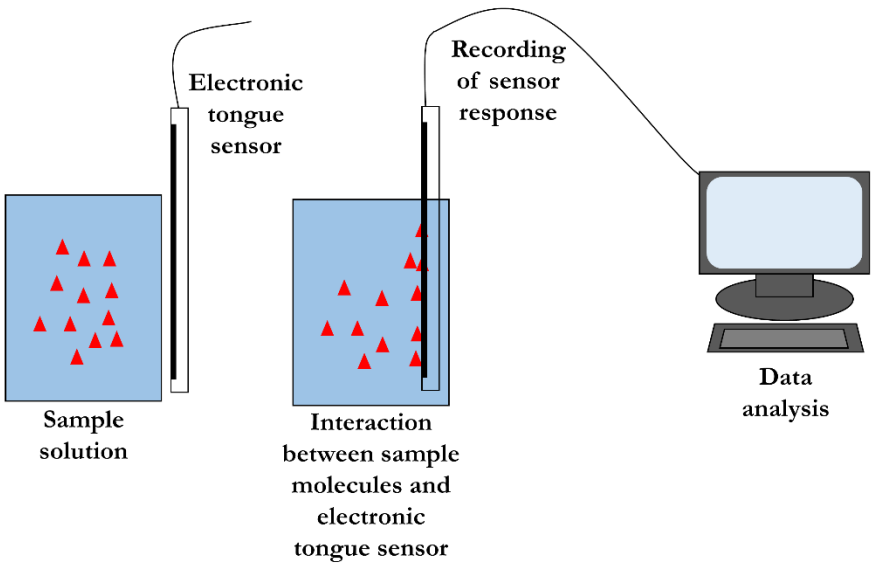

Figure 5: Schematic drawing of a common electronic tongue set-up.

Electronic tongues are mostly based on electrochemical measurement procedures like potentiometry [53, 54] or voltammetry [55, 56] but can also include mass sensitive [57] or optical devices [58]. Commonly, all measurement principles are based on the interaction of the sample molecules of a multicomponent solution with the sensing unit resulting in

changes of the sensor signal. Potentiometric electronic tongue sensors are frequently used as they follow a well-established analytical method with the opportunity to develop a wide range of different and individual sensor arrays. In general, the sensor signal is the result of a change in the potential difference between a working electrode and a corresponding reference electrode.

The membrane electrodes used for electronic tongue sensor arrays typically consist of a plasticized membrane of different kind. The composition of the membrane is responsible for the sensor response, whereby the membrane is developed in a way to interact with as many different tastant as possible. The capability of the membrane components to bind to taste substances can cause a change in the electric potential comparable to the depolarization of the human taste receptor [40]. For example, one commercially available electronic tongue, namely the Insent taste sensing system (Insent Inc, Atsugi-Shi, Japan) (Fig. 6), comprises of a sensor array of membrane electrodes based on artificial lipids [53]. A sensor response is obtained in aqueous media by interactions of the lipid membrane of the electrode with the different sample molecules. Dependent on the analyte, the negatively charged lipids in the membrane of the electrodes can be either prevented of dissociation by hydrogen ions in the sample solution, or bigger lipophilic molecules (e. g. positively charged quinine) can be adsorbed onto the hydrophobic parts of the membrane. Among others, these interactions cause changes in the membrane potential that can be differentiated from each other [40]. This phenomenon of a low selectivity of the membrane electrodes is called global selectivity since the sensor can respond to various components of a sample solution.

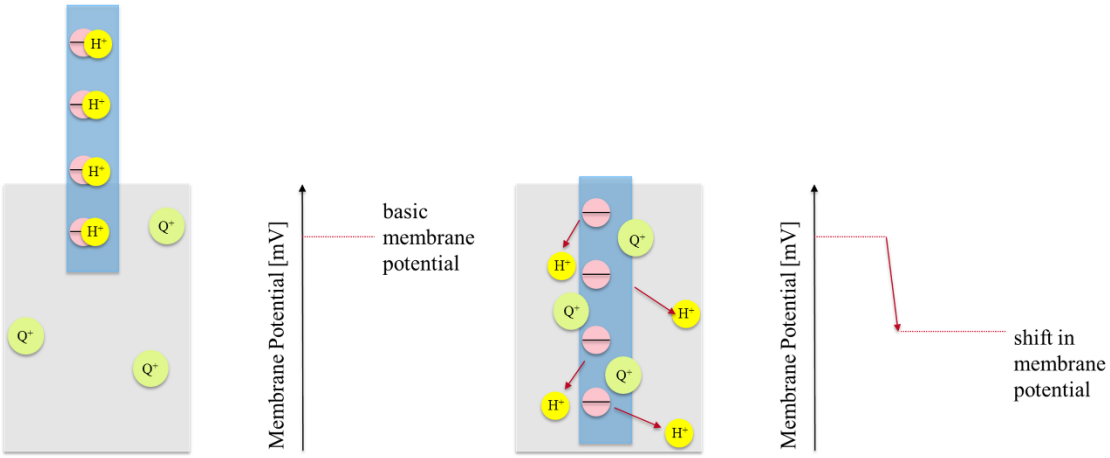

Figure 6: Example of a sample-sensor-interaction between an aqueous quinine solution and membrane electrodes with incorporated artificial lipids causing a change in the membrane potential, which is recorded as the sensor response.

Electronic tongues allow the assessment of liquid or dissolved pharmaceutical products and the comparison of drug formulations. Electronic tongues are implemented as analytical tools in the development of oral pharmaceuticals and especially for the formulation of taste-masked products. The comparison between different drug formulations containing the same API but different excipients can support the decision for one or the other formulation due to its taste pattern. The next chapter (see 1.2) is therefore focused on the different types of sensors arrays, the measurement principles, the data evaluation and interpretation and the relevance of electronic tongues in the field of pharmaceutical sciences.

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1.2 About data interpretation, sensor performance evaluation and unsolved tasks of polymer membrane based electronic tongues for pharmaceutical application

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1.2.1 Abstract

The application of electronic tongues for the characterization of oral pharmaceuticals is well-established to get first information about the taste of a drug product. Therefore, several different sensor arrays have been developed and used for pharmaceutical analysis during recent years. However, most electronic tongue systems are not qualified according to standardized protocols (such as the ICH Q2 guideline) for this purpose and besides, no standardized recommendations considering the sensor performance are given by responsible authorities. The evaluation of different potentiometric electronic tongue systems such as ion-selective electrodes, the taste sensor with global selectivity and ion-selective field-effect transistors, provides a summary of quality attributes for the sensors regardless of the sensor array design. Different strategies of data interpretation by mathematical models like principal components analysis and partial least square regression can offer purposive recommendations for quantitative and qualitative taste analysis. The use of electronic tongues for the correlation with clinical data, the determination of bitterness thresholds or the assessment of taste-masking requires reproducible and reliable data and therefore this review emphasizes the strong need for standardized quality attributes defined for the sensor performance of all electronic tongue systems in pharmaceutical analysis. Beside this aspect, the detailed evaluation of different sensor systems enables to formulate open demands for electronic tongues and suggests the introduction of a joint information pool for electronic tongue sensors. This pool could help approving electronic tongues as analytical tools as they provide many advantages such as their individual composition and properties to answer unsolved pharmaceutical questions.

1.2.2 Introduction

Electronic tongues are frequently used analytical tools in the taste assessment of oral pharmaceuticals. Taste is a major critical attribute for the compliance of orally taken drugs. Considering known drawbacks of human taste panels, such as ethical concerns, low

reproducibility or high costs [1], electronic tongues have been introduced as promising tools for the quantitative analysis of tastants and for the qualitative analysis of drug formulations [2]. An early assessment of the taste patterns of pharmaceutical formulations supports the development of palatable drugs, resulting in improved patients' compliance and therapeutic success. However, the physiology of human taste perception is of high complexity and difficult to simulate. Electronic tongues aim to imitate the interactions between taste substances and the taste receptors and as a consequence to mimic the human taste sensing mechanism [3]. Due to the high number of different molecules in a multicomponent solution, this cannot successfully be done by one single sensor. This is why an array of multiple sensors is applied and electronic tongues are basically defined as multisensory arrays comprised of nonspecific, low selective, chemical sensors showing overlapping signals combined with an appropriate mathematical method such as pattern recognition or multivariate data analysis for data processing [4-6]. The sensor based imitation of the human taste sensation on the tongue can be divided into two basic approaches; the taste sensor on the one hand and the electronic tongues on the other. The taste sensor is designed with global selectivity and aims to mimic the human taste sensation [7], which is unique in the field of electronic tongues. However, sensor arrays functioning as electronic tongues in general give a wide range of information about a multicomponent solution that is not necessarily comparable to the human taste but qualifies the sample characteristics [8]. The main criteria for those sensors are low selectivity and high cross-sensitivity [5, 9]. A second criteria, which is of major importance for a sensor array, is the difference in the individual sensor responses of the array [5]. Thus, the sensor array generates a "spectrum like" [5] response in a multicomponent medium. This response can be interpreted by multivariate data analysis as a sensor response pattern, which is representative for the sensor array in a specific medium. These response patterns allow for a quantitative analysis or qualitative recognition of these media [5]. A sensor array of an electronic tongue can comprise of 4-40 sensors [10], dependent on the task and the availability of different sensors. Increasing the number of sensors in a sensor array or combining different sensor systems result in more distinctive sensor response patterns and can improve the resolution of the multicomponent samples [11, 12].

Since the EU legislation on medicines for children of 2007 came into force [13, 14], taste has become a critical variable for the compliance and acceptance of drugs. Thus, taste and

taste-masking of orally taken solid dosage forms play an important role in the development of new pharmaceuticals and taste is dealt as a quality attribute of oral pharmaceuticals in general [15]. Demanded by the authorities, special requirements for the assessment of the taste can be expected. In general, high quality standards with regard to validation and qualification are required for pharmaceutical applications by the responsible authorities. This is why performance qualifications (PQs) according to ICH guideline Q2 have been performed for the two commercially available electronic tongues, the αAstree electronic tongue (Alpha MOS, Toulouse, France) and the Insent taste sensing system (Insent Inc., Atsugi-Shi, Japan) [16, 17]. Besides, many different non-commercial electronic tongue systems have been applied to individual pharmaceutical questions without providing data of a successful PQ [1, 11]. In this regard it should be be noted that due to the variety of electronic tongue approaches, general quality parameters are hardly definable, though this would be beneficial for pharmaceutical application. Therefore, at least detailed knowledge about how different electronic tongues are utilized for the characterization of oral pharmaceuticals is necessary to assess the requirements for such sensor systems.

Besides providing a detailed view on different electronic tongue systems and particular vocabulary, this article therefore critically reviews the different commercial and non-commercial sensor arrays with regard to quality requirements and unsolved task for pharmaceutical product characterization.

1.2.3 Electronic tongues: overview of sensoring units and their performance tests

The sensoring unit of electronic tongues (exemplarily shown in Figure 1) comprises a sensor array following different measurement principles including electrochemical and optical techniques as well as mass-sensitive devices [18]. The sensor array is the central element of an electronic tongue and combines several approaches for the assessment of a multicomponent sample. Among the others, electrochemical techniques are the most prominent measurement principles of electronic tongues, like potentiometry [7, 9] and voltammetry [6, 19]. Furthermore, systems based on conductometry [20] like impedance measurements are also reported. Optical sensor arrays are based on the absorption of light at specific wavelengths and can be applied for analytes with low or non-electrochemical activity that are hardly to detect with electrochemical devices [21]. Electronic tongues

based on mass sensors use the piezoelectric effect and respond to the adsorption of an analyte to chemosensitive material on the surface of a quartz crystal that reacts to changes in the chemical composition of the analyte [22].

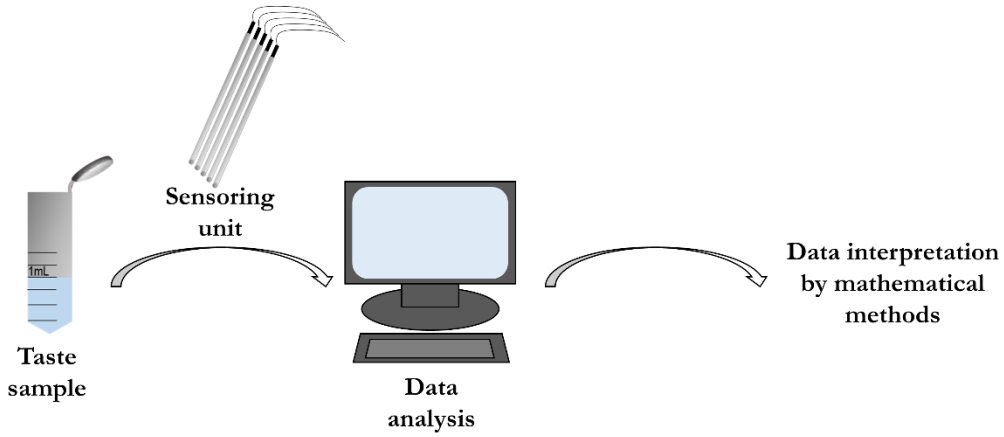
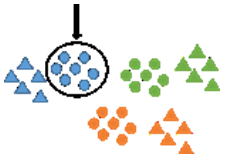
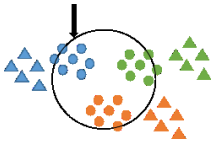
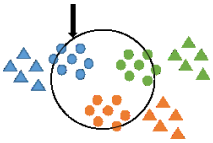
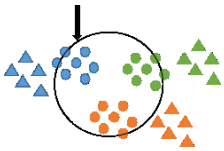
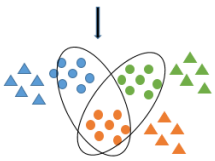
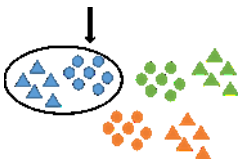


Figure 1: Schematic overview on electronic tongue analysis procedure.

However, most electronic tongue systems applied for the analysis of pharmaceuticals employ electrochemical sensors and therefore this review focuses on different electrochemically based sensors systems used for pharmaceutical applications. Several research groups introduced non-commercial and also commercial sensor arrays for the analysis and characterization of oral pharmaceuticals. The process of taste assessment begins with the collection of absolute sensor responses that are caused by the interactions between the sample molecules and the sensoring unit. For better comprehensibility of the following chapters, wording and interpretation of different selectivities / sensitivities are summarized in Table 1.

Table 1: Explanation of wording used by different authors to describe sensor characteristics. Symbols and colors are surrogates for different analytes, which are more or less different/comparable in the chemical behavior. The arrow and marking illustrate a possible detection.

Wording	Explanation	Scheme/Figure	Expert Opinion	Reference
Discrete selectivity	Sensors are selective towards one specific ion species			Legin et al. [23], Mostafa et al. [24]
Low selectivity	Sensors are selective towards a number of different analytes ↔ discrete selectivity		Contrary to discrete, comparable to overlapping selectivity	Toko et al. [7, 25, 26], Kobayashi et al. [27]
Overlapping selectivity	Sensors are selective towards a number of different analytes ↔ discrete selectivity		Contrary to discrete, comparable to low selectivity	Riul et al. [28]
Cross-selectivity	Sensors are selective towards a number of different analytes ↔discrete selectivity		Comparable to low selectivity	Vlasov et al. [5, 9], Winquist et al. [6, 8], Legin et al. [10, 23]

Wording	Explanation	Scheme/Figure	Expert Opinion	Ref.
Cross-sensitivity	Sensors show responses to a number of different analytes with distinguishable and reproducible sensor signals			Vlasov et al. [2, 5, 9], Legin et al. [10, 29]
Global selectivity	Sensors respond consistently to the same taste species			Toko et al. [7, 25, 26], Hayashi et al. [30], Kobayashi et al. [31]

To ensure reliable and reproducible data, most sensor arrays employ a sensor performance test prior to the measurement of the samples, which will be introduced in the following. These performance tests, however, are not standardized and only applied to the specific sensor array and include tests to confirm the stability of the sensor signal and the sensitivity towards the sample molecules.

1.2.3.1 Sensor arrays following a potentiometric measurement principle

Even though many different measurement principles are reported in literature, for the e-tongue based assessment of pharmaceuticals, electrochemical detection procedures in general and potentiometry in particular are most commonly used [5]. These also include the two commercially available electronic tongue systems, the αAstree electronic tongue (Alpha MOS, Toulouse, France) and the Insent taste sensing system (Insent Inc., Atsugi-Shi, Japan) as well as sensor arrays based on ion-selective electrodes introduced by Russian and Italian working groups [23, 24].

Potentiometric measurements are based on the potential difference between two electrodes, the working electrode and a reference electrode and is monitored under a zero current flow regime when an equilibrium state is reached. The working electrode and the reference electrode form an electrochemical cell developing a potential as the result of the changes in the free energy that is caused if the chemical phenomenon reach an equilibrium [25, 26]. This also involves the formation of an ion concentration gradient across a semi-permeable membrane generating a potential without an explicit redox reaction, which is the principle of ion selective electrodes (ISEs) [26]. In general ISEs are developed with membranes showing a discrete selectivity (Table 1), interacting with only one target ion to which the sensor is highly selective. The concentration of the analyte can be determined by the Nernst equation (Eq. 1) that describes the logarithmic dependency of the sensor response on the activity of the analyte [27, 28]. Regardless of the applied system, potentiometric e-tongue sensors measure the potential over charged membranes prepared with different materials [25].

Equation 1:

$$E = E^0 + \frac{RT}{zF} \cdot \ln a_i$$

where E = electrode potential, E^0 = standard electrode potential, R = universal gas constant, T = temperature, z = ionic valence of the substance, F = Faraday constant, a_i = activity of the substance

However, electronic tongue sensors show low selectivity (Table 1). If sensors are dedicated to specific taste sensations, which can be caused by several groups of molecules [7, 29-31], low selectivity can be defined as global selectivity. On the other hand, low selectivity can be an acronym for cross-selectivity, whereby each sensor is sensitive to a wide range of taste substances [5, 6, 8, 9]. This allows their implementation in complex solutions but has the drawback that the Nikolsky-Eisenman equation (Eq. 2) might not be applicable anymore [5].

Equation 2:

$$E = E^0 + \frac{RT}{z_i F} \cdot \ln[\, a_i + \sum_j K_{ij} \left(a_j\right)^{z_i/z_j}$$

where E = electrode potential, E^0 = standard electrode potential, R = universal gas constant, T = temperature, $z_{i/j}$ = ionic valence of the substance, F = Faraday constant, $a_{i/j}$ = activity of the substance, K_{ij} = selectivity coefficient

Mathematical procedures for signal processing that consider these requirements could be used to overcome these problems implementing multivariate data analysis, such as principal component analysis (PCA) or artificial neural networks (ANN) and pattern recognition [5].

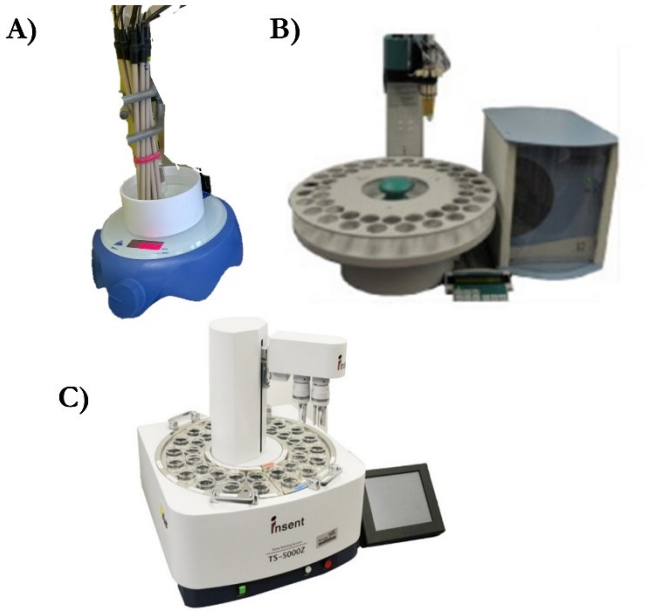


Figure 2: Pictures of three different potentiometric electronic tongue systems. A) Sensor array as example for electronic tongues based on ion-selective electrodes (St. Petersburg), B) Taste sensing system TS-5000Z as example for the commercially available edition of the "Taste sensor" (Insent Inc., Atsugi-Shi, Japan), C) αAstree liquid and taste analyzer based on ISFET technology (Alpha MOS Inc., Toulouse, France).

Ion-selective electrodes

The first sensor arrays based on ion-selective electrodes (ISEs) were launched in 1996 as a result from a collaboration of a Russian and Italian working group by Legin et al. [24] and Di Natale et al. [23]. The development of an electronic tongue, comprising different potentiometric chemical sensors, emerged from the idea to improve the analytical application of highly selective sensors by less selective and cross-sensitive sensor arrays combined with an appropriate data analysis approach [2]. The sensors can be prepared with different materials, such as chalcogenide glasses with added metals or polyvinylchloride (PVC) as polymer with incorporated plasticizers and ionic additives, such as ionophores and are therefore supposed for individual development. The sensing units functioned as working electrodes and were combined with a silver/silver chloride electrode as reference electrode [9, 32]. In comparison to ion selective electrodes with discrete selectivity, the electronic tongue showed a five times higher selectivity and a three times lower detection limit and therefore the electronic tongue was found to be superior in the analysis of liquid samples [33].

The preparation of these nonspecific electrodes can be performed according to ISEs as they are based on the same principles. Therefore, the electrodes can consist of glass, solid state or liquid membranes in general [34]. However, most ion-selective electrodes that are functionalized as electronic tongues are based on polymeric membranes [34]. According membranes are composed of PVC (e.g. 33% (w/w)) as polymer combined with a plasticizer (e.g. 66% (w/w)) and ionophores (e.g. 1% (w/w) [35]) or other ionic additives for active recognition of sample molecules mixed with THF as organic solvent [26]. Ionophores can be ion exchanger or a macrocyclic compound, which can surround the target ions in a cavity-like environment [26]. As the selectivity of an ion-selective electrode can be defined by the binding strength between the ionophore or other ionic additives to the target ion [26], the use of less selective ionophores can change an ion-selective electrode to an electrode with cross-sensitivity towards a wide range of species with different sensitivity. The potential membrane materials, types of plasticizers, ionophores and ionic additives are numerous, which allows the individual preparation of membrane electrodes for different applications.

The parameter describing all approaches of electronic tongue sensors based on ISEs is the cross-sensitivity. Vlasov et al. proposed three parameters for the characterization of this attribute [9]: the average slope ($s > 25$ mV/pX), the average signal-to-noise ratio for sensor stability ($K > 2$) and the "non-selectivity parameter" that describes the distribution of sensitivity for different cations ($F > 0.5$). Using these parameters, the sensor performance can be evaluated with regard to quality attributes of the sensor array before implementation as electronic tongue.

The "Taste sensor"

The first electronic tongue based on a taste sensor with global selectivity was developed by Toko et al. and was published in 1990 [31, 36]. Since then the potentiometric multichannel taste sensor composed of lipid membrane sensors was further improved and commercialized as the Insent taste sensing system that is purchased by Insent Inc. (Atsugi-Shi, Japan) nowadays.

The principle of the taste sensor is based on the physiology of the human taste perception. To simplify the recent findings on the composition and the signal transduction of human taste receptors the fluid mosaic model that was suggested in the 1970 [37] was used as a model for the development of lipid/polymer membranes [30]. According to Kobayashi and Ikezaki [38], the membranes comprise of different types of artificial lipids and plasticizers combined with polyvinylchloride (PVC) as immobilizing polymer. In general the membranes were prepared by mixing the lipids and plasticizers in tetrahydrofuran (THF) before PVC is added. The mixtures are then poured into petri dishes and the formed polymer films were dried for three days resulting in membranes of approximately 200 µm thickness. Depending on the used lipids and plasticizers either positively charged, negatively charged or combined membranes were produced. The successful compositions were used for the preparation of the membrane electrodes functioning as the working electrodes of an electrochemical cell together with a silver/silver chloride reference electrode.

The mechanism of the taste sensor response is explained by the Gouy-Chapman theory [39, 40] and is based on the electrical double layer that is formed on a charged membrane. With regard to the composition of the membrane, the electrical double layer that is formed

when immersing the sensor in an aqueous solution results in distinguishable membrane potentials for different samples [41]. For the advanced form of the taste sensor four requirements were defined by the authors [30]: *1) The threshold of the sensors has to be similar to the human taste threshold. 2) Global selectivity, meaning that the sensors should respond consistently to the same taste similar to the human taste receptors that respond selectively to the same taste compounds. 3) The information obtained by the taste sensors has to be a clearly defined unit. 4) Interactions between taste substances have to be detected by the taste sensors* (Table 2). Considering the physiological and technical requirements, taste sensors dedicated to bitterness, sourness, sweetness, saltiness, umami and astringency have been developed and are purchased by Insent Inc. To ensure the accurate performance of the sensors different quality attributes were defined. Therefore, the sensors can be checked by an automated evaluation of basic taste samples, a so called maintenance measurement. For each sensor and taste sample a range for the sensor response is defined and verifies the sensor performance. Furthermore, a stability criterion is specified and checked prior to each measurement to ensure a stable sensor signal within the defined range. Sensors that does not show a stable signal within the specified range (e.g. 0.5 mV) are marked and can be excluded from the data evaluation.

Ion-selective field-effect transistors (ISFETs)

ISFETs technology was established in the 1970s [42] and is based on an ion-selective membrane attached to an electrode. Since the composition of the ion-selective membranes are supposed to be of large variety, sensor arrays with a low selectivity, also described as overlapping selectivity, can be produced [43]. Basic principle of ISFETs combines the technologies of ISEs and solid state microelectronics whereas the metal gate of conventional MOSFET (metal oxide semiconductor FET) is replaced by the ion-selective membrane [44]. The gate potential is therefore a result of the interaction between the membrane and the ions in solution. A potential is then applied to the drain and causes a current between the drain and the source that is dependent from the charge of the semiconductor surface, which is controlled by the gate potential [43].

The commercially available electronic tongue from Alpha MOS Inc (Toulouse, France) is called αAstree liquid and taste analyzer and is based on chemically modified field effect transistor technology (ChemFET) that is further similar to the ISFET technology.

The employed sensors comprise of a field-effect transistor made of silicon that is coated with different organic materials [43]. A special sensor set for pharmaceutical application is available as well as one set for bitterness intensity measurement. The sensor sets differ in the composition of the silicon transistor coatings that are responsible for electrochemical recognition of liquid samples. Thereby, the sensors were developed with regard to a good repeatability, sensitivity and selectivity [45]. However, no further recommendations were made for appropriate sensor performance testing by the supplier. Even though the composition of the sensors are not fully reported, it is known that they also consist of a polymer matrix, a plasticizer and several ionophore-like substances to ensure electrochemical sensitivity of the sensors [46]. Each sensor set consists of seven sensors that show cross-selectivity, meaning that each sensor respond to the five basic tastes, sourness, sweetness, bitterness, saltiness and umami, but with different intensity [45]. Cross-selectivity of ISFET sensors and cross-sensitivity of ISEs follow the same recognition principle.

Table 2: Electronic tongue principles and sensor specific performance tests.

Electronic tongue principle	Sensor performance check by	Limits	Reference
Ion-selective Electrodes (ISEs) with low selectivity and cross-sensitivity	1) average slope by multilinear regression	s > 25 mV/pX	Vlasov et al. [9]
	2) sensor stability by signal-to-noise ratio	K > 2	
	3) "non-selectivity parameter" that describes the distribution of the sensors sensitivity	F > 0.5	
Taste sensor with global selectivity	1) Threshold		Toko et al. [26]
	2) Global selectivity		
	3) obtained information as a defined unit		
	4) taste sensors respond to interactions between the analytes		
	5) sensor check	0.5 mV	
Ion-selective field-effect transistors (ISFETs)	1) Repeatability		Zheng et al. [45]
	2) Sensitivity		
	3) Selectivity		

1.2.4 Evaluation and interpretation of the sensor responses in pharmaceutical development

Even though several additional sensor arrays are described in literature to be used as electronic tongues [47-49], for pharmaceutical application the presented approaches are mostly used. The utilization of electronic tongues in the field of pharmaceutics is generally based on the taste assessment of drug substances or drug formulations. This application

offers an instrumental approach for the classification of drug product candidates according to their predicted palatability with a high reproducibility and biorelevant aspects. In this manner, different taste-masking approaches or formulation strategies can be characterized to develop an adequate drug product for the target population such as pediatrics. Several studies dealing with the taste or taste-masking assessment of oral pharmaceutical formulations were conducted during the last years and are also summarized in review articles [1, 43, 50-52]. These articles report the frequent use of electronic tongues for the characterization of oral pharmaceutical formulations or the implementation of this analytical tool in the development of such formulations in consideration of taste, taste-masking and palatability in general.

The obtained sensor responses of the whole sensor array have to be interpreted by appropriate mathematical methods to process the information of each sensor at once. As the analysis of a multicomponent solution is a complex issue the sensors could not be interpreted by oneself and the theoretical equations such as Nernst law or Nikolsky-Eisenman equation are not always applicable. Therefore, pattern recognition methods (PARC) or multivariate data analysis are used for the evaluation of the sensor outputs [5]. According to the purpose of the analysis, the sensor responses can be evaluated qualitatively or quantitatively by partial least square regression (PLS), principal component analysis (PCA) or artificial neural networks (ANNs) [53-59]. Commonly, a log-linear relation between the sensor response pattern and the concentration of the main analyte is documented for the assessment of a drug compound in order to proof the sensitivity of the sensor array towards the analyte prior to the taste assessment of a drug formulation [1, 17, 45] or for the prediction of bitterness scores in correlation with human data [60].

The qualitative comparison of different drug formulation candidates and their corresponding placebo samples or pure drug solutions by PCA aims for the determination of similarities and differences in the sensor response pattern indicating taste differences due to the composition of those samples. For the taste assessment of oral pharmaceuticals this method is frequently used in order to predict the most palatable drug formulation [11, 61-66]. In this context, similarities and dissimilarities between the taste patterns of the analyzed samples can be expressed as distances in a mathematical model, the principal components analysis (PCA). During the measurement, each sample is described by each

sensor of the array. The sensor responses can be displayed in a multidimensional coordinative system where every sensor is one variable and the sensor responses are the corresponding observations. To reduce the high amount of data in the matrix, a new coordinative system is determined. The new coordinative system is built of principal components (PCs) that are used for the description of the data. The first principal component (new x-axis) is defined by the direction of the data with the highest variances. The second principal component (y-axis) is orthogonal to the first and if a third principal component is defined, it will expand the two-dimensional space with a third dimension (z-axis), the same applies for any further principal component [67]. The aim is a reduction of the dimensions that describe the observations and therefore less principal components as variables are commonly used, in most cases only two or three.

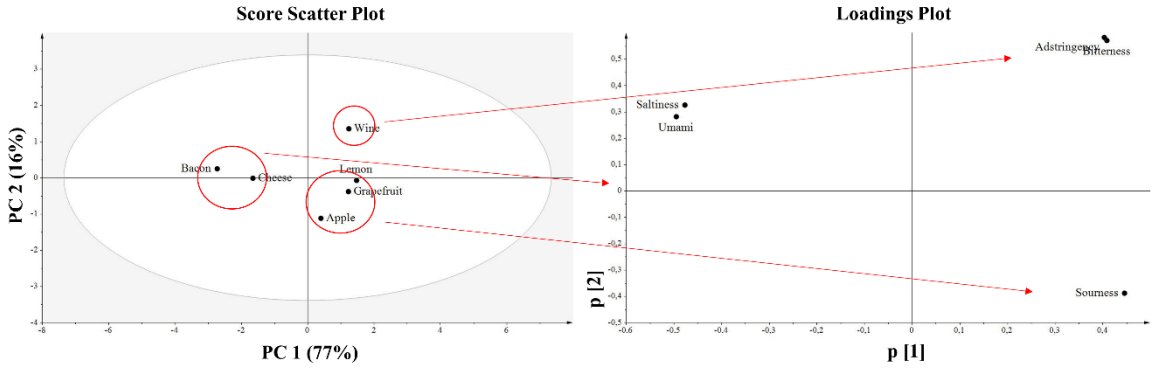


Figure 3: Example of a score scatter plot and the corresponding loadings plot for strong tasting food products. Sour tasting products (lemon, grapefruit, apple) are arranged in an area that is characterized by sourness, whereas the bitter and astringent tasting wine is located in the according quadrant and cheese and bacon are arranged in the area dominated by umami and salty taste sensation.

The observations, arranged in the new coordinative system of principal components, are data points defined as vectors containing the information of the transformation of the data from the original coordinative system into the new one and are called scores [67]. The aim of the data reduction is to maintain the descriptive information and to display the similarities between the samples in one model. The results of a principal component analysis is displayed in a score scatter plot and its corresponding loadings plot whereas the mathematical model is described by the amount of principal components that were used,

the residuals (R^2), which are the explained variation that represents the fraction of the sum of squares that is explained by the model and the fraction of the total variation of all x-variables that can be predicted by the model (Q^2).

In a 2D score scatter plot two principal components are shown (e.g. PC 1 and 2) and all analyzed samples are displayed in the plot as the scores. The location of the scores in or outside the confidence ellipse at a defined significance level and their arrangement to each other within the plot can be interpreted. The loadings plot displays the variables of the model and their impact on the observation vectors within the score scatter plot. A principal component analysis is often used if different drug formulation candidates are compared, in particular, to identify the most suitable formulation with the least change in taste pattern by addition of the bad tasting API. For an instance, Immohr et al. determined the taste-masking capability of different child-appropriate beverages on liquid drug formulations for pediatric use [11]. The sensor response pattern of the pure beverages have been compared to the beverages mixed with a liquid drug formulation by principal component analysis (Fig. 4).

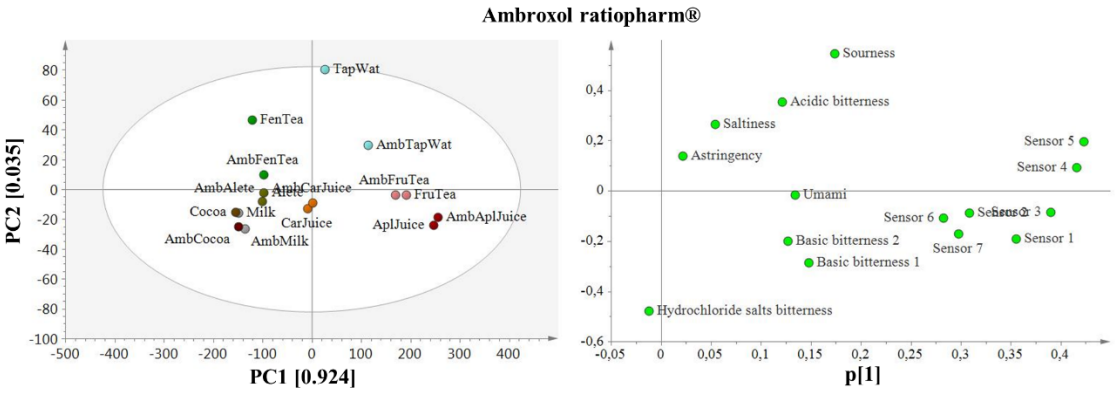


Figure 4: Score Scatter Plot and corresponding loadings plot of the taste-masking assessment of different child appropriate beverages (e.g. apple juice, fruit tea and milk) on the liquid oral drug Ambroxol ratiopharm®. The principal component analysis was performed using two principal components, (R2=0.959, Q2=0.913), containing the information of all samples based on the detection by the 15 employed sensors; each sample was measured in triplicate but displayed as mean; data is ctr scaled.

The differences in the sensor response pattern were interpreted as the differences in the taste pattern. Samples of the pure beverage and its corresponding drug mixture that showed highest similarities in their taste pattern were located closest to each other in the score scatter plot of the principal component analysis. In order to define these similarities and dissimilarities, the distances in the score scatter plot can be calculated as the Euclidean distances with an absolute value. This strategy of the Euclidean distances were used to detect the taste-masking capabilities in several other studies [11, 63, 64] whereby low distances indicated a high level of similarities in the taste pattern and therewith good taste-masking capabilities. This principle can also be applied for the electronic tongue guided formulation development of oral pharmaceuticals [61, 65, 66] such as done by Woertz et al., who developed a taste-masked oral liquid formulation of quinine hydrochloride by screening the effect of sweetening agents, ion exchange resins and complexation agents [61]. Implementation of a stepwise development resulted in a successful formulation of a taste-masked oral liquid.

Quantitative analysis by PLS can be a useful tool for the evaluation of bitterness thresholds for an instance. If a threshold of a specific API has been defined in a human taste study, this threshold might be used as the target concentration that is not to be exceeded in a certain formulation. Therefore, a calibration of the sensor array with the pure API is performed and a multilinear regression model is calculated. For a reliable calibration model, a calibration and a validation data set as well as a cross-validation of the model is used. The different formulation candidates can be screened and the free amount of the API in the sample can be analyzed. If the detected amount of API by the sensor array is reduced within the formulation even though the absolute amount is confirmed by another method (e.g. HPLC) a reduction in the bitter taste can be concluded. A further possible application area might be in drug dissolution studies. Thereby, the released amount of the bitter or bad tasting API within different media and time frames could be evaluated. If the release of the API can be inhibited or reduced within the environment of a simulated oral cavity, a suitable taste-masking for the administration time of oral drugs might be achieved. In a proof of concept study, this approach was shown by Khaydukova et al [68], Figure 5. In this study, electronic tongue sensors were used for the quantitative analysis of quinine hydrochloride and ibuprofen sodium in different artificial saliva media. Beside the suitability of the sensors for the application as an in-line detector in drug dissolution studies, their superiority against common UV

detection was demonstrated. Contrary to a UV probe, the electronic tongue sensors were capable to analyze the released drug amount in biorelevant artificial saliva, which was a turbid medium.

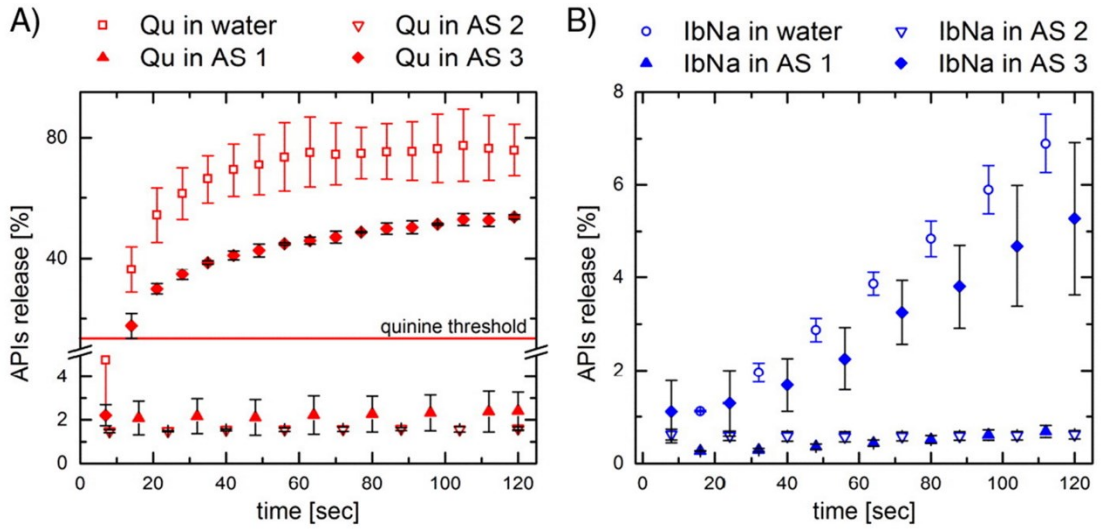


Figure 5: Evaluation of drug dissolution profiles in different artificial saliva media (AS1-3) by electronic tongue sensors and PLS analysis used for the prediction of a bitter taste sensation with the help of bitterness thresholds of the investigated APIs [68].

An adequate interpretation of the evaluated data of electronic tongues is the most important step to gain benefit from the obtained data. Misinterpretation or over-interpretation might cause wrong decisions on drug formulations and can lead to negligible usage of the whole taste assessment study. It is necessary to point out the difficulties in the correlation and interpretation of the in-vitro data to in-vivo results. The aim of such electronic tongue study is the reduction of human taste panels and a more reproducible and cost-effective taste assessment. However, due to the high complexity of the human taste sensation physiology, by now a sufficient imitation of the human taste buds is not imaginable. Therefore, it is of major importance to improve the instrumental approach by in-vivo data to gain more information on the correlation of the obtained electronic tongue results for a better interpretation of the in-vitro data. Only if a sensor array is authorized to work according to a human taste panel, its results are usable for further evaluation and interpretation. This aspect is in particular of major importance with regard to pediatric pharmaceuticals. It is of common knowledge that children's taste perception is different to that of adults as it was already described by Charles Darwin [69].

Furthermore, the taste of pediatric oral pharmaceuticals is of particular importance but at the same time little is published on correlated in-vivo and in-vitro results for the taste assessment with children. Most human taste panels are performed with adults and are often focused on the evaluation of the taste of common bitter molecules such as quinine [70] or paracetamol [62]. Correlation of data with new chemical entities or a database with the commonly orally administered drug are missing but urgently needed.

1.2.5 Unsolved tasks for pharmaceutical applications of electronic tongues

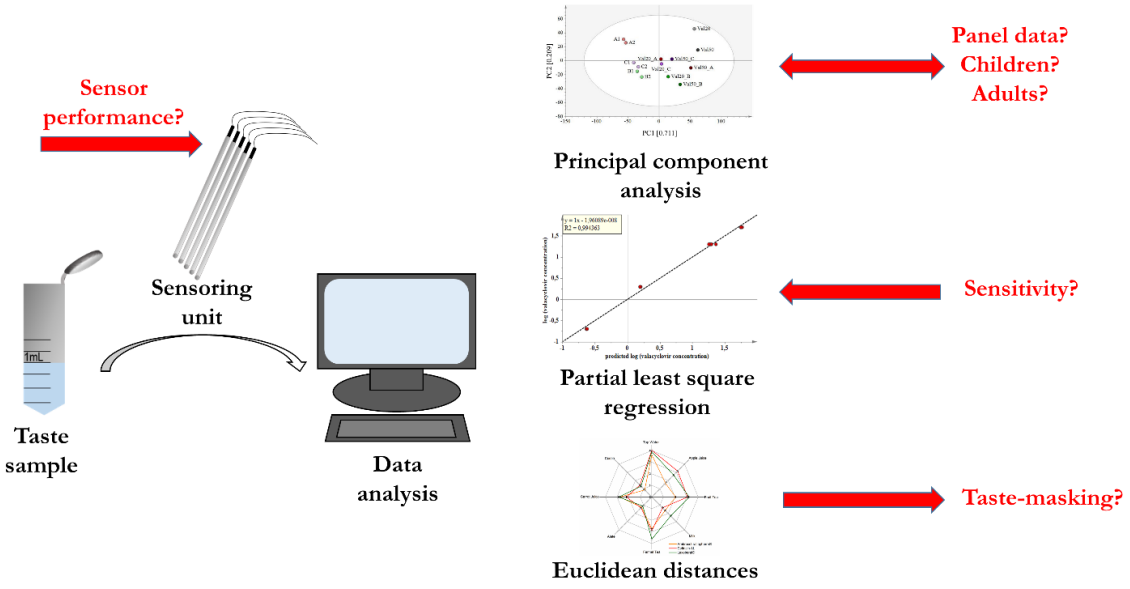


Figure 6: Analytical units and unsolved tasks for the subunits of electronic tongue systems.

Taking the high number of pharmaceutical taste assessment studies into account [1, 43, 50-52], the relevance and significance of electronic tongues as an analytical tool in the characterization of oral pharmaceuticals is proven by its frequent usage. The registration of a drug product, however, requires the validation of any analytical instrumentation that is used for the characterization of the drug. Even though the two commercially available electronic tongue systems (αAstree electronic tongue (Alpha MOS, Toulouse, France) and the Insent taste sensing system (Insent Inc., Atsugi-Shi, Japan)) were shown to be suitable to perform according to ICH guideline Q2 [16, 17], only the whole instruments, including

one batch of sensors, were qualified. The individual sensors were not evaluated according to their single sensor performance. Due to the various approaches for electronic tongue sensors that were launched by several different research institutes, a great variation in the performance of the sensors is assumed. Nevertheless, generally applicable recommendations for the sensor performance that can be adapted for the different kinds of electronic tongue sensors would be preferable. Especially for sensors that are based on the same measurement principle, shared quality criteria could support the effort to authorize electronic tongues as analytical tools for the characterization of oral drugs.

The validation of a common analytical procedure for the characterization of a drug product is meant to demonstrate its suitability for the intended purpose, as it is described by ICH guideline Q2. The recommended procedure for validation is, however, not applicable for electronic tongues as it is for HPLC analysis for instance. Nevertheless, the definition of validation should be adopted to electronic tongue systems. Electronic tongues are intended to assess the taste of oral pharmaceuticals and are used for the classification of appropriate drug formulations in order to identify and predict the most palatable one. In this manner, the suitability of the sensor array can be defined as the capability to differentiate between the taste patterns of drug formulation candidates. This includes a quantifiable sensor response of the targeted drug compound, as its bitter taste is aimed to be altered for a better palatability of the drug formulation. Only if the sensors are sensitive towards the drug compound that was found to be responsible for a bad taste, a reduction of this compound can be registered by the electronic tongue [71].

With regard to the requirements of electronic tongues to characterize the taste of oral pharmaceuticals, quality attributes for the sensor array might be defined that are applicable for all types of sensors. If this approach is successful, electronic tongues could be utilized in the development of oral pharmaceuticals with legalization of the registration authorities. These quality criteria should comply with the specifications of the drug compound and have to be confirmed for each substance prior to the taste assessment study. Taking the definition of suitability into account, the sensor array has to be evaluated with regard to its sensitivity towards the drug compound, the stability of the sensor signal and the diversity of the array. In this manner, the quality is independent from the measurement principle or the design of the sensor array as it is only defined by the performance of the sensors. This furthermore, enables the combination of different electronic tongues to improve the

discrimination capabilities [12]. One of the most important factors of electronic tongues is the capability to detect alterations in taste evoked by interactions between the components of a drug formulation, which was already described by Toko [30]. At the same time it is necessary to prove, that the sensor response is of log-linear correlation to the drug concentration to ensure that a change in the sensor response is not caused by an absolute alteration in the drug concentration but by the related taste. In this manner, Valsov et al.[9] proposed a minimum value for the slope ($s > 25$ mV/pX) within the range of the drug concentration for a suitable sensitivity in the detection of the drug compound. Furthermore, a stable sensor signal is of major importance and might be assessed by evaluation of the relative standard deviation of the sensor response towards the same sample [72]. This criterion has been described by Vlasov et al. and was calculated as the signal-to-noise ratio [9]. In addition, the "non-selectivity" parameter was used to ensure the cross-sensitivity of the sensor array [9]. Beside the evaluation of the performance of one sensor array, according criteria of a representative number of sensor arrays of the same type have to be defined in order to verify the individual production of the sensors as valid. Only if the development and production of the sensors lead to reproducible results, the whole system can be qualified as it is suitable for a repetitive application of the same purpose to reproduce data and provide reliable new data on an established product.

The consideration and implementation of such quality attributes for electronic tongue sensors that are based on the sensory outcome is suitable to provide a pool of possibilities to answer to pharmaceutical challenges. Even though, validation and qualification of the systems for a specific purpose have to be adopted to the targeted API and formulation excipients, it could afterwards be used for the authorized characterization of taste related properties of the drug formulation.

One of the most prominent advantages of the potentiometric sensor arrays is the high individuality of materials that can be used for the preparation of the sensors, irrelevant if either based on ISEs or ISFETs. Therewith, the individual preparation of sensors in consideration of special pharmaceutical questions is guaranteed. However, this benefit is not sufficiently utilized so far. By incorporation of special excipients that interact with a targeted molecule structure in addition to the low selective components, a special sensitivity towards challenging samples can be achieved. This might apply for new chemical entities or low electrochemically active ingredients but also for a differentiation

of stereoisomers. The development of stereoselective electronic tongue sensors would open a completely new application area as significant differences in taste are reported for some enantiomers such as L-praziquantel and D-praziquantel [73]. In this case, the bad tasting enantiomer, D-praziquantel, is the pharmacologically non-active component, which confirms the potential benefit of stereoselective electronic tongue sensors in the early stage of pharmaceutical formulation development. Even though the successful differentiation on amino acids of different tastes was reported by Toko [7], no suitable sensor array for the discrimination between the stereoisomers of the amino acids was presented by the authors. Certainly, the differences in taste of D- and L-amino acids are supported in literature [74, 75] but systematically developed and validated stereoselective electronic tongue sensors are not commercially available so far even though they are investigated [72].

To answer the open demands for electronic tongues and to utilize all of the collected data, the introduction of an information pool on electronic tongue sensors and its properties together with the molecules that can be characterized by the sensor arrays and a correlation to in-vivo data might provide the missing link between the different research groups and approaches. A united information board could further bundle the different sensor approaches and introduce recommendations for standardized guidelines on sensor performance and validated production methods.

1.2.6 Conclusion

The eligibility of electronic tongues for the characterization of oral pharmaceuticals is undisputed. However, the sensor performances of the several sensor approaches, the obtained data and the evaluation and interpretation of this data is controversial as no standardized and unified guidelines exist for the characterization of pharmaceutical products by electronic tongues. It is unquestionable that the variety of different sensor systems and the possibility to develop individual sensor arrays for special pharmaceutical questions is a great chance for the assessment of oral drug formulations that often contain critical components such as new active pharmaceutical ingredients and a wide range of different excipients of potentially harmful substances. Therefore, a performance qualification of the sensor array could confirm the individual sensors according to standardized quality attributes that are not only dedicated to the electronic system but the

single sensors. Evaluation of the measurement principles of the most commonly used electronic tongue systems provides the possibility to develop harmonized outcome orientated quality attributes for a successful sensor qualification in the future.

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1.3 Aims of the thesis

The application of electronic tongues for the characterization of oral pharmaceuticals increasingly gained importance during the last years since taste has become a critical quality attribute in pharmaceutical development. The large number of different commercial and non-commercial electronic tongue systems introduced for pharmaceutical applications points out their high relevance and frequent use. However, the knowledge on sensor technologies has not been used for a correlation to sensor performance and justification of electronic tongues for the characterization of pharmaceuticals with approval of regulatory authorities so far. Therefore, the overall aim of this thesis was the comprehension of sensor systems in consideration of the development of electronic tongues sensors, the critical evaluation of the sensor performance and the application of self-developed sensors for special pharmaceutical questions.

In particular, aims of the thesis can be defined as follows:

Development of potentiometric membrane electrodes compatible to the taste sensing systems of Insent Inc. (Atsugi-Shi, Japan) for the preparation of individual electronic tongue sensors; according sensors should be capable to expand the existing sensor arrays for special pharmaceutical requirements, in particular stereoselective sensors.

The critical evaluation of the sensor performance and the definition of quality attributes that contribute to the establishment of electronic tongues as analytical tools for the characterization of oral pharmaceuticals, approved by the authorities. This includes the systematic investigation of the membrane composition on quality attributes such as the stability of the sensor response, the sensitivity of the sensors, the shelf-life of the sensors or the robustness of the membrane electrodes towards potentially harmful substances.

The application of commercial and self-developed sensors for the correlation of in-vitro data to human taste panel data in order to assess the suitability of the sensors and further implement them for the assessment of taste-masking properties of pharmaceutical formulations and the analysis of challenging pharmaceutical questions.

2 Development and critical evaluation of potentiometric electronic tongue sensors

Evaluation of recently published data on electronic tongues used for the characterization of oral pharmaceuticals illustrated the urgent need for systematic investigations on quality and performance of the sensor arrays. Knowledge on the composition and correlation between the manufacturing and performance of the sensors is essential for an application of the sensors as analytical tools in the strictly controlled field of pharmaceutics. Therefore, this chapter focuses on the manufacturing process of membrane electrodes for the use as electronic tongue sensors as well as the performance and robustness of the sensor array. Comprehension on quality aspects like the reproducibility of the manufacturing process, the stability and sensitivity of the sensor response and the impact of potentially harmful substances was generated. The gained knowledge allowed for the manufacturing of self-developed electronic tongue sensors working according to defined quality criteria and generating reproducible and reliable data for the analysis of oral pharmaceuticals. In addition, this knowledge facilitated the development of sensors with individual properties in order to answer to special pharmaceutical requirements such as the production of stereoselective electronic tongue sensors.

The subsequent research paper has been published by Sensors & Actuators: B. Chemical (Impact factor 2016:5.401) in 2017. The presented research has been conducted as the basic work for the development and comprehension of self-developed electronic tongue sensors. The first author, Laura Isabell Immohr (University of Duesseldorf) is responsible for the concept, experimental work, data evaluation and interpretation as well as writing of the manuscript. The second and senior author, Miriam Pein-Hackelbusch (University of Applied Sciences Ostwestfalen-Lippe) is responsible for the idea, concept and revision of the manuscript.

2.1 Development of stereoselective e-tongue sensors considering the sensor performance using specific quality attributes – a bottom up approach

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2.2 Data for a pre-performance test of self-developed electronic tongue sensors

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2.3 Impact of sodium lauryl sulfate in oral liquids on e-tongue measurements

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3 Clinical relevance of electronic tongue data

The clinical relevance of electronic tongue data for the assessment and development of oral pharmaceuticals is based on the suitability of the sensor arrays to mimic the human taste perception. Only if the in-vitro data is capable to represent in-vivo results of a human taste panel, electronic tongues can receive the authorization to function as an alternative analytical method to human taste studies. Therefore, the correlation of in-vitro and in-vivo data is of high relevance in order to justify the application of electronic tongues in the taste assessment of pharmaceutical products. This chapter focuses on the investigation of human taste data and the correlation to electronic tongue results. This section is written to feature different approaches of electronic tongues in the development process of pharmaceutical formulations and to emphasize the benefit of an instrumental taste assessment method.

The following research paper has been published by the International Journal of Pharmaceutics (Impact factor 2016: 3.649) in 2017. The presented research has been conducted in the context of a collaborative work between the University of Duesseldorf and F. Hoffmann-La Roche Ltd, Basel. All listed authors were involved in the project and responsible for different parts of the research. The first author, Laura Isabell Immohr (University of Duesseldorf) is responsible for the experimental work, data evaluation and interpretation, the concept and writing of the manuscript. The second authors, Peter Kühl and Angela Dischinger are responsible of the idea and concept of the research, data evaluation, writing and revision of the manuscript. Heidemarie Kletzl, Stefan Sturm and Andreas Guenther as second authors are involved in concept, conduct and evaluation of the clinical work as well as revision of the manuscript. Senior author, Miriam Pein-Hackelbusch (University of Applied Sciences Ostwestfalen-Lippe) is responsible for the idea, concept, experimental work, data evaluation, writing and revision of the manuscript.

3.1 Early pediatric formulation development with new chemical entities: Opportunities of e-tongue besides human taste assessment

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3.2 In vivo and in vitro palatability testing of a new paediatric formulation of valaciclovir

Diane ET Bastiaans[1], Laura I Immohr[2], Gertrude G Zeinstra[3], Riet Strik-Albers[4], Miriam Pein-Hackelbusch[5], Michiel van der Flier[4], Anton FJ de Haan[6], Jaap Jan Boelens[7], Arjan C Lankester[8], David M Burger[1], Adilia Warris[9]

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4 Application electronic tongue sensors for special pharmaceutical questions

With growing capability to develop robust electronic tongue sensors working according to defined quality attributes, the clinical relevance was demonstrated by correlation of the obtained in-vitro data with human data. In the field of pharmaceutics, the sensors are faced with special demands in particular concerning the sample medium, purpose and design of the study. The application of electronic tongue sensors as detection system in turbid media such as beverages, foodstuff or artificial saliva could fill the gap in the direct assessment of APIs in biorelevant and natural media without complex sample preparations. Thereby, electronic tongues could not only be used for the assessment of taste and taste-masking effects but also for the concentration dependent determination of drug release profiles in order to monitor taste relevant drug concentrations in the oral cavity. This is why the following chapter focuses on the applications of electronic tongue sensors for the assessment of taste during concrete pharmaceutical problems and provides data that verifies and supports the advantages of electronic tongues as analytical tools in pharmaceutical analysis.

The following research paper has been published by the AAPS PharmSciTech in 2016 (Impact factor 2015: 1.954). The presented results were generated within the scope of an advanced practical training of pharmacy students. All four authors of the article were involved in the project and are listed according to their contribution. Laura Isabell Immohr as first author was responsible for the idea, concept, practical work, data evaluation and writing of the manuscript. Second authors, Claas Hedfeld and Arthur Lang were substantially involved in the practical and data evaluation as participating pharmacy students. Miriam Pein-Hackelbusch, listed as senior author was responsible for the idea, concept, data evaluation, writing and revision of the manuscript.

4.1 Suitability of e-tongue sensors to assess taste-masking of pediatric liquids by different beverages considering their physico-chemical properties

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The following research paper has been published by the European Journal of Pharmaceutical Sciences in 2017 (Impact factor 2015: 3.773). The presented research was performed in the context of the cooperational work between the working groups of Dmitry Kirsanov and Miriam Pein-Hackelbusch. The published data is part of a scientific exchange project between St. Petersburg State University, University of Duesseldorf and the University of Applied Sciences, Ostwestfalen-Lippe to join research on the development and application of self-developed electronic tongue sensors. Scientists of both working groups profited from the Russian-German cooperation and took the chance to visit each other's institutes and expand the research project to an intercultural exchange of the scientists. In this manner, Maria Khaydukova conducted part of the practical work at the University of Duesseldorf, L. Isabell Immohr at the Institute of Chemistry of St. Petersburg State University and Venera Gilemkhanova at the Life Science Technology Department of the University of Applied Sciences, Ostwestfalen-Lippe. Part of the research was funded by St. Petersburg State University, DAAD "Dmitry Mendeleev", The Government of Russian Federation and SEPAWA. First author, Maria Khaydukova, was responsible for the concept, practical work, data evaluation and writing of the manuscript. Second authors, L. Isabell Immohr and Venera Gilemkhanova were involved in the practical work, data evaluation, writing and revision of the manuscript whereas Dmitry Kirsanov and Miriam-Pein Hackelbusch were responsible for the idea, concept of the work, data evaluation as well as writing and revision of the manuscript. The senior author, Andrey Legin, was responsible for the ideas and revision of the manuscript.

4.2 Critical view on drug dissolution in artificial saliva: a possible use of in-line e-tongue measurements

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5 Conclusion and future perspectives

5.1 Quality attributes for electronic tongue sensors

The development of polymer based membrane electrodes for electronic tongue measurements improved comprehension about the manufacturing process and the composition of the membranes. This knowledge was used for a critical evaluation of the sensor performance and further enabled to define quality attributes such as the stability of the sensor response or the sensitivity of the sensors. These quality attributes demonstrated the suitability of the sensors for the analysis of multicomponent mixtures maintaining a certain sensitivity and selectivity at the same time to ensure the reliable detection of interactions between critical samples with the sensors and among each other. The definition of quality attributes and moreover, the recommendation of specification limits for the quality attributes could be used for the harmonization and standardization of regulatory requirements for electronic tongues by the authorities. This standardization could open the way for electronic tongues as validated and authorized analytical tools in the characterization of pharmaceutical products. The manufacturing of electronic tongue sensors according to specified quality criteria is the key for an individual development of sensors for special pharmaceutical questions and contributes to a wide range of new application areas of electronic tongues in the field of pharmaceutical sciences.

5.2 Benefit of self-developed electronic tongue sensors

The obtained comprehension of critical attributes that define the quality of electronic tongue sensors enabled the evaluation of self-developed sensors with regard to their pharmaceutical application. In this context, the capability of the self-developed sensors to analyze critical samples containing inter alia surfactants that potentially harm the lipid-based polymer membrane of the sensors was presented. Additionally, self-developed sensor arrays were successfully used to analyze the taste-masking effect of a multicomponent medium, such as common beverages, on a multicomponent drug formulation; an application where most other analytical methods fail. One of the most noticeable advantages of electronic tongue sensors is their suitability to detect a drug compound in a concentration dependent manner beside the low selectivity of the sensor

array. This approach is only possible using a large sensor array with sensors showing different selectivity patterns expressed by their diverse responses to basic taste samples. A well-considered combination of sensors with different sensor response patterns for a specific application can provide optimal capacity of the sensor array.

The progress in sensor development and manufacturing furthermore demonstrated that the individual preparation of electronic tongue sensors enabled the sensors to expand their analytical capabilities for particular pharmaceutical applications in comparison to commercially available electronic tongue systems. As one of the most challenging pharmaceutical questions is based on the discrimination between the different enantiomers of a drug compound, the successful development of electronic tongue sensors, capable to distinguish between the two enantiomers, can be dealt as one of the most important achievements of this thesis. The possible differences in taste and pharmaceutical activity of enantiomers can be analyzed with specially prepared sensors ensuring a growing comprehension on the generation of taste as well as on the discrimination between pharmacologically active and non-active compounds. Since the human taste perception is capable of stereoselective discrimination it is reasonable that artificial taste sensors should be developed with similar properties, which was successfully demonstrated.

5.3 Clinical relevance of electronic tongues

One of the most critical issues about electronic tongues is their questionable clinical relevance. Not only due to limited technical capacity but also due to missing comprehension about human taste perception, the artificially simulation of taste receptors by electronic tongues has to face several drawbacks in taste assessment. However, it is recognizable that a correlation of human taste data with electronic tongue data was able to demonstrate the relevance of sensor arrays for the assessment of oral drug formulations. The reconciliation of in-vivo and in-vitro data as the first step to justify the use of electronic tongue sensors as analytical tool for taste assessments is essential. Certainly, if this correlation is demonstrated as it was during this work, the implementation of electronic tongues can provide valuable information on the taste pattern of drug formulations that can further be used to guide the development of a taste-masked pharmaceutical product.

5.4 Critical considerations

Even though electronic tongues aim for the imitation of the human taste sensation and several sensor approaches try to mimic the taste receptors, a conclusion of a defined taste cannot be drawn by electronic tongue analysis. Regardless from the sensor array, the sensor response pattern can only be interpreted as a relative pattern of taste and no absolute information on the taste can be stated. However, with the use of a large sensor array, a huge quantity of information on the multicomponent analyte can be detected and by application of an adequate mathematical method for data evaluation and interpretation, electronic tongues can be used to guide the formulation development of oral pharmaceuticals.

Beside this, the limitation of electronic tongues to liquid samples is one of the most challenging and disadvantageous properties of electronic tongues. The electronic tongue sample preparation of initial solid samples by dissolution often results in less biorelevant experimental environments and could further negatively or positively influence the outcome. Therefore, the development of sensors sensitive to solid samples that are in direct contact with the moistened sensor membrane would improve the concept of a biorelevant and instrumental analytical tool for the imitation of the human tongue.

5.5 Future perspectives

The scientific achievements of this work with regard to individual sensor manufacturing and elaboration of new application areas was set into context to common requirements of the authorities concerning the use of electronic tongues as analytical tools for the characterization of pharmaceuticals. A successful implementation of electronic tongues supported by the authorities could be defined as a further aim to which this thesis contributed. Especially since taste has become a critical parameter of oral pharmaceuticals and is required to be considered during the development of oral drug formulations, the taste assessment of pharmaceuticals has become actuality. The authorization of electronic tongues and the development of a standardized protocol for taste assessment would be of major benefit for pharmaceutical companies during the registration of new drug formulations. If in future, the preparation of individual electronic tongue sensors is standardized and authorized due to defined quality attributes, the value of such sensor

arrays in applied research will grow and will contribute to the development of safe and compliant drug products.

6 Summary

Taste assessment of oral pharmaceutical products for the development of palatable drug formulations has recently become common practice since taste was defined as a critical parameter for those dosage forms. Electronic tongues are well-established instruments for the objective and reproducible evaluation of taste patterns without ethical concerns. In the last years, numerous studies have been performed on the implementation of electronic tongues for the characterization and guidance of the development of oral pharmaceutical formulations. Therewith, a performance qualification of commercially available electronic tongue systems proved the analytical relevance of the obtained data. Due to the emerging use and interest in electronic tongues as analytical tools in the characterization of oral dosage forms for the authorization of pharmaceutical products, the need for systematic investigation of the different sensor systems is still growing.

In this thesis, the urgent need for the definition of quality attributes for electronic tongue systems on the basis of the sensor array was addressed. The preparation of self-developed polymer-based membrane electrodes improved comprehension on sensor composition, manufacturing, performance and applications. This knowledge could generally be used to define quality attributes for electronic tongue sensors regardless from the method of manufacturing or basic measurement principle. The definition of quality attributes including the stability of the sensor responses, the reproducibility, robustness and the sensitivity of the sensors on a sensor-based level enables the discussion for the legislation of electronic tongues by regulatory authorities.

Beside the quality aspect of the sensor performance, the knowledge on the manufacturing of self-developed sensors was used to answer unsolved pharmaceutical and analytical questions. Comprehension on the composition of membrane electrodes for the characterization of a multicomponent solution enabled the individual manufacturing of specialized electronic tongue sensors. The incorporation of special molecules in the polymer membrane of the sensors, facilitated the interaction with the target molecules in the sample solution. Therewith, the incorporation of cyclodextrins enabled the discrimination of enantiomers by stereoselective sensor membranes providing an appreciable achievement for the characterization of enantiopure pharmaceuticals since

differences in taste and pharmacological activity of enantiomers were dealt as one major challenge in the characterization of oral pharmaceuticals.

During this work, the correlation of the obtained in-vitro taste data with results of human taste panels increased the clinical relevance of electronic tongues and furthermore enabled their application for the development of taste-masked pharmaceuticals and justified the implementation of electronic tongues for additional analytical pharmaceutical questions. The functionalizing of polymer-based sensor arrays for the detection of drug release profiles in biorelevant artificial saliva opened a new application field of electronic tongues. The drawbacks of turbid and multicomponent sample media for the analytic by UV spectroscopy were overcome. In-line monitoring of the released drug amount could be used for correlation with taste perception thresholds of the bitter tasting API, providing a real-time control of the palatability of solid dosage forms. This approach further facilitates the application of electronic tongue sensors for the taste-masking assessment of different multicomponent beverages for the administration of liquid pharmaceuticals in children.

Taking the achievements in the comprehension of electronic tongue sensors and their application areas into account, this thesis offers the opportunity of standardizing the characterization of oral pharmaceuticals by electronic tongue systems with defined quality criteria for a future approach to approve electronic tongues by regulatory authorities. Furthermore, the superiority of the sensor arrays for special pharmaceutical and analytical applications was demonstrated and the relevance of electronic tongues as analytical tools in the field of pharmaceutical sciences was strengthened.

7 Zusammenfassung

Die Bewertung des Geschmacks oral verabreichter Arzneiformen zur Entwicklung wohlschmeckender Arzneimittel ist gängige Praxis seitdem Geschmack zu einem kritischen Parameter oraler pharmazeutischer Produkte geworden ist. Elektronische Zungen stellen eine etablierte Methode zur instrumentellen und reproduzierbaren Bewertung von Geschmacksmustern ohne ethische Bedenken dar. In den letzten Jahren haben zahlreiche Studien den Einsatz von elektronischen Zungen zur Geschmacksbewertung und Entwicklung geschmacksoptimierter Arzneimittel dokumentiert. Damit wurde durch die Qualifizierung kommerzieller Systeme die analytische Relevanz der erzeugten Daten bestätigt. Aufgrund des steigenden Interesses und des Einsatzes elektronischer Zungen als analytisches Werkzeug zur Charakterisierung oraler Arzneiformen für die Zulassung pharmazeutischer Produkte, wächst die Nachfrage nach einer systematischen Untersuchung der verschiedenen Sensorsysteme stetig.

In der vorliegenden Arbeit wurde die große Nachfrage nach einer Formulierung von Qualitätsattributen für elektronische Zungen auf Basis der Sensorsysteme adressiert. Die eigene Entwicklung von polymerbasierten Membran-Elektroden ermöglichte ein Verständnis der Zusammensetzung, der Herstellung, der Performance und den Einsatzgebieten elektronischer Zungen. Dieses Wissen konnte für die Definierung von Qualitätsattributen auf Sensorbasis genutzt werden, welche die Bereiche der Stabilität des Sensorsignals, die Reproduzierbarkeit, Robustheit und die Sensitivität der Sensoren umfassen und eine Diskussion zur Zulassung elektronischer Zungen von den Behörden ermöglicht.

Neben den Qualitätsaspekten der Sensorperformance, konnte das Wissen über die Herstellung eigener Sensoren zur Beantwortung spezieller pharmazeutischer und analytischer Fragestellungen genutzt werden. Verständnis über die Zusammensetzung von Membranelektroden für die Charakterisierung von Mehrstoffgemischen ermöglichte die individuelle Entwicklung spezieller Sensoren. Die Verarbeitung bestimmter Moleküle in der Polymermembran der Sensoren, ermöglichte die Interaktion mit den Zielmolekülen der Probenlösung. Auf diese Weise konnte die Verwendung von Cyclodextrinen in der Polymermembran, eine Unterscheidung von Enantiomeren durch stereoselektive Sensoren erzeugen und stellt damit eine große Errungenschaft für die Charakterisierung

enantiomerenreiner pharmazeutischer Zubereitungen dar. Die Unterschiede im Geschmack und der pharmakologischen Aktivität von Enantiomeren stellen einer der großen Herausforderungen für die Charakterisierung von oralen Arzneiformen dar.

Darüber hinaus wurde in dieser Arbeit die klinische Relevanz von elektronischen Zungen durch die Korrelation von erzeugten Ergebnissen mit Daten von Geschmacksstudien am Menschen aufgezeigt, welche ferner den Einsatz elektronischer Zungen für die Entwicklung geschmacksmaskierter Arzneimittel ermöglichen und die Verwendung für spezielle pharmazeutische Fragestellungen rechtfertigt. Der Einsatz von polymerbasierten Sensorsystemen für die Analyse von Freisetzungsprofilen in biorelevanten künstlichen Speichel eröffnete ein neues Einsatzgebiet von elektronischen Zungen. Damit konnten die Nachteile eines trüben und mehrkomponentigen Probenmediums für UV-spektroskopische analytische Methoden überwunden werden. Das in-line Monitoring freigesetzter Arzneistoffmengen kann weiter für die Korrelation mit Grenzwerten für die Geschmacksempfindung bitterer Arzneistoffe genutzt werden und ermöglicht eine Kontrolle des Geschmacks oraler Arzneiformen in Echtzeit. Dieser Ansatz konnte auch für den Einsatz von Sensorsystemen zur Bewertung der geschmacksmaskierenden Eigenschaften verschiedener Getränke zur Verabreichung flüssiger Arzneizubereitungen für Kinder verwendet werden.

Eine abschließende Betrachtung des, in dieser Arbeit erlangten, Verständnisses über Sensoren für elektronische Zungen und ihre Einsatzgebiete eröffnet die Möglichkeit einer Standardisierung in der Charakterisierung oraler Arzneiformen durch elektronische Zungen mit definierten Qualitätskriterien für eine zukünftige Zulassung ebendieser Sensorsysteme durch die Behörden. Des Weiteren konnte die Relevanz elektronischer Zungen als analytische Werkzeuge im Bereich der pharmazeutischen Wissenschaften gestärkt werden.

8 List of original publications

L. I. Immohr, C. Hedfeld, A. Lang, M. Pein-Hackelbusch

Suitability of e-tongue sensors to assess taste-masking of pediatric liquids by different beverages considering their physico-chemical properties

AAPS PharmSciTech, 2017, 18, 330-340

L. I. Immohr, R. Turner, M. Pein-Hackelbusch

Data for a pre-performance test of self-developed electronic tongue sensors

Data in Brief, 2016, 9, 1090-1093

L. I. Immohr, R. Turner, M. Pein-Hackelbusch

Impact of sodium lauryl sulfate in oral liquids on e-tongue measurements

International Journal of Pharmaceutics, 2016, 515, 441-448

M. Khaydukova, D. Kirsanov, M. Pein-Hackelbusch, L. I. Immohr, V. Gilemkhanova, A. Legin

Critical view on drug dissolution in artificial saliva: a possible use of in-line e-tongue measurements

European Journal of Pharmaceutical Sciences, 2017, 99, 266-271

J. Ritzer, T. Lühmann, C. Rohde, M. Pein-Hackelbusch, L. I. Immohr, U. Schedler, T. Thiele, S. Stübinger, B. von Rechenberg, J. Waser, F. Schlottig, M. Merli, H. Dawe, M. Karpíšek, R. Wyrwa , M. Schnabelrauch, L. Meinel

Diagnosing peri-implant disease using the tongue as a 24/7 detector

Nature Communications, 2017;8, 264

D. E. Bastiaans, L. I. Immohr, G. G. Zeinstra, R. Strik-Albers, M. Pein-Hackelbusch, M. van der Flier, A. F. de Haan, J. J. Boelens, A. C. Lankester, D. M. Burger, A. Warris

In vivo and in vitro palatability testing of a new paediatric formulation of valaciclovir

British Journal of Clinical Pharmacology, 2017, open access, doi: 10.1111/bcp.13396

L. I. Immohr, M. Pein-Hackelbusch

Development of stereoselective e-tongue sensors considering the sensor performance using specific quality attributes – a bottom up approach

Sensors and Actuators B: Chemical, 2017, 253, 868-878

M. Münster, A.H.A. Mohamed-Ahmed, L.I. Immohr, C. Schoch, C. Schmidt, C. Tuleu, J. Breitkreutz

Comparative in vitro and in vivo taste assessment of liquid praziquantel formulations

International Journal of Pharmaceutics, 2017, 529, 310-318

L. I. Immohr, A. Dischinger, P. Kühl, H. Kletzl, S. Sturm, A. Guenther, M. Pein-Hackelbusch

Early pediatric formulation development with new chemical entities: opportunities of e-tongue besides human taste assessment

International Journal of Pharmaceutics, 2017, 530, 201-212

9 Contributions to meetings

Oral presentations

I. Immohr, M. Khaydukova, A. Legin, D. Kirsanov, M. Pein-Hackelbusch

Development of a Dissolution Test Model for Orodispersible Pharmaceuticals

Science conference between North Rhine-Westphalia and the Russian Federation, 26th April 2016, Münster

I. Immohr, M. Pein-Hackelbusch

Influence of Polymer Film Properties on Membrane Electrode Quality for E-Tongue Sensors

8th PSSRC Annual Symposium Ljubljana, 17th September 2014

Poster

I. Immohr, A. Lang, C. Hedfeld, M. Pein-Hackelbusch

Taste-Masking Evaluation of Liquid Pediatrics Mixed with Beverages

10th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Glasgow 2016

I. Immohr, R. Turner, M. Pein-Hackelbusch

Applicability of an External Standard for E-Tongue Measurements

AAPS Annual Meeting, Orlando, FL, USA, October 26th to 29th 2015

I. Immohr, R. Turner, M. Pein-Hackelbusch

Assessing the Effect of Sodium Lauryl Sulphate on the Performance of E-Tongue Sensors

7th EuPFI Conference 'Formulating Better Medicines for Children', 16th to 17th September 2015, Antwerp, Belgium

I. Immohr, M. Pein

Critical Evaluation of Self-Developed Membrane Electrodes

8th Polish-German Symposium on Pharmaceutical Sciences, 29th to 30th May 2015, Kiel, Germany

M. Khaydukova, L. I. Immohr, A. Legin, D. Kirsanov, M. Pein

Influence of Artificial Saliva on taste Masking Studies

1st European Conference on Pharmaceutics, Reims 2015

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11 Erklärung

Hiermit erkläre ich gemäß §3 Absatz 2 der Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät der Heinrich-Heine-Universität Düsseldorf, dass ich die vorliegende Arbeit eigenständig und ohne unerlaubte Hilfe angefertigt habe und diese in der vorgelegten oder in ähnlicher Form noch bei keiner anderen Institution eingereicht habe.

Düsseldorf,