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CRITICAL REVIEW

Recent advances in bitterness evaluation methods

Zhaobin Chen,[a] Jinhui Wu,*[a] Yang Zhao,[a] Feng Xu[bc] and Yiqiao Hu*[a]

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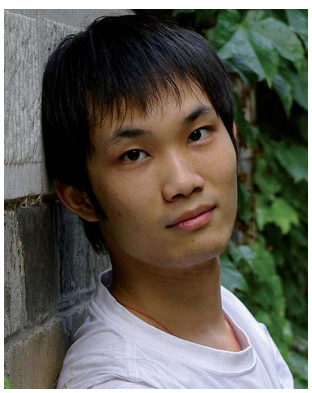
Many active pharmaceutical ingredients (APIs) are bitter and the bitterness intensity is usually in direct proportion to the treatment efficacy of APIs. However, the bitterness of some APIs induces an unpleasant taste or odor that has been a hurdle to their commercialization. One potential solution to address this is to mask the unpleasant taste, the success of which depends on the precise evaluation of bitterness. Currently, various methods have been developed for bitterness evaluation, such as the human gustatory test, the animal gustatory test, calcium imaging and electronic tongues (taste sensors). In this paper, we review state-of-the-art bitterness evaluation methods and discuss their distinctive advantages and potential challenges for use in the pharmaceutical and food industries.

Introduction

It is generally accepted that mammals can discern five basic taste attributes, *i.e.*, bitterness, umami, sourness, sweetness and saltiness.[1,2] Within these five tastes, bitterness is an implication of noxious stimulus as it induces aversion in mammals.[3,4] The unpleasant flavor and aversion induced by bitter substances make them difficult to swallow. Many foods elicit a bitter flavor naturally (*e.g.*, grapefruit juice, beer)[5] and many active pharmaceutical ingredients (APIs), such as acetaminophen, macrolide antibiotics and alkaloids are too bitter to be administrated orally.[6–8] Therefore, bitterness is one of the greatest challenges for food and drug commercialization due to lack of patient compliance, especially in pediatric patients.[9–11] Thus a suitable bitter masking method is necessary to ensure the acceptability and commercialization of APIs, and many bitter evaluation methods have also been developed for evaluating the efficiency of bitter masking.

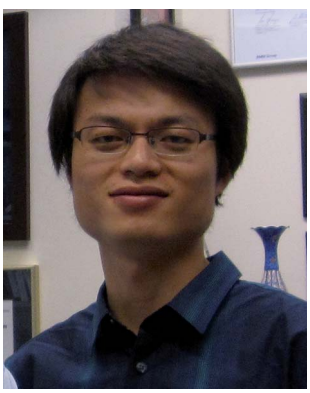
Currently, many bitter evaluation methods have been reported (Table 1). Among them, the human gustatory test is the most direct method for bitter evaluation. A similar method, the animal gustatory test, is also widely used. However, both human and

[a]*State Key Laboratory of Pharmaceutical Biotechnology, Nanjing University, Nanjing, 210093, P.R. China. E-mail: wuj@nju.edu.cn; huyiqiao@nju.edu.cn; Fax: +86-025-83596143; Tel: +86-025-83596143*
[b]*The Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an, 710049, P.R. China*
[c]*Biomedical Engineering and Biomechanics Center, Xi'an Jiaotong University, Xi'an, 710049, P.R. China*



Zhaobin Chen

Zhaobin Chen received B.S. in Biotechnology from Nanjing University in 2010. He is now pursuing an M.S. in Pharmaceutics at Nanjing University. His research interests are mainly focused on bitterness evaluation.



Jinhui Wu

Jinhui Wu received B.S. and M.S. in Pharmacology from China Pharmaceutical University in 2003 and 2006, respectively, and a Ph.D. in biology from Nanjing University in 2010. He joined the faculty of Nanjing University in 2010 and has been affiliated with the State Key Laboratory of Pharmaceutical Biotechnology. His research interests include bitterness analysis, protein nanocarriers for chemical drug delivery and microfluidic-based high throughput drug screening. He has 18 peer reviewed publications and has applied for 9 patents.

animal gustatory tests are qualitative and safety concerns limit their application.[12] Recently, with advances in fluorescence quantitative analysis and electrochemical detection, several *in vitro* bitter evaluation methods have emerged, such as calcium imaging and electronic tongues (ET).[12,13] These *in vitro* methods hold great potential to address the challenges associated with human or animal gustatory tests. In this review, we present state-of-the-art methods for bitterness evaluation and discuss the distinctive advantages and potential challenges of these methods from the viewpoint of the pharmaceutical and food industries.

Human gustatory test

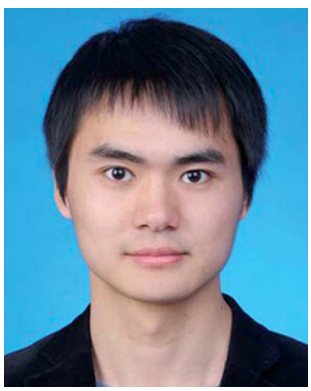
In vivo, bitterness is perceived by a family of G protein-coupled receptors called hT2Rs (human bitter taste receptors), with 25 members present on the surface of the tongue.[14,15] The human gustatory test is the most direct method for bitter evaluation and can directly express taste perception exactly after taste tests.

Normal subjects without genetic defects in bitter perception can qualify bitterness evaluation without taste training. For instance, 10 healthy volunteers (no taste training given) participated to evaluate the bitterness of acetaminophen in a wax matrix system. In the gustatory test, acetaminophen was diluted to 20, 25, 30, 35 or 40 μg mL^{-1} with an adequate amount of water, and was detected as bitter by more than half of the volunteers at the concentration of 35 μg mL^{-1}.[8] However, results from gustatory tests are reliable only after statistical analysis and results from individuals sometimes contain errors. For example, one volunteer who identified 25 μg mL^{-1} as bitter did not identify 30 μg mL^{-1}. In another example, the bitterness threshold of roxithromycin was evaluated to be in a wide range between 19.8 to 29.7 μg mL^{-1} by volunteers.[16] Although bitterness evaluation without taste training can reduce the costs and time involved in the evaluation, it is a low accuracy method. This problem can be reduced by using trained subjects.

In the food industry, the recruitment of volunteers for bitterness evaluation is rigorous and it is necessary to follow national industry standards. For instance, according to German Industry Standards (DIN10961),[17] subjects will be asked to judge the taste impressions of 10 aqueous standard solutions, including sweet (6 mg mL^{-1} sucrose), bitter (0.3 mg mL^{-1} caffeine), sour (0.4 mg mL^{-1} citric acid), astringent (0.3 mg mL^{-1} tannin) and salty (1.3 mg mL^{-1} sodium chloride). To enter the test stage, the accuracy rate of each test subject in the training stage needs to reach 70%.[18] A triangle test method (called force-choice discrimination) has also been developed. In the triangle test, subjects are given two or three samples that contain tastants and reference. Subjects who can identify the tastant sample can be recruited.[19,20] After the training process, the accuracy of bitter evaluation can be significantly increased.

Besides the subject training, there are also other factors that affect the human gustatory test such as sample position, touch, rinsing and psychological effects. Taste cells distribute unevenly on the surface of the tongue. They are mainly located on the margins and the root of the tongue.[3,21] This spatial distribution has a significant effect on bitterness evaluation. For example, the sensitivity of quinine evaluated at the root was 2-fold higher than that at the tip.[2] The sensitivity of the taste cells is also affected by tactile interaction, especially at low concentration levels of the bitter stimulus.[22] To minimize operator errors, suitable procedures should be established by taking tactile stimulation into consideration. For example, a pipette should be used to drop the samples directly onto the middle of the tongue to avoid tactile stimulation on taste cells. Subjects keep the sample in the mouth for a few seconds and then spit out (called sip-and-spit).[20]

Although the rigorous regulation of the human gustatory test can reduce the error of evaluation, the psychological and



Yang Zhao

Yang Zhao received his Bachelors degree in Aquiculture from Shanghai Ocean University in 2009, and since then has attended Nanjing University to study for his Masters degree. Currently, Yang Zhao is a research fellow at the State Key Laboratory in Nanjing University. His research is focused on drug metabolism and new drug development.

Feng Xu

Feng Xu received his Bachelors degree in Thermal & Power Engineering and Industry Engineering in 2001, and his Masters degree in Mechanical Engineering in 2004, both from Xi'an Jiaotong University. In 2008, he received his PhD degree in Engineering from the University of Cambridge. Dr. Xu is currently a Professor at School of Life Science and Technology in Xi'an Jiaotong University, China. His research is focused on nano and microscale cell engineering, for multiple applications including regenerative medicine, novel in vitro *disease models,* etc. *Dr Feng Xu has published over 70 journal papers, and over 70 conferences papers/abstracts.*

Yiqiao Hu

Yiqiao Hu received a B.S. in Pharmacy from China Pharmaceutical University in 1982 and a Ph.D. in biology from Nanjing University in 2000. She is a professor at the School of Life Sciences in Nanjing University. Her research interests include bitterness analysis and masking, and self-assembling protein nanoparticles for drug and gene delivery.

Table 1 Comparison of different bitterness evaluation methods

	Testing time	Quantitative	Throughput	Cost	Ref
Human gustatory test	Long[a]	Yes	Low	High[c]	106,114
Animal gustatory test	Long	Yes	Medium	Low	25,30
Calcium imaging	Long[b]	Yes	High	Medium	13,33
E-tongue	Short	Yes	High	High	42,55,59

[a] including rigorous taste training. [b] including cell transfected. [c] including rigorous taste training.

physical statuses of test subjects are impossible to match (*e.g.*, the genetic difference of taste sensitivity). This is the major shortcoming for the human gustatory test.[23] The rigorous training programs also make the human gustatory test hard to perform due to the high cost and long testing time. The potential safety and ethical problems also need to be considered.

Animal gustatory test

As an alternative to the human gustatory test, animals (*e.g.*, rodents, dogs and some other mammals) can also be used for bitterness evaluation in view of their similar bitter taste receptors to those of human beings.[2,24] Compared with other animals, rats and mice are much easier to handle and thus are the preferred animals in bitterness evaluation. Since rats intake greater amounts, measurement errors are lower in rats than in mice. The two-bottle choice paradigm and the brief access test are the two most commonly used methods.

Two-bottle choice paradigm

The two-bottle choice paradigm (also called the two-bottle taste preference test) is a simple method and does not require expensive instruments.[25] In this method, the intake difference between sample and reference is used to show the bitterness intensity. To minimize measurement errors, water deprivation is applied 24 h before the initiation of the test.[26] The position of the bottles is also switched in the middle of the test.[27] After each test, water is available to rats for 24 h to exclude the effect of the last test prior to the next one.[28] An obvious advantage is that no special instruments or operator training are required in this method.

Brief-access test

Different from the two-bottle choice paradigm, the licks performed by rats within the same time (typically 5–30 s) are counted to reflect the bitterness. This test requires a special instrument ("Davis Rig", Fig. 1), which is commercially available. This instrument consists of a computer, a test closet for rats and a shelter with several little bottles. The shelter and closet are separated by a plate, in which there is a shutter for spouting solutions of a sample one at a time. The shutter is connected to a computer to control the delivering order and interval time.[29] When a rat licks, an imperceptible electric signal is generated and sent to the computer, which is counted as one lick. Generally, a thirsty rat will lick the spout delivering water 30 to 50 times in 5 s.[25] The trial begins immediately after the first lick and if no lick has happened within a certain time (*e.g.*, 300 s), this trial ends and the next will begin.[30] The trials should be conducted within a 30 min session.[30,31] To avoid routine behavior, the order of the sample delivery should be random. Compared to the two-bottle choice paradigm, this method is more efficient without post-ingestive effects (*e.g.*, satiety or toxicity).[30]

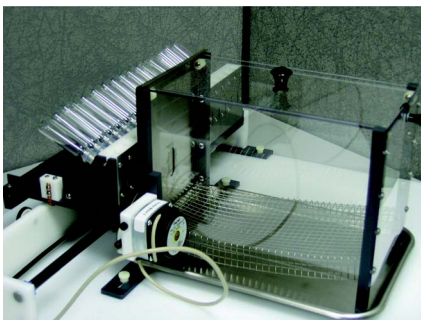


Fig. 1 Davis Rig "Lickometer" for the brief-access taste assay. Rodents that are mildly water-deprived lick solutions from up to sixteen bottles arranged in the "lick block". Spouts from each bottle are presented one at a time through the port in the front of the cage. A "lick" is recorded each time the rodent's tongue makes contact with the spout. Reproduced with permission from ref. 25.

Besides rats, other rodents can also be used for bitterness tests, where the intake can reach almost 100 mL to reduce random error. However, the results of animal gustatory tests should be treated cautiously since they only reflect the intensity of aversion, which is an indirect reflection of bitterness. Besides, inter-species differences have been reported where the results from animal gustatory tests may not reflect the human response. For instance, cycloheximide could only be recognized by mice and rats, but not by humans and rabbits.[26] In most cases, animal gustatory tests are used to establish a dose–response relation for a known bitter substance, but not for unknown compounds. Besides, a conditioned environment (20–22 °C, 12h : 12h light–dark cycle) is required during the tests.[26,32] Overall, animal gustatory tests are a useful method for bitter evaluation at present due to their easy recruitment, simple procedures and low costs.

Calcium imaging

Molecular biology has demonstrated that human bitter taste receptors (T2Rs) belong to the class of G protein-coupled receptors (GPCRs) and the G protein signaling pathway plays a crucial role in bitterness perception.[24,25] When bitter compounds contact with T2R, the G-protein splits into two parts: α-gustducin and $\beta 3/\lambda 13$ complex. The latter then activates phospholipase *C* (PLC), an enzyme that hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG)

and inositol 1,4,5-trisphosphate (IP_3). This hydrolysis process results in high levels of IP_3 and induces the release of Ca^{2+} from the endoplasmic reticulum. Ca^{2+} in turn stimulates TRPM5 channels and generates action potentials.[2] In this pathway, the release of Ca^{2+} from the endoplasmic reticulum to the cytoplasm is a key step in inducing the downstream reaction. Based on the discovery of the bitter signaling mechanism, calcium imaging technology has been introduced into bitterness evaluation.

The dyes used for calcium imaging can be classified as ratiometric dyes and non-ratiometric dyes. For non-ratiometric dyes, such as Fluo-4, the absolute concentration of calcium is measured by the increase in fluorescence. However, due to the circulation of endochylema, it is sometimes difficult to achieve uniform and repeatable distribution of these dyes, which directly affects the quantification of Ca^{2+} concentration. To minimize the effect induced by non-uniform dye distribution, the fluorescence responses normalized to background can be used, which is defined as $\Delta F/F = (F - F_{base})/(F_{base} - F_{back})$ with F_{base} being fluorescence responses before stimulation and F_{back} being background noise.[33,34] In the ratiometric method, the wavelengths of excitation and/or emission of dyes change when the dyes bind with calcium. For example, the excitation wavelength of Fura-2 shifts from 380 nm to 340 nm when it binds calcium. The relative calcium concentration is then measured by a ratio formulation ($[Ca^{2+}] = k_{eff}*(R - R_{min})/(R_{max} - R)$, $R = F_1/F_2$, F_1, the fluorescence at wavelength one; F_2, the fluorescence at wavelength two; R_{min}, R at zero Ca^{2+} concentration; R_{max}, R at saturating Ca^{2+} concentration; k_{eff}, effective binding constant.). In this way, the drawback of the non-ratiometric method mentioned above is excluded. However, to obtain a stable value of R_{min} and R_{max} for the same dye, a complex calibration is necessary. In this case, calcium concentration can be compared from experiment to experiment.[35,36]

Usually, a bitter substance only partly stimulates the bitter receptors. For example, aristolochic acid, a known bitter substance, can only stimulate three bitter receptors: T2R14, T2R31 and T2R43.[4,33,34] Therefore, to avoid false bitterness evaluation, the stimulated receptor (T2R14, T2R31 or T2R43) needs to be transfected into cells for the bitterness evaluation of aristolochic acid. In transfection, the bitter receptor cDNA is usually modified with some additional sequences. For example, to improve membrane targeting, the cDNA can be modified with ssr-3 (somatostatin receptor type 3 sequence), and to easily detect the expressed bitter receptor, the cDNA can be modified with HSC (herpes simplex virus glycoprotein D).[37,38] Using this method, Meyerhof established relationships between 104 bitter chemicals and 25 human bitter receptors.[13] Different hT2Rs obtained from GenBank can be modified and then transfected into cells for bitterness evaluation. The HEK 293T cell line that stably expresses the chimeric G-protein subunit (mostly expressed Gα16gust 44) is the most commonly used in transfection. The calcium responses of the transfected cells can be measured after the addition of test substances.[4,33,34] To exclude false positive responses, a concentration–response relation should be established before the initiation of the study and the concentration of test substance should be lower than the artificial signal threshold.

Different from the human and animal gustatory tests, calcium imaging technology extends the insight of bitter evaluation technologies from a behavior level to a cell and molecular level. Recently, calcium imaging has also been applied to high-throughput screening for mining hT2Rs inhibitors. For instance, an inhibitor called GIV3727 was found from 17 854 compounds *via* a calcium imaging method.[34] This discovery opens a new door for bitterness masking in the pharmaceutical industry. It is envisioned that more bitter inhibitors will be discovered soon, especially with the advances in cell-based high throughput screening methods.[39] However, in spite of all this potential, some challenges remain. The inhibitors filtered out only specific bitter receptors and it is still hard to find inhibitors that can tune all bitter receptors. The complex procedures of calcium imaging technology also limit its applicability. In addition to plasmid construction, there are still obstacles in cell transfection and the selection of dyes with low photobleaching. In addition, it is still challenging to use calcium imaging to assess substances with a strong response in the control set, and substances with poor solubility in water.

Electronic tongue

In recent decades, electronic sensor technology has taken remarkable steps towards mimicking human sensations, such as taste and olfactory responses. The electronic tongue (ET) has become commonly used in taste evaluation in both food and pharmaceutics. According to the definition of the IUPAC (International Union of Pure and Applied Chemistry) technical report, the ET represents a multisensory system, which consists of a number of low selectivity sensors and uses advanced mathematical procedures for signal processing based on pattern recognition (PARC) and/or multivariate analysis.[40] Selectivity and sensitivity are two important criteria for ETs. Compared to the enormous amount of chemically different bitter substances, the number of electrodes (*i.e.*, sensors) is limited. Thus, ETs need to be cross-selective (also called global-selective), which means not specific for a particular substance but universal to various bitter substances.[40–42] However, for a particular target, sensors with unique selectivity may be more useful, such as in the detection of trace biomolecules.[43] In addition, cross-sensitivity is given as the ability of the ET to respond stably and reproducibly to multispecies solutions under test.[40] These capabilities are directly determined by the material of the electrodes. Since no universal material can compromise cross-selectivity and high selectivity, a considerable strategy for electrode fabrication is to choose materials that are suitable for the chemicals of interest. The distribution of chemicals of interest over the surface of the electrodes should be specific to facilitate the pattern recognition. Still, with the development in selectivity and sensitivity, the ET becomes a promising instrument in bitterness evaluation and is an ideal alternative due to the lack of safety issues compared with human panel tests.[44,45] Furthermore, the ET also has the advantages of being economical and time saving compared with human panel tests in bitterness evaluation.

Common principles of sensors for ETs

Since the glass electrode was first developed in 1909,[40] electrodes for sensor arrays have been investigated intensively.[46–49] The detection principle of electrodes can be generally divided into

potentiometric, voltammetric, impedimetric, optical and mass sensors.

Potentiometric sensors

Potentiometric sensors are among the most popular sensors adopted into ETs, and this type of ET converts the electric potential signals into taste attributes. A potentiometric system usually consists of several working electrodes and a reference electrode. The potentials of sample solutions are detected by all these electrodes and recorded as V_s (samples) and V_r (reference) respectively. The V_s recorded by different working electrodes is always characterized and can then be classified into different taste attributes and intensity by pattern recognition methods.

Nowadays, various materials are used in the fabrication of electrodes and adequate materials are necessary to obtain satisfactory sensitivity and selectivity. Lipids, polymers and various organic molecules have been used to modify membranes while metals, chalcogenide glasses and semiconductors, *etc*, have been used as electrode bodies.[42,50–52] These materials were chosen according to the test purposes. For instance, Hayashi reported that the electrodes modified by decyl alcohol, oleic acid and dioctyl phosphate showed an increased electric potential to the bitter substances quinine and $MgSO_4$. However they responded oppositely to phenylthiourea, indicating that these materials were not suitable to evaluate the bitterness of this substance.[49] The potentiometric response depends on the distribution of ions (or neural molecules) on the sensor membrane.[53] Different charged ions in the test solution may affect the potentiometric response and hence may change the final test conclusion. For example, comparatively stronger signals were induced by quinine than salicylic acid for the positive membrane, because it was more attractive to basic molecules than acidic drugs in Uchida's experiment. In addition, both positive and negatively charged membranes were not sensitive enough to amphiphilic molecules, such as acetaminophen.[54] In general, both the properties of electrodes and solutions should be taken into account and pretests would be necessary before choosing suitable electrodes to assemble a sensor array.

To date, several potentiometric sensor systems have been reported and applied in the bitterness evaluation of beverages and drugs.[44,53–56] A series of taste sensor systems developed by Intelligent Sensor Technology Co. Ltd. has already been commercialized, including SA401, SA402, SA402B and the latest TS-5000Z. This series of ETs has been widely used in the investigation of bitterness suppression and quantitation of drug bitterness.[6,12,42,45,54,56–59] Uchida used SA402B to evaluate the bitterness of 9 antibiotics and found that the results were similar to those from human panel tests, suggesting this ET was capable of predicting the bitterness intensity.[56]

Astree, developed by Alpha M.O.S., is another distinguished commercial ET system.[60] The working electrodes in Astree are fabricated by ChemFET (*i.e.* chemically modified field-effect transistor) and coated with different polymers according to the different test objectives.[60–63] This system has been applied to evaluate the taste of food or pharmaceutical products.[62–66] For example, Astree has been applied to compare the bitterness of original and generic products of famotidine orally disintegrating tablets. A good correlation was obtained between Astree results and a human panel test.[65] Another interesting experiment in the discrimination of orange juices based on this system was conducted by Baldwin and co-workers. Results suggested that Astree was capable of discriminating different orange juices and exhibited similar conclusions to a human panel test.[67] All these studies suggested that Astree was a reliable device for bitterness evaluation.

Based on FET and various membrane materials, many other sensors have been also developed.[68] For instance, aptamers and carbon nanotubes were used to modify FET by a Korean group, resulting in a system capable of real time detection of thrombin, at concentrations as low as 7 pM.[69] Enzyme nanoparticles were introduced to the gate surface of the FET by Vijayalakshmi. This ENFET was shown to be effective in pH detection of triglyceride solutions.[70] A novel ENFET was developed by Premanode and co-workers, in which creatininase, creatinase and urease were attached to the transistor for real time monitoring of creatinine and urea.[71] Braeken reported another ENFET which showed high sensitivity and long-term stability in the detection of glutamate due to the coating of glutamate oxidase layers.[43] (It should be noted that, the ENFET mentioned here are not only potentiometric sensors.) The significant advantages of these ENFET include high selectivity, sensitivity and stability. However, in most cases, these ENFET were only designed for particular applications. Thus, improvement still could be achieved regarding selectivity (*i.e.*, capability of measuring substances with different structures).

Voltammetric sensors

Similar to potentiometric sensors, voltammetric sensors have been also intensively used in the detection of beverages, food and water pollution.[72–76] In most cases, voltammetric sensors were designed based on the detection of electric current between the working electrode and the counter electrode. However, it is difficult to maintain a stable potential in two-electrode systems, in which the counter electrode takes the roles of supplying electrons and referencing potential. To avoid this problem, the roles of the counter electrode are replaced by two electrodes, reference electrode and counter, resulting in a so-called three-electrode system.[77]

Noble metals have been extensively used as working electrodes for voltammetric sensors and show high selectivity in most cases.[76,78–80] To obtain cross-selectivity and better performance, various materials including polymers, graphite-epoxy, phthalocyanines and doping agents have been utilized as coating membranes.[72,73,75] For instance, Rodriguez-Mendez used polypyrrole and phthalocyanines to improve the electrochemical behavior of electrodes in bitterness evaluation.[46,75] A novel ET containing five modified graphite-epoxy electrodes was developed by Del Valle to analyze cava wines.[73] Although good selectivity has been reported, voltammetric sensors are still insufficient for the detection of solutions with high resistance.

Voltammetric analytical methods include cyclic, stripping and pulse voltammetry.[80] Cyclic voltammetry has been used in the evaluation of wines, beers and bitter substances.[46,72,73] Pulse voltammetry, including large amplitude pulse voltammetry (LAPV) and small amplitude pulse voltammetry (SAPV), has been used in the discrimination of teas, fruit juices and milk.[74,76,78–80] For instance, Ivarsson found that in the discrimination of teas, better

results were obtained by the combination of LAPV and staircase voltammetry (the potential sweep is a series of stair steps) than using single waveforms.[74] Thus, to obtain accurate results, the analytical methods are extremely important. Specifically, using only one waveform in different conditions is not adequate.

Impedimetric sensors

Besides potential and currency, impedance has also played an important role in ET development. Impedimetric sensors take the voltage–current ratio at a particular frequency as the detection signal and simultaneously another important interfacial property—capacitance—can be also detected. To date, impedance sensors have been widely used in taste discrimination and this method has proven to be a feasible and effective method due to its cross-selectivity, high sensitivity and reproducibility.

Various electrodes have been studied and used as impedimetric sensors recently. Electrodes made from alumina substrate with gold contacts were fabricated by Pioggia.[81] Carbon nanotubes, carbon black, hydrogel and several polymers were applied in layers. These sensors were robust and capable of responding to various solutions. Another type of electrode made from a poly(vinyl chloride) (PVC) tube body, copper disk and graphite-epoxy composite has been studied by Bonanni.[82] In this platform, avidin bulk was used to immobilize biotinylated oligonucleotides to detect the complementary DNA sequence. For example, *Salmonella* spp was successfully detected by the IS200 probe sequence modified electrode. Similarly, H1N1 swine flu correlated DNA sequence was detected by the complementary DNA modified sensor.[83] To extend the applications of sensors based on this platform in complex solutions, a dual-genic hybridization sensor was fabricated by Bonanni. Satisfactory results were achieved in discrimination of poly T oligonucleotides, poly C oligonucleotides and their mixtures.[84] This result elicited the possibility to analyze real DNA samples by this method with low cost and simplified procedures.

Interdigitated electrodes are also extensively used in impedimetric sensors.[85] This type of sensor (a metal electrode coated with weakly conducting material) can be considered as an equivalent circuit and its theoretical description has been presented by Taylor.[86] Various materials have been used in modification of interdigitated electrodes for different purposes. An impedance ET modified with polypyrrole, polyaniline and stearic acid and their mixtures was capable of discriminating basic tastes and mineral waters.[87] This ET was also able to classify red wines and beverages.[88,89] Besides the coating materials, there exists a negative correlation between the thickness of the film and the sensitivity of polymer sensors.[90] The correlation between thickness and capacitance has been reported in the case of polypyrrole and polyaniline membranes.[91]

Based on the development of various materials and types of electrodes, impedimetric sensors are now on the road towards miniaturization, faster detection and lower cost while maintaining good performance in measurements.

Optical sensors

Optical techniques used for detection in methods such as HPLC have proven to be powerful with high sensitivity. Therefore the optical sensor technique is proposed as a promising approach for chemical sensing. Parameters (refractive index, absorbance or photocurrent, *etc.*) of optical electrodes will change when they contact with the analytes with lighting.[92,93] Materials with various properties are used to modify the surface of electrodes. For instance, a photocurable membrane that is capable of passing through photocurrents was successfully made by adding Irgacure 651 (photoinitiator) into a mixture of oligomers and diluting agents. This photocurable membrane was proven to be selective to surfactant anions.[93] A semiconductor combined with Chalcogenide glass was studied and used in the fabrication of optical sensors by Richardson and co-workers, which was certified for the detection of isopropanol. In experiments performed by these sensors, the refractive index was measured.[92] To detect optical absorbance, tetraphenylporphyrin zinc was used as an optical indicator and polymers such as PVC and hydrophilic thermoplastic polyurethane (TPU) were used.[94]

Although the application of optical sensors is limited by shortcomings in their preparation and durability, much progress has been achieved. For example, an optical sensor modified by dye/silicon was developed and used to evaluate basic taste substances by Lee and co-workers, resulting in satisfactory properties and thus was suitable for taste evaluation.[95] Another classic experiment was performed by Anslyn and co-workers. In this experiment, an optical indicator and chiral receptors were immobilized on the sensors, and the enantiomers of Leu, Val, Trp and Phe were well separated.[96] Obviously, this successful combination of receptors and optical indicator in sensor fabrication sheds light on the possibility of mimicking the human taste by human taste receptor-modified sensors.

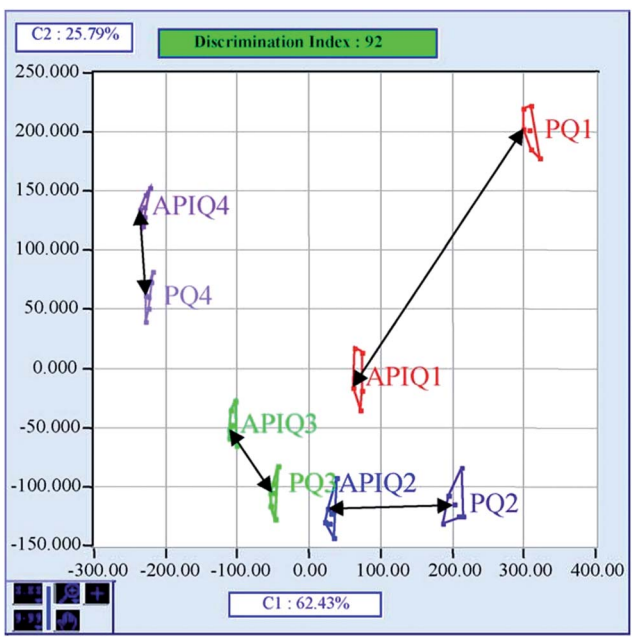


Fig. 2 A PCA map for quinine in the presence of different levels of Acesulfame K. Key: PQ1–water, APIQ1–0.2 mM quinine; PQ2–0.1 mM Acesulfame K (Ace K), APIQ2–0.1 mM Ace K + 0.2 mM quinine; PQ3–1.0 mM Ace K, APIQ3–1.0 mM Ace K + 0.2 mM quinine; PQ4–5.0 mM Ace K, APIQ4–5.0 mM Ace K + 0.2 mM quinine. Reproduced with permission from ref. 66.

Mass sensors

The mass sensor was proposed and developed as a new method for taste evaluation, based on the mass changes before and after contact with analytes. Within this principle, surface acoustic waves (SAW) and bulk acoustic waves (BAW) are two major approaches for detection. Devices based on SAW can operate at higher frequency and thus can achieve higher sensitivity. However, the signal of SAW devices falls severely in liquid sensing, limiting the applications in liquid samples.[97] Quartz crystal microbalance (QCM) is a typical representative of BAW, in which the piezoelectric effect is the basis. In other words, a current appears while the QCM sensor interacts with analytes, due to its mass change. To minimize the interference caused by other components in complex solutions (*i.e.*, to improve the selectivity), membranes with particular selectivity are usually necessary in modification of sensors. For instance, high selectivity in the detection of damaged DNA amongst normal DNA was obtained by a QCM model modified by biotinylated tDNA.[98] Okahata has synthesized a lipid multibilayer film that is capable of discriminating bitter chemicals from a number of structurally different chemicals.[99] Fung developed a new method based on molecularly imprinted polymers (MIP). The coating made from MIP contains an appropriate cavity and interacts with its complementary functional group of the target molecule, so that the interference of other substances in complex solution can be minimized. Based on this coating, a QCM sensor for liquid sensation of taste substances in food was developed and high selectivity was obtained.[100] Although numerous studies have

Table 2 Summary of electronic sensors including principle, materials pattern recognition and applications

	Materials	Applications	PARC	Ref
Potentiometry	Chalcogenide glasses, PVC	Heavy metal	ANNs	52
	Lipid analogs, PVC, dioctyl phenylphosphonate	Basic taste substances	PCA	49
	Chalcogenide and oxide glasses, PVC	Red wines	PLS-DA	103
	Lipophilic additive, graphite powder, dibutyl phthalate	Pancuronium bromide		105
	Dioctyl phosphate, oleyl amino, decyl alcohol, *etc.*	River water	PCA	55
	PVC, dioctyl sebacate, dibutyl sebacate, *etc*,	Ammonium, potassium	ANNs	50
	Polyurethane, PVC, *etc.*	Teas, beers	PCA	51
	Dioctyl phenylphosphate, Hexadecanoic acid, *etc.*	Medical plants, Chinese medicines	PCA	6
	Oleic acid, cetyl alcohol, *etc.*	Propiverine hydrochloride and other commercial medicines	PCA	10,12,54
Voltammetry	Noble metals	Drinking water, baby food, teas, beverages	PCA	74,78,80
	Polypyrrole, phthalocyanines, *etc.*	Oleuropein, ligstroside, alcohol in beers	PCA, PLS	46,72
	Graphite-epoxy	Wines	PCA, ANN	73
	Phthalocyanines, polypyrrole and perylenes	Red wines	PCA	75
Impedance	polypyrrole	Water and taste substances	PCA	115
	Polyaniline, polypyrrole	Beverages	PCA	89,91
	Polypyrrole, stearic acid, *etc.*	Red wines	ANN, PCA	88
	Polylactic acid, carbon black, polyvinyl alcohol, *etc.*	Standard taste solutions	PCA	81,102
Optics	Urethane-acrylate, 2-cyanophenyl	Anionic surfactants		93
	Mono- and diacrylates	Ion-selective		116
	Porphyrin	Organics, alcohol, amines	PCA	94
	Chalcogenide glass and semiconductors	*N*-methylaniline, isopropanol		92
	Dye/silicon and dye/lipid/ PVC-PVAc-PVA	Basic taste solutions	PCA	95
	Pyrocatechol violet (PCV), chromoxane cyanin R (CCR) and chrome azurole S (CAS) and chiral receptors	Enantiomers of Leu, Val, Trp and Phe	PCA	96
Mass	QCM, thiols, DNAs	Damaged DNA		98
	QCM, chiral porphyrin diad	Enantiomers of limonene		117
	QCM, lipid multibilayer matrix	Bitter substances		99
	QCM, silver	Benzene		118
	QCM, MIPs	Food		100
	Interdigital transducer (IDT), $LiTaO_3$ wafers	Basic taste solutions	PCA	119

been presented in mass sensor development, few of them focused on taste evaluation of solutions with unknown intergradient, which suggests that the improvement in cross-selectivity with a high signal-to-noise ratio of sensors is still needed.

Pattern recognition methods and calibration

To mimic natural taste perception, pattern recognition methods (PARC) are usually utilized, which convert electrical signals into readable maps. Principal component analysis (PCA) is the most widely used pattern in ETs.[44,46,55,58,65,78,80,91] An advantage of PCA is the reconstruction of the raw data, in which data will be summarized into several uncorrelated principal components, resulting in a PCA map such as that shown in Fig. 2.[66] In Fig. 2, the small cluster of each sample represents good reproducibility; and a distinct discrimination between different sample pairs (active *versus* placebo) is observed, representing clear discrimination of taste attributes. Similar to PCA, partial least square regression (PLS) is also an intensively used linear multivariate method, of which performances depends on the numbers of components.[44,60,63,65,79] Moreover, it can be used to calibrate ETs before real testing and quantification. To handle the non-linear data, artificial neural networks (ANNs) are frequently used methods,[41,50,73,87] such as back propagation and self-organizing map (SOM).[52] All these methods have been described in the literature.[40,44,80,101]

It should be noted that ETs do not measure real human perceptions, although the outputs of ET can be shown as patterns in which different taste attributes separate well. Thus, calibration with human panel tests is usually necessary. For instance, to confirm the accuracy of SA402 in the bitterness evaluation of berberine, a calibration curve of berberine at different concentrations resulting from the human gustatory test was made and was then used to calibrate the output of the sensor.[6] A similar calibration was performed in evaluation of pediatric drugs.[10]

Even in the detection of ions and biological molecules, training and calibration is still needed. In some cases, standard samples for calibration may be unknown or difficult to prepare, which suggests that calibration by external samples is unavailable.[84,102] Under these conditions, a method called leave-one-out can help significantly in calibration. Briefly, every sample will be taken out from all the samples in turn and after each is taken out, PARC is then performed. All the results obtained from each round will finally be compared to each other (so called cross-validation) to justify the usefulness of the PARC used. PARC such as multi-linear regression (MLR),[103] PLS[67,103] and ANNs[41,50,79] are intensively used in calibration.

The successful detection of ions, heavy metals and specific molecules and evaluation of fruit juices, beverages and drugs, *etc*, have indicated ETs as a promising tool in different fields of application.[41,47,52,67,73,104,105] However, the ideal ET that can realize the human taste sensation is still yet to be developed. Currently, the improvement of ETs is mainly focused on the aspects of reproducibility, selectivity and sensitivity, mostly by exploiting new materials or mixtures of regular ones (the materials and applications, *etc*, according to principles are summarized in Table 2.) In most cases, cross-selectivity and high sensitivity stand opposing due to the properties of the materials used in the electrodes, such as the case mentioned before.[49] An ET system may solve this problem theoretically by embracing more electrodes to achieve high sensitivity and cross-selectivity simultaneously. However, the amount of data will probably be incalculable and difficult to process by pattern recognition methods such as PCA. Besides, great efforts are also needed in lowering costs, miniaturization and realizing real-time and high throughput detection. With the procedures of training or calibration, ET technology offers a choice for taste evaluation with high efficiency and without safety concerns.

Conclusions and future perspectives

Since an essential standard to evaluate the efficiency of bitterness masking is the real sensation of human taste, the closer to human perception the better the bitterness evaluation method will be. Moreover, human gustatory tests can be performed without any high-tech instruments, providing an easy method of bitterness evaluation in a short time. Combined with adequate data analysis, the human gustatory test can obtain the most reliable evaluation of tastants under certain standards. Thus, this method becomes a prerequisite in the pharmaceutical and food industries before the commercialization of products.[17,106–109] To avoid the limitation of complicated procedures and high cost, this method is usually simplified in laboratory research when rigorous results are not necessary.[11,16,34]

Animal gustatory tests are another method of bitterness evaluation commonly used due to their ease of application. However, it takes a long time to perform animal gustatory tests and the results can only be regarded as indirect evidence. Therefore, this method is usually applied to evaluate the factors affecting the taste perception or to develop the dose–response relation.[27,28,32]

In contrast to human and animal gustatory tests, safety problems are not a concern for *in vitro* methods (*e.g.*, calcium imaging). New entity molecules and compounds with extreme toxicity can be evaluated without any risks *via* calcium imaging methods. Furthermore, these methods can even screen a bitter substance from a library comprising thousands of chemical candidates in a high throughput manner.[8,37] The signal-to-noise ratio in this method can be enhanced by elaborating several new calcium indicators and the improvement of fluorescence detection instruments.[26,28] Besides the utilization of screening, calcium imaging can also be helpful to provide a deeper insight into bitter receptors.[37,38,110] However, this method is still indirect and its operating procedure is laborious and complicated.

An ideal bitter evaluation technology should possess the characteristics of high speed and accuracy in qualitative and quantitative evaluation without safety concerns. So far, E-tongues are the most widely investigated instruments for bitter assessment. Lipid and polymers take a prominent position among various materials used for electrode manufacture due to their similarity to biomembranes. In addition, materials such as biomolecules are also exploited to increase the selectivity of sensors. Recently, the combination of selective and partially selective electrodes has become more popular with improved evaluation results. Biosensors containing specific ligands are embraced by many new systems, aiming to attain high sensitivity in detection. Besides, new pattern recognition methods are also under evolution, focusing on simplification, accuracy and

becoming more convenient to operators without training. However, it is still difficult to compromise cross-selectivity and high sensitivity. In addition, the reproducibility and durability of electrodes, the miniaturisation of the ET system, the relatively high cost and the delicate fabrication obstruct the widespread use of ETs. To date, E-tongues have been applied not only in the pharmaceutical industry, but also to the evaluation of water pollution, quality analysis in wine, and culture of microbes.[111–113] In the near future, bionic E-tongues are expected and ideal technologies for bitterness evaluation would be within reach.

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