Challenges in using Electronic tongue to study *rasa* of plants: II. Impact of solvent and concentration on sensor response and taste ranking

Dushyant Kumar 1, Aruna Singh 1, Rama Jayasundar*

Department of NMR, All India Institute of Medical Sciences, New Delhi, India

**Abstract**

**Background:** Although Electronic tongue is used in pharmaceutical, food and beverage industries for objective evaluation of taste, its use in medicinal plants from an ayurvedic perspective is novel. Control experiments are therefore necessary to standardise and optimise parameters.

**Objective:** The aim is to optimise the use of solvent and standardise sample concentration for study of plants from an ayurvedic standpoint of *rasa*. The major objectives are two-fold: (i) evaluate sensor response to different types of solvent water (ii) explore use of E-tongue in taste ranking of medicinal plants used in ayurveda.

**Materials and methods:** Single, double and triple distilled, reverse osmosis and milliQ waters were evaluated separately and as a medium for preparing plant extracts. For taste ranking, standard addition method using D-glucose as sweet taste standard was used for different brands of mango juices (case in point study) and eight medicinal plants from sweet category. The effect of sample concentration and taste standard on taste ranking were evaluated.

**Results:** MQ and TD water demonstrated similar organoleptic properties whereas plant extracts prepared in DD and MQ water showed maximum taste-based differentiation. The mango juices were taste discriminated by E-tongue and ranked based on their sweetness scores. The relative ranking of plant samples showed concentration dependence and also varied with the concentration range of taste standard.

**Conclusion:** Milli-Q and double distilled water can be used for E-tongue studies of medicinal plants. While the results open up the possibility of taste ranking of medicinal plants, they also demonstrate the importance of standardising and optimising the concentration of samples and taste standards in the context of ayurvedic *rasa* based studies.

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hence soluble in saliva [7]. All taste measurements with E-tongue therefore use water as the solvent. Type and quality of water play an important role in sensor response and hence should be chosen with care.

E-Tongue is also a promising technology for taste ranking. For example, tea samples have been taste ranked and correlated with phenol contents and chemovariations due to differences in their geographical origin [8]. Different brands of fruit juices have also been graded according to taste and correlated with their tannin and polyphenol contents [9]. Taste ranking is generally carried out with reference to specific taste standards such as glucose and sucrose for sweet [10], quinine and berberine hydrochloride for bitter [11,12], and tannic acid for astringent [8,9] and citric acid for sour [9]. A crucial parameter governing sensor response in taste ranking is the concentration of solute.

The aim of this study is to optimise the use of solvent and standardise sample concentration for study of plants from an ayurvedic standpoint of rasa. The objectives of this article are hence threefold: (i) analysis of E-tongue sensor response to different types of laboratory grade water, (ii) establishing taste ranking via case in point studies prior similar analyses in plant extracts, (iii) exploring taste ranking in medicinal plants using information of their rasa mentioned in Ayurveda texts. For the first objective, E-tongue sensor response to laboratory grade waters as standalone samples and solvent for plant extracts are studied. While the second objective deals with important preliminary studies for taste ranking plant samples, the third one explores the relationship between taste ranking, and concentration of plant samples and taste standards.

2. Material and methods

2.1. Laboratory grade water types

Five different types of laboratory grade water were used: (i) Single Distilled (SD) (Widson Scientific Works, India) (ii) Double Distilled (DD) (Widson Scientific Works, India) (iii) Triple Distilled (TD) (Harrison Pharma Machinery, India) (iv) Reverse Osmosis (RO) (Merck Millipore, USA) (v) Milli-Q (MQ) (Merck Millipore, USA) water.

2.2. Sample preparation

2.2.1. Plant samples for extraction with different types of laboratory grade water

The following plants were grouped under different taste categories in ayurveda and extracted with the different types of water: Fruits of Phoenix sylvestris (PS) (sweet), Citrus limon (CL) (sour), Momordica charantia (MC) (bitter), Capsicum annuum (CA) (pungent) and Ficus bengalensis (FB) (astringent). These were purchased from local market and prepared as the stock solution. Fifty grams each were soaked separately in 100 ml of different types of water at room temperature. The samples were washed, cut into half and squeezed to express the juice, which was filtered (Whatman 3) and used as the stock solution.

2.2.2. Samples for taste ranking

Four brands of mango juice were obtained from the local market: Maaza mango (F1), Paperboat aamras (F2), Frooti (F3) and Hello (F4). The ingredients of all the brands are mentioned in Table 1. For taste ranking of plants, those mentioned under the sweet category in ayurveda [13] were used: P. sylvestris (PS) (fruit), Musa paradisiaca (MP) (fruit), Phaseolus trilobus (PhA) (seed), Tribulus terrestris (TT) (fruit), Cocos nucifera (CN) (fruit), Vitis vinifera (VV) (fruit), Prunus amygdalus (PA) (seed) and Euryale ferox (EF) (seed). These were purchased from local market and prepared as follows: 10 g each of the plant samples were soaked separately in 100 ml MilliQ water (Merck Millipore, USA) for 24 h, cold macerated, filtered using Whatman filter paper (no. 3), and 10 ml of the filtrate diluted to 100 ml. Glucose (Sigma Aldrich, USA) was used as the reference taste standard for sweet taste.

2.3. Conductivity and pH measurements

Conductivity (Horiba Scientific, Japan) and pH (Oakton pH 700) of the samples were measured in triplicate prior to E-tongue studies.

2.4. Evaluation using E-tongue

E-tongue (z-Astree II, Alpha MOS, France) used in this study consisted of an autosampler with a circular platform for holding 16 beakers of 125 ml capacity each, a silver/silver chloride reference electrode, sensor set (sensor array # 5), data acquisition system and a workstation with AlphaSoft software. The sensors work on the principles of ChemFET (Chemical modified Field Effect Transistor). The sensor array had seven sensors (S1–S7) with S1, S3 and S4 specific to sour, salty and umami tastes, respectively. Responses from all seven sensors were stored as data matrix, integrated and used for the final multivariate analysis. The acquisition parameters were: 120s acquisition time; 10s sensor cleaning time; 100 mL sample volume; 5 replicates per sample. The first and fifth data points were excluded from the analysis to prevent pre-conditioning and saturation errors in the sensors, respectively. The sensors were cleaned after each measurement using MQ water to prevent cross-contamination.

2.4.1. Sensor response to types of laboratory grade water

Fig. 1a shows the arrangement of samples in the 16 autosampler. Beakers 2, 4, 6, 8, 10 contained the test water samples (SD, DD, TD, RO, MQ) and those in positions 1, 3, 5, 7, 9 held water for cleaning the sensors after each measurement. Beakers 11–16 were empty. For data acquisition, the following measuring sequence was employed:

\[(2, 1)_{S} - (4, 3)_{S} - (6, 5)_{S} - (8, 7)_{S} - (10, 9)_{S}\]

The numbers in the brackets indicate the sample position and the order of measurement. For example, (2, 1) refers to measurement of sample in beaker 2 followed by cleaning of sensors in beaker 1. The subscript indicates the number of times (five in this case) the sequence was repeated.

Table 1

| Ingredients                          | F1   | F2   | F3   | F4   |
|--------------------------------------|------|------|------|------|
| Mango pulp                          | 19.5%| 45%  | 19.5%| Not known |
| Added sugars                         | 13%  | 8.4% | 13.3%| 12%  |
| Acidity regulator                   | +    | +    | +    | +    |
| Antioxidant                          | +    | +    | +    | +    |
| Preservative                         | +    | +    | +    | +    |
| Synthetic colours                   | +    | +    | +    | +    |
| Artificial flavours                  | +    | +    | +    | +    |
| Spices and condiments                | -    | +    | +    | +    |
| Water                                | +    | +    | +    | +    |

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2.4.2. Sensor response to plant samples extracted in different types of laboratory grade water

The objective of this study is to identify the lab grade water (used for sample extraction) which would maximise the differences between the sensors’ response and help in taste-based differentiation of plants. Based on prior studies, a sample concentration of 1.0% (1.0 ml plant sample + 99.0 ml water) was prepared from the stock solution of each of the five plant extracts separately in MQ, SD, DD and RO water. While beakers in position 1, 3, 5, 7, 9 contained water for cleaning the sensors, those in locations 2, 4, 6, 8, 10 contained the plant samples (Fig. 1a). The measuring sequence for data acquisition was the same as before (section 2.4.1).

2.4.3. Taste ranking of mango juice

Standard addition method was used for taste ranking. For this, one of the sample was taken as the reference. Five to six 100 ml aliquots of this reference sample were prepared and the taste standard (glucose in this study) was added in linearly increasing concentration. These samples (termed the training set) with added glucose were used for calibration and training the sensors to sweet taste. The calibrated sensor responses from the training set were compared with the samples to be ranked.

For taste ranking of mango juice, ten ml each of the four brands of juice (F1–F4) were diluted to 100 ml with MilliQ water. F1 was used as the reference sample and d-glucose (31.25 mg, 62.5 mg, 125 mg, 250 mg, 500 mg and 1000 g corresponding to 0.03%, 0.06%, 0.125%, 0.25%, 0.5% and 1%) was added separately to six 100 ml samples of F1 to get the training set of samples (F1a – F1f) with different concentrations of taste standard (Table 2).

Fig. 1b shows the positioning of samples in the autosampler for this experiment. Beakers 2–11 contained ten samples - six F1 samples with increasing concentration of d-glucose and four samples (F1–F4) without addition of glucose. Beaker 1 had MilliQ water for cleaning the sensors and beakers 12–16 were empty. The measurement sequence was as follows:

\[(2, 1)_{5} - (3, 1)_{5} - (4, 1)_{5} - (5, 1)_{5} - (6, 1)_{5} - (7, 1)_{5} - (8, 1)_{5} - (9, 1)_{5} - (10, 1)_{5} - (11, 1)_{5},\]

where the numbers within the parenthesis indicate the sequence of measurements and the subscript, the number of repetitions of the sequence. The sensors were cleaned after each measurement and water replaced after each cleaning.

2.4.4. Taste ranking of medicinal plants – Effect of concentration of sample and taste standard

In this experiment, concentrations of both taste standard (d-glucose) and samples were varied. The concentration ranges used for d-glucose were 0.6%–1% (0.06%, 0.125%, 0.25%, 0.5% and 1%) and 0.125%–2% (0.125%, 0.25%, 0.5%, 1% and 2%). Five sample concentrations, 10%–50% in steps of 10%, were used. P. sylvestris was used as the reference sample/training set for addition of d-glucose. There were 13 samples in total - five samples of PS with different concentrations of d-glucose (training set) and eight plant extracts without glucose. The sample arrangement in the autosampler was

Table 2

| Samples      | Sample volume (ml) | Quantity of d-glucose added (mg%) | Final sample volume (ml) |
|--------------|--------------------|-----------------------------------|--------------------------|
| F1a          | 10                 | 31.25/0.03                        | 100                      |
| F1b          | 10                 | 62.5/0.06                         | 100                      |
| F1c          | 10                 | 125/0.12                          | 100                      |
| F1d          | 10                 | 250/0.25                          | 100                      |
| F1e          | 10                 | 500/0.50                          | 100                      |
| F1f          | 10                 | 1000/1.00                         | 100                      |
| F1 (reference)| 10                 | 0.00                              | 100                      |
| F2           | 10                 | 0.00                              | 100                      |
| F3           | 10                 | 0.00                              | 100                      |
| F4           | 10                 | 0.00                              | 100                      |
similar to that shown in Fig. 1b, and only beakers 15 and 16 were empty. The measurement sequence was as follows:

\[(2, 1)S - (3, 1)S - (4, 1)S\] ..........................  (13, 1)S

2.5. Data analysis

Principle Component Analysis (PCA) was carried out on sensor responses to evaluate the taste differences between the various samples. For PCA of data from different brands of mango juice, those from samples which did not have added glucose (F1–F4) were used. For studies on water, Taste Discrimination Analysis (TDA) was used for quantitative evaluation of the sensors’ response in organoleptic units (OU). TDA measured similarity/dissimilarity in the taste of samples with respect to that of reference. MQ water was used as the reference for studies on laboratory grade water. The sensor data was converted to organoleptic distance (OD) and plotted on the y-axis with sample data points on the x-axis. Discrimination with respect to the reference was thus measured quantitatively in OU. Deviation from the reference indicated dissimilarity. For analyses on plants extracted with different laboratory grade water, sensor response from C annuum expressing in terms of organoleptic distance was used as the reference.

For taste ranking studies, calibration obtained from samples (training set) with varying concentrations of the taste standard were used to rank the samples without the taste standard. The response of all the sensors were integrated by the analysis software to generate ranking on a relative scale of increasing intensity (1–12). For studying the impacting roles of concentration, the taste ranking data was analysed in two ways: (i) maintaining the sample concentration constant and varying the D-glucose concentration, (ii) keeping the D-glucose concentration constant and changing the sample concentration.

3. Results and discussion

3.1. Conductivity and pH of different grades of laboratory water

The conductivity and pH readings of the different types of water are shown in Table 3. It can be seen that SD water showed the maximum conductivity followed by RO, DD, TD and MQ water. The pH of RO water was the highest followed by SD, DD, MQ and TD, although all the values were within the normal range [14,15].

During the distillation process, water is boiled and the steam recondensed with the resulting water containing minerals, organics and volatile traces. The number of times the water is taken through this process defines if it is single, double or triple distilled water. Increasing the number of distillations would cause a progressive decrease in the amount of solids, salts and minerals in the water.

In reverse osmosis, water is passed through various filters (e.g. sediment, carbon and membrane) resulting in purified water containing cations and anions. In milliQ water, RO purified water is passed further through ion exchange filters and dispensed through a 0.22 μm membrane filter. The resulting ultra-pure water is free from salts, solids and ions. The conductivity of water results from the ions present in it. So, as the ion content decreases, the purity of water increases. This can be inferred from the conductivity of the different types of laboratory grade water (Table 3). Based on the acceptable range for conductivity of water for E-tongue (1–5 μs/cm) [personal communication, AlphaMOS, France], MQ, TD and DD water are suggested for Electronic tongue based studies.

3.2. Evaluation using E-tongue

3.2.1. Sensor response to types of laboratory grade water

Fig. 2a shows the 2D PCA plot of the sensor responses differentiating types of laboratory grade water (Discrimination index = 81 with 96.7% PC1 and 2.6% PC2). All three data points for each sample are shown in the plot. MQ and TD water had overlapping coordinates in the PC1 dimension indicating their equivalence and showed very little dispersion in the PC2 axis denoting their close similarity, which were also reflected in their conductivity and pH readings. DD was closer to TD and MQ water as compared to SD and RO water. The sensor responses to both SD (highest conductivity) and RO (highest pH) water were well distant from those of other water types, although conductivity of the RO water was in the acceptable range for E-tongue studies. Fig. 2b shows the quantitative taste discrimination analysis for different types of laboratory grade water with MQ as the reference. The X axis shows the sensor response data points in triplicate for each sample and the Y axis indicates the distance between the sensor responses in OU. The grey area marked around zero OU in the graph specifies the acceptable range for similarity between sensor responses with respect to the reference. Although TD did not fall within the acceptable range, it showed the least distance from MQ (−586.66 ± 23.09 OU). On the other hand, the organoleptic distance of SD (12.200 ± 1058.30 OU), DD (3400 ± 1058.30 OU) and RO (15,033.33 ± 1530.79 OU) from MQ were significantly large indicating their dissimilarity with MQ. SD and RO were distanced similarly from the reference. This analysis indicated the similarity between TD and MQ water quantitatively, although the dispersion of data points in MQ was less compared to those in TD. These results also correlated with the PCA plot, and the conductivity and pH of the water samples. The data therefore suggests that MQ and TD water can be used interchangeably and MilliQ water was chosen for further studies.

3.2.2. Sensor response to plant samples extracted in different types of laboratory grade water

Fig. 3 shows the sensor response in organoleptic units for the plant extracts from four different taste groups (C. annum – pungent; P. sylvestris – sweet; F. bengalensis – astringent; M. charantia – bitter) prepared in different types of laboratory grade water. Differentiation between the plant samples was best with DD water followed by RO and MQ water. For example, in samples prepared with double distilled water, increase in organoleptic distance with respect to C. annum was 28% for F. bengalensis, 62.3% for P. sylvestris and 56.8% for M. charantia. Although plants prepared in SD water also showed better differentiation, especially between CA and PS, and CA and FB, single distilled water was excluded since its conductivity was beyond the acceptable range for E-tongue studies. Results from RO water were also not considered since it too had high conductivity and pH.

There are reports on use of deionised [12,16] and plain distilled [10,17,18] water for E-tongue studies. However, based on the results in this study, both DD and MQ water are considered suitable for E-tongue based studies on rasa of plants. The previous results have shown the critical role solvent plays in sensor response and the

| Water type          | Conductivity (μS/cm) | pH     |
|---------------------|----------------------|--------|
| Single Distilled    | 8.66 ± 0.57          | 7.21 ± 0.02 |
| Double Distilled    | 3.33 ± 0.57          | 7.16 ± 0.02 |
| Triple Distilled    | 2.00 ± 0.00          | 7.00 ± 0.02 |
| Reverse Osmosis     | 4.33 ± 1.15          | 7.41 ± 0.01 |
| MilliQ              | 2.00 ± 0.00          | 7.04 ± 0.01 |
importance of choosing the right type of water as solvent before planning the E-tongue experiments on medicinal plants.

3.2.3. Taste ranking of mango juice

Fig. 4 shows the radar plot of all the sensors’ response for the four samples of mango juice. Although the basic composition of all the mango juices were similar (water, mango pulp, added sugar, antioxidant and preservatives), there were also differences in their quantity and those of other ingredients such as acidity regulator, synthetic colours, artificial flavours and spices (Table 1). Furthermore, variances due to the source of mango pulp in the beverage would also be a cause for differences in the final taste of the juice. All these factors govern the grade of sweetness and these are evident from the PCA plot of the sensor responses and taste ranking. The 2D PCA plot (Fig. 5) discriminated the sensor responses to the four different brands of mango juice with a discrimination index of 93% (PC1 = 93.04% and PC2 = 6.06%). The differences reflect the variations in the ingredients affecting the sweetness of the samples. These were also mirrored in taste ranking: F4 (7.3) had the highest score followed by F3 (7.2), F2 (6.9) and F1 (2.6).

Different brands of mango juices were taken for this study with each make varying in their exact composition, thus modulating the taste of the final product. The objective was to explore the use of E-tongue to differentiate samples with slightly differing ingredients/flavours but all under the same taste category. This was successfully demonstrated as can be seen from the results. Since mango juice also has complex matrix like plant

![Fig. 2. Evaluation of E-tongue sensor response to different types of laboratory grade water using (a) PCA plot (b) Taste Discriminant Analysis. SD-Single Distilled, DD-Double Distilled, TD-Triple Distilled, RO-Reverse Osmosis, MQ-MilliQ.](image-url)
extracts, this study is considered a prelude to understanding the behaviour of sensors to different plant extracts belonging to the same taste group.

3.2.4. Taste ranking of medicinal plants - Effect of concentration of sample and taste standard

Table 4 shows the ranking (1–8 in ascending order) and scores for sweetness for the different plant extracts for five sample concentrations (10%–50%) at two different D-glucose (taste standard) concentration ranges. With the D-glucose in the concentration range of 0.6%–1%, sample concentrations of 20% and 30% gave similar ranking patterns with *E. ferox* ranking first and *P. sylvestris* last (outlined in Table 4). Except for the interchange in the ranks of *P. trilobus* and *P. amygdalus*, the ranking order for the rest of the samples was the same. Further increase in concentration (40% and 50%) displayed different ranking patterns. The reason for variations in taste ranking could be the concentration dependent threshold sensitivity of the sensors. For example, a sample concentration of 10% may have been too low to elicit a stable response and concentrations of 40% and 50% too high causing saturation of sensors. The sensor responses were stable at 20% and 30% sample concentration.

At the higher concentration range of glucose (0.125%–2%), the ranking for sweet taste was similar for 10% and 20% (outlined in Table 4). As the sample concentrations increased to 30% and 40%, *P. sylvestris* and *E. ferox* continued to score the lowest and highest, respectively, but the order of ranking of other samples changed. Although the least (PS) and the highest (EF) in taste ranking remained the same at both concentration ranges of glucose, the order between them were different. These studies demonstrate the concentration dependent sensor responses for taste ranking. At low glucose concentration range, 20% or 30% sample concentration gave stable response. On the other hand, with increased range of glucose concentration, a corresponding decrease in sample concentration (i.e. 10% and 20%) was required to get reliable taste ranking. Further in-depth studies are required to understand and conclude the taste ranking capability of E-tongue in the context of plant extracts.

4. Conclusion

E-tongue is used mainly in pharmaceutical, food and beverage industries for taste suitability, matching placebo, improving drug palatability, taste masking, etc. However, applications of E-tongue in the field of ayurveda and medicinal plants are novel. This study highlights the important role of control experiments while exploring E-tongue for novel applications in ayurvedic pharmacology. The water/solvent dependent sensor response and the importance of choosing the right type of water for studies of medicinal plants from an ayurvedic standpoint have been highlighted. Our results have shown that while triple distilled and MQ waters can be used interchangeably, double distilled followed by MQ water highlighted better the taste differences between the plant extracts from different taste groups. Since both ion content and purity of water play a role in sensor response, conductivity of the samples should be measured prior to E-tongue analyses. Based on the results of this study, MQ and DD water are considered suitable for studies on medicinal plants from an ayurvedic stance.

For E-tongue to be useful in taste ranking of medicinal plants, choice of taste standards, their concentrations and those of sample are very important. These parameters should be optimised with application-specific requirements. The present study has shown
the importance of concentration of samples and taste standards for training the sensors for a specific taste, especially in the context of complex matrices like plants. The concentration of samples should be such that it induces stable and reproducible sensor response but should not be high enough to saturate the sensors or too low to prevent a measurable response. The concentration range of the taste standard should be chosen in a way that uniform and reproducible ranking patterns are produced.

Taste ranking of plants has important implications for ayurveda, where rasa or taste is an important parameter for not only identifying the therapeutic and nutritional properties of plants but also as a quality factor [13,19,20]. More in-depth studies are required to explore if E-tongue can reliably rank the taste of medicinal plants. If it can, this may open up immense opportunities and possibilities in ayurvedic pharmacology such as quality evaluation, identifying rasa-based plant substitutes, understanding the taste variability of plants and water from different geographical locations and sources, all of which have important implications for ayurvedic practice.

**Table 4**

| Sample concentration (%) | D-glucose concentration range | Ranking of sweet taste (lowest to highest) | Sweetness scores/Plants |
|--------------------------|-------------------------------|------------------------------------------|-----------------------|
| 10%                      | 0.06–1%                      | 1.4/PS, 6.0/TT, 5.5/MP, 4.1/EF, 4.2/TT   | Phoenix sylvestris, MP – Musa paradisiaca, PT – Phaseolus trilobus, TT – Tribulus terrestris, CN – Cocos nucifera, VV – Vitis vinifera, PA – Prunus amygdalus, EF – Euryale ferox. |
| 20%                      | 0.125–2%                     | 2.2/PS, 4.4/VV, 5.1/CN, 5.6/PT, 4.9/PT   |
| 30%                      |                               | 4.7/PT, 7.2/TT, 5.8/MP, 6.4/PT, 5.3/PS   |
| 40%                      |                               | 7.2/PA, 6.2/TT, 5.6/PA, 6.8/TT, 5.7/PT   |
| 50%                      |                               | 7.3/MP, 6.5/PT, 6.8/MP, 6.2/PA            |
|                          |                               | 7.4/PA, 7.3/PT, 6.9/PA, 7.3/TT            |
|                          |                               | 8.0/EF, 8.0/EF, 9.5/EF, 9.7/EF, 10.4/EF   |

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**Conflict of interest**

None.

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