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Research Article

EVALUATION OF TASTE MASKING OF ONDANSETRON USING INSENT TASTE SENSING SYSTEM

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ABSTRACT

The most important evaluation required for taste masked dosage forms is the complete masking of bitter or unacceptable taste of drug. It is very important to evaluate and quantify the bitter taste of drug and masking effects of taste masking agents used in formulation. Ondansetron was studied as model bitter drug. Insent Taste Sensing system was used to assess the bitterness of drug, taste masking potential of Neotame and its comparison to human sensory taste panel. Bitterness sensor specially designed by Insent for Ondansetron was used to measure its bitterness. Ondansetron solutions showed linear response to this sensor in range of 0.008 mM to 0.164 mM. Bitterness of Quinine hydrochloride solution was compared with Ondansetron solutions and sensor response indicated 0.01mM of Ondansetron corresponds to 0.1mM of Quinine hydrochloride. Quinine as standard bitter drug was taste evaluated in the concentration of 0.013 mM to 0.1 mM using human sensory panel and bitterness score was assigned. The concentration in the range of 0.045 mM to 0.1 mM corresponding to Bitterness score 4, 5, and 6 was evaluated with different Neotame concentrations (0.001 to 0.0003 mM). Suitability of Neotame as taste masking agent for Ondansetron was evaluated and it was observed that it reduces bitterness intensity measured as Change in Membrane Potential (CPA values) due to adsorption perceived as "After taste" from 22.47 mv to 16.54 mv.

Keywords: Ondansetron; Taste masking; Sensors; Quinine; Neotame.

INTRODUCTION

The need for taste masking is more prominent when formulating an oral dosage form for children and elderly patients. Taste being subjective perception, it varies amongst the individuals. The necessity to achieve taste masking and ensuring that complete taste masking is being achieved becomes an important deliverable for formulators working on development of these dosage forms. Ondansetron is a serotonin $5HT_3$ antagonist; anti-emetic drug. Serotonin receptors of the $5\text{-}HT_3$ type are present both peripherally on vagal nerve terminals and centrally in the chemoreceptor trigger zone of the area postrema. The drug is indicated for prevention of nausea and vomiting which is associated with emetogenic cancer chemotherapy[1]. Ondansetron is highly bitter drug and hence there is a need to taste mask especially when administrating to pediatrics and geriatrics population. Literature reports several taste masking technologies for development of suitable dosage form. Few of these techniques are complexation with Ion-exchange resins[2], complexation with hydroxyl betacyclodextrins[3] Ondansetron fast dissolving tablets using Eudragit polymers[4], Ondansetron microspheres prepared using spray drying technique[5], ionotropic gelation technique[6], Ondansetron films using polymer and sweeteners[7]. However, the effectiveness of these taste masked dosage forms is evaluated using human volunteers or in vitro using simulated saliva studies. Taste assessment of pharmaceutical preparations typically requires a large, trained taste panel, and sophisticated instrumentation and proper interpretation. The tests may require the same health safeguards as a clinical trial. The use of sensory panels is a complicated procedure due to the subjectivity of panel members and also exhibits the potential toxicity of drugs[8].

There are several methods available to evaluate the achievement of taste masking of formulations. These are Taste panel studies, Electrophysiological methods, Animal taste preference tests, In vitro drug release studies, In vitro Assay method and Biomimetic taste sensing systems[9]. Biomimetic taste sensing systems involve electrochemical sensors; these are electronic taste sensing systems which are based on different underlying techniques for example, electrochemical measurements, like Potentiometry, Amperometry, and Voltammetry, or Impedance spectroscopy[10]. Therefore, the current research work was aimed to study the effectiveness of Taste masking capability of Neotame for Ondansetron drug using Insent Taste Sensing System TS-5000Z. The principle behind taste evaluation is based on potentiometric measurement and system is equipped with different sensors, each lipid membrane sensor is claimed to be associated with a specific taste stimulus. Taste Sensing System models SA401, SA402, and SA402B were sold in Japan in 1993, 1996, and 2000, respectively. TS 5000 Z system can accommodate upto 8 sensors and can together detect various taste qualities such as sourness, saltiness, umami, bitterness, astringency[11]. Researchers of Intelligent Sensor Technology Inc., Japan have studied efficiency of taste masking or bitterness suppression for high potency sweeteners such as aspartame and sodium saccharine using Quinine hydrochloride (Quinine HCL) as the bitterness standard[12]. The experimentation flow and techniques designed for the current research work is based on the similar lines. For the current study, sensor BT0 was provided by Insent for taste masking evaluation of Ondansetron. BT0 sensor is found to have high correlation with bitterness of quinine HCl and other hydrochloride salts. Quinine HCl is used as standard bitter drug. Thus, bitterness intensity of many hydrochloride-salt drugs can be translated into corresponding concentration of Quinine HCl. Also, validation-correlation with human taste panel is carried out for comparative assessment of sensor BT0 and evaluation of taste masking of ondansetron orodispersible films.

TASTE SENSING SYSTEM: INSENT TS- 5000 Z

The instrument comprises of the taste sensors which are made up of an artificial lipid membrane that causes electrostatic or hydrophobic interactions with various taste substances, allowing them to sense "taste". The taste sensors can evaluate "Initial taste" and "After taste". Initial taste is the taste perceived when food or pharmaceutical formulation makes its first contact with tongue. Whereas, as the name suggests, 'After taste' is the taste which is remaining after food or medicine is swallowed. The Insent system considers potential of a reference solution as standard value 'Zero'. The difference in these potential values are applied to understand 'Initial Taste". Similarly, the difference in potential values observed after rinsing the sensors is considered as 'After Taste". Insent system recommends to use a solution containing 30 mM Potassium Chloride and 0.3 mM Tartaric acid as "Reference solution" as this solution is a substitute solution for human saliva. The reference solution itself has no taste[13]. In this study, bitterness intensity of ondansetron as drug was translated into corresponding concentration of Quinine HCL. The mV values obtained especially of BT0 sensor are applied to bitterness masking evaluation.

MATERIALS AND METHODS
Chemicals and Reagents

Potassium chloride (Analytical Grade), Quinine hydrochloride Dihydrate Ph. Eur., Tartaric acid (Ph. Eur.) and Absolute Ethanol (purity 99.98%) were purchased from Sigma Aldrich. Potassium hydroxide (Analytical Grade) was purchased from Merck and was prepared of 0.1 mol/L).Hydrochloric acid solution was prepared of 1 mol/L. and The inner solutions for sensors and reference electrodes of the taste sensing system TS-5000Z consisting of 3.33 mol/L Potassium chloride in saturated Silver chloride solution and it was provided by Insent Inc. Purified water for preparation of test and standard solution was generated in-house using water purification system of Siemens (Lobastar 7TWF).

Drug Substances and Drug Formulations

Ondansetron; drug substance was provided as gift sample from Dr. Reddy's laboratories (**lot no: ADCH001739**). Drug formulation comprised of Orodispersible film (ODF) of Ondansetron. Ondansetron ODF were formulated and described in detail in section 2.2.5. Neotame from The NutraSweet company (**Lot No: B103301245**) and Mannitol (Pearlitol ® Flash) was obtained from Roquette Pvt Ltd.

TASTE SENSING SYSTEM TS- 5000Z

Taste sensing system TS -5000 Z installed at **S. Zhaveri Pharmachem Pvt Ltd**, Analytical laboratory, India was used for current study.

Sensors

All measurements were performed using the taste sensing system TS-5000Z (Insent Inc., Japan). This electronic tongue was equipped with four lipid membrane sensors labelled according to the different taste qualities and corresponding four reference electrodes. Bitterness sensor 1 (CPA1 CO0) for acidic bitterness, Bitterness sensor 2 (CPA1 AN0) and Bitterness sensor 3 (CPA1 BT0). Both AN0 and BT0 for basic bitterness. The fourth sensor represented the gustatory stimuli such as astringency (CPA1 AE1). BT0 sensor was specially designed by Insent Inc., Japan for the current study. BT0 comprises of phosphoric acid di-n-decyl ester as artificial lipid and Bis (1-butylphenyl) adipate as plasticizer. It is specially recommended for bitter hydrochloride salts, Ondansetron is one such bitter drug. The "aftertaste" can be measured for bitterness, umami and astringency. In current study, these were detected using sensors AN0 and BT0. 0.2 ml of inner solution was filled into each sensor before beginning of the experiments. All sensors were preconditioned in standard solution for one day before the measurement.

Preparation of Standard and Washing Solutions

The experimental method followed for preparation of standard and washing solutions were as per requirement of Insent system. As sensors are positively charged and negatively charged, both the sensors require two separate washing solutions. Washing solution for positively charged sensor comprised of 100 mmol/L Potassium chloride and 10 mmol/L Potassium hydroxide. Whereas, negatively charged sensor compromised of ethanol (diluted to 30% from Absolute Ethanol) and 100 mmol/L Hydrochloric acid. A standard solution can also be used as cleaning solution. Reference solution acts as substitute for human saliva and it was prepared using 30 mmol/L Potassium chloride and 0.3 mmol/L Tartaric acid in distilled water.

Preparation of Stock Solution

10mM KCL solution and 1N HCL were prepared separately. 120 mg of Ondansetron was dissolved in 100 ml of 10mM KCL and 1 ml of 1N HCL solution. 10mM KCL was used for final volume make up and it was made upto 500 ml. This solution was used as Stock Solution (0.656 mM). The remaining solutions having concentration in the range of 0.008mM-0.656 mM were prepared for linearity and details are in **Table 1**. These were prepared by diluting stock solution to 100 mL with 10 mM KCL solution.

Electronic Tongue System and Measurement Set Up

Before the measurements on Insent Tasting System TS 5000 Z, the system was qualified before actual measurement of formulations. Linearity range for Ondansetron was established by studying different concentrations.

The handling of sensors was as per instructions provided from Insent TZ system. Prior to the beginning of experiments, each sensor (TecLabS Europe, Germany) was filled with 0.2 mL of saturated Silver chloride solution, also called inner solution. The reference electrode was completely filled up with inner solution. All sensors were preconditioned in standard solution for one day before actual measurements. Sensors were regularly checked for their mV values as per requirement of the system. Actual measurement step involves first measuring mV values for a reference solution (Vr) and then the actual sample (Vs) (Solution to be evaluated for taste). These values indicates the "Initial Taste" for the given samples. In order to detect "After taste" electrode is rinsed first with washing solution and again measuring mV values (Vr'). Thus, each sample is measured four times. Cleaning procedure of sensor involves its rinsing with washing solution. It causes desorption of the some of the adsorbed substance and hence change in membrane potential after cleaning gives values of "After taste". Initial taste is indicated by relative value (Vs-Vr). Whereas, Vr'-Vr indicates "After taste" of the sample. Later value is referred as CPA (Change in Membrane Potential caused due to adsorption) values.

Concentration dependence of various sensors and its response to Ondansetron was established in order to determine the suitable sensor for bitterness estimation of Ondansetron. For this, drug solutions were prepared in 10mM KCL solution at different concentration ranges and at 25 °C using Ondansetron as a stock

solution. The prepared solutions were sonicated intermittently. Results of electric response of sensors with respect to Ondansetron concentrations is presented in Table 2, Figure 1. Quinine hydrochloride solution at 0.5 mM concentration was prepared as external standard in demineralized water as this concentration was in the linear range for all the sensors used in experiment.

$$R = Vs - Vr \quad \text{Equation 1}$$

$$CPA = Vr' - Vr \quad \text{Equation 2}$$

Validation – Correlation with Human Taste Panel

Human sensory taste panel study was conducted by Insent Inc., Japan and data was provided for comparison purpose. The human study compromised of total of 14 volunteers. There were 6 males and 8 female volunteers. The age groups of volunteers were between that of 20-50 years. There were total of five volunteers in the age group of 20-30 years and 9 volunteers in the age group of 40-50 years. The test conducted was the blind test. First, human panelists tasted different quinine solutions as standard samples and then evaluated bitter intensity of samples composed of Quinine and Neotame. Neotame concentrations selected for study 0.03% w/v, 0.0010 % w/v and 0.0003% w/v. Taste evaluation was judged based on the bitterness score ranging from 1 to 6 respectively based on the Quinine concentrations.

Formulation of Ondansetron ODF

Ondansetron ODF were formulated by solvent casting method. Sample solutions were prepared by dissolving the Ondansetron films in demineralised water (Table 3). All samples were prepared in 10 mM KCl solution with target final Ondansetron concentration of 0.1 mM. The analytical procedure was performed for all the samples and repeated four times. All the samples were sonicated for 3 minutes using Ultrasonic bath. These were filtered using 0.22 micron Whatmann filter G4 and investigated by the electronic tongue system and analyzed using UV spectroscopy at 310 nm for Ondansetron formulations. The mV readings obtained from first run was discarded as per requirement of Insent System (This ensures the conditioning of sensors). All the data obtained for each sample were treated statistically by in-built software in the system.

Reporting Results

Results were expressed as raw data in terms of mV as Relative Values and CPA Values of the sample. Sensor signal results were either evaluated directly or multivariate data analysis was performed. Multivariate analysis, i.e. Principal Component Analysis (PCA), was used to reduce the multidimensional space without losing information. Using PCA, the most abundant information contained in the original data could be transformed into the first principal component (PC-1), and the second most abundant information is transformed into the second component (PC-2). In this way, information from the raw data can be extracted in order of importance. For the placebo sample and the sample with test compound, a cluster could be obtained in a PCA map by plotting PC-1 against PC-2. For multivariate data analysis, raw data were pre-treated by mean centering and scaling to unit variance. Data processing, graphical illustration and statistical interpretation of the results were carried out using in-built software system of Insent- TS- 5000Z.

Table 1: Sample Preparation for Linearity Evaluation of Ondansetron

Ondansetron concentrations (mM)	Amount of Stock solution added (ml)
0.008	1.2 ml
0.033	5.0 ml
0.164	25 ml
0.328	50 ml
0.492	75 ml

Table 2 : Electrical Response of Sensors to Ondansetron

Ondansetron concentration (mM)	Electrical Response of Sensors			
	BT0	CO0	AN0	AE1
Characteristics	Basic groups with hydrochloride salts	Acidic groups	Basic Groups	Astringent
0.008	9 ± 0.12	0	0	0
0.033	21± 0.52	0.9±0.62	2±0.65	0.9 ±0.58
0.164	35±1.25	0.7±0.85	7±0.95	0.7±0.96
0.328	35±2.36	0.8±0.65	8±0.36	0.8±0.56
0.492	35±3.25	0.9±1.08	9±0.65	0.9±0.58
0.656	35±3.28	0.9±0.85	9±0.36	0.9±1.08

Table 3: Ondansetron Solutions for Taste Analysis

Batches	Concentration (mM)	Original CPA (BT0)(mV)	Estimated CPA(BT0)(mV)
OF1 (Placebo batch)	0.1	0.41± 0.12	- 5.52 ± 0.3
OF2(Ondansetron hydrochloride)	0.1	24.39 ± 0.26	24.39 ± 0.71
OF 3 (Ondansetron oral film without Neotame)	0.1	22.17 ± 0.04	22.17 ± 0.8
OF 4 (Ondansetron oral film with Neotame)	0.1	22.47 ± 0.88	16.54 ± 0.6

Table 4: Comparison of Bitterness Score and Sensor Response for Quinine Hydrochloride

Concentration of Quinine hydrochloride (mM)	Bitterness Score	CPA (BT0)(mV)	Calculated Bitterness Score
0.013	1	8.15	1.81
0.02	2	12.33	2.29
0.03	3	17.36	2.87
0.045	4	24.05	3.63
0.0675	5	31.27	4.46

Table 5: Samples for Human Sensory Testing

Combination solutions for human sensory panel	Quinine concentration (mM)	Neotame concentration (% w/v)
Bitterness score 4 + low concentration of Neotame	0.045	0.0003
Bitterness score 5 + low concentration of Neotame	0.0675	0.0003
Bitterness score 6 + low concentration of Neotame	0.1	0.0003
Bitterness score 4 + Moderate concentration of Neotame	0.045	0.003
Bitterness score 5 + Moderate concentration of Neotame	0.0675	0.003
Bitterness score 6 + Moderate concentration of Neotame	0.1	0.003
Bitterness score 4 + High concentration of Neotame	0.045	0.001
Bitterness score 5 + high concentration of Neotame	0.0675	0.001
Bitterness score 6 + High concentration of Neotame	0.1	0.001

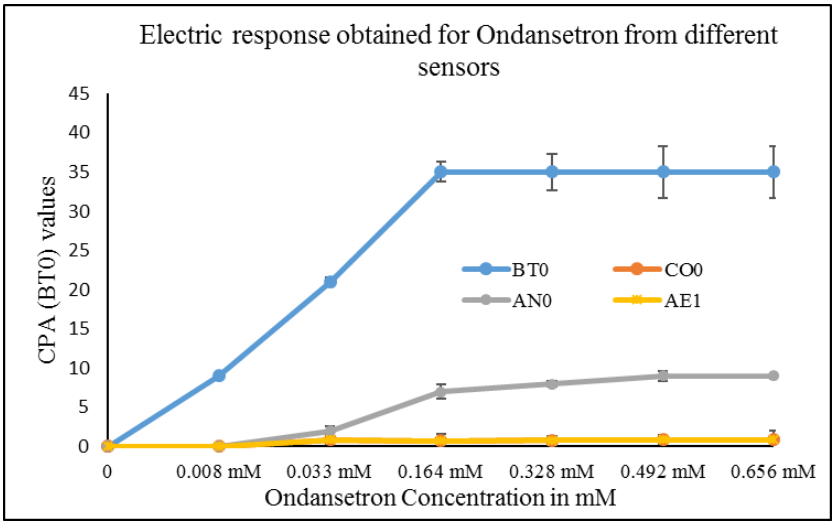

Figure 1: Sensitivity of Ondansetron for BT0 sensor

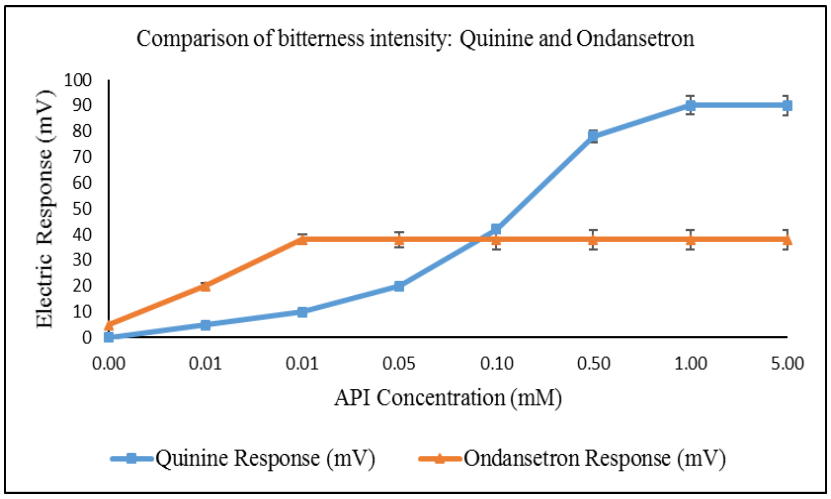

Figure 2: Bitterness intensity comparison of Ondansetron and Quinine HCl.

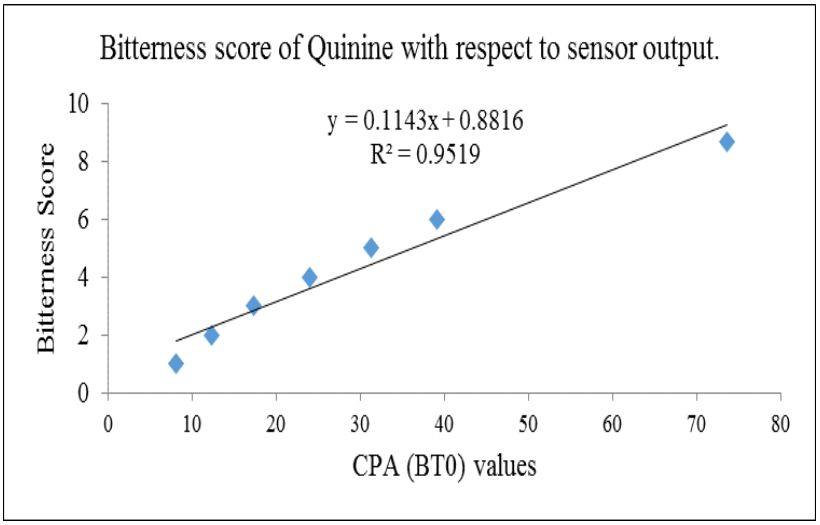

Figure 3: Bitterness scores of Quinine Hydrochloride and CPA (BT0) output

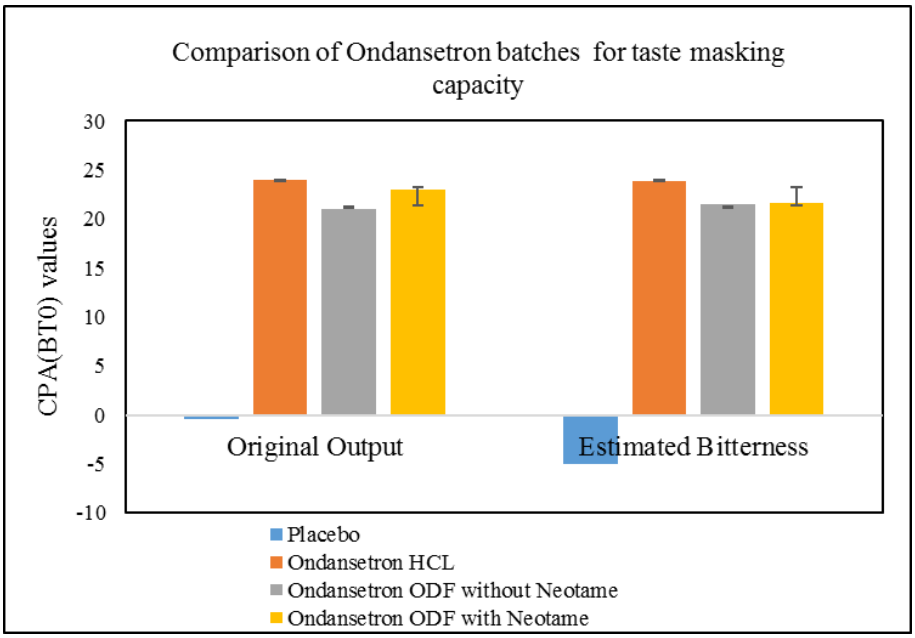

Figure 4: Original sensor outputs and estimated bitterness masking effect

RESULTS AND DISCUSSION

Concentration Dependence of Ondansetron for Sensors

The linearity concentration range selected was from 0.008 mM to 0.656 mM. These freshly prepared ondansetron solutions measured using Insent Taste Sensing System as per method in section 2.2.2 . The electrical responses in mV obtained from each sensor (Table 4). This response from BT0 indicates the highest intensity of bitterness. Also, electrical responses depend on the concentration and an increase in response is observed with increase in concentration upto 0.164 mM. Later on, response does not show much increase. Thus, linear response of drug is in the range of 0.008 mM to 0.164 mM range. The response of BT0 sensor for Ondansetron and Quinine HCl was established. The response of Ondansetron was found to be saturated around 0.01 mM that would correspond to the bitterness of Quinine HCl at 0.1mM (Figure 2). Based on this observation, the requirement of 0.1mM concentration for quinine hydrochloride and 0.01mM concentration for Ondansetron hydrochloride was found to be suitable for the current study.

Taste Analysis of Ondansetron ODF

Ondansetron ODF compromised of excipients such as Sweetening agents, Neotame, Polymer, and Plasticizer. Placebo ODF was prepared with all the excipients except the drug. Neotame was added in the current formulation as taste masking agent. Neotame is chemically (N- [N- (3, 3- dimethyl butyl)-L-aspartyl]-L-phenylalanine 1-methylester) is a derivative of dipeptide composed of the amino acids, aspartic acid and phenyl alanine. Neotame is 8000 times sweeter than sugar[14]. Thus, it is used as artificial sweetener and flavour enhancer for taste masking of bitter drugs. All samples were prepared in 10mM KCL solution with target final Ondansetron concentration of 0.1 mM.

Validation-Correlation with Human Taste Panel

Each human volunteer tasted 6 quinine samples which were prepared of different concentrations and its intensity of

"Bitterness" perceived by volunteers was scored between 1 to 6. Bitterness score of "1" corresponded to the threshold concentration of Quinine hydrochloride (minimum concentration), and it was observed that as the score increased, at each incremental step, it showed that the concentration was approximately 1.5 times higher than that of the earlier score. For example, bitterness score of "1" was given to the concentration of 0.013 and that of bitterness score "2" was given to that of the quinine hydrochloride (1.5 x 0.013 = 0.0195 ~ 0.02) and similar concentration relationship existed for bitterness score 2,3,4,5 and 6 with respect to quinine concentration. Next samples for taste evaluation by human volunteers involved the testing of total 9 samples (Table 5); 3 different concentrations of Quinine were selected based on bitterness score obtained. (Bitterness score towards higher side i.e. 4, 5, 6 were selected).

Derivation of Evaluation Formula based on Measurement of Samples

The current work studied the comparison of bitterness of human sensory taste panel with the electric responses observed for electronic tongue sensors, so it was utmost important to derive an equation indicating the relationship of mV values with the bitterness scores. This equation was derived by calculating the regression equation between bitterness scores of the human sensory test and corresponding sensor outputs, the bitterness scores become correlated with BT0 (CPA) outputs as shown below. The electric response obtained from bitterness sensor BT0 in mV. The score indicates the "bitterness score" as provided by human volunteers of sensory taste panel. The Figure 3 is a plot of bitterness score against the mV values of BT0 sensor. The straight line plot obtained fits into an equation of Y= mX + C, where m is the slope and C is intercept value. Thus, equation can be written as

Bitterness score = 0.1143×CPA1 (BT0) [mV] ＋0.8816 …..(a)

Thus, the bitterness score obtained for Quinine from human Sensory taste panel and using Insent's BT0 sensor is quite different.
Total bitterness score can be calculated as additive effect of output from bitterness sensor BT0 and score obtained from human sensory test. Thus, equation can be written as,

Total bitterness score ＝ Bitterness score ＋Change in bitterness score …… (b)

As for current experiment, both the mV outputs are known i.e. for CPA (BT0) of API and the concentration of Neotame; the total bitterness score can be estimated. Further, an estimated sensor output can be obtained by putting an estimated total bitterness score (equation b) in the equation (a) and it is modified as:

Estimated sensor output [mV] ＝Total bitterness score – 0.8116 / 0.1143 …..(c)

Formulation samples included Ondansetron mouth dissolving films prepared with and without taste masking agent. Since basic bitterness sensor (BT0) exhibited the highest intensity of bitterness and clear concentration dependency among the other sensors used for "After Taste" CPA (BT0) output in mV was evaluated.

Figure 4 represents electric response (output) values provided by BT0 sensor against that of formulations with Ondansetron. 1 indicates Placebo formulation, 2 indicates the Ondansetron (Pure drug as such). 3 and 4 indicates Ondansetron ODF without and with Neotame respectively. Also, the masking effects of the formulations were assessed with the evaluation formula. The evaluation formula was based on the bitterness intensity derived from Quinine Hydrochloride as a standard for bitterness. It means that the tested samples were assumed to have similar response to Quinine Hydrochloride. There is no significant change observed with CPA (BT0) values indicating that formulation with Neotame is not sufficient and it requires to be improved with respect to taste masking characteristics. The Ondansetron film containing Neotame i.e. OF 4 showed higher mV values as compared to that of estimated CPA values out indicating the sensitivity of Sensor. An evaluation formula was derived from human sensory test for a high intensity sweetener Neotame. This evaluation formula was based on after taste values of BT0 sensor CPA (BT0) and human sensory scores of samples contained different concentrations of Neotame. With respect to Ondansetron, it was found that Neotame is sufficient for taste masking the bitter taste of ondansetron (Considering the estimated CPA values). However, the actual output indicates that there is a need to further improve the formulations with respect to taste masking of Ondansetron. Thus, taste evaluation by electronic tongue provides a benchmark for evaluation of complete taste masking of proposed formulations.

Thus, from the current experimental work, it can be concluded that BT0 sensor is suitable for studying the taste masking of Ondansetron hydrochloride. It exhibits the linear response and it is directly proportional to the Ondansetron concentrations. It is in the range of 0.008mM to 0.164 mM. The 0.1mM concentration can be used for taste masking evaluation with respect to Ondansetron and its formulations. The present taste masked formulation did not show significant change in the bitterness score with respect to the plain API. The masking capacity of Neotame was estimated based on the equation derived from the human sensory test. From the estimation, the bitterness intensity of Ondansetron was expected to be decreased from CPA (BT0) 22.47 mV to 16.54 mV. Thus, Neotame can be used as a taste masking agent for Ondansetron. However, concentration of Neotame needs to be further optimized. Further, we can conclude that the taste sensing system is a useful tool in taste assessment and masking studies for an intensely bitter substance such as Ondansetron. The Taste Sensing Equipment can be useful in screening different taste masking agents as well as establishing their optimum concentration. It can be applied for the best taste masking effect of the unpleasant API especially in the early stage of drug and formulation development.

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