

Artificial lipid membrane: surface modification and effect in taste sensing

Saurav Kumar^{1, 2}, Amol P Bhondekar^{1, 2*}, Prateek Jain³, Sudeshna Bagchi^{1, 2}
Anupma Sharma^{1, 2}, Ritesh Kumar^{1, 2}, Sunita Mishra^{1, 2}

¹CSIR-Central Scientific Instruments Organisation, Sector-30 C, Chandigarh

²Academy of Scientific and Innovative Research (ACSIR), New Delhi-110025, India

³National Agri-Food Biotechnology Institute (NABI), Mohali, Punjab-140308

E-mail: amolbhondekar@csio.res.in; amol.bhondekar@gmail.com; Tel.: +91-0172-2657190(489)

Abstract. All articles *must* contain an abstract. The abstract text should be formatted using 10 point Times (or Times Roman, or Times New Roman) and indented 25 mm from the left margin. Leave 10 mm space after the abstract before you begin the main text of your article. The text of your article should start on the same page as the abstract. The abstract follows the addresses and should give readers concise information about the content of the article and indicate the main results obtained and conclusions drawn. As the abstract is not part of the text it should be complete in itself; no table numbers, figure numbers, references or displayed mathematical expressions should be included. It should be suitable for direct inclusion in abstracting services and should not normally exceed 200 words. The abstract should generally be restricted to a single paragraph. Since contemporary information-retrieval systems rely heavily on the content of titles and abstracts to identify relevant articles in literature searches, great care should be taken in constructing both.

1. Introduction

Taste is one of the five fundamental sensory perception identified in humans. The sensory apparatus by which we can identify the signal i.e. tongue, can distinguish five fundamental tastants which are saltiness, sourness, umami, sweetness and bitterness [1, 2]. Among these tastes, sweetness is a signal of presence of carbohydrates that serve as an energy for living organisms. Biologically speaking, taste substances interact with the bio-membrane on the taste buds and sweetness is received by T1R2 and T1R3 taste receptors, which are G-Protein coupled receptors (GPCRs) [3–5]. Sweetness is a pleasant taste for most of the people and it is the major attribute for the value of food and its demand. Therefore, evaluation of sweetness is an important task for most of the food industry. Sweet taste substances are commonly categorized as sugars, which is of a different kind as per their chemical structure and degree of sweetness. Major sugars -Sucrose, Fructose, and Glucose are carbohydrates with aldehyde or ketone group or their derivatives [6]5. Since sugars are principally nonionic and in the aqueous phase exhibit their molecular structure without charge, hence potentiometric measurement with lipid/polymer membrane shows low sensitivity to sugars compared to other basic taste substances like NaCl, HCl, MgCl₂, etc [7].

The taste sensor has the characteristic of using in common partially-selective sensor electrodes and measuring the analytes. The development of taste sensor need to fulfill following aspects: (a) the response of the taste sensor should be comparable to that of human taste; this fact is called global selectivity; (b) the taste sensor threshold for each taste should accord with the human sense; (c) the unit of taste information from the taste sensor should be clearly defined; (d) the taste sensor can detect interactions between taste substances or taste qualities such as a suppression effect that appears between sweetness and bitterness [8]. The taste sensor can discriminate the samples into the five basic taste qualities [1,9]. The taste sensor may utilizes lipid/polymer membranes as the taste-sensing part and outputs a change in the membrane potential caused by the interaction between the lipid/polymer membrane and taste substances. Lipid/polymer membranes are composed of a polymer support, a



lipid, and a plasticizer [10]. The lipid and plasticizer are responsible for controlling hydrophobic and electrical properties of the membrane surface, whereas the polymer support plays the role in encapsulating and immobilizing the lipid. Lipid/polymer membranes are designed and developed so that they can selectively respond to each taste quality. The lipid/polymer membrane responds to taste substances in sample solutions (i.e. said as analytes), and this response is called relative value, which is the difference between the membrane potential for the sample solution and that for the reference solution as the source. The properties of the lipid membrane formed from hydrocarbon solvents are identical in all the aspect to those of biological membrane resistance, which is generally much lower in cell membrane- and is there attributed to the presence of special translocators that locally modify the bilayers. However, the resistance of the experimentally prepared membrane can also be lowered to that of cell membrane by the insertion of translocator and protein or similar structure which could bring similar change. These molecules, actually disturb the fluidity and permeability of the membrane and further bring change to its surface charge density [11]. The same can also be achieved by the application of nanoparticles which is biocompatible and better catalytic activity.

Gold nanoparticles (AuNPs) have become increasingly attractive for nano-biotechnological, biomedical and pharmaceutical applications due to their unique optical, electronic and thermal properties as well as their straightforward synthesis, stability and the simple incorporation with lipid membrane and proteins [12][13]. The nanoparticles show similar dimensions to those of biomolecules, and the integration of nanoparticles with biomaterials exhibits unique recognition, catalytic, and inhibition properties, yields novel hybrid nano-bio materials for synergetic properties and functions [14]. The use of nanostructured thin films of conducting polymers and a lipid-like material coating gold microelectrodes, it is possible to mimic the human perception of taste. An electronic tongue composed of nanostructured films of lipid and plasticizer, these material acts as a transducers as it provide signals on interaction with electrolytes [15–17].

It is important to determine the necessary and sufficient conditions for fabricating a sensor, which may provide possible understanding for the reaction mechanism. This may be a basis for creating sensors that can respond to natural substance based on their type. The studies on the interaction between metal cations and bio-membranes have attracted considerable interest in recent years. Up to now, studies have been made on the interaction of Ca^{+2} , Cu^{+2} [14, 16]. Researchers have found that charged particle penetration into the lipid membrane (like AuNP) had effect on the fluidity of the membrane lipid and its charge density. Due to the physicochemical interaction, AuNP have considerable influence on the electrical properties of artificial lipid membranes [15, 18]. The interaction of nanoparticles (here, AuNP) and artificial lipid membrane depends upon the surface charge. When positively charged AuNPs are attached to the negatively charged membrane, then the membrane would try to maintain the original charge distribution [19].

During the process, membrane probably would lose its rigidity, and its morphology would be changed and this statement suggest that the fluidity and permeability of the membrane might increase. Neutral and negatively charged nanoparticles hardly adsorb onto negatively charged cell surface due to low adhesion [20,21]. Conversely, TDAB offers positively charge on the membrane surface, and citrate coated AuNP holds negative charge which might offer different interaction behavior as explained above and hence it definitely offer higher adhesion and stronger interaction.

In this experiment, we fabricated sweet sensitive AuNP modified artificial lipid membrane to sense the sweet taste analyte. The developed sweet-sensitive lipid/polymer membrane composed of tetra-dodecylammonium bromide, di-n-octyl-phenylphosphonate and polyvinylchloride [2,10,22,23]. Modification of lipid membrane was done with AuNP synthesized by citrate method. The taste sensor was studied for different taste substance from low concentration of 0.01mM (i.e. 10 μ M) to 1000mM. The modified lipid membrane's response has been compared with non-modified lipid membrane at different concentration of taste substances on gold electrode. It was tried during the experiment to monitor how the interaction of nanoparticles (AuNP) affects the sensitivity and selectivity of artificial lipid membrane. Further investigation towards the interaction of AuNP with artificial lipid membrane has been made from UV-Vis spectroscopic studies and the explanation was provided in its mechanism.

2. Materials and Methods

2.1 Reagents

Tetradodecylammonium bromide (TDAB), di-octyl-phenylphosphonate (DOPP), polyvinyl chloride (PVC), tetrahydrofuran (THF) and trisodium citrate and tetrachloroauric acid (HAuCl_4) was purchased from Sigma-Aldrich, Inc. The taste substance, i.e. analytes sucrose, D+-glucose, fructose were purchased from Himedia laboratory Pvt Ltd, Mumbai; Magnesium chloride (MgCl_2) purchased from Loba Chemie Pvt Ltd, Mumbai. Chemicals like sodium chloride (NaCl), hydrogen chloride (acid) (HCl) and sodium-L-glutamate (MSG) obtained from Chem Lab NV, Industrieterrein "De Arend" 2 B-8210 Zedelgem (provided by Alpha MOS in the testing kit). The glassware used for synthesizing GNPs was thoroughly cleaned and siliconized using 1% 3-glycidoxypolytrimethoxysilane solution (GOPS) (Sigma, India). Water was purified with a Milli-Q ultra-pure system (Millipore, India) having a resistivity $>18 \text{ M}\Omega/\text{cm}$.

2.2 Lipid/polymer membrane

The lipid/polymer membrane is composed of tetradodecylammonium bromide (TDAB) as the lipid, di-octylphenylphosphonate (DOPP) as the plasticizer and Polyvinyl chloride (PVC) as the polymer. The concentration used herein in the experiment has already been used in the commercial application of taste sensor and other earlier work. The reported work showed that the CPA value of the sensor is highly sensitive, a potential drop due to absorption onto membrane was reported smaller than -3mV for all the major taste analyte [7]. The procedure followed for fabricating the membrane was in accordance with previously reported work (reference). The membrane carries a positive charge because TDAB is positively charged in aqueous solution. The membrane was designed to contain lipid concentrations in the predefined ration of 1:2:3 with plasticizer and PVC represented in table 1. Because of the ratio, the properties of the membrane, i.e. the surface charge density, its fluidity, and hydrophobicity, which affect the potential value across the membrane [10]. The amount of the analyte substance adsorbed onto the lipid /polymer membrane depends upon lipid concentration, because it gives the site in the form of the charge layer to enable interaction (hydrophobic).

2.3 Synthesis of aqueous gold nanoparticles

An aqueous solution of GNPs was prepared by reducing tetrachloroauric acid (HAuCl_4) with glutamic acid under refluxing to yield colloidal gold particles. For this, 50 ml of 0.01% tetrachloroauric acid in Milli-Q water was taken in a reaction flask and refluxed for 10 min with 50 ml aqueous solution of trisodium citrate of 5 mM molarities was used [24]. The reduction of the gold metal ions (Au^{3+}) to yield GNPs (AuNP) was confirmed by the appearance of a dark cherry-red to blue color in the different colloidal solutions.

2.4 Modification of Lipid membrane

The lipid/polymer membrane modified to study the response behavior of sweet analyte comprised of the same discussed in section 2.2. The chemical species of AuNP are introduced at the time of preparation of the lipid/polymer membrane and concentrations of lipid and plasticizer kept same. The modification of lipid/polymer membrane with AuNP brings about charges in the surface charge density, fluidity and hydrophobicity of the membrane surface.

2.5 Measurement using Taste Sensor

The relative value of the variation of membrane potential was measured using an electrochemical work station, (Zahner elektrik electrochemical workstation IM6) composed of a gold working electrode and a common reference electrode. The sensing electrode to which lipid/polymer membrane (modified and

non-modified) is attached measures the changes in the membrane potential that is generated when the electrode is immersed in a sample. The membrane prepared deposits on the electrode. The details of the sample used in the study are summarized in table 1. The measurement procedure is as follows (Figure 2). The sensor electrode was immersed in the reference solution, and the membrane potential is measured (V_r), this process is followed by membrane potential in the sample solution (V_s). After this once again, the sensor electrode is treated with the reference solution. The highest value achieved during the sample solution (90% of the highest value) and the initial value of reference solution (V_r), the difference is taken as relative change due to the sample solution interaction with the membrane. In the end, the rinsing solution, which consists of 100mM KCl, 10mM KOH, 30% vol of Ethanol was used to rinse the sensor electrode. The process was repeated for five times and mean value of the consistent response is taken into consideration to avoid possible human error during the experiment.

Table 1. Analytes used during the taste sensing

Taste Sample	Analyte	Composition
Control	PBS	137mM NaCl, 2.7mM KCl, 10mM Na ₂ HPO ₄ , 1.8mM KH ₂ PO ₄
Sweet	Glucose	0.01mM -1000mM + control
	Sucrose	
	Fructose	
Salty	NaCl	
Sour	HCL	
Bitter	MgCl ₂	
Umami	MSG	

2.6 UV-Vis Spectroscopic Investigation

The UV-Visible absorption spectra were obtained using Lambda 25, Perkin Elmar, UV/VIS Spectrometer with the slit width of 10 nm and the measurement were performed in the range of 200 to 700 nm.

3. RESULT AND DISCUSSION

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.

3.1. Chemical characteristics of modified artificial lipid membrane

The taste sensor based on artificial lipid membrane, was synthesized by using hydrocarbon compound was analyzed for all basic tastes. The artificial lipid membrane was modified by using gold nanoparticles (AuNPs) which may to affect the surface property of the lipid membrane. The synthesis process was a chemical process comprised of lipid part: plasticizer part and supporting polymer part in the ratio of 1:3:2 [1–3]. Figure 1 shows the chemical structure of the lipid molecule (tetradodecylammonium bromide i.e. TDAB), plasticizer molecule (dioctyl phenyl phosphonate i.e. DOPP) and supporting polymer (polyvinyl chloride i.e. PVC). The concentration of TDAB on the membrane surface is a factor causing the surface of the lipid/polymer membrane to be positively charged because TDAB is a positively charged lipid [1,4,5]. During the reaction, lipid part which acts as ammonium salt provide bromide ion to plasticizer and give rise to the appearance of positive surface charge over the membrane surface of TDAB. The AuNP which was prepared by the process mentioned in the section 2.3, consists of the process of refluxing with citrate solution. This process gives rise to the appearance of partial negative charge on the AuNP surface [6]. The modified artificial lipid which is achieved by the doping of AuNP during the synthesis of artificial lipid membrane gives rise to a membrane which is more conjugated. The interaction between the AuNP and artificial lipid membrane brought charges rearrangement within the membrane, this eventually affects the fluidity, charge density and permeability of the membrane. The physico-chemical behavior of the lipid membrane depends upon the surface charge of the membrane [6–8], here hydrophilic part which is outside comprised of ammonium ion which is partially positive and the natural adherence is achieved to the citrate coated AuNP which is partially negative. In this situation the membrane probably would lose its rigidity, and its morphology would be changed and this reason suggests that the fluidity and permeability of the membrane might increase [9–11].

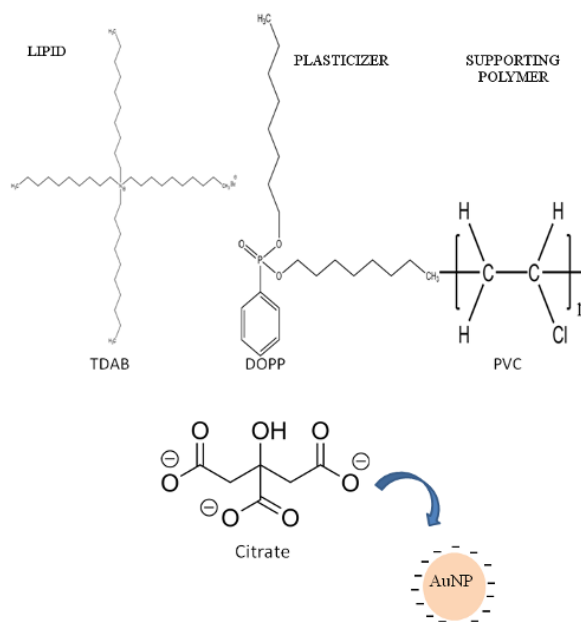


Figure 1. Structure of the lipid, plasticizer and supporting polymer used for the lipid-polymer membranes. Gold nanoparticles (AuNPs) with negative charge on the surface.

3.2. Sensing characteristics of modified artificial lipid membrane

In the Figure2, the process of sensing mechanism is illustrated, where the relative values were taken as the electronic response. Baseline measurement were carried by using the control solution (mentioned in the table 1). The values of open circuit potential changes in the presence of different analytes. It was observed that the baseline shift towards higher electronic response with in the presence of sugar molecules in the solution while the decrement was obtained in case of salt, sour and bitter taste sample in the solution.

Table 2. List of chemicals used during the synthesis of lipid membranes

Chemical used with abbreviation	Weight of chemicals used
tetradodecylammonium bromide/TDAB	100mg
dioctyl phenyl phosphonate/DOPP	310 μ L
polyvinyl chloride/PVC	200mg

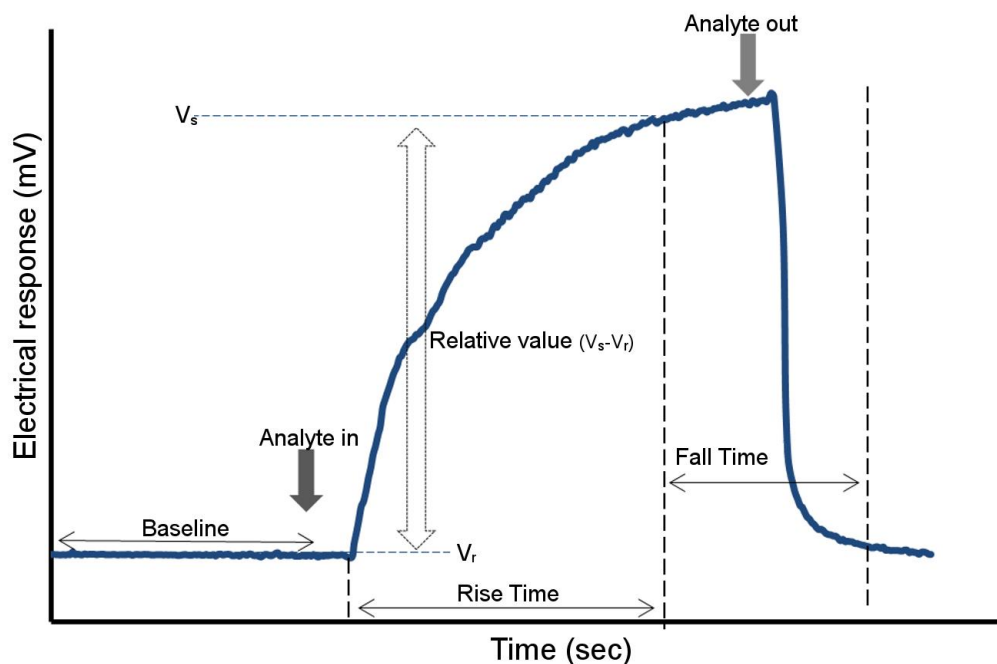


Figure 2. Schematic representation of taste sensing measurement process

3.3. Spectroscopic characteristics of the modified artificial lipid membrane

In Figure 3, the UV-Vis graph shows the change in the absorption spectra of artificial lipid membranes due to its modification with AuNP. Inset figure depicts the absorption peak of the AuNP at 520 nm. This AuNP was doped during the synthesis process of the artificial lipid membrane. Nanoparticles, which increase the electron density of lipid membranes, it consequently increases the interaction between part of the lipid membrane based on its polarity [10,12,13]. THF which was used during the UV-Vis measurement appears to be a good solvent in which the chains are relatively extended, so the light can be absorbed without much mutual interference. The absorption peak at $300\text{ nm} \pm 5\text{ nm}$ may arise due to $n \rightarrow \pi^*$ transition of $\text{C}=\text{O}$, the similar transition effect might occur in the complex of TDAB-DOPP with PVC. The peak wavelength shows the tendency to shift towards long wavelength as the size of the conjugated system increases [14]. In Figure 3, the absorption peak of 300 nm shift towards longer wavelengths shows the presence of the larger size of the conjugated system in the modified artificial lipid membrane. The additional band appeared in the modified structure likely to be attributed due to the covalent interaction of AuNP to the polymers 25. The strong absorption band centered at 520 nm arises due to the presence of AuNP in a dielectric medium, localized surface plasmon give rise to such peaks. During the lipid membrane synthesis this dielectric medium changed and hence peak got suppressed and a small hump is visible with the small red shift. This suppression in the intensity may also suggest the decrease in the free AuNP in the solvent after self assembly with artificial lipid membrane.

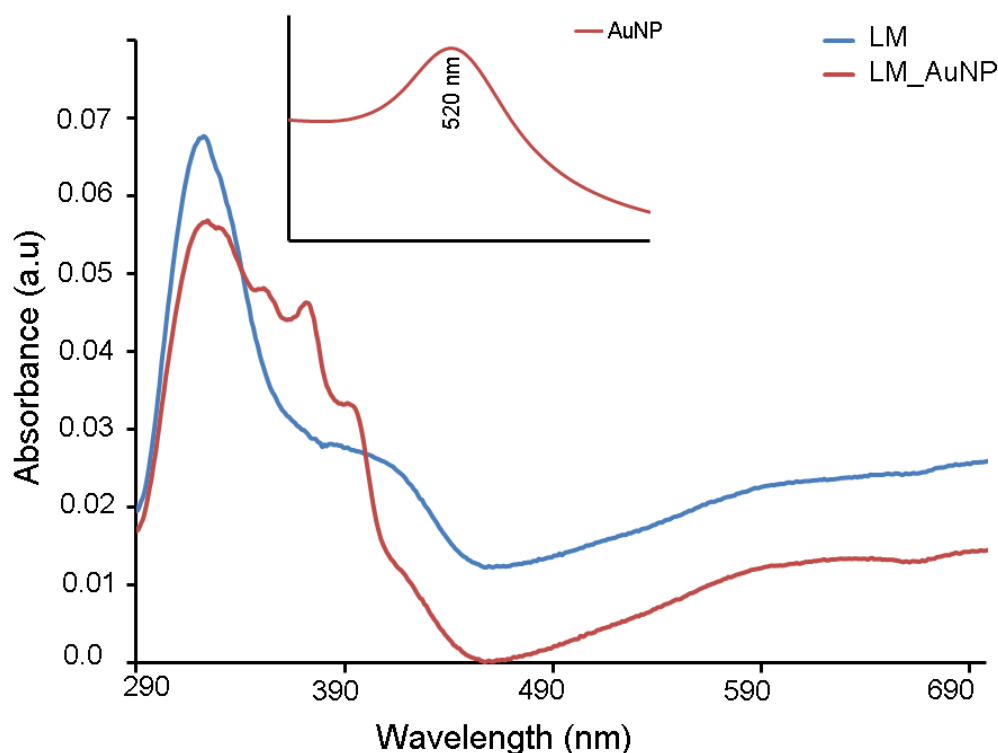


Figure 3. UV-Vis spectra of modified artificial lipid membrane (LM_AUNP) and TDAB-DOPP based artificial lipid membrane (LM). The inset shows the UV-Vis graph of AuNP.

3.4. Taste sensing

The response study behavior was studied subsequently using developed open circuit potential within the synthesized artificial lipid membrane deposited on the electrode. Figure 4 shows the response graph of AuNP modified artificial lipid membrane (LM_AuNP) and non-modified artificial lipid membrane (LM), in the different concentrations, range between 10 μ M to 1000 mM. depicted in (a) to (f). Relative values were considered during the response measured, where it was found that sugar molecules showed enhanced response behavior towards the modified artificial lipid membrane which might be due to dissociation state of -COOH of the AuNP incorporated with the lipid membrane [5,6,15,16]. It was observed earlier that the carboxyl or phosphatate group in the lipid/polymer membranes is essential for the sugar (sucrose) response of the sweetness sensor, and the hydroxyl group may not hold the necessary position for such event. Although, carboxylate groups of citrate molecule may react with the -OH group of sugars [17]. Among the sugars, the response behavior for modified lipid membrane was more prominent for the fructose sugar rather than conventional sucrose, this may be due to the presence of 'keto' group of fructose and it is also known to us, The response behavior for the compounds like NaCl, HCl and MgCl₂ showed consistent decrease in the increment in potential across the artificial membrane. The relative value of the NaCl increases negatively with the lipid membrane due to the screening effect of electrolyte Cl⁻ anions [18]. The similar kind of behavior can be suggested for HCl and MgCl₂.

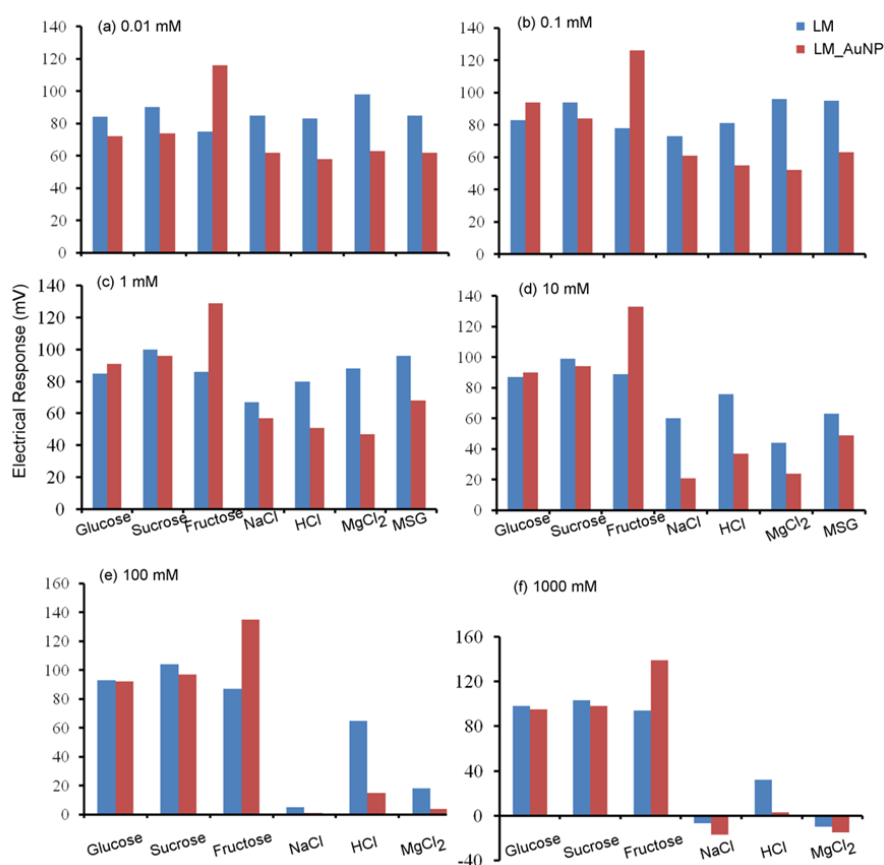


Figure 4. Taste response analysis of AuNP modified artificial lipid membrane (LM_AuNP) and TDAB-DOPP based artificial lipid membrane (LM) on basic taste analytes for sweet (glucose, sucrose and fructose), salty (NaCl), sour (HCl), bitter (MgCl₂) and umami taste (MSG). (a) 0.01 mM; (b) 0.1 mM; (c) 1 mM; (d) 10 mM; (e) and (f) shows 100 mM and 1000 mM respectively for sweet (glucose, sucrose and fructose), salty (NaCl), sour (HCl), and bitter (MgCl₂) taste.

In the results, it was also observed that the steady relative response between modified and unmodified lipid membrane was found in the MSG compound, which might be due to its neutrality in an aqueous medium [19,20].

Figure 5 showed the radar plot for the analysis of sensitivity towards sweet analytes, and in the result, it was observed that the LM_AuNP artificial lipid membrane showed enhanced sensitivity towards fructose molecule. Based on the response graph (Figure4) and radar plot (Figure5), we can suggest that, the behavioral difference in sensitivity of modified lipid membrane (LM_AuNP) and non-modified lipid membrane (LM). The increased response to fructose, the behavior of NaCl towards Au doped TDAB-DOPP (LM_AUNP) based artificial lipid membrane electrode is a matter of investigation and we are conducting further experiments to ascertain these facts.

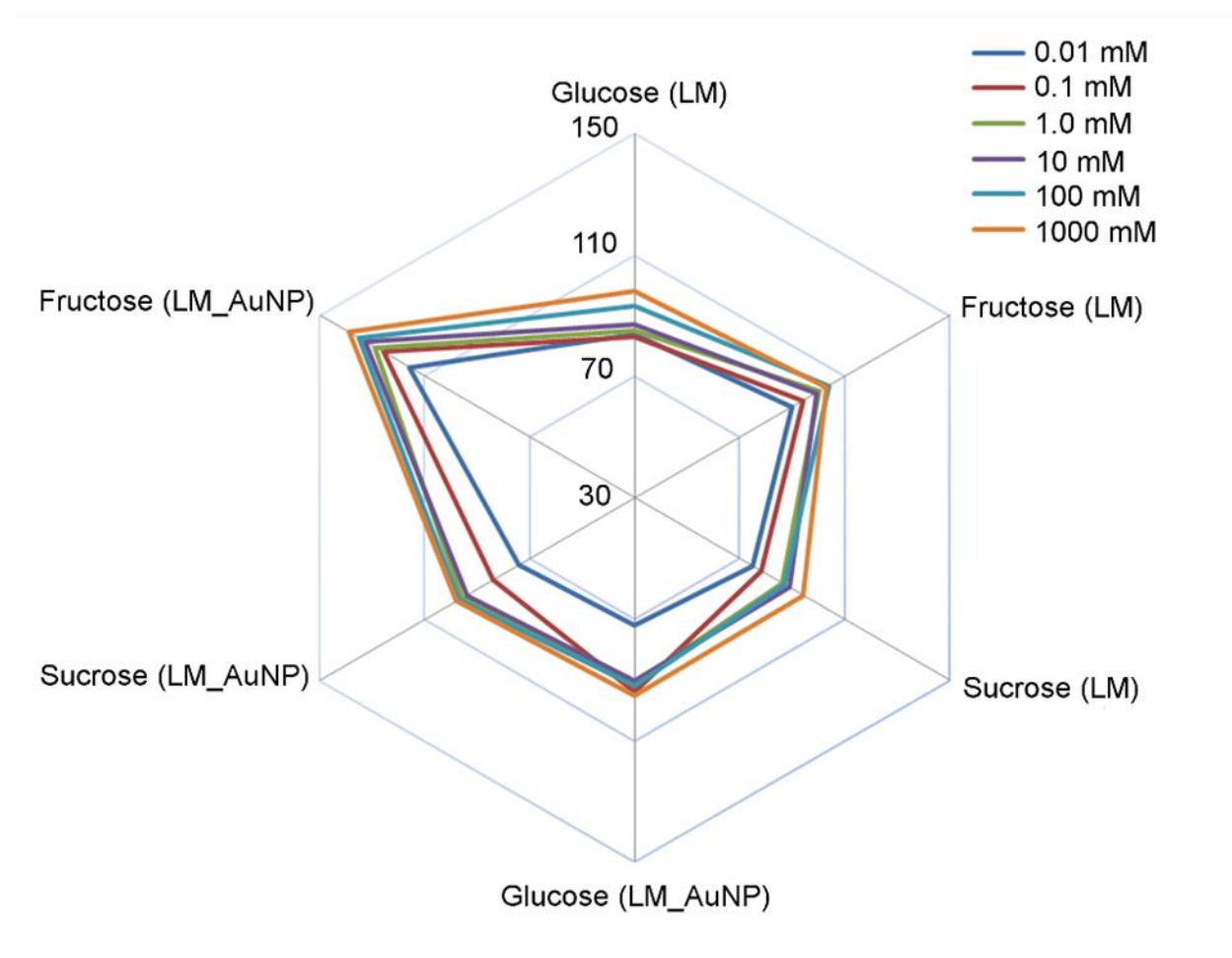


Figure 5. Radar plot representing the relative values in AuNP modified artificial lipid membrane (LM_AuNP) and TDAB-DOPP based artificial lipid membrane (LM) for sweet taste analytes (glucose, sucrose, and fructose).

4. Conclusions

In this study, a lipid membrane with AuNP modification was developed, which shows high sensitivity towards sugar molecules. The UV/Vis graph clearly showed the presence of conjugation due to the AuNP, and decrease in the free AuNP into the system. The interaction between hydrophilic ammonium part and the AuNP might be positive which leads to the change in the physicochemical behavior of the modified lipid membrane. The comparison of the response of TDAB-DOPP (artificial lipid membrane) with AuNP with unmodified TDAB-DOPP, showed higher sensitivity to fructose molecules. The results from the other analyte's response suggested that the surface charge changed due to the doping, which results in the variation of response for different taste analytes like salty, sour and bitter. Comprehensively, we can state that the observed response in the presence of AuNP, which is the citrate coated, showed enhanced sensitivity to fructose. We are conducting a further experiment to clarify the relationship between modified the artificial lipid membrane and change in its sensitivity towards the same taste analytes groups.

Acknowledgments:

The authors extend their sincere thanks to the Director, "CSIR-Central Scientific Instruments Organisation", to laboratory members of FPIL group of Agrionics Division.

Author Contributions:

Saurav Kumar and Amol P. Bhondekar conceived and designed the experiments; Saurav Kumar, Amupma Sharma performed the experiments; Ritesh Kumar, Sudeshna Bagchi analyzed the data; Prateek Jain contributed to characterization; Saurav Kumar wrote the paper; Amol P. Bhondekar, Sunita Mishra edited the paper. All authors have read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest

5. References

- [1] Toko K 1996 Taste sensor with global selectivity *Mater. Sci. Eng. C* **4** 69–82
- [2] Yamafuji K, Toko K and Hayashi K 1996 Taste sensing system using artificial lipid membranes
- [3] Krull U J and Thompson M 1987 Chemo-receptive lipid based membrane transducers
- [4] Toko K 2000 Taste sensor *Sensors Actuators B Chem.* **64** 205–15
- [5] Toyota K, Cui H, Abe K, Habara M, Toko K and Ikezaki H 2011 Sweetness sensor with lipid/polymer membranes: sweet-responsive substances *Sens. Mater* **23** 465–74
- [6] Cho E C, Xie J, Wurm P A and Xia Y 2009 Understanding the role of surface charges in cellular adsorption versus internalization by selectively removing gold nanoparticles on the cell surface with a I2/KI etchant *Nano Lett.* **9** 1080–4
- [7] Verma A and Stellacci F 2010 Effect of surface properties on nanoparticle–cell interactions *Small* **6** 12–21
- [8] Tatur S, Maccarini M, Barker R, Nelson A and Fragneto G 2013 Effect of functionalized gold nanoparticles on floating lipid bilayers *Langmuir* **29** 6606–14
- [9] Brockman H 1994 Dipole potential of lipid membranes *Chem. Phys. Lipids* **73** 57–79
- [10] Broda J, Setzler J, Leifert A, Steitz J, Benz R, Simon U and Wenzel W 2016 Ligand-Lipid and Ligand-Core Affinity control the Interaction of Gold Nanoparticles with Artificial Lipid Bilayers and Cell Membranes *Nanomedicine Nanotechnology, Biol. Med.*
- [11] Hanemann T and Szabó D V 2010 Polymer-nanoparticle composites: from synthesis to modern applications *Materials (Basel)*. **3** 3468–517
- [12] Behera M and Ram S 2013 Spectroscopy-based study on the interaction between gold nanoparticle and poly (vinylpyrrolidone) molecules in a non-hydrocolloid *Int. Nano Lett.* **3** 1–7

- [13] Chitte H K, Bhat N V, Karmakar N S, Kothari D C and Shinde G N 2012 Synthesis and characterization of polymeric composites embedded with silver nanoparticles *World J. Nano Sci. Eng.* **2** 19
- [14] Ionescu D, Popescu A, DRĂGUȘIN M, DIMA M, IFTIME A and GANEA C 2007 Modulation by quercetin of the effect of certain Hofmeister anions on artificial lipid bilayers *Rom J Biophys* **17** 85–90
- [15] Yasuura M, Okazaki H, Tahara Y, Ikezaki H and Toko K 2014 Development of sweetness sensor with selectivity to negatively charged high-potency sweeteners *Sensors Actuators, B Chem.* **201** 329–35
- [16] Habara M, Beppu D, Cui H, Ikezaki H and Toko K 2007 Detection of sugars using lipid/polymer membranes *Sens. Mater* **19** 325–31
- [17] Wittmann V 2006 *The Organic Chemistry of Sugars*. Edited by Daniel E. Levy and Péter Fügedi.
- [18] Kobayashi Y, Habara M, Ikezaki H, Chen R, Naito Y and Toko K 2010 Advanced taste sensors based on artificial lipids with global selectivity to basic taste qualities and high correlation to sensory scores *Sensors* **10** 3411–43
- [19] Corradini D 2010 *Handbook of HPLC* (CRC Press)
- [20] Daub C D, Leung K and Luzar A 2009 Structure of Aqueous Solutions of Monosodium Glutamate *J. Phys. Chem. B* **113** 7687–700