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Fourier analysis of potential oscillations of a liquid membrane for the discrimination of taste substances

Dana Cucu [a], D. Mihailescu [a], Gabriela Mihailescu [b], D.P. Nikolelis [c], Maria-Luiza Flonta [a], P.T. Frangopol [d]

[a] *University of Bucharest, Faculty of Biology, Membrane Biophysics Group, Spl. Independentei 91–95, 76201Bucharest, Romania*
[b] *Institute of Physics and Nuclear Engineering, P.O. Box MG-6, R-76900Magurele-Bucharest, Romania*
[c] *University of Athens, Department of Chemistry, Laboratory of Analytical Chemistry, Panepistimiopolis-Kouponia, 15771-Athens, Greece*
[d] *University "Al. I. Cuza", Faculty of Physics, Department of Biophysics and Medical Physics, P.O. Box 1637, R-6600Iasi-7, Romania*

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Abstract

Electrical potential oscillations were obtained across a liquid membrane composed of nitrobenzene/picric acid placed between two aqueous phases in the presence of various taste (i.e. salty, sweet and bitter) substances. The influence of these compounds on electrical oscillations was studied using Fourier analysis to establish a "fingerprint" of the substance that can be correlated with its taste index. Various concentrations of each substance were tested to obtain a Fourier spectrum with discrete peaks which can be further processed. The electrical oscillations consisted of a number of weak damped oscillators, and the Fourier spectra of these signals were found to have a number of discrete peaks of decreasing amplitude at low frequencies (0–0.5 Hz). A correlation of the frequency of the first peak of the Fourier spectrum with the taste index was found for bitter substances, whereas for salty substances the amplitude of the first two peaks of the spectrum was correlated with the taste index.

Keywords: Fourier spectrum; Liquid membrane; Self-sustained oscillation; Taste sensor

1. Introduction

Taste sensation is triggered in biological systems by a variation in the electrical potential of gustatory receptor membranes induced by the chemical stimuli. The induced potential variation (usually depolarisation) generates impulses in gustatory nerve fibres. For example, when chemical substances such as sugars and amino acids interact with receptor membranes, the membrane potential is changed (depolarised), and the depolarisation is propagated electrically to the synaptic region or impulse-generating area of taste cells [1,2]. Recently, the reception of taste and odour has been correlated to the hydrophobicity of the chemical stimuli and to its distribution between water and oil, as set by its partition coefficient [3]. Studies focused towards this direction suggested that lipids play an important role, and bitter and odour substances may be detected by direct adsorption to the lipid bilayer matrix without specific receptor proteins, which cause the membrane potential in biological cells [2,4–6]. A model for the dynamic response of taste receptor cells to salty stimuli has recently been proposed which takes into

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consideration the function of the lipid bilayer in the apical membranes acting as an agent which produces the response to the stimulation [7].

Electrical potential oscillations obtained in a liquid or a lipid-impregnated membrane were very promising as a model phenomenon in excitatory biological membranes [8]. The capability of such systems to discriminate various substances having similar chemical structure has encouraged the hope that a possible mechanism of taste or olfactory response can eventually be established. Furthermore, results from the studies of potential oscillations can be very useful to design biosensors [16].

The identities of the specific chemicals that excite different taste receptors are still incomplete. For practical reasons in taste analysis, the receptor capabilities have been collected into four categories called the primary sensation of taste; these categories comprise sour, bitter, salty and sweet. All the taste substances have a threshold of stimulation which is dependent on concentration. The threshold for stimulation of the salty taste by sodium chloride is 0.001 M and that for stimulation of the sweet taste by sucrose is 0.01 M. The reciprocal of the taste thresholds provides the taste index. For example, sucrose has a taste index of 1, whereas this value for glucose is 0.8, indicating that sucrose is sweeter than glucose. Sodium chloride is set as the reference among salty substances with a taste index of 1 [9].

An important aspect of studies and investigations in the field of the taste index is the analysis of electrical potential oscillations. Usually, there are two routes of analysis, which include simple frequency mediating [8] and chaotic attractor or fractal chaos [10,11]. The former technique, however, has the disadvantage of a large value for the standard deviation of the mean, whereas the latter, which includes aspects of the modern theory of chaos, offers parameters that are not very intuitive. Fourier analysis has rarely been used as a tool to study potential oscillations [12–14], despite the fact that it has been extensively used in electrical engineering for signal analysis and in spectroscopy.

In the present studies, the Fourier transform and signal analysis have been used to study the fast Fourier spectrum of the electrical oscillations obtained on a liquid membrane. The studies were focused on a finite number of parameters, such as the amplitude and frequency values of the Fourier spectrum, which represent a "fingerprint" of a substance at a given concentration. Furthermore, an effort was made to explore the discriminative ability of a liquid membrane based on correlation of the amplitudes and frequencies of the peaks of the Fourier spectrum with the taste index.

2. Experimental

The equipment used in the present studies was similar to that reported previously by Shaw and Coddington and by Yoshikawa (Fig. 1) [1,17]. A solution of nitrobenzene (4 ml) containing 1.5 mM picric acid was placed at the bottom of a U-shaped glass tube as the organic phase. Aqueous solutions (5

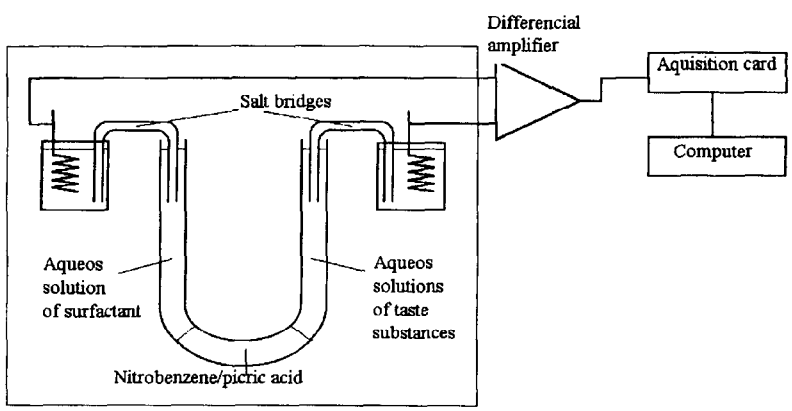

Fig. 1. Experimental equipment.

ml) were poured simultaneously into both arms of the cell onto the organic phase without stirring. The left aqueous phase contained a surfactant agent (sodium dodecyl sulphate) plus an alcohol (isobutyl alcohol). Solutions of the substances which were examined as potent inducers of electrochemical potential oscillations were placed in the right arm of the tube. The following substances were tested: (i) DL-alanine, sucrose and glucose for the sweet taste; (ii) NaCl, KCl, NaI and NH_4Cl for the salty taste; (iii) caffeine, strychnine and atropine for the bitter taste. The concentration of the substances ranged between 10 μM and 1 M.

The solutions were connected with two electrodes by salt bridges. The electrical potential oscillations were recorded and processed by a computer via an analogue–digital interface (PC Lab card, maximum acquisition rate: 100K samples per second). Each recording containing 12 000 data points was stored in a file. User-friendly software for recording, display and analyses was written in our laboratory in C code. The recording started immediately after the addition of the active substance and was continued for > 20 min. All measurements were carried out at 20 ± 1°C and were performed three times.

3. Results and discussion

3.1. Electrical potential oscillations

Fig. 2 shows various types of self-sustained oscillations. These spontaneous oscillations started about 3 min after the organic phase (nitrobenzene/picric acid) first made contact with the aqueous solutions; this time delay is probably due to the diffusion process and establishment of the related equilibrium. The amplitude values had a range of ≈ 10–30 mV. Potential oscillations gradually decreased, then stopped, both in amplitude and frequency. Direct discrimination of the various types of substances based on the potential oscillations as obtained above could not be achieved owing to the large dispersion of both frequency and amplitude of the oscillations. Therefore, in an effort to find a means to correlate the oscillations with the type of substance, a Fourier analysis of these oscillations was attempted.

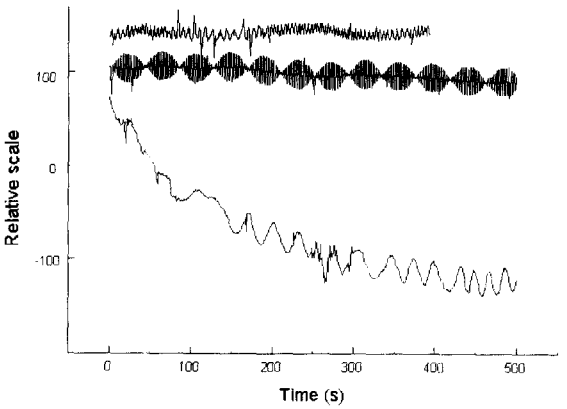

Fig. 2. Different types of self-sustained electrical oscillations: top, 10^{-4} M NH_4Cl; middle, 10^{-3} M glucose; bottom, 1 M alanine. Each oscillation has its own offset in electrical potential which is not shown. The vertical axis is in relative scale because each oscillation has its own offset. This offset value affects the first coefficient in the fast Fourier transform, a value disregarded in this study.

3.2. Fourier series and harmonic analysis

A single damped harmonic oscillator can be described by the following equation

$$\Psi(t) = \exp(-1/2\Gamma t)\cos\omega_1 t \quad (1)$$

where Γ is the damped constant and $\omega_1^2 = \omega_0^2 - 1/4\Gamma^2$, ω_0 being the characteristic frequency of the free oscillator.

The function $\Psi(t)$ can be expressed by a superimposition of harmonic oscillators described by a Fourier series

$$\Psi(t) = \int_0^\infty A(\omega)\sin\omega t\,d\omega + \int_0^\infty B(\omega)\cos\omega t\,d\omega \quad (2)$$

$A(\omega)$ and $B(\omega)$ are the imaginary and the real part of the Fourier transform, respectively.

In the case of a weak oscillator the intensity (amplitude) I, which is equal to $B^2(\omega) + A^2(\omega)$, is proportional to $L(\omega)$

$$L(\omega) = (1/2\Gamma)^2/\left[(\omega_0 - \omega)^2 + (1/2\Gamma)^2\right] \quad (3)$$

where $L(\omega)$ represents a typical Lorentzian curve. The maximum of this function is for $\omega = \omega_0$ (resonance frequency). Therefore, the frequency of the

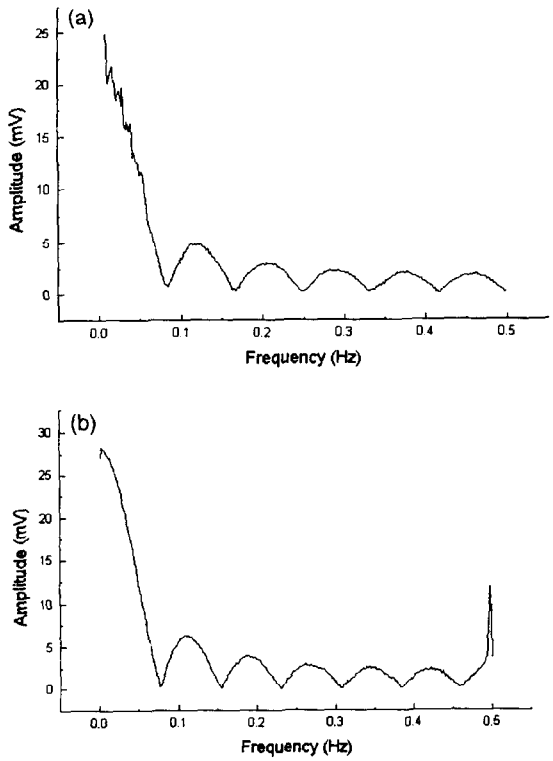

Fig. 3. (a) The power Fourier spectra for 1 M alanine; (b) the power Fourier spectrum for glucose at 10 μM. Even though there seem to be multiples of the same frequency, the intervals between maxima are not equal (see also the frequencies from the columns of Table 1). So there is not a set of fundamental frequencies and its higher harmonics.

maximum intensity value in the "power spectrum" (i.e., the intensity of the Fourier transform as a function of frequency) provides the resonance frequency which is characteristic of the weak damped oscillator. The above theory can be extended to a superimposition of any number of damped weak oscillators, and the intensity of the Fourier transform would be the sum of many Lorentzian curves.

3.3. Fourier form of potential oscillations of sweet, bitter and salty substances

A large variety of sweet, bitter and salty substances (as reported above in Section 2) was tested experimentally to obtain electrical potential oscillations. The Fourier spectrum of each substance was studied at various concentration levels (in the range 1 μM to 1 M). Fig. 3 provides typical spectra obtained by using the fast Fourier transform for two sweet substances, i.e. glucose at a concentration level of 10 μM (Fig. 3(a)) and alanine at a concentration of 1 M (Fig. 3(b)). It is seen in this figure that there are distinct peaks, and each of them is well fitted with a Lorentzian curve with a correlation better than 0.96.

A fast Fourier transform has provided for each of the examined substance a spectra containing five to ten distinct maxima with slightly decreasing amplitude (i.e., similar to weak damped oscillators). The

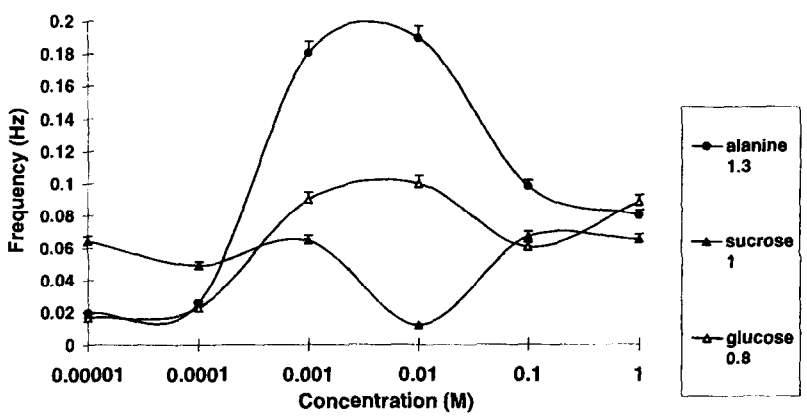

Fig. 4. Frequency positions of the dominant peak in the Fourier spectra as a function of concentration for: alanine (taste index 1.30, sucrose (taste index 1), and glucose (taste index 0.8).

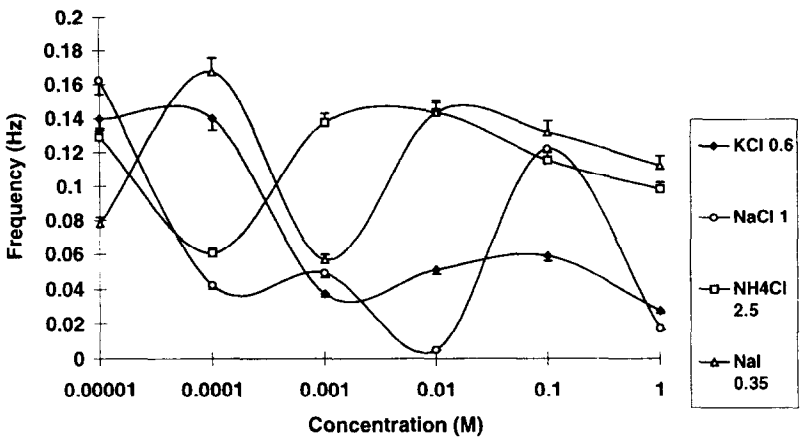

Fig. 5. Frequency dependence on concentration for (a) KCl (taste index 0.6); (b) NaCl (taste index 1); (c) NH_4Cl (taste index 2.5); NaI (taste index 0.35).

range of frequencies which was examined was 0–0.5 Hz, as the peaks obtained in this range had amplitudes with measurable values and, furthermore, peaks of much smaller amplitude obtained at higher frequencies are most likely due to electrical noise; therefore, as the processes to obtain the electrical potential oscillations are slow because of diffusion and adsorption at the liquid interface, only low frequencies are important to modelling the taste sensor. Indeed, the frequencies of the peaks obtained in the range up to 0.5 Hz can be considered characteristic for each substance and the concentration tested. Table 1 contains the frequencies at which there are maximum values of the real part of the fast Fourier transform of electrical potential oscillations for the four examined salty substances and the respective amplitudes. These sets of dominant frequencies provide a "fingerprint" of the substance at a given concentration.

An examination of the dependence of these dominant frequencies on the concentration of the substance (including all three types of taste) was also made. Fig. 4 shows plots of the frequency of the first maximum peak obtained in the Fourier spectra for alanine, sucrose and glucose, which have a taste index of 1.3, 1 and 0.8, respectively. It can be seen in this figure that there is an increase of frequency values with concentration in the range between 10 μM and 0.001 M for all sweet substances examined and the frequency of this first peak is maximised for concentrations of ≈ 0.001 M; above this concentration there is a gradual decrease of frequency values. The threshold concentration to obtain this phenomenon can also be calculated from this figure; i.e., for glucose this concentration is $\approx 3 \times 10^{-5}$ M.

The typical number of peaks in Fourier spectra for salty substances is larger than for sweet substances. Fig. 5 shows the frequency dependence vs. concentration for four salty substances, i.e. KCl, NaCl, NH_4Cl and NaI. All salty substances provide a maximum value at a concentration of ≈ 10 μM, whereas the frequency decreases with an increase of concentration up to ≈ 0.01 M levels and an abrupt increase of frequency values is again noticed for concentrations larger than 0.01 M.

All bitter substances examined provided a maximised frequency value of the first dominant peak at

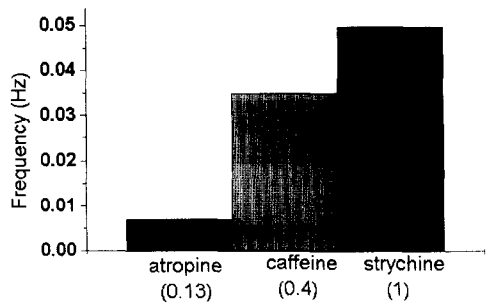

Fig. 6. Histogram of frequency position of the main peak in the Fourier spectra for some bitter substances. The taste indexes are given in parentheses.

Table 1
The amplitudes and their frequency position extracted from Fourier power spectra for salty (NaCl, KCl, NaI, NH_4Cl) and sweet (alanine, glucose, sucrose) substances

	Alanine						Sucrose						Glucose			
Taste index	1.3						1						0.8			
Concentration	1 M		10^{-2} M		10^{-3} M		1 M		10^{-1} M		10^{-2} M		10^{-4} M		10^{-5} M	
	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude
	0.1230	4.8766	0.1210	3.4678	0.1269	2.3477	0.0507	3.5912	0.1093	6.2956	0.1210	4.7220	0.0253	5.7881	0.0312	4.0090
	0.2070	3.0038	0.2031	2.2712	0.2031	1.3461	0.0820	1.3156	0.1894	3.9407	0.2070	3.0385	0.2636	1.2468	0.0585	3 6960
	0.2851	2.2988	0.2226	1.8928	0.2949	1.1290	0.1152	0.8806	0.2617	2.9594	0.2910	2.4023	0.2851	1.3126	0.1445	2.5857
	0.3789	2.0861	0.2910	1.8970	0.3769	0.9772	0.1464	0.6805	0.33984	2.5394	0.3027	2.0757	0.3261	1.0679	0.1484	2.5527
	0.4648	1.9107	0.3808	1.6137	0.4589	0.9355	0.1796	0.5924	0.4218	2.4808	0.3780	1.8941	0.4238	0.4348	0.2011	2.0195
			0.4492	1.3556			0.2128	0.5021	0.4980	11.957	0.4511	1.7425	0.4453	0.5699	0.2304	2.1252
	0.4628	1.3333			0.2441	0.4493						0.4960	0.6015	0.2402	1.9951	
							0.2773	0.4178							0.2812	1.5361
							0.3066	0.3865							0.3867	1.0241

	KCl		NaCl						NaI						NH_4Cl			
Taste Index	0.6		1						0.35						2.5			
Concentration	10^{-2} M		10^{-5} M		1 M		10^{-1} M		10^{-5} M		10^{-1} M		10^{-3} M		10^{-2} M		10^{-5} M	
	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude	frequency	amplitude
	0.0253	30.986	0.1289	4.3229	0.0273	8.5869	0.0332	20.962	0.1074	9.3897	0.1191	5.4136	0.1269	2.3477	0.1210	4.7720	0.1113	2.3787
	0.1230	7.9571	0.1328	3.9663	0.1210	2.0747	0.1230	6.0094	0.1933	5.6678	0.2050	3.3409	0.2031	1.3461	0.2070	3.0385	0.2089	1.3936
	0.2070	4.8083	0.2050	2.7585	0.2089	1.2889	0.2089	3.6620	0.2597	4.3028	0.2890	2.4910	0.2949	1.1290	0.2910	2.4023	0.2851	1.0461
	0.2890	3.9371	0.2949	2.0138	0.2968	1.0106	0.2910	2.7588	0.3418	3.7433	0.3789	2.0359	0.3769	0.9772	0.3027	2.0757	0.3652	0.9957
	0.375	3.2093	0.3710	1.7391	0.3691	0.9102	0.3691	2.3376	0.4179	3.3570	0.4609	2.0453	0.4589	0.9355	0.3789	1.8941	0.4628	0.8384
	0.4589	3.1523	0.4609	1.6309	0.4589	0.8332	0.3867	2.1685	0.4375	3.2477					0.4511	1.7425		
							0.4570	2.2153	0.4941									
							0.4726	1.9450	0.6820									
									0.4847									

0.001 M concentration levels. Fig. 6 shows histograms of the frequency positions of the dominant peak in the Fourier spectra for atropine, caffeine and strychnine at the above concentration level. It should be noted that this is the only case among sweet, salty and bitter substances in which a correlation of the maximised frequency values with taste index was noticeable; i.e., an increase of the frequency value is found with an increase of the index value.

In order to compare the characteristic frequencies of the Fourier spectra of various salty substances, our studies were made at equilibrium, i.e. when the distribution (partition) equilibrium was reached. When a substance partitions between two phases, the chemical potential of this substance must be equal in the two phases when the distribution equilibrium is established

$$\mu_{i,w} = \mu_{i,o} \tag{4}$$

For a dilute solution, this equation becomes the Nernst distribution law

$$\beta_i = c_{i,o}/c_{i,w} = \exp\left[\left(\mu^0_{i,w} - \mu^0_{i,o}\right)/RT\right] \tag{5}$$

where w and o denote the aqueous and the organic phase, respectively, c_i is the concentration of the species i, μ^0 is the standard chemical potential and β_i is the partition coefficient of i. The standard chemical potential of a substance in various solvents depends on the degree of the interaction of the substance with the solvent. The difference in the standard (molar) Gibbs transfer energy of solvation of the species i comprising the substance is equal to the difference in the standard chemical potential

$$-\Delta G^0_i = \mu^0_{i,w} - \mu^0_{i,o} \tag{6}$$

By applying the above equations for distribution of an electrolyte BA between two immiscible solvents, i.e. water and another solvent of high permittivity, we obtain

$$\beta_{BA} = \left[(a_{B,o}a_{A,o})/(a_{B,w}a_{A,w})\right]^{1/2} = a_{\pm,o}/a_{\pm,w} = \exp\left[\left(\mu^0_{BA,w} - \mu^0_{BA,o}\right)/RT\right] \tag{7}$$

where $a_{B,o}$, $a_{B,w}$ and $a_{A,o}$, $a_{B,w}$ are the activities of the species B and A, respectively, in the organic solvent (o) and in water (w), and $a_\pm$ is the activity of the electrolyte BA in the organic solvent (o) and in water (w). For dilute solutions activities can be replaced by concentrations, and thus we obtain

$$\beta_{BA} = \left[(c_{B,o}c_{A,o})/(c_{B,w}c_{A,w})\right]^{1/2} = c_{\pm,o}/c_{\pm,w} = \exp\left[\left(\mu^0_{BA,w} - \mu^0_{BA,o}\right)/RT\right] \tag{8}$$

In analogy to Eq. (6), the difference of the standard chemical potential of the species i in the two solvents is given by the difference in the Gibbs transfer energy of solvation

$$-\Delta G^0_{BA} = \mu^0_{BA,w} - \mu^0_{BA,o} \tag{9}$$

The standard Gibbs energies of ion transfer from water to nitrobenzene are 32.5, 34.5, 24.3, 27.4, 30.5 and 18.8 kJ mol^{-1} for hydrogen, sodium, potassium, ammonium, chloride and iodide ions, respectively [15]. We have chosen substances which contain a common ion, i.e. NaI, NaCl, KCl, and NH_4Cl, in order to explore whether cations or anions play the more important role for transfer in nitrobenzene. We calculated the standard Gibbs energies for each substance as the sum of the standard Gibbs energies of its ions, equal to 53.3, 65.0, 54.8 and 57.9 kJ mol^{-1} for NaI, NaCl, KCl and NH_4Cl, respectively. However, there was no systematic correlation and these substances provided very different electrical potential oscillations. Therefore, both ions making up the substance are important for its transfer to the organic solvent.

The Fourier spectra of NaI, NaCl, KCl and NH_4Cl at various concentrations were obtained, and the amplitudes of the first two peaks (at frequencies of ≈ 0.02 and 0.1 Hz) of the oscillations were compared (Table 2). Furthermore, a correlation was made

Table 2
The amplitudes from Fourier spectra at frequencies of 0.1 Hz, A_1 and A_2, for salty substances. The concentrations in the aqueous phase were so chosen that the concentrations of ions in nitrobenzene were the same and equal to 5×10^{-6} M (see text)

Substance	Taste index	Concentration in aqueous media/M	A_1	A_2	A_1/A_2
NaI	0.35	0.01	4.72	3.04	1.55
KCl	0.6	0.01	5.41	3.34	1.62
NaCl	1	0.01	20.96	3.66	6.73
NH_4Cl	2.5	0.01	30.98	4.80	6.45

with the taste index of these four salty substances (Table 2). As can be seen in this table, the amplitudes at these frequencies increase as the taste index increases.

Similar studies ware made for sweet and bitter substances, but we were not able to establish a correlation between the amplitudes at the above frequencies of oscillations and the taste index.

In conclusion, the present studies show that the electrical potential oscillations obtained at the equilibrium distribution of a substance between water and an organic solvent can be considered as a combination of various weak damped harmonic oscillations, and this provides the opportunity of using Fourier transform and harmonic analysis to obtain a Fourier spectrum of the oscillations. The Fourier spectrum offers the potential for discrimination between different types of substances. Each substance has its own set of frequency positions of peaks in the Fourier spectrum which can be a "fingerprint" for the substance at a given concentration. Furthermore, for bitter substances, there is a correlation between the frequency of the first peak and the taste index of the substance. For salty substances a correlation was established between the amplitudes at two frequencies of the first two peaks in the Fourier spectra at a given concentration and the taste index.

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