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Bitterness Evaluation of H_1-Receptor Antagonists Using a Taste Sensor

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The objective of this study was to evaluate an improved bitterness sensor, which, it is postulated, will allow a more precise and sensitive prediction of the bitterness of active pharmaceutical substances. The bitterness sensor, BT0, has a membrane surface with improved and optimized hydrophobicity, and was developed to enhance the hydrophobic interaction between the membrane and basic bitter substances. The bitterness of eight H_1-receptor antagonists was measured using a multichannel taste-sensing system incorporating sensor BT0. Three variables, relative value (*R*), change in membrane potential caused by adsorption (*CPA*) and adsorption ability (*CPA*/*R*), were used in the evaluation. For sample solutions of the eight H_1-receptor antagonists, higher sensor output values of *R* and *CPA* were observed with sensor BT0, in comparison with a conventional bitterness sensor, AN0. The higher output values seem to be due to the superior hydrophobic interactions between the BT0 sensor membrane and basic bitter substances, as sensor BT0 also showed higher *CPA/R* values. The data suggest that sensor BT0 provides a more sensitive bitterness evaluation, being able to detect bitterness in sample solution concentrations as low as 0.01 mg/ml. The eight H_1-receptor antagonists could be categorized into three groups by principal component analysis using data from sensor BT0. Sensor output from sensor BT0 could be used to discriminate effectively between drugs without the need for performing laborious gustatory sensation tests.

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1. Introduction

H_1-receptor antagonists are widely used in the treatment of histamine-mediated allergic conditions. Although they can be administered topically by nasal or ophthalmic routes, oral administration is more convenient and preferred by patients. Most H_1-receptor antagonists are known to be bitter, which can hinder therapeutic management and lead to patient noncompliance. The quantitative and qualitative prediction of the bitterness of such active pharmaceutical entities would be very helpful in the early stages of formulation development, and allow strategies for bitterness masking to be developed.

The multichannel taste sensor is composed of functional sensors with artificial lipid membranes of different compositions.[1,2] Each sensor is able to identify a specific taste, i.e., saltiness, sourness, sweetness, bitterness, and umami, in a manner similar to human gustatory sensation. Taste evaluation has been performed in various foods and beverages (such as coffee, beer, mineral water, milk, rice, and vegetables) using the taste sensor. In the pharmaceutical industry, bitterness evaluation using the taste sensor is also attracting increasing attention.[3–5] Bitterness evaluation studies have been reported for various basic bitter drugs, such as quinine hydrochloride.[6–8] In addition, we have recently reported a quantitative analytical method for the evaluation of the bitterness of antibiotics such as clarithromycin,[9] Chinese medicine,[10] and bitterness-masked famotidine orally disintegrating tablets.[11]

The bitterness prediction method using the taste sensor has already been established in principle in several studies, particularly with regard to basic bitter drugs. Some bitter substances, however, show lower sensor outputs and require an improved bitterness sensor with respect to sensitivity and accuracy. The improved bitterness sensor, BT0, has recently been developed by optimizing the hydrophobicity of the membrane surface and enhancing the hydrophobic interaction between the membrane and basic bitter substances.

The bitterness of eight H_1-receptor antagonists, which are known to be basic bitter substances, was evaluated using the newly developed sensor BT0, and the data obtained were compared with the data from a conventional bitterness sensor, AN0. Three variables, relative value (R), change in membrane potential caused by adsorption (CPA), and adsorption rate (CPA/R), were used in the data analysis.

2. Materials and Methods

2.1 *Chemicals*

Quinine hydrochloride and eight H_1-receptor antagonists, cetirizine dihydrochloride, diphenhydramine hydrochloride, chlorpheniramine maleate, epinastine hydrochloride, ketotifen fumarate, olopatadine hydrochloride, fexofenadine hydrochloride, and azelastine hydrochloride were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan. All other reagents were of special reagent grade.

2.2 *Taste-sensing system*

The taste-sensing system, SA402B, of Intelligent Sensor Technology Inc. (Atsugi, Japan) was used to measure the electric potential of various concentrations of the eight

H_1-receptor antagonists, as shown in Fig. 1. The detecting sensor part of the equipment, which is attached to a mechanically controlled robot arm, consists of a reference electrode and multiple sensors acting as working electrodes. Four different types of sensor, BT0, AN0, C00, and AE0, were used for this study. Sensors AN0, C00, and AE0 have typically been used for bitterness evaluation to identify basic bitter materials, acidic bitter materials, and astringent materials, respectively. Sensor BT0 is a new and improved bitterness sensor, developed especially to detect basic bitter materials. The hydrophobic interaction between the membrane and basic bitter substances has been enhanced by optimizing the hydrophobicity of the membrane surface on sensor BT0.

Each sensor is composed of a unique artificial lipid-based membrane. The lipid components of the sensors are listed in Table 1. The lipid was mixed in a test tube containing plasticizers, dissolved in tetrahydrofuran, and dried on a glass plate at 30°C to form a transparent thin film. A Ag/AgCl electrode and an inner solution containing 3.33 M KCl and saturated AgCl were used for the reference electrode and sensors. When the taste substances are adsorbed by the sensors, a potential change occurs in the artificial lipid membrane, in the same manner as in the human tongue. The difference between the electric potential of the working electrode and the reference electrode was measured using a high-input impedance amplifier connected to a computer.

The procedure used to measure the sensor output values produced by the adsorption of the samples is summarized in Fig. 2. In the first step, a reference solution (corresponding to saliva) is measured and the obtained electric potential (mV) is defined as V_r. Then,

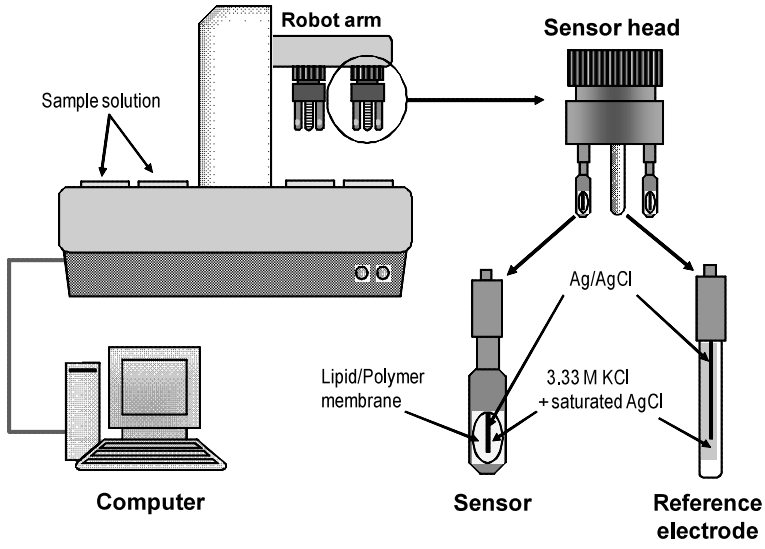

Fig. 1. Overview of the taste sensing system (SA402B).

Table 1
Lipid components of the sensor membranes.

Sensor	Lipid	Plasticizer
BT0 (Bitterness, basic)	Phosphoric acid didodecyl ester	Bis (1-butylpentyl) adipate tributyl o-acetylcitrate
AN0 (Bitterness, basic)	Phosphoric acid didodecyl ester	Dioctylphenyl phosphate
C00 (Bitterness, acidic)	Tetradodecyl ammonium bromide	2-Nitro phenyloctyl ether
AE1 (Astringency)	Tetradodecyl ammonium bromide	Di-*n*-octylphenyl phosphonate

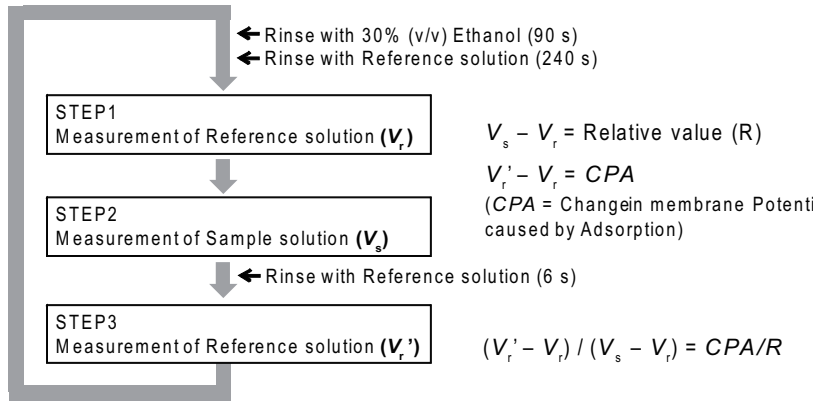

Fig. 2. Taste measurement procedure.

a sample solution is measured and the electric potential is defined as V_s. The relative sensor output is represented by the difference ($V_s \pm V_r$) between the potentials of the sample and the reference solution. The electrodes are subsequently rinsed with fresh reference solution for 6 s. When the electrode is dipped into the reference solution again, the new potential of the reference solution is defined as V_{r0}. The difference ($V_{r0} \pm V_r$) between the potentials of the reference solution before and after sample measurement is the change in the membrane potential caused by adsorption (*CPA*) and corresponds to the so-called 'aftertaste.' In this experiment, the measurement time was set at 30 s. After the measurement of each sample, the electrodes are rinsed first with 30% (v/v) ethanol for 90 s and then with fresh reference solution for 240 s. The rinsing steps are intended to exclude cross-contamination among samples, and are performed after each set of measurements. The measurement of each sample is repeated four times and the average value of the last three measurements is used in the data analysis.

2.3 *Sample preparation*

The H_1-receptor antagonists were completely dissolved in 10 mM KCl aqueous solution. The reference solution, corresponding to saliva, which is tasteless, was composed of 30 mM KCl and 0.3 mM tartaric acid.

3. Results and Discussion

3.1 *Sensitivity of sensors*

CPA of quinine hydrochloride, a typical standard bitter substance, and eight H_1-receptor antagonists, as measured using sensors BT0 and AN0, is shown in Table 2. Using BT0, *CPA* was 10 mV or higher in all the samples except cetirizine and olopatadine, even at low sample concentrations of 0.01 mg/ml. Using AN0, *CPA* was below 5 mV for all the samples except epinastine hydrochloride, and it was demonstrated that bitterness evaluation at concentrations at or below 0.01 mg/ml was no longer reliable. The BT0 membrane was 6.9 times more sensitive, on average, than the AN0 membrane. *CPA* was crealy obtainable in a lower concentration of sample solution using sensor BT0. At a 0.1 mg/ml sample concentration, a relatively high concentration, sensor BT0 still showed higher output values, suggesting, on average, a threefold higher sensitivity. Therefore, sensor BT0 offers a useful bitterness sensor at any concentration of sample solution.

Overall, for basic drug solutions such as the H_1-receptor antagonists, the sensor output values obtained from BT0 were significantly larger than those obtained from AN0; these larger output values are advantageous for not only qualitative but also quantitative taste evaluation of various medicines.

Table 2
CPA of quinine hydrochloride and H_1-receptor antagonist solutions measured using BT0 and AN0.

Drug	*CPA* (mV) of sample solution (0.01 mg/ml)		*CPA* (mV) of sample solution (0.1 mg/ml)	
	BT0	AN0	BT0	AN0
Quinine hydrochloride	12.72	2.36	63.26	17.27
Cetirizine dihydrochloride	8.01	4.56	45.46	28.43
Diphenhydramine hydrochloride	23.61	4.86	71.35	25.38
Chlorpheniramine maleate	18.83	0.84	56.20	8.48
Epinastine hydrochloride	25.57	10.47	71.78	39.31
Ketotifen fumarate	12.84	3.20	42.97	16.15
Olopatadine hydrochloride	4.53	3.90	37.12	24.50
Fexofenadine hydrochloride	15.54	3.50	64.43	24.19
Azelastine hydrochloride	20.12	1.33	82.68	21.07

3.2 *Linearity of sensor output*

To confirm the linearity of the sensor output, Fig. 3 shows the relationship between the drug concentration and *CPA*, obtained using sensors BT0 and AN0, of eight

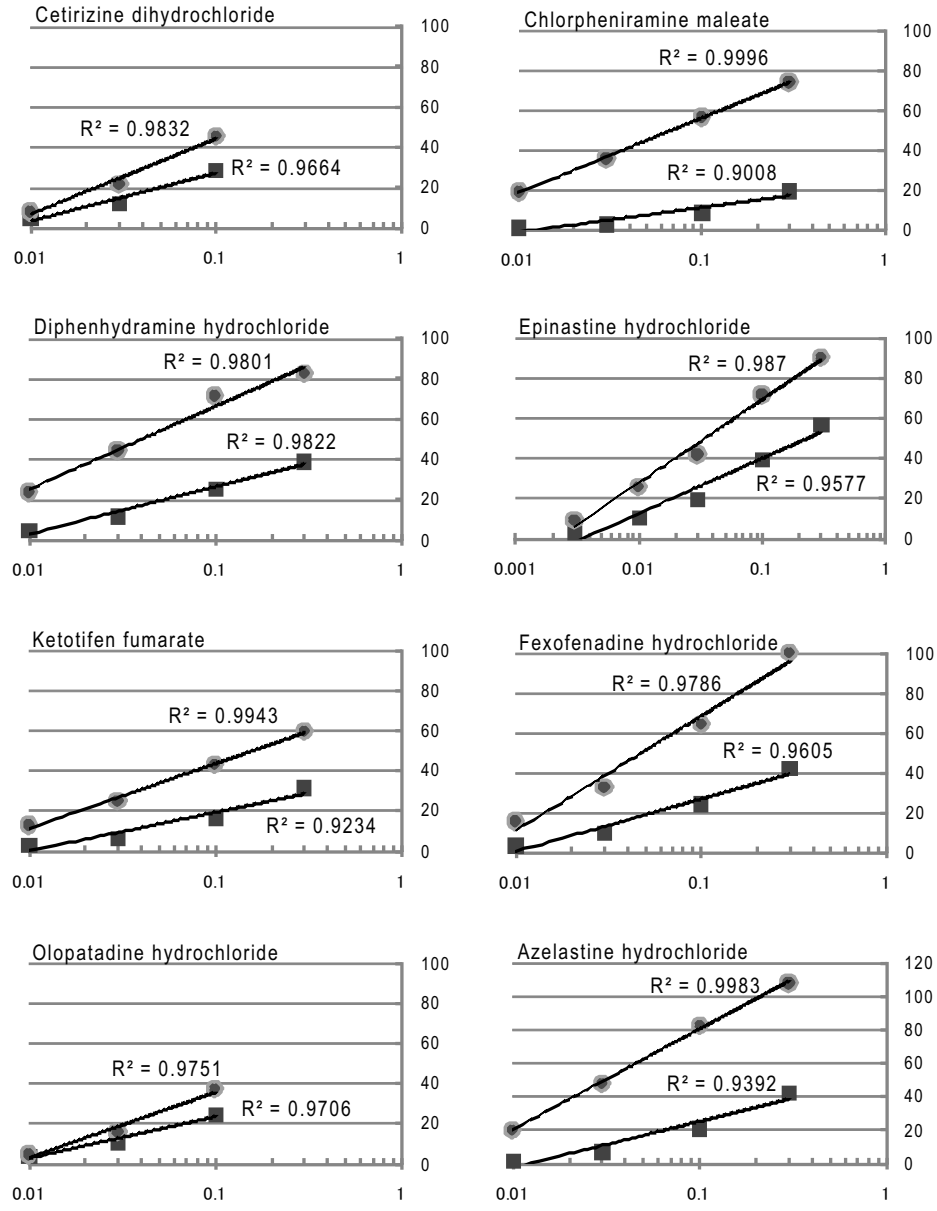

Fig. 3. Relationship between drug concentrations and *CPA*. *x*-axis, drug concentration (mg/ml); *y*-axis, *CPA* (mV). ●, BT0 sensor; ■, AN0 sensor.

H_1-receptor antagonists. For all the compounds, better correlation coefficients were calculated using the data from sensor BT0. Sensor BT0 showed a significantly higher linearity and from 1.52- to 3.03-fold steeper slopes on the charts. Therefore, it was concluded that sensor BT0 offers a more precise bitterness evaluation at a wider range of sample concentrations than sensor AN0.

3.3 *Adsorption ability*

The *CPA*/*R* values, which characterize the ability of a bitter substance to adsorb onto the sensor surface, are shown in Table 3. The *CPA*/*R* values measured with sensor BT0 were higher than those measured with sensor AN0. Therefore, it was concluded that the hydrophobic interaction between the BT0 membrane and the H_1-receptor antagonists was enhanced. To interpret this difference in *CPA*/*R* between drugs, we looked at the relationship between the *CPA*/*R* value and log *P*, the partition coefficient, which is known to be correlated with hydrophobicity. However, no relationship between log *P* and *CPA*/*R* could be determined. This may be due to the fact that these eight H_1-receptor antagonists have similar hydrophobic properties.

3.4 *Principal component analysis*

Principal component analysis (PCA) is a multivariate analytical method that reduces the dimensional space without loss of information. As shown in previous studies,[12,13] discrimination by PCA of the taste sensor output seems to allow for a better understanding of the obtained data. We conducted PCA using three variables, *R*, *CPA*, and *CPA*/*R*, obtained using taste sensors BT0 and AN0 for the eight H_1-receptor antagonists at two different concentrations (0.01 and 0.1 mg/ml) plus four different concentrations of quinine solution as reference. The PCA data are shown in Figs. 4 and 5. The relative contributions of PC1 and PC2 are described in the chart. The factors PC1 and PC2 can be assumed to represent the bitterness intensity and adsorption ability, respectively.

Table 3
Adsorption ability (*CPA/R*) of H_1-receptor antagonists.

Drug	Log *P**	*CPA/R* (BT0)	*CPA/R* (AN0)
Cetirizine dihydrochloride	3.58	0.37	0.19
Diphenhydramine hydrochloride	2.84	0.54	0.31
Chlorpheniramine maleate	3.58	0.47	0.19
Epinastine hydrochloride	3.07	0.54	0.40
Ketotifen fumarate	3.35	0.52	0.28
Olopatadine hydrochloride	3.48	0.53	0.21
Fexofenadine hydrochloride	5.67	0.48	0.50
Azelastine hydrochloride	4.04	0.57	0.45

*Log *P* was calculated by MarvinSketch.

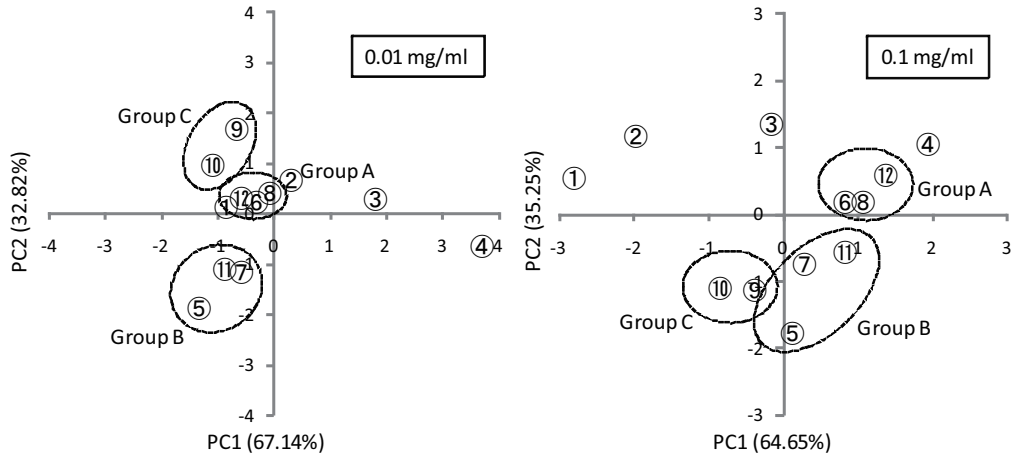

Fig. 4. PCA of data from eight H_1-receptor antagonists using sensor BT0: (1) quinine, 0.01 mg/ml, (2) quinine, 0.03 mg/ml, (3) quinine, 0.1 mg/ml, (4) quinine, 0.3 mg/ml, (5) cetirizine, (6) diphenhydramine, (7) chlorpheniramine, (8) epinastine, (9) ketotifen, (10) olopatadine, (11) fexofenadine, (12) azelastine.

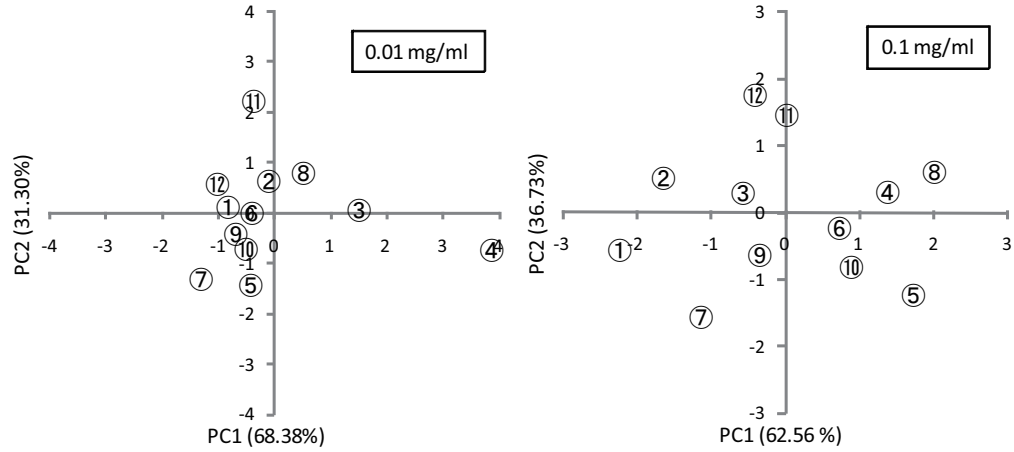

Fig. 5. PCA of data from eight H1-receptor antagonists using sensor AN0: (1) quinine, 0.01 mg/ml, (2) quinine, 0.03 mg/ml, (3) quinine, 0.1 mg/ml, (4) quinine, 0.3 mg/ml, (5) cetirizine, (6) diphenhydramine, (7) chlorpheniramine, (8) epinastine, (9) ketotifen, (10) olopatadine, (11) fexofenadine, (12) azelastine.

In Fig. 4, which shows PCA data from the BT0 membrane, the eight H_1-receptor antagonists can be seen to fall into three groups: group A (diphenhydramine, epinastine, and azelastine), group B (chlorpheniramine, cetirizine, and fexofenadine), and group C (ketotifen and olopatadine). This grouping was not affected by the concentration of the sample solutions. We have not yet precisely determined the basis for this grouping, but it seems likely to be related to the chemical structure. The two drugs in group C have a tricyclic structure, while two of the three compounds in group B, cetirizine and fexofenadine, have a diphenylmethane structure. Therefore, chemical structure appears to be one of the critical factors affecting the taste of the H_1-receptor antagonists. However, as we used only eight H_1-receptor antagonists and multiple parameters can affect the grouping of the drugs, further studies will be necessary to interpret this grouping fully. Meanwhile, it was not possible to obtain any output value from sensor AN0, which seems capable of discriminating between the drugs tested in this PCA.

In PCA conducted using data from the sensor AN0, shown in Fig. 5, the data could not be used to characterize the different groups.

4. Conclusions

The following conclusions were obtained from this study.

(1) At a sample solution concentration of 0.01 mg/ml, sensor BT0 showed 6.9 times greater sensitivity, on average, than sensor AN0. Therefore, sensor BT0 can be used for the evaluation of bitterness at lower concentrations of sample solution.
(2) The highest correlation was obtained between the *CPA* obtained from sensor BT0 and the sample solution concentration. The slope of the linear curve for sensor BT0 was also greater. Therefore, sensor BT0 could predict the bitterness of drugs with much better accuracy than the conventional sensor AN0.
(3) The larger output values for sensor BT0 seem to be due to the stronger hydrophobic interactions between the sample solutions and the membrane surface of sensor BT0. This characteristic seems to be the most positive aspect of the newly developed sensor compared with the conventional one.
(4) PCA using data obtained from sensor BT0 enabled the eight H_1-receptor antagonists to be classified into three groups. The output data from sensor BT0 may allow the characterization of basic bitter drugs without the need for laborious gustatory sensation tests.

Thus, it is concluded that bitterness evaluation of H_1-receptor antagonists using sensor BT0 is more sensitive and more reliable than that using sensor AN0. The use of sensor BT0 would therefore improve the accuracy of bitterness predictions of various types of basic active pharmaceutical compounds.

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