The Bitterness Intensity of Clarithromycin Evaluated by a Taste Sensor

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The purpose of this study was to evaluate the ability of a quantitative prediction method using a taste sensor to determine the bitterness of clarithromycin powder suspensions of various concentrations and of a commercial clarithromycin dry syrup product (Clarith® dry syrup, Taisho Pharmaceutical Co., Ltd., Tokyo) containing aminoalkyl methacrylate polymer as a taste-masker. The bitterness of the clarithromycin dry syrup product dissolved in various beverages was also evaluated in gustatory sensation tests and using the taste sensor. In the sensor measurements, three variables were used to predict bitterness in single and multiple regression analysis: relative sensor output (R), the change of membrane potential caused by adsorption (CPA), and CPA/R ratio. The CPA values for channel 3 of the sensor predicted well the bitterness of clarithromycin powder suspensions and their filtered solutions. For Clarith® dry syrup, the sensor output was small, suggesting that aminoalkyl methacrylate polymer was successful in almost complete masking of the bitter taste of the dry syrup product. When the bitterness intensities of mixtures of 1 g of Clarith® dry syrup with 25 ml of water, coffee, tea, green tea, cocoa, milk, and a sports drink were examined, a good correlation was obtained between the results from human taste tests and the predicted values calculated on the basis of multiple regression analysis using CPA data from channel 4, and the CPA/R ratio from channel 3 of the taste sensor ($r^2=0.963$, $p<0.005$). Co-administration of 1 g of Clarith® dry syrup with an acidic sports drink was found to be the most bitter using either method.

Key words taste sensor; bitterness intensity; clarithromycin; Clarith® dry syrup; taste-masking; sports drink

The bitterness of human pharmaceutical medicines plays a critical role in patient compliance, as the oral administration of bitter drugs is often hampered by their unpleasant taste, leading to noncompliance and thus decreasing therapeutic efficacy, especially in children and the elderly. The quantitative evaluation of the bitterness of medicines is, therefore, an important factor in drug formulation design.

The macrolide clarithromycin is active against penicillin-susceptible and penicillin-intermediate pneumococci, as well as *Moraxella catarrhalis*, and is used to treat various diseases, especially *Helicobacter pylori*[1] and pediatric infections.[2] Clarithromycin treatment has been reported to result in good bacteriological eradication and high overall clinical efficacy. Due to the high bitterness intensity of clarithromycin, however, several approaches have been taken to masking its bitterness in pharmaceutical formulations.[3—5]

In the present study, our aim was to evaluate the bitterness of clarithromycin quantitatively using a taste sensor. The taste sensor, an 'electric tongue' with global selectivity, was developed by Toko and has been applied to evaluation of the taste of foodstuffs. It comprises several different lipid/polymer membranes which are able to transform information about substances producing taste into electrical signals.[6—9] The sensor output has been shown to produce similar patterns for groups of chemical substances with similar tastes.

We have previously evaluated the bitterness of several human pharmaceuticals using the taste sensor and have suggested that the sensor could be useful in providing quantitative predictive data on the bitterness of commercial medicines.[10—14]

In the present study, we firstly evaluated the bitterness of various concentrations of clarithromycin, in solution and suspension, using the taste sensor. Secondly, we determined the bitterness of a commercial dry syrup product (Clarith® dry syrup, Taisho Co. Ltd., Tokyo), which contains aminoalkyl methacrylate polymer as a taste-masking agent, in order to investigate the effect of taste-masking on the sensor output. Lastly, the effect of mixing Clarith® dry syrup with various beverages (water, coffee, tea, green tea, cocoa, milk, and sports drink) on perceived bitterness, was also evaluated in gustatory sensation tests and using the taste sensor.

Experimental

Materials Clarithromycin powder and clarithromycin dry syrup (Clarith® dry syrup) containing 100 mg clarithromycin per 1-g sachet, were donated by Taisho Co. Ltd., Kyoto, Japan. Clarithromycin powder was suspended in 10 mM KCl solution and the concentration was adjusted within the range 0.01 to 3.0 mM. Mixtures of Clarith® dry syrup and various beverages were prepared as follows: 1 g of Clarith® dry syrup was dissolved in 10 mM KCl and then suspended in 25 ml of water, coffee (blend 114, UCC, Kobe), tea (day&day tea bag, Nittoh Tea, Tokyo), green tea (Ooi-ocha, Kirin Beverages, Tokyo), cocoa (Van Houten Cocoa, Kataoka Bussann Co. Ltd., Tokyo), milk (Noukyo Gyuunyu, Tokyo), and sports drink (Aquarious®, Japan-coca-cola, Tokyo) for 1 min. The test samples were mixed well before being used for sensor measurements or gustatory sensation tests. Quinine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.), dissolved, and diluted to produce solutions between 0.0667 and 1.0 mM. All other reagents were of special reagent grade.

Determination of the Solubility of Clarithromycin Clarithromycin powder suspensions of various concentrations (0.01, 0.03, 0.1, 0.3, 0.5, 1.0 mM) were filtered through a membrane filter of 0.45-μm pore size and the clarithromycin concentrations of the filtered solutions were determined using HPLC: 100 μl was injected onto a chromatograph (Shimadzu LC-10A, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-10AV), an integrator (Shimadzu C-R6A), and a reversed-phase column (Cosmosil 5C18-AR, 4.6×150 mm, Nacalai Tesque Co., Ltd., Kyoto). The following mobile phase system was used: A, 1/15 M monobasic potassium phosphate; B, acetonitirile; (A : B=13 : 7). The flow rate was adjusted so that the retention time for the clarithromycin peak was about 8 min. The wavelength was set at 210 nm.

Gustatory Sensation Test The gustatory sensation tests were performed with human volunteers according to a previously described method.[15,16] The standard quinine hydrochloride concentrations used were 0.0667, 0.01, 0.03, 0.10, 0.30, and 1.00 mM and the corresponding bitterness scores were defined as −1, 0, 1, 2, 3, and 4, respectively. Before testing, the volunteers ($n=9$) were asked to keep the above standard quinine solutions in

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their mouths, and were told the concentrations and bitterness scores of each solution.

Clarithromycin powder suspensions of various concentrations (0.01, 0.03, 0.1, 0.3, 0.5, 1.0, 3.0 mM), their corresponding filtered solutions, and suspensions of Clarith® dry syrup in various beverages (1 g in 25 ml of water, coffee, tea, green tea, cocoa, milk, or a sports drink) were evaluated in human gustatory sensation tests. After tasting each study sample, the volunteers were asked to give the sample a bitterness score. All samples were kept in the mouth for 15 s. After tasting the sample, subjects gargled well and waited for at least 20 min before tasting the next sample.

Sensor Measurements of Clarithromycin Powder and Clarith® Dry Syrup Suspensions The taste sensor system and the lipid components of the sensor used in the present study are essentially same as those described in a previous paper.[11,12] The taste sensor system, SA402B of Intelligent Sensor Technology Co., Ltd., Atsugi, Japan, was used to measure the electric potential of clarithromycin. The electrode set was attached to a mechanically controlled robot arm. The detecting sensor part of the equipment consists of eight electrodes composed of lipid/polymer membranes. Each lipid was mixed in a test tube containing poly(vinylchloride) and dioctylphenylphosphonate as a plasticizer, dissolved in tetrahydrofuran, and dried on a glass plate at 30 °C to form a transparent thin film, almost 200 μm thick. The electrodes consisted of an Ag wire whose surface was plated with Ag/AgCl, with an internal cavity filled with 3 M KCl solution. The difference between the electric potential of the working electrode and the reference electrode was measured by means of a high-input impedance amplifier connected to a computer.

Samples of clarithromycin suspensions in the concentration range 0.01—3.0 mM, their filtered solutions, and Clarith® dry syrup (1 g suspended in 25 ml of water, coffee, tea, green tea, cocoa, milk, or a sports drink), were evaluated. Fresh 30 mM KCl solution containing 0.3 mM tartaric acid (corresponding to saliva) was used as the reference sample (Vr) and also to rinse the electrodes after every measurement. The method used to measure the sensitivity and the selectivity of adsorption of the samples is summarized in Chart 1. The electrode is first dipped into the reference solution (Vr) and then into the sample solution or suspension (Vs). The relative sensor output is represented as the difference (Vs−Vr) between the potentials of the sample and the reference solution. When the electrode is dipped into the reference solution again, the new potential of the reference solution is defined as Vr′. The difference (Vr′−Vr) between the potentials of the reference solution before and after sample measurement is defined as CPA (change of membrane potential caused by adsorption) and corresponds to aftertaste. Each measuring time was set 30 s, and the electrodes were rinsed after each measurement. In the present study, relative sensor output values (R), CPA values, and CPA/R were used to predict the bitterness of clarithromycin powder and dry syrup suspension.

Statistical Analysis The difference between the bitterness intensity of clarithromycin powder suspensions and that of Clarith® dry syrup suspensions was analysed using the Student's unpaired t-test. A value of $p<0.01$ was accepted as indicating a significant differences between values.

S-PLUS 2000J (Mathematical Systems, Inc.,Tokyo, Japan) was used for regression analysis.

Results and Discussion

The Response Electric Potential Patterns for Clarithromycin Powder and Clarith® Dry Syrup Suspension The change of membrane potential caused by adsorption, representing the after-taste profile of bitterness, has been used as a criterion for bitterness intensity.[11,13] Figure 1 shows the response electric potential pattern of CPA values for 3 mM clarithromycin powder suspension (A) and 1 g of Clarith® dry syrup (containing 100 mg of clarithromycin) suspended in 25 ml of water (B). When 100 mg of clarithromycin powder was directly suspended in 25 ml of water, its concentration was calculated to be 5.35 mM. Thus the control concentration for the dry syrup suspension should have been 5.35 mM, almost twice that of the 3 mM powder suspension.

As shown in Fig. 1A, the clarithromycin powder suspension shows positive CPA values in channels 1 to 4 but no electric responses in channels 5 to 8. The positive value in

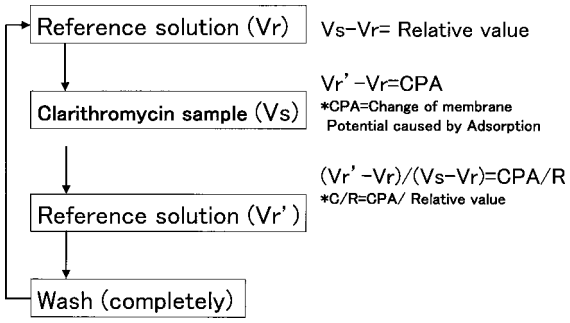

Chart 1. Measuring Procedure in This Study

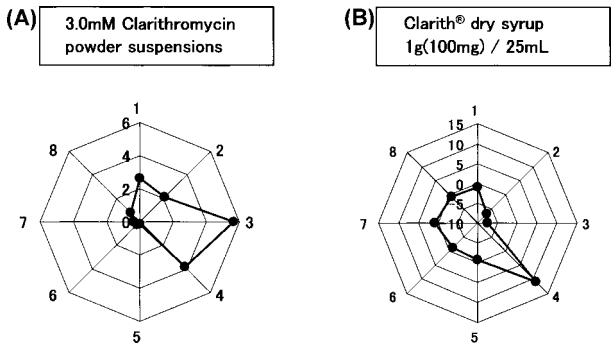

Fig. 1. The Response Electric Potential Pattern of CPA Values for (A) 3 mM Clarithromycin Powder Suspension and (B) 1 g of Clarith® Dry Syrup (Containing 100 mg Clarithromycin) Suspended in 25 ml of Water

channels 1 to 4 are due to the positive charge of clarithromycin was gave to the surface on sensor membrane. The CPA value in channel 3 shows the largest sensor output.

The dry syrup suspension shows small or slightly negative CPA values in almost all channels (Fig. 1B). A positive charge, which is unlikely to be due to clarithromycin itself, was observed only in channel 4. The CPA values for channel 3 of the dry syrup suspension were significantly smaller than those of the 3 mM clarithromycin powder suspension, even though the clarithromycin concentrations of the two samples are similar (*i.e.*, if it is assumed that all the clarithromycin is completely dissolved). Thus the clarithromycin itself shows the comparatively large response to channels 3 and 4, whereas the response of dry syrup product to channel 3 was small. This low value of channel 3 in dry syrup is due to the well controlled-released characteristics of the dry syrup product, and the data means the clarithromycin was more spectic to channel 3 rather than channel 4. The sensor ouput of channel 4 in dry syrup product belongs to the clarithromycin and additives such as sweeteners. As described in the information sheet for Clarith® dry syrup, the product contains alkaline additives which maintain the alkaline pH of clarithromycin suspensions. Under alkaline conditions, the solubility of the clarithryomycin is very low. The sensor data thus reflect the controlled-release characteristics of the dry syrup product. The taste sensor data of channel 3 in dry syrup product means coating effectiveness for the bitterness.

Gustatory Sensation Tests of Clarithromycin Powder and Clarith® Dry Syrup Suspension Clarithromycin is poorly soluble and has a low solubility rate. In these experiments, the clarithromycin powder was in suspension rather than solution, even though considerable physical means (heat for 1 h at 50 °C, agitation, and sonication) were used to try to

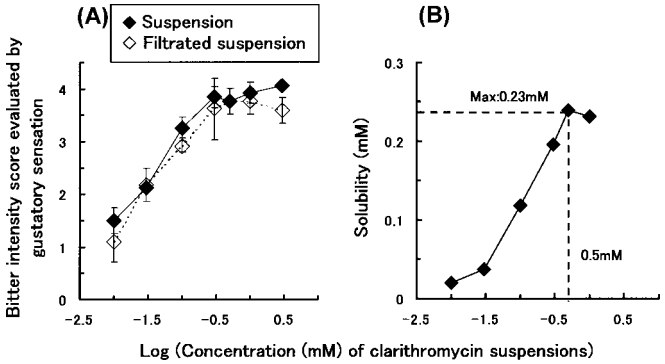

Fig. 2. (A) Gustatory Sensation Test Results for Various Concentrations of Clarithromycin Powder Suspensions and Their Filtered Solutions

The *Y*-axis shows the bitterness scores, using quinine as reference drug, while the *X*-axis represents the log concentration of the powder suspensions. For further explanation, see text.

(B) Solubility Data for Various Concentrations of Clarithromycin Suspensions

The data are the means of duplicate samples.

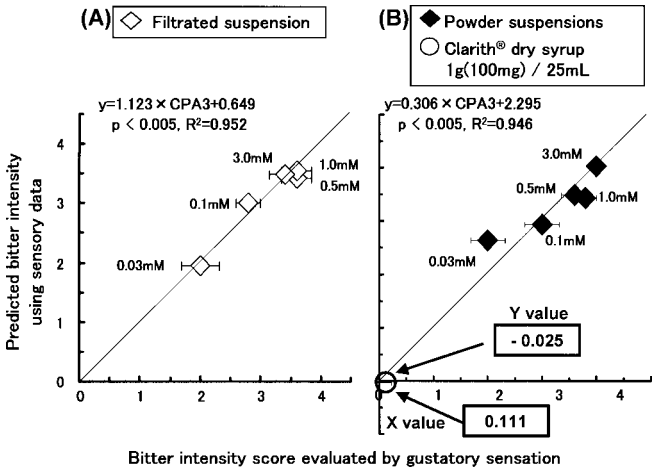

Fig. 3. The Relationship between Bitterness Intensity Scores Obtained in Human Gustatory Sensation Tests and the Predicted Bitterness Scores Derived from the Taste Sensor Output (CPA Value of Channel 3)

Figure 3A shows data from the filtered solutions, while Fig. 3B shows data from the clarithromycin suspensions and Clarith® dry syrup. For further explanation, see text. Error bars represents the mean plus standard deviation ($n=9$).

dissolve it. (Drug stability during the above treatment was confirmed; data not shown).

Figure 2A shows the gustatory sensation results for various concentrations of clarithromycin powder suspensions and their filtered solutions. As the concentration of clarithromycin increased up to 0.5 mM, so the intensity of bitterness increased, up to a maximum value of 4.0. Over 0.5 mM, the bitterness intensity remained almost constant. In the figure, the closed symbols represent the data from the suspensions while the open symbols represent the filtered solution samples. As expected, the bitterness intensity of the powder suspension was the same or a little greater than that of its filtered solution. The pattern of increase in bitterness intensity with concentration was very similar for the suspensions and their filtered samples.

Figure 2B shows the solubility data for various concentrations of clarithromycin suspensions. Solubility increased with concentration up to 0.5 mM, at which point solubility became constant (at about 0.23 mM). These solubility data reflect quite well the bitterness intensity data obtained in gustatory sensation tests. Drug saturation was achieved in suspensions containing 0.5 mM clarithromycin and above. The bitterness intensity of the dry syrup product was very low (0.111) in the gustatory sensation test.

Bitterness Prediction for Clarithromycin Powder and Clarith® Dry Syrup Suspensions Using the Taste Sensor Figures 3A and B show the relationship between the bitterness scores obtained in gustatory sensation tests and the predicted bitterness intensity calculated from the sensor output using the CPA value of channel 3 (when we used channel 4 data, simultaneous good relationship was also demonstrated: data not shown). The data from the filtered solutions are shown in Fig. 3A, while Fig. 3B shows the data from the powder suspensions and Clarith® dry syrup. In Fig. 3A, the derived regression equation was $Y=1.123\times\mathrm{CPA3}+0.649$ ($r^2=0.952$, $p<0.005$), where Y and X represent the predicted taste sensor value and the observed gustatory bitterness score, respectively. The bitterness of clarithromycin filtered solutions could be predicted with good accuracy using taste sensor data for CPA of channel 3 at concentrations up to 0.5 mM clarithromycin. At higher concentrations the filtered samples were presumably saturated, as the observed and predicted bitterness intensities for filtered solutions of 0.5 mM suspensions and above were essentially the same.

For different concentrations of clarithromycin powder suspensions and dry syrup product suspensions, shown in Fig. 3B, the observed and predicted bitterness intensities were very similar. The derived regression equation was $Y=0.306\times\mathrm{CPA3}+2.295$ ($r^2=0.946$, $p<0.005$), where Y and X represent the predicted and observed bitterness scores, respectively. For the Clarith® dry syrup suspension, the predicted bitterness intensity calculated on the basis of this regression equation was -0.025, which was very close to the value of 0.111 obtained in the gustatory sensation tests. Thus, the bitterness intensity of the dry syrup product was significantly less than that of equivalent clarithromycin powder suspensions.

In addition, as shown in Figs. 3A and B (Also in Fig. 2A), it can be seen that the obtained and predicted bitterness scores of the suspensions were almost same or a little larger than those of the corresponding filtered solutions. Even though we did not know the precise reason for the difference between suspension and filtered solution, possibilities were shown as follows: the first possibility : release rate of clarithromycin may depend on concentration of suspensions. The second possibility is supersaturation in high concentrated clarithromycin powder suspension. The third different idea is the adhesion of undissolved drug particle on the surface of sensor membrane. In this case, high concentrated drug might be exposed to the surface of the artificial membrane. In related to above speculations, we have no evidence yet.

There have been several attempts to achieve taste masking of clarithromycin by physical means, using microspheres[17,18] or coating.[19] In general, macrolide compounds are known to be strongly bitter. There have been reports of taste-masking of clarithromycin using a polymer carrier system, and absorption to Carbopol (a high molecular weight polyacrylic acid).[3] However, insufficient taste suppression was achieved using these techniques. Another approach has been reported by Yajima *et al.*[4,5] These authors have used a simpler preparative method involving spray-congealing, and their technique

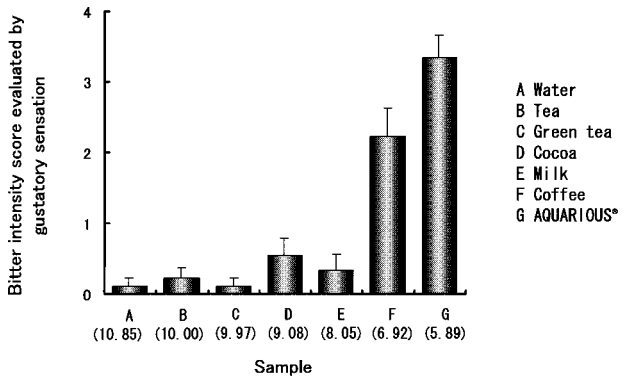

Fig. 4. Bitterness Intensity Scores Obtained in Human Gustatory Sensation Tests for 1 g of Clarith® Dry Syrup Suspended in 25 ml of Water, Coffee, Tea, Green Tea, Cocoa, Milk, or Sports Drink

Figure in parenthesis give the pH of the resulting mixture. Error bars represents the mean plus standard deviation (n=9).

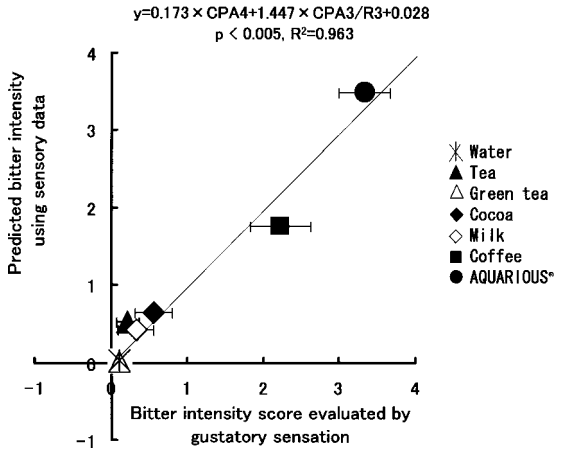

Fig. 5. The Relationship between the Bitterness Intensity Scores Obtained from Human Volunteers and the Predicted Values Calculated from the Equation Derived from Multiple Regression Analysis Using CPA Value of Channel 4, and CPA/R Value for Channel 3

Y and X represent the predicted and observed bitterness scores, respectively. For further explanation, see text. Error bars represents the mean plus standard deviation (n=9).

has already yielded products for the commercial Japanese market (Clarith® dry syrup). They also demonstrated that release of clarithromycin in the mouth is initially very limited. The present taste sensor data obtained for Clarith® dry syrup supports these findings. Thus, the use of the taste sensor may reduce the requirement for gustatory sensation data for taste-masking experimentation, and thereby decrease the number of volunteers needed for gustatory sensation tests. The taste sensor seems to be useful for predicting the bitterness of suspensions for many pharmaceutics.

Prediction of Bitterness Intensity of Suspensions of Clarith® Dry Syrup in Various Beverages Figure 4 shows the observed and predicted bitterness intensities of 1 g of Clarith® dry syrup suspended in 25 ml of water, coffee, tea, green tea, cocoa, milk, and a sports drink. Figures in parenthesis on the X axis show the pH of the resulting mixtures. In general, as the pH of the mixture decreases, so the bitterness intensity increases. Although Clarith® dry syrup product was shown to be a well taste-masked formulation, co-administration with coffee or the acidic sports drink considerably enhanced the bitterness of the preparation. In particular, the acidic sports drink was found to give rise to a strongly bitter taste, as predicted in the information sheet for Clarith® dry syrup.

This phenomenon is predominantly due to the fact that clarithromycin is a basic drug, whose solubility increases with decreasing pH. The pH of clarithromycin suspensions seems to be a critical factor in bitterness perception, and co-administration of any acidic drink such as orange juice or yoghurt with the dry syrup will make the product taste more bitter. In the case of the mixture of coffee and Clarith® dry syrup, the enhanced bitterness may also include a component due to the inherent bitterness of the coffee.

Figure 5 shows the correlation between the gustatory sensation and taste sensor data. Good correlation was obtained between observed bitterness intensity in human volunteers and the predicted values calculated on the basis of multiple regression analysis using the CPA value of channel 4, and CPA/R value for channel 3. The derived regression equation was $Y=0.173\times CPA4+1.447\times CPA3/R3+0.028$ ($r^2=0.963$, $p<0.005$), where Y and X represent the predicted and observed bitterness scores, respectively.

We do not know precisely how the taste sensor detects components which enhance or suppress the bitterness of clarithromycin. In coming study, we would like to measure clarithromycin concentration in the mixture of dry syrup product with various beverages, and to examine wheather the taste sensor are able to predict or not predict the effect of the components involved in beverages on the bitterness of released clarithromycin in various beverages.

In the present study, we did not compare the sensitivity of taste sensor with conventional method such as HPLC method. This issue will be overcome in near future. Nevertheless by using taste sensor, we could discriminate beverages which enhance the bitterness of clarithromycin and those does not enhance the bitterness.

In conclusion, the taste sensor was capable of quantitatively predicting the bitterness of clarithromycin powder suspensions of various concentrations. The bitterness of a commercial clarithromycin dry syrup product (Clarith® dry syrup), when taken with various beverages (water, coffee, tea, green tea, cocoa, milk, and a sports drink) could also be predicted by the taste sensor.

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