

Preparation and Evaluation of Poly- γ -Glutamic Acid Hydrogel Mixtures with Basic Drugs or Acidic Drugs: Effect on Ease of Swallowing and Taste Masking

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Abstract

The purpose of this study was to prepare a poly- γ -glutamic acid hydrogel (PGA gel), to examine its ease of swallowing using texture profile analysis (TPA) and to evaluate its taste-masking effects on basic or acidic drugs using the artificial taste sensor. Using TPA, 0.5% and 1.0% PGA gels, 0.5% and 1.0% agar and 1.0% ι -carrageenan in the absence of drug was examined the hardness, adhesiveness and cohesiveness, ranked according to permission criteria published by the Japanese Consumers Affairs Agency. 0.5% PGA gel and 1.0% agar were classified into grade II. In the taste sensor measurement, the bitterness suppressions by 0.5% PGA gel were larger than that by 1.0% agar in all drugs and the bitterness suppressions of basic drugs in 0.5% PGA gel were more potent than those of acidic drugs in 0.5% PGA gel. ^1H -nuclear magnetic resonance spectroscopic analysis was carried out to examine the difference in mechanism of bitterness suppression between basic drugs and acidic drugs mixed with PGA gel. The signals of the proton nearest to the nitrogen atom of basic drugs shifted clearly upfield, suggesting an interaction between the amino group of basic drugs and the carboxyl group of PGA gel. In conclusion, PGA gel is expected to be a useful excipient in formulations contained various drugs, especially basic drugs; it also has advantage for not only increasing ease of swallowing but also masking the bitterness of drugs even though a small amount of a single drug dose might be preferred.

Keywords

Poly- γ -Glutamic Acid Hydrogel, Bitterness, Taste Sensor

1. Introduction

Taste and ease of swallowing are important factors used to determine the acceptability of oral pharmaceutical formulations. The bitter taste of an active pharmaceutical ingredient may cause poor palatability [1] and consequently reduce drug efficacy through non-compliance, especially in pediatric patients. As many active pharmaceutical ingredients exhibit bitterness, taste masking is an important step in formulation development. There are various strategies for this. Traditionally, bitterness has been masked by the addition of sweeteners or flavours [2] [3]. At the cognitive level, the perceived inhibition of bitterness occurs in the central nervous system in the brain via taste-taste interactions. An alternative approach is to prevent bitterness perception peripherally, using techniques such as encapsulation, molecular inclusion of cyclodextrins [4] [5], complexation with ion-exchange resins [6], tannate [7] [8], fatty acids [9] or food proteins [10] or the addition of bitterness-masking substances such as chlorogenic acid. Electrostatic interactions between basic bitter active ingredients and chlorogenic acid attenuate the binding of the bitter active ingredients to bitterness receptors [11] [12]. A third strategy is the application of bitter taste receptor blockers [13] [14].

Ease of swallowing for medicines in each patient is another critical factor influencing adherence for oral medicines, especially in geriatric populations. The elderly have been reported to exhibit less accurate speech movements [15], more variable orofacial movements [16] [17], and slower and more variable tongue movements during swallowing [18]. Aspiration pneumonia is a common form of pneumonia with especially high mortality in the elderly who are more susceptible due to a deterioration of the swallowing function. It is therefore necessary to develop oral formulations, which can be easily swallowed by elderly patients.

Oral jellies are generally easy to swallow and be also useful to prevent the drug refusal in pediatric population. But their manufacturing method is complicated due to the need for transition from a solid by heating to a gel by cooling, while being molded into an appropriate shape.

Murakami *et al.* reported the preparation of a biodegradable poly- γ -glutamic acid (PGA) gel by cross-linking of PGA with L -Lysine (L -Lys) by amide linkage in an environmentally benign aqueous solvent. PGA is a biodegradable polymer, produced by *Bacillus subtilis* var. *natto*. The key feature of PGA gel is that gelation occurs immediately on the addition of water to a powdered form of the PGA gel. It has been reported that the water absorption of PGA gel ranged from 300 to 2100 g/g [19].

Therefore, active pharmaceutical ingredients which are vulnerable to heat can be made into solid oral formulations using the powdered form of PGA gel and could be gelated easily by simple addition of water.

PGA gel consists of poly- γ -glutamic acid and L -Lysine hydrochloride. Acceptable Daily Intake (ADI) of poly- γ -glutamic acid is 26 - 27 mg/kg/day which described in Japan's Specifications and Standards for Food Additives (JSFA).

ADI of L-Lysine hydrochloride is 34 - 40 mg/kg/day which described in safety dates of Japan Pharmaceutical Excipients Council (JPEC). The amount of 0.5% PGA gel was defined 25 mg/5 mL/single dose. Therefore, PGA gel used in single dose in human could be judged to be safe in terms of the toxicity.

PGA is already known to be a safe excipient and is used as a food additive to assist the absorption of minerals [20] [21]. To our knowledge, however, there have been no reports in which PGA gel has been used in a pharmaceutical preparation to enable taste-masking and improve ease of swallowing.

In this study, we focused on the use of PGA gel in formulations for geriatric and pediatric patients as the gel was expected to enable easy swallowing. In addition, Tsuji *et al.* reported that commercial gel formulations could suppress drug bitterness by protecting the active ingredient from direct exposure to the tongue by creating a physical barrier [22].

In the present study, we chose four drugs as model drugs, *i.e.*, benazepril hydrochloride (BEN) and ambroxol hydrochloride (AMB) as basic drugs and diclofenac sodium (DIC) and etodolac (ETO) as acidic drugs, since we previously confirmed that these four drugs have bitterness [23] [24] [25]. BEN is used in the elderly to treat hypertension at a single dose of 5 - 10 mg. AMB is used to treat acute bronchitis and bronchial asthma at a single dose of 15 mg and has dry syrup and liquid syrup as oral formulation for pediatric patients. DIC and ETO are non-steroidal anti-inflammatory drugs and widely used to treat a pain, at a single dose of 25 mg and 200 mg, respectively.

The use of an “electronic tongue” or taste sensor for pharmaceutical purposes is an innovation which reduces dependence on human gustatory sensation testing. We have previously evaluated the bitterness of several registered basic or acidic medicines using the taste sensor [12] [23] [25]-[31].

The purpose of this study was to prepare a PGA hydrogel, to evaluate its ease of swallowing using texture profile analysis (TPA) and to examine its taste-masking effect when loaded with basic or acidic drugs using the artificial taste sensor.

In TPA, the hardness, adhesiveness and cohesiveness of 0.5% w/v and 1.0% w/v PGA gels, 0.5% w/v and 1.0% w/v agar and 1.0% w/v ι-carrageenan were examined in the absence of drug, and determined whether or not these formulations were within the range of permission criteria for people with difficulty swallowing, as published by the Japanese Consumers Affairs Agency.

In order to predict the bitterness of four drugs mixed with 0.5% w/v PGA gel and 1.0% w/v agar, the sensor outputs of AN0, the basic bitterness-sensitive sensor and C00, the acidic bitterness-sensitive sensor in the artificial taste sensor, were measured. The results of these tests confirmed the potent bitterness-suppressing effect of PGA gel containing BEN, AMB, DIC and ETO.

Finally, ¹H-nuclear magnetic resonance (NMR) spectroscopic analysis was carried out to examine the difference in mechanism of the bitterness suppression of four drugs when mixed with PGA gel.

2. Material and Methods

2.1. Materials

PGA, average molecular weight 200,000 - 500,000, L-Lysine monohydrochloride (L-Lys), 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride n-hydrate (DMT-MM), Diclofenac sodium and Etodolac were obtained from Wako Pure Chemical Industries, Ltd. (Japan), Benazepril hydrochloride from Tokyo Chemical Industry Co. (Japan), Ambroxol hydrochloride from LKT Labs, Inc. (USA) and agar purified powder from Nacalai Tesque, Inc. (Japan). Quinine hydrochloride (Qui), used as a standard of basic bitterness and iso-alpha-acid, used as a standard of acidic bitterness were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and Intelligent Sensor Technology Inc. (Atsugi, Japan), respectively. The structure of BEN, AMB, DIC and ETO are shown in **Figure 1**.

2.2. Preparation of PGA Hydrogel

PGA gel was prepared according to the method described in the report of Murakami *et al.* [19]. The method can be summarized as follows: PGA was placed in a glass vessel and dissolved in 1.0 M aqueous sodium hydroxide (NaOH) solution. L-Lys was dissolved in water and adjusted to pH 8.9 by the addition of 1.0 M NaOH. The solution of L-Lys was added to the solution of PGA and the mixture stirred for 30 minutes. DMT-MM dissolved in water was then added to the solution of PGA/L-Lys and the mixture stirred for four hours at 25°C. The reaction product was then immersed in 0.1 M phosphate buffer (pH 7.0) for 24 h, before adjusting the pH to 8.0 by adding 1.0 M NaOH.

The swelled product was transferred into a nylon mesh bag and filtered. The product, which became PGA gel, was washed by immersing the bag in purified water, which was changed once a day for a week. Powdered PGA gel was obtained by lyophilization of the PGA gel.

2.3. Water Absorption of PGA Gel

The water absorption of PGA gel was measured using the method described in a previous report [19]. The powdered PGA gel was weighed and placed in a nylon mesh bag, similar to that used for washing the gel. The bag was immersed in purified water for 24 h at 25°C. After 24 h, it was hung for 10 min to remove excess water and weighed. In order to exclude water absorption by the nylon mesh bag, the operation was carried out using an empty nylon bag as reference (n = 3). The water absorption (g/g) of PGA gel was calculated using the following equation:

$$\text{Water absorption} = (W_s - W_p) / W_p$$

W_s : the weight of the swelled PGA gel.

W_p : the weight of the powdered PGA gel.

2.4. Texture Profile Analysis (TPA)

TPA was carried out to evaluate the physical properties of the gel using TA.XT *plus C* texture analyzer (Stable Micro Systems, Surrey, UK). Samples tested were

measurements were performed as follows, according to our previous articles [12] [23] [25]-[31].

The electrode set was attached to a mechanically controlled robot arm. The detecting sensor part of the equipment consists of a reference electrode and a working electrode composed of lipid/polymer membranes. The electrodes have an internal cavity filled with 3.3 M KCl solution. 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. Fresh 30 mM KCl solution containing 0.3 mM tartaric acid (corresponding to saliva) was used as the reference solution and also to rinse the electrode after every measurement.

The measurement procedure is as follows: The electrodes are dipped first into the reference solution and the electric potential obtained (mV) is defined as V_{r_0} . Then a sample solution is measured and the electric potential obtained defined as V_s . The relative sensor output (R), represented by the difference between the potentials of the sample and the reference solution ($V_s - V_{r_0}$), and corresponds to the “taste immediately after putting in the mouth”. The electrodes are subsequently rinsed with a fresh reference solution for 6 s. When the electrodes are dipped into the reference solution again, the new potential of the reference solution is defined as V_{r_1} . The difference between the potentials of the reference solution before and after sample measurement ($V_{r_1} - V_{r_0}$) is defined as the “Change in the membrane Potential caused by Adsorption” (CPA), and corresponds to the so-called “aftertaste”. CPA is a specific expression of bitterness. In this study, the CPA of AN0 and C00 were used to evaluate bitterness and the R was used to evaluate each taste except bitterness. These concentrations, 0.03% w/v monosodium glutamate (MSG) for umami, 0.0012% w/v tartaric acid (Tar) for sourness, 0.25% w/v sodium chloride (NaCl) for saltiness, 0.001% w/v Qui for bitterness of basic substances, 0.01% w/v iso-alpha-acid for bitterness of acid substance and 0.075% w/v tannic acid (Tan) for astringency, represent the threshold for control substances for each taste.

Firstly, we measured the predicted taste (umami, sourness, saltiness, bitterness of basic and acidic substances and astringency) of 0.5% w/v PGA gel using 1.0% w/v agar as a reference. Secondly, we evaluated bitterness-masking of four drugs by the two different gels individually.

For prediction of taste of PGA gel, PGA gels at concentrations of 0.5% w/v were prepared by adding purified water. Agar (1.0% w/v) was prepared by dissolving 50 mg of agar purified powder in 5 mL purified water and heating.

For evaluation of bitterness-masking, BEN (5 mg), AMB (15 mg), DIC (25 mg) and ETO (200 mg) were added to 0.5% w/v PGA gel and 1.0 % w/v agar, respectively, and mixed for 10 seconds. In order to examine simulated bitterness in the oral cavity in a time-dependent manner, mixtures of the drug and gel were placed in a mesh filter and immersed in 20 mL purified water (37°C). Solutions were tested in the taste sensor after immersion for 5, 15 and 30 seconds.

2.6. Determination of Concentrations of Drugs Eluted from Two Different Gels

In order to examine drugs elution from the two gels in a time-dependent manner, the concentration of drugs was determined using high-performance liquid chromatography (HPLC; LC-2010C, Shimadzu Corp., Japan), an integrator (LC solution; Shimadzu Corp., Japan) and a reverse-phase column (CAPCELL PAK C18 UG120 S5: 150 mm × 4.6 mm i.d.; Shiseido Co., Ltd., Japan) according to the previous reports [24] [32] [33] [34]. For HPLC of BEN, the mobile phase composition was 20 mM phosphate buffer (pH 4.5): acetonitrile (53:47, v/v) and the flow rate was 1.0 mL/min. The ultraviolet detection wavelength and the column temperature were set at 242 nm and 25°C. For AMB, the mobile phase composition was acetonitrile: water (40:60, v/v) and the flow rate was 1.0 mL/min. The ultraviolet detection wavelength and the column temperature were set at 215 nm and 40°C. For DIC, the mobile phase composition was acetonitrile: methanol (80:20, v/v) and the flow rate was 1.0 mL/min. The ultraviolet detection wavelength and the column temperature were set at 276 nm and 25°C. For ETO, the mobile phase composition was methanol: water (20:80, v/v) and the flow rate was 1.0 mL/min. The ultraviolet detection wavelength and the column temperature were set at 220 nm and 27°C. Samples were prepared in the same way as for taste sensor measurement.

2.7. ¹H-NMR Spectroscopic Analysis

¹H-NMR spectra were measured on a JEOL 500 MHz spectrometer using dimethyl sulfoxide (DMSO-*d*₆) as solvent and tetramethylsilane (TMS) as internal standard. Sample solutions of drugs were prepared with/without powdered PGA gel. The mixing ratios of powdered PGA gel to drugs in the sample solution were 1:5 w/w for BEN, 1:1.667 w/w for AMB, 1:1 w/w for DIC and 1:0.125 w/w for ETO corresponding to each drug in 0.5% w/v PGA gel.

2.8. Statistical Analysis

BellCurve for Excel® (Social Survey Research Information Co., Ltd., Japan) was used for statistical analysis. Tukey's test and Dunnett's test were used for multiple comparisons. Correlation was examined using the Pearson product-moment correlation coefficient. The 5% level of probability was considered significant.

3. Results and Discussion

3.1. Water Absorption of PGA Gel

The water absorption of powdered PGA gel, prepared according to the method described in a previous report [19], was 653 ± 87 g/g (n = 3, mean ± SD). This suggests that the PGA gel was prepared successfully, as the water absorption was within the range described previously.

3.2. Evaluation of the Physical Properties of Five Gels Using TPA

Figure 2 shows the permission criteria according to which foodstuffs may be considered suitable for persons with difficulties swallowing, as published by the Japanese Consumers Affairs Agency [35] and the physical properties (hardness, adhesiveness or cohesiveness) of five gels.

The permission criteria are divided into three grades, defined as I, II and III, with grade I describing the most suitable conditions. The overall grade of each sample is decided on the permission criteria of its highest grade for any parameter (hardness, adhesiveness or cohesiveness). For hardness, the value of grade I is from 2500 (N/m²) to 10,000 (N/m²), the value of grade II is from 1000 (N/m²) to 15,000 (N/m²), the value of grade III is from 300 (N/m²) to 20,000 (N/m²). For adhesiveness, the value of grade I is 400 or less (J/m²), the value of grade II is 1000 (J/m²) or less, the value of grade III is 1500 (J/m²) or less. For cohesiveness, the value of grade I is from 0.2 to 0.6, the value of grade II is from 0.2 to 0.9, the value of grade III is not defined.

All five gels were within the range of permission criteria for persons with difficulty swallowing, with grades varying between I and III for the various parameters. Overall, 1.0% w/v PGA gel, 0.5% w/v agar and 1.0% w/v ι-carrageenan were considered grade III, while 0.5% w/v PGA gel and 1.0% w/v agar were grade II.

When values for hardness and adhesiveness are small, the property of the sample is similar to that of a liquid. The values for hardness and adhesiveness of 0.5% w/v agar and 1.0% w/v ι-carrageenan were both small. A grade I product should ideally have a high value for hardness and a low value for adhesiveness. Of the five gels studied, 0.5% w/v PGA gel and 1.0% w/v agar were closest to grade I.

Agar purified powder can be transformed into agar gels that have melting temperatures close to physiological temperatures, while gelation of ι-carrageenan needs not only water but also cation. Conversely, PGA gels are easy to prepare by the addition of water to the powdered form. PGA gel preparation is also a safe procedure, glutamic acid and L-Lys, its main components, being naturally occurring products.

By the following all experiments, we used only 0.5% w/v PGA gel and 1.0% w/v agar.

3.3. Taste Evaluation of PGA Gel in the Absence of Drug by Taste Sensor Measurement

Figure 3 shows sensor responses to 0.5% w/v PGA gel using 1.0% w/v agar as reference for all six sensors. The largest response was from AAE, which was developed specifically to detect umami. The sensor output of AAE was equivalent to the threshold for umami (0.03% w/v MSG). It was considered that the umami was probably derived from the glutamic acid component of PGA. The sensor outputs of the other five sensors were significantly lower than the thresholds for

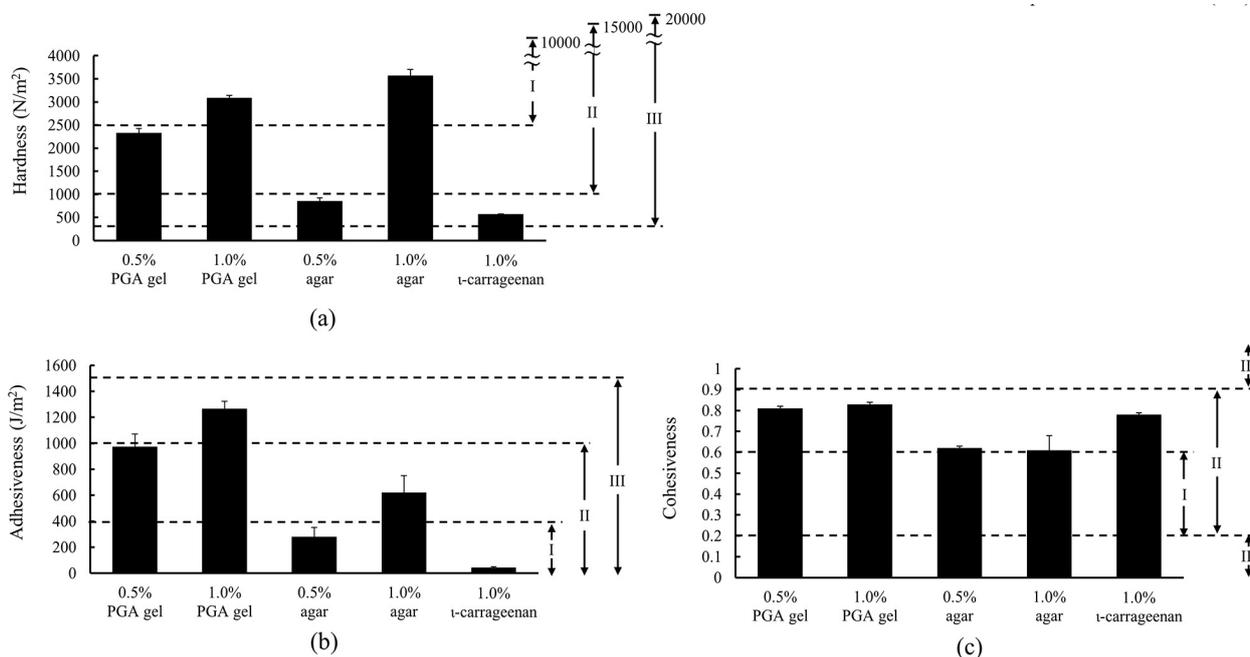


Figure 2. The permission criteria according to which foodstuffs may be considered suitable for persons with difficulties in swallowing, as published by the Japanese Consumers Affairs Agency and the physical properties of five gels; (a) Hardness, (b) Adhesiveness and (c) cohesiveness. Data are expressed in mean \pm SD ($n = 3$).

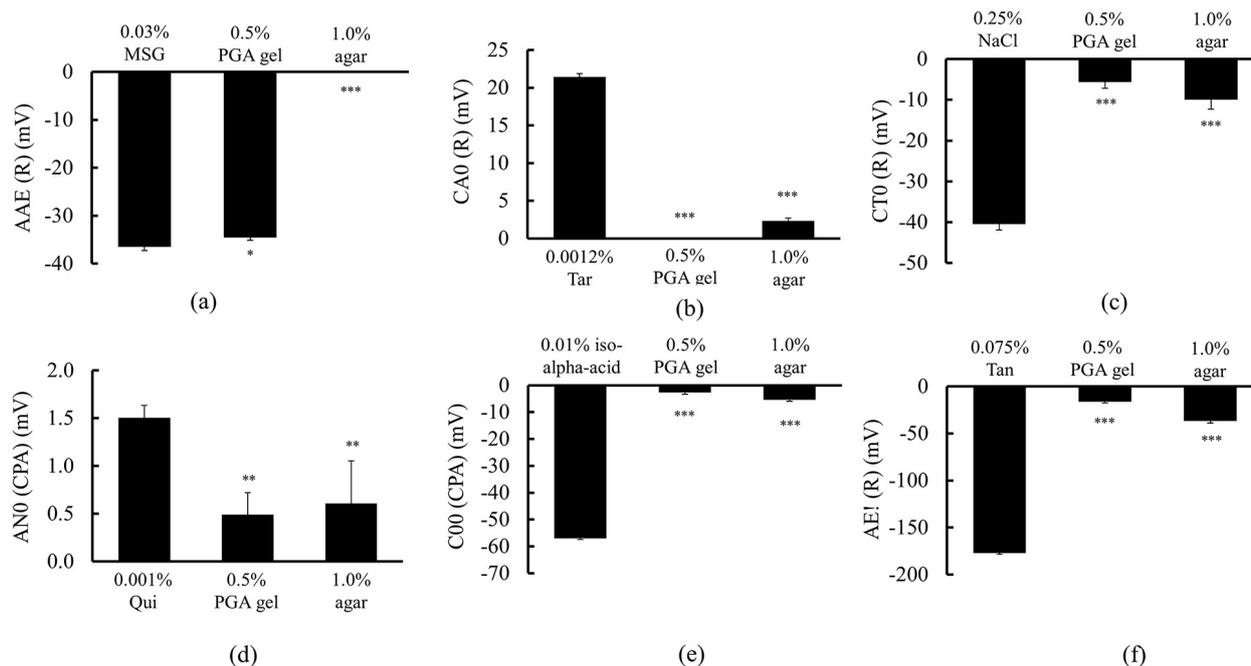


Figure 3. Taste sensor outputs in response to 0.5% w/v PGA gel and 1.0% w/v agar. (a) AAE, (b) CA0, (c) CT0, (d) AN0, (e) C00 and (f) AE1. Data are expressed in mean \pm SD ($n = 3$). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ versus control substances for each taste (Dunnett's test).

each taste. Consequently, PGA gel was suggested to have a slight flavor of umami, and confirmed the acceptability for use in oral pharmaceutical preparations in terms of texture.

3.4. The Influence of Two Different Gels on the Bitterness of Four Drugs: Taste Sensor, Determination of Drug Concentrations

Figure 4 shows the influence of mixtures of four drugs (BEN, AMB, DIC and ETO) with the two different gels on AN0 or C00 sensor outputs. For two basic drugs which are BEN and AMB, the sensor outputs in response to BEN (5 mg per sample) and AMB (15 mg per sample) were significantly reduced in mixtures with each gel. The CPA value of AN0 for a quinine solution with $\tau = 1$, the basic bitterness threshold, was about 2 mV. The sensor outputs in response to BEN mixed with 0.5% w/v PGA gel was equivalent to or beneath the basic bitterness threshold at all times and 0.5% w/v PGA gel suppressed the CPA value of AN0 than 1.0% w/v agar. The sensor outputs in response to AMB mixed with 0.5% w/v PGA gel was higher than the basic bitterness threshold at all times, 0.5% w/v PGA gel, however, suppressed the CPA value of AN0 than 1.0% w/v agar as with BEN. On the other hand, for two acidic drugs which are DIC and ETO, the CPA value of C00 for an iso-alpha-acid solution with acidic bitterness threshold was about 50 mV. The sensor outputs in response to DIC (25 mg per sample) was significantly reduced in mixtures with each gel and those in response to DIC mixed with each gel were beneath the acidic bitterness threshold at all times except a sample of 1.0% w/v agar after 30 seconds. 0.5% w/v PGA gel suppressed the CPA value of C00 than 1.0% w/v agar. The sensor outputs in response to ETO (200 mg per sample) was significantly reduced in mixtures with 0.5% w/v PGA gel, while not reduced in mixtures with agar except a sample of 5 seconds later. The sensor outputs in response to ETO mixed with 0.5% w/v PGA gel were beneath the acidic bitterness threshold at all times.

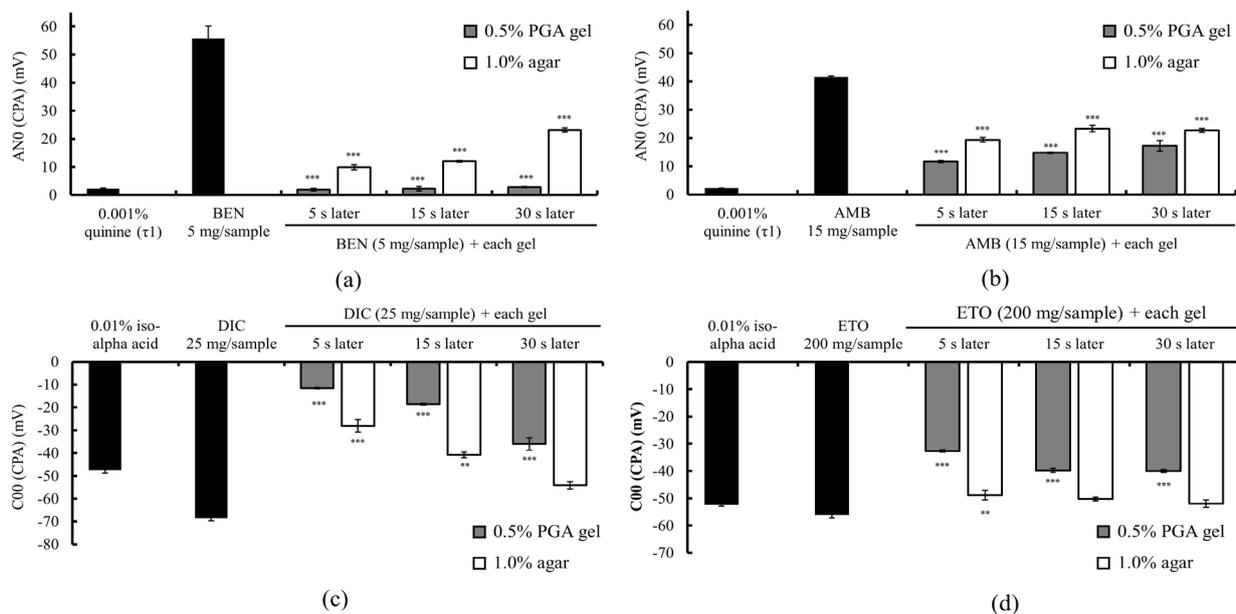


Figure 4. The dose-dependent increase in AN0 (CPA) of (a) BEN, (b) AMB and C00 (CPA) of (c) DIC, (d) ETO mixed with two different gels. Data are expressed in mean \pm SD. (n = 3). **p < 0.01; ***p < 0.001 versus control substance of each taste (Tukey's test).

Figure 5(a), Figure 5(c), Figure 5(e) and Figure 5(g) show concentrations of four drugs eluted from each gel in a time-dependent manner. The amount of BEN eluted increased in a time-dependent manner from 0.5% w/v PGA gel. 14.49 ± 0.02 $\mu\text{g/mL}$ of BEN in 1.0% w/v agar was eluted within five seconds; the amount eluted did not change between five and 15 seconds, with 43.00 ± 0.01 $\mu\text{g/mL}$ of BEN in 1.0% w/v agar being eluted between 15 and 30 seconds. For AMB, 2.59 ± 0.01 $\mu\text{g/mL}$ of AMB in 0.5% w/v PGA gel was eluted within five seconds and 4.28 ± 0.07 $\mu\text{g/mL}$ of AMB in 0.5% w/v PGA gel was eluted within 15 seconds; the amount eluted did not change between 15 and 30 seconds. 15.16 ± 0.01 $\mu\text{g/mL}$ of AMB in 1.0% w/v agar was eluted within five seconds and 55.50 ± 0.04 $\mu\text{g/mL}$ of AMB was eluted within 15 seconds; the amount eluted did not change between 15 and 30 seconds. For DIC, the amount of DIC eluted was confirmed to increase in a time-dependent manner from each gel. For ETO, the amount of ETO eluted was confirmed to increase in a time-dependent manner from 0.5% w/v PGA gel. 28.56 ± 0.34 $\mu\text{g/mL}$ of ETO in 1.0% w/v agar was eluted within five seconds; the amount eluted did not change between five and 30 seconds. There seems to be no significant difference in the suppression rate of the sensor outputs between AMB and DIC. The concentrations of AMB or DIC were 750 $\mu\text{g/mL}$ or 1250 $\mu\text{g/mL}$, respectively, if the full amounts of drugs were eluted from gels. For AMB, 4.19 ± 0.02 $\mu\text{g/mL}$ of AMB in 0.5% w/v PGA gel was eluted within 30 seconds and be about 1/180 of the full amounts. On the other hand, 55.34 ± 0.10 $\mu\text{g/mL}$ of AMB in 1.0% w/v agar was eluted within 30 seconds and be about 1/14 of the full amounts. For DIC, 423.20 ± 0.41 $\mu\text{g/mL}$ of DIC in 0.5% w/v PGA gel was eluted within 30 seconds and be about one third of the full amounts. On the other hand, 643.08 ± 0.35 $\mu\text{g/mL}$ of DIC in 1.0% w/v agar was eluted within 30 seconds and be about one half of the full amounts. The concentration of DIC eluted to 30 seconds later was about seven times larger than that of AMB in 1.0% w/v agar, while the concentration of DIC eluted to 30 seconds later was about 60 times larger than that of AMB in 0.5% w/v PGA gel. These results suggested that the effect of bitterness suppression of DIC was smaller than that of AMB since a greater amount of DIC eluted from 0.5% w/v PGA gel was larger than that of AMB eluted from 0.5% w/v PGA gel. In addition, it was suggested that the quantity of a drug mixed gels was related these results. It was difficult to wrap all drugs in the case that the quantity of a drug per single dose was a large quantity such as ETO.

Figure 5(b), Figure 5(d), Figure 5(f) and Figure 5(h) show the correlation between logarithm of concentrations of four drugs eluted from two different gels and the taste sensor output (CPA) of AN0 or C00 in response to drugs mixed with the two different gels, respectively. There were good correlations between logarithm of concentrations of each drug eluted from two different gels and the taste sensor output (CPA) of AN0 or C00 in response to drugs mixed with two different gels, respectively (BEN; $r = 0.94$, AMB; $r = 0.96$, DIC; $r = -0.96$, ETO; $r = -0.95$, Pearson product-moment correlation coefficient, $p < 0.01$). These results show that

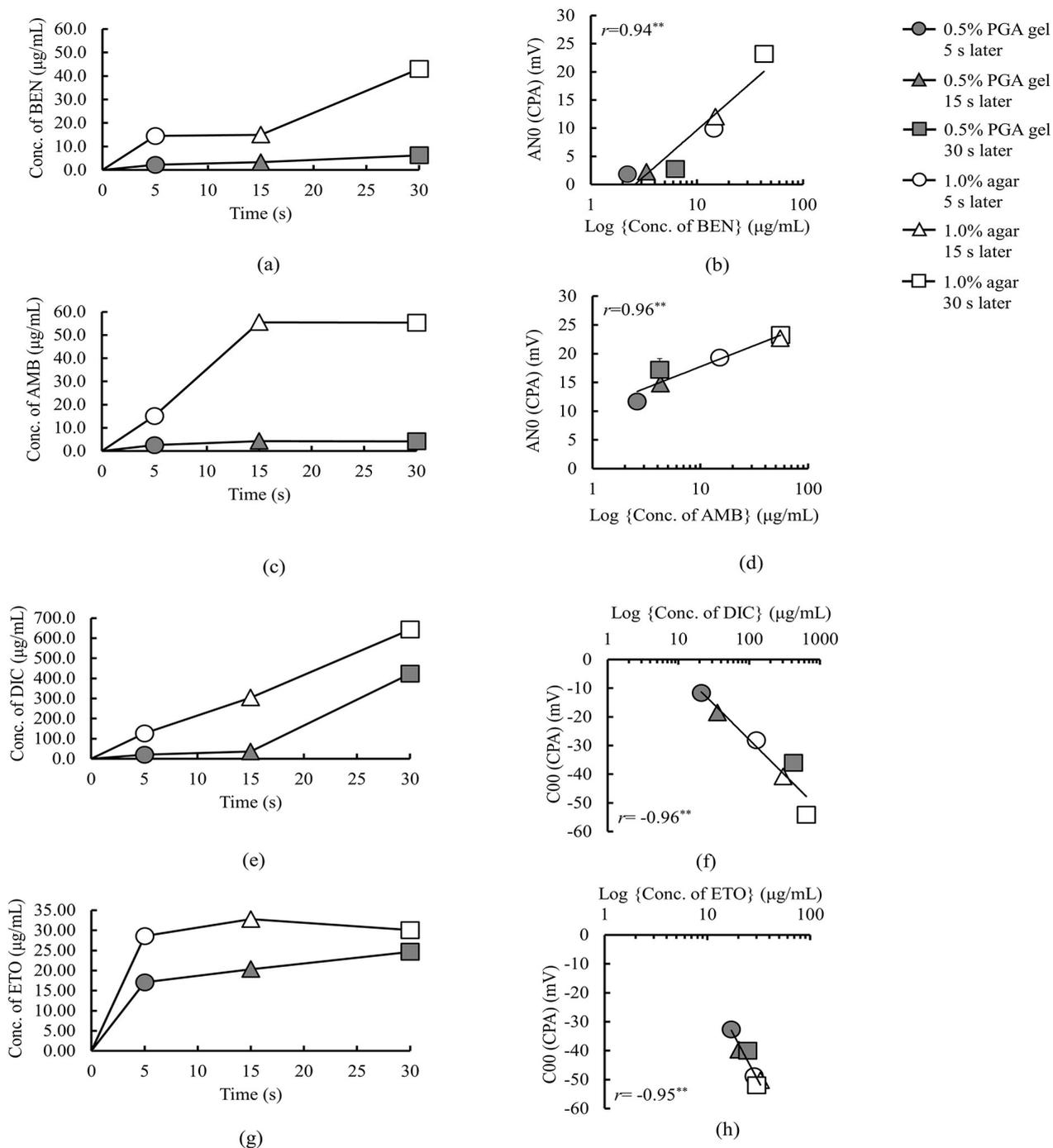


Figure 5. Determination of concentrations of four drugs eluted from two different gels; (a) BEN, (c) AMB, (e) DIC and (g) ETO, and the correlation between logarithm of concentrations of each drug eluted from two different gels and taste sensor outputs (CPA) of ANO or C00 in response to drugs mixed with the two gels; (b) BEN, (d) AMB, (f) DIC and (h) ETO. Data are expressed in mean \pm SD. (n = 3). **p < 0.01 is regarded as significant (Pearson product-moment correlation coefficient).

the sensor outputs in response to drugs were in direct proportion to the logarithm of drug concentrations, according to the Weber-Fechner law [36] and drug concentrations were accurately predicted by the taste sensor. The results suggest that 0.5% w/v PGA gel may be the best gels for bitterness-masking.

3.5. ¹H-NMR Spectroscopic Analysis of the Interaction between Four Drugs and PGA Gel

BEN and AMB, basic bitter drugs, are adsorbed on the negatively charged and hydrophobic part of the taste sensor membrane and cause a change in membrane potential by altering the charge density of the taste sensor output. In previous studies, we have demonstrated a taste-masking effect when a basic bitter drug is combined with an acidic compound, for example, diphenhydramine hydrochloride as basic bitter drug with chlorogenic acid as acidic compound [11] or zopiclone as basic bitter drug with citric acid as acidic compound [37]. In this study, ¹H-NMR analysis suggested an electrostatic interaction between the amino group of the basic bitter drug and the carboxyl group of the acidic compound. It was suggested that the electrostatic interaction reduced the membrane adsorption of the basic bitter drug in the taste sensor.

An interaction between the amino group of BEN or AMB and the carboxyl group of glutamic acid in PGA gel was expected to reduce the membrane adsorption of BEN or AMB in the taste sensor. ¹H-NMR was used to evaluate the interaction between four drugs and PGA gel. Chemical shifts (δ) of each proton of drug alone or with PGA gel (at ratios of 1:5, 1:1.667, 1:1 and 1:0.125 to correspond to BEN, AMB, DIC and ETO, respectively) obtained from ¹H-NMR analysis are shown in **Table 1**. The difference between the chemical shifts ($\Delta\delta$) of drug alone and drug with PGA gel was calculated by the following equation:

$$\Delta\delta = \delta_{\text{drug with PGA gel}} - \delta_{\text{drug alone}}$$

In the ¹H-NMR spectra of BEN, the signals of proton 5 and 15 (proton No. shows in **Figure 1**) of BEN were at 3.73 - 3.83 ppm for BEN alone, 3.57 - 3.63 ppm (Δ -0.20) for BEN with PGA gel. The signals of proton 5 and 15 near to the nitrogen atom of BEN, shifted clearly upfield when BEN was mixed with PGA gel. In the ¹H-NMR spectra of AMB, the signal of proton 4 of AMB were at 3.07 ppm for AMB alone, 2.78 ppm (Δ -0.29) for AMB with PGA gel, while the signal of proton 7 of AMB were at 4.09 ppm for AMB alone, 3.93 ppm (Δ -0.16) for AMB with PGA gel. The signals of proton 4 and 7, near to the nitrogen atom of AMB, shifted clearly upfield when AMB was mixed with PGA gel. On the basis of these results, it is suggested that the upfield shift was due to a shielding effect. Lan *et al.* reported that the proton of cation moieties experiences a chemical shift and up-field drift caused by the addition of an anion, with the possible formation of an electrovalent bond between the cation and anion [38]. Their results support the results described in the present paper. On the other hand, the signal of all protons in DIC and ETO which were acidic drugs was not clearly shifted.

It is postulated that PGA gel suppresses the bitterness of basic drugs such as BEN and AMB by electrostatic interaction between a positive ion-charged amino group of BEN or AMB and a negative ion-charged carboxyl group of PGA gel.

4. Conclusions

Taste and ease of swallowing are important factors determining the acceptability

Table 1. Chemical shifts of each proton of four drugs, with or without PGA gel, obtained using $^1\text{H-NMR}$ in $\text{DMSO-}d_6$; (a) BEN, (b) AMB, (c) DIC and (d) ETO.

(a)		
Proton No.	BEN	BEN:PGA gel = 1:5
2	4.47, 4.63	4.39, 4.53
5, 15	3.73 - 3.83	3.57 - 3.63
6, 7, 17	2.22 - 2.29, 2.42 - 2.49 2.58 - 2.64, 2.69 - 2.77	2.15 - 2.19, 2.33, 2.54 - 2.58
9 ~ 12, 19 ~ 23	7.18 - 7.37	7.14 - 7.31
16	2.08	2.19
24	4.06, 4.12	3.94
25	1.12	1.02

(b)		
Proton No.	AMB	AMB:PGA gel = 1:1.67
1	3.39	3.38
2, 3, 5, 6	1.18, 1.46, 1.91, 2.11	1.16, 1.31, 1.87, 2.02
4	3.07	2.78
7	4.09	3.93
11	7.62	7.55
13	7.50	7.39
O1	4.57	4.50
N1	5.64	5.61
N2	8.88	-

(c)		
Proton No.	DIC	DIC:PGA gel = 1:1
2	3.37	3.39
5	6.22	6.22
6	6.90	6.91
7	6.71	6.72
8	7.03	-
11, 13	7.42	7.42
12	7.04	7.05

(d)		
Proton No.	ETO	ETO:PGA gel = 1:0.125
2	2.76, 2.90	2.76, 2.89
5	3.88 - 3.98	3.88 - 3.98
6	2.58 - 2.71	2.58 - 2.71
7	7.22	7.22
8	6.90	6.90
9	7.87	7.87
11	10.4	10.4
12	2.05	2.05
13, 15	0.65, 1.26	0.65, 1.26
14	2.84	2.84

of oral pharmaceutical formulations. Therefore, taste-masking and minimum critical viscosity must be considered in dosage form design of oral pharmaceutical formulations. The PGA gel prepared in this study in the absence of drug had a slight taste of umami and was without bitterness or astringency. The 0.5% w/v PGA gel was assigned grade II in the permission criteria according to which foodstuffs may be considered suitable for persons with difficulties in swallowing by the Japanese Consumers Affairs Agency, and had adequate hardness and adhesiveness to enable easy swallowing. The 0.5% w/v PGA gel containing drugs sustainably suppressed the basic bitterness sensor outputs (CPA) of AN0 to BEN or AMB to a greater degree than 1.0% w/v agar. It can therefore be concluded that the bitterness of BEN and AMB are suppressed by mixing with PGA gel. The mechanism underlying bitterness suppression of basic drugs such as BEN and AMB by PGA gel is suggested to be electrostatic interaction, confirmed by ¹H-NMR spectroscopic analysis, in addition to creating a physical barrier by the PGA gel. On the other hand, the 0.5% w/v PGA gel also suppressed the acidic bitterness sensor outputs (CPA) of C00 to DIC or ETO than 1.0% w/v agar, however the effect of bitterness suppression of DIC and ETO which were acidic drugs was smaller than that of BEN and AMB which were basic drugs. It is suggested that the mechanism underlying bitterness suppression of acidic drugs such as DIC by PGA gel is the only physical barrier by the PGA gel-like agar.

PGA gel is therefore a novel gelling agent, which does not require heating or cations. Furthermore, it may be useful in oral pharmaceutical formulations to enable easy swallowing and the masking of bitterness of various drugs, especially basic drugs.

The methods of bitterness masking are classified into four kinds of methods as follows: physical (e.g., encapsulation), chemical (e.g., complexation with ion-exchange resins), biochemical (e.g., bitter taste receptor blockers) and sensual (e.g., the addition of sweeteners or flavours) masking. The taste-masking using PGA gel seems to have an additive effect of physical masking and chemical masking. In addition, PGA gel has benefit in the point of not only the ease of swallowing but also the masking of bitterness.

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Conflicts of Interest

The authors declare no conflict of interest regarding the publication of this paper.

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