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Research Papers

Preparation, characterization and taste-masking properties of polyvinylacetal diethylaminoacetate microspheres containing trimebutine

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Abstract

The objectives of this study were to produce acid soluble, polyvinylacetal diethylaminoacetate (AEA) microspheres containing trimebutine (as maleate), using a water-in-oil-in-water (w/o/w) emulsion solvent evaporation method, to characterize their in-vitro release properties, and to evaluate the taste-masking potential of this formulation in human volunteers. The pH of the external aqueous phase was the critical factor in achieving a high loading efficiency for trimebutine in the microencapsulation process; nearly 90% (w/w) loading efficiency was obtained at above pH 10. Trimebutine was completely released from AEA microspheres within 10 min in a dissolution test at pH 1.2, simulating conditions in the stomach, whereas at pH 6.8, the pH in the mouth, only small quantities of trimebutine were released in the initial 1–2 min. The results of a gustatory sensation test in healthy volunteers confirmed the taste-masking effects of the AEA microspheres. Finally, an attempt was made to encapsulate the salts of other basic drugs (lidocaine, imipramine, desipramine, amitriptyline, promethazine and chlorpheniramine) into AEA microspheres using the w/o/w emulsion evaporation method. The loading efficiencies were ranked in almost inverse proportion with the solubility of the drugs in the external aqueous phase. This study demonstrated the possibility of masking the taste of salts of basic drugs by microencapsulation with AEA using a w/o/w emulsion solvent evaporation method.

Introduction

In recent years, the importance of patient compliance, not only in drug efficacy per se, but also in the overall economics of healthcare, has been increasingly recognized. Efforts to improve patient compliance have included attempts to improve the palatability of orally administered pharmaceutical agents. In particular, a bitter taste is known to decrease patient compliance, and thus reduce effective pharmacotherapy.

In the present study, we investigated the possibility of masking the bitterness of trimebutine, a drug used as a treatment for irritable bowel syndrome (Saivin et al 2000; Laugier et al 2001; Birrer 2002). Trimebutine has an extremely unpleasant bitter taste, reported to be 70–80% of the bitterness of quinine, which is the accepted standard for bitterness (Uchida et al 2000). As this is highly likely to give rise to non-compliance when administered orally, it would be a considerable advantage to be able to mask the bitterness of oral formulations containing trimebutine.

Various technologies have been used to mask the bitterness of pharmaceuticals, including the use of cyclodextrin (Miyaji et al 1992), ion-complex resin (Agarwal et al 2000) and film coating (Chopra et al 2002). In the present study, we have used a microsphere system involving a water-in-oil-in-water (w/o/w)-type emulsion solvent evaporation technique previously reported by our laboratory (Ogawa et al 1988; Uchida et al 1998, 2001).

Our goal was to completely mask the taste of trimebutine by encapsulation in microspheres, while allowing the complete release of the drug under the acidic conditions of the stomach (pH 1.2). The pH inside the oral cavity has been reported be about 6.8 (Murray et al 1985), and this was confirmed by our own findings, which showed a range of 6.3–7.2 in 11 volunteers. We selected polyvinylacetal diethylaminoacetate (AEA), an acid-soluble polymer, for the encapsulation of trimebutine. It has been reported that this polymer is insoluble in media with pH above 5.8 (Koyama et al 1990; Shimano et al 1995). The properties of the AEA microspheres were examined in dissolution tests at various pH values and in gustatory sensation tests in human volunteers. Finally, acid salts of other basic drugs (hydrochlorides of lidocaine, imipramine, desipramine, amitriptyline and promethazine and chlorpheniramine maleate) were encapsulated in AEA microspheres using the w/o/w emulsion evaporation method, and the relationship between loading efficiencies and critical factors such as solubility in the external aqueous phase was examined.

Materials and Methods

Materials

AEA was kindly donated by Sankyo Co Ltd (Tokyo, Japan). Polyvinyl alcohol (MW 85000–146000, 87–89% hydrolysed) was supplied by Aldrich Chemical Co. Ltd (Milwaukee, WI, USA). Trimebutine maleate was kindly donated by Yamanouchi Co Ltd (Tokyo, Japan). The structures of trimebutine and AEA are shown in Figure 1. Chlorpheniramine maleate, lidocaine hydrochloride, desipramine hydrochloride, amitriptyline hydrochloride, imipramine hydrochloride, and promethazine hydrochloride were purchased from Sigma Co Ltd (St Louis, MO, USA). Other reagents were all of special grade.

Solubility of trimebutine and other basic drugs in the external aqueous phase

As a medium, pH 10 buffer (0.1 M glycine/0.1 M sodium hydrochloride buffer) was used. Excess amounts of each drug (1.0–5.0 g) were put into 30-mL flasks; 5 mL medium was added to each flask and shaken at 80 rev min^{-1} at 25°C for 10 h. We had confirmed in a pilot study that equilibrium was reached within 10 h. The suspension was filtered using a Millipore filter and the filtered solution was diluted with medium. The drug concentration of the diluted sample was determined using a high-performance liquid chromatography (HPLC) method as described below. The above experiment was repeated four times and the mean ± s.e.m obtained.

Preparation of AEA microspheres containing trimebutine or other basic drugs

For the preparation of AEA microspheres containing trimebutine and other drugs, a w/o/w emulsion solvent evaporation method was adopted (Ogawa et al 1988; Uchida et al 1998, 2001). For example, 20 mg of the drug (corresponding to a theoretical loading of 10%) was suspended in purified water. This medium, as the internal aqueous phase, was emulsified with 5 mL of methylene chloride containing 180 mg of polyvinyldiethylaminoacetate for 1 min using an ultrasonic disruptor (UD-200; Tomy Seiko Co Ltd, Tokyo, Japan). This w/o emulsion was poured into 200 mL of 0.5% (w/v) polyvinylalcohol solution (dissolved in buffers of various pH) as the external aqueous phase. Emulsification was continued using a homogenizer (Physcotron; Nichion Irikakikai Co Ltd, Tokyo, Japan) at 3000 rev min^{-1} for 1 min. This dis-

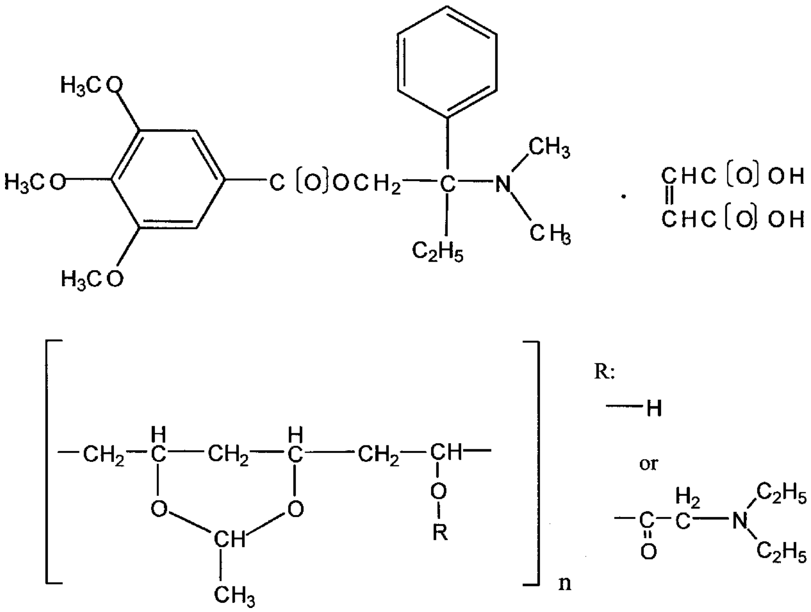

Figure 1 Chemical structures of trimebutine maleate (top) and polyvinylacetal diethylaminoacetate (bottom).

persion was gently agitated in a 500-mL beaker on a stirring plate with a 3.9-cm stirring bar for 4 h at room temperature. The microspheres were collected by centrifugation at 3000 rev min^{-1} for 15 min, washed with purified water and freeze-dried (FD-5N; Tokyo Rikakikai Co Ltd, Tokyo, Japan) for at least 4 h. The pH of the external aqueous phase was varied between pH 6 and 11. Yields were determined as the percentage of weight of the recovered microspheres after drying divided by the initial amount of enteric polymer and drug used.

A morphological study was performed using a biological microscope and scanning electron microscope (JSM-5400; Nihon Dennshi Co Ltd, Tokyo, Japan).

Drug loading for AEA microspheres

Five mL of pH 1.2 buffer was added to 5 mg of microspheres. The solution was agitated until the microspheres were completely dissolved. Then, 1 mL of this solution was further diluted with 4 mL of pH 1.2 buffer solution up to 5 mL. The diluted sample was then centrifuged at 3000 rev min^{-1} for 15 min, and 50 mL of the filtered sample was injected onto a chromatograph (Shimadzu LC-10A; Shimadzu, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD-10AV), an integrator (Shimadzu C-R4A) and a reversed-phase column (Cosmosil 5C18-AR, 4.6×150 mm; Nakalai Tesque Co Ltd, Kyoto, Japan).

The following mobile phase systems were used for all drugs, except lidocaine hydrochloride: solution A, water phase containing 0.77% sodium lauryl sulfate (adjusted to pH 3.0) by diluted perchloric acid (0.085% v/v) and diluted ammonium acetate (0.1% v/v), and solution B, acetonitrile (A/B, 50:50). The flow rate was 1.0 mL min^{-1} and the wavelength was set at 254 nm. The loading was calculated from the weight of the initial microspheres and the amount of drug incorporated. In the case of lidocaine, the mobile phase used was: solution A, 0.1% phosphoric acid and solution B, (acetonitrile/methanol, 10:3), (A/B, 85:15), the flow rate was 1.5 mL min^{-1} and the wavelength was set at 265 nm. In the HPLC study, the recovery of trimebutine was over 99.5%, and its deviation was within 1.5% of the measurement concentration range. The detection limit was 0.02 mg mL^{-1} and was much lower than the sample concentration.

In-vitro drug release from AEA microspheres

The in-vitro release profiles of trimebutine and the other basic drugs from the AEA microspheres were determined according to the method described in the Japanese Pharmacopeia (XIII) using the rotation basket method. Four different media at different pH were used in the dissolution test: pH 1.2 (hydrochloric acid/sodium hydroxide solution), pH 5.0 (citric acid/sodium citrate buffer), pH 6.0 (water), and pH 10.0 (glycine buffer). An appropriate amount of AEA microspheres containing 5 mg of drug were suspended in 500 mL of buffer solution, and 3-mL samples were withdrawn at 1, 5, 10, 15, 30, 45 and 60 min. The samples were centrifuged (3000 rev min^{-1} for 5 min), and the concentration of the substrate in the supernatant analysed by the HPLC method described above.

Gustatory sensation test

The standard quinine hydrochloride concentrations used were 0.010, 0.0158, 0.0251, 0.0398, 0.0631, 0.10, 0.159, 0.251, 0.398, 0.631 and 1.0 mM, corresponding to bitterness scores of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively. Before the start of testing, the volunteers (n = 11) were instructed to keep 5 mL of the above standard solutions in their mouths for 15 s, and were told their bitterness scores. The volunteers were then asked to taste 5 mL of the test sample solutions and to give them a bitterness score. The test samples contained various concentrations of trimebutine standard solution (10, 20, 50, 100, 250 and 500 mg mL^{-1}) or AEA microspheres containing 100 mg of trimebutine in 50 mL of water. In the case of AEA microspheres, the sample was kept in the mouth for 1 min; all other solution samples were kept in the mouth for 15 s. After tasting the samples, subjects gargled well and waited for at least 20 min before tasting the next sample (in our pilot study, aftertaste disappeared within 20 min for sample solutions).

Statistical analysis

Significant differences for loading efficiency among microspheres were analysed using the Student's unpaired *t*-test. A value of $P < 0.05$ was accepted as indicating a significant difference between values.

Results and Discussion

Optimization and characterization of trimebutine-loaded AEA microspheres

The generation of a stable w/o/w type emulsion seems to be essential for obtaining a high loading efficiency (Kentepozidou & Kiparissides 1995). The addition of electrolytes such as sodium chloride to the external water phase, or raising the osmotic pressure of the external water phase, have also been reported to prevent leakage of the drug from the internal water phase. In the present study, we examined the effect of the external aqueous phase pH on the loading efficiency of trimebutine. Microencapsulation was performed at a theoretical loading of 10%, using 400 mL of internal aqueous phase.

In general, the solubility of a basic drug can be represented as follows:

$$S = S_0(1+10^{pK_a-pH})$$

where S is the solubility, S_0 is the solubility of unionized drug, and pK_a is the dissociation constant of the drug. Using a pK_a value for trimebutine of 6.25, the ratio of solubility at pH 8.0 compared with pH 6.0 could be calculated as follows:

$$\begin{aligned} &S_0(1+10^{pK_a-pH})/S_0(1+10^{pK_a-pH}) \\ &\quad = (1+10^{6.25-8})/(1+10^{6.25-6}) \\ &\quad = 1.018/2.778 = 0.367 \end{aligned}$$

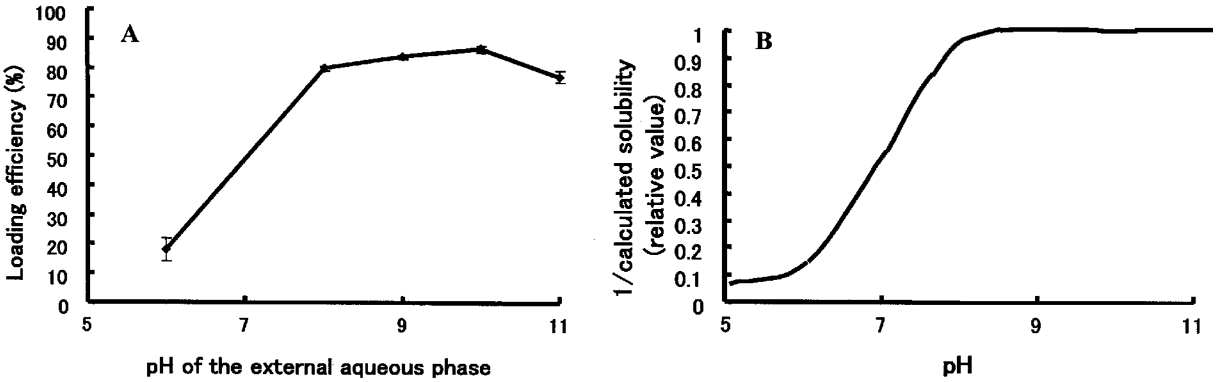

Figure 2 (A) Effect of the pH of the external aqueous phase on the loading efficiency of trimebutine in polyvinylacetal diethylaminoacetate microspheres. Error bars represent s.e. (n = 4–5). (B) Reciprocal curve of solubility of trimebutine (relative value).

Thus, an increase in the pH of the external aqueous phase will give rise to a decrease in the solubility of trimebutine, such that the drug is less partitioned or leaked from the internal to the external aqueous phase. This will give rise to an increase in loading efficiency.

Figure 2A and B shows the effect of pH on the obtained and simulated loading efficiencies of trimebutine in AEA microspheres, respectively. As shown in Figure 2A, a low loading efficiency (18%) was obtained at pH 6.0 (water), whereas above pH 8.0, the loading efficiency was dramatically enhanced. A comparatively high loading efficiency (86%) was observed at pH 10. Figure 2B shows the simulated pH reversed solubility profile (as the normalized line) for trimebutine. As criteria for drug leakage, a reversed function of solubility ($S = S_0\ (1 + 10^{pK_a - pH})$) was adopted. The simulated line (Figure 2B) almost coincided with the actual pH-loading efficiency profile; this phenomena suggests the importance of the external aqueous phase pH on trimebutine loading.

The effect of the volume of the internal aqueous phase on loading efficiency was also investigated when the external aqueous phase pH was fixed at 10. At internal aqueous phase volumes of 60, 100, 200, 400 and 800 mL, corresponding loading efficiency values (mean ± s.e.m.) were 82.7 ± 1.9, 88.9 ± 1.7, 85.1 ± 1.3, 86.2 ± 1.1 and 84.9 ± 0.7% w/w, respectively. There were no significant differences (Student's unpaired *t*-test) in loading efficiency when the internal aqueous phase volume was changed from 60 to 800 mL. AEA microspheres with highest loading efficiency (8.9%, w/w), prepared using 100 mL of internal aqueous phase and pH 10 buffer as an external aqueous phase, were employed in the following study. The mean diameter of AEA microspheres ranged from 30 to 40 mm for all batches prepared and the yields were over 90% in all cases.

Release test and gustatory sensation of AEA microspheres with 8.9% w/w trimebutine

A dissolution test using various media at different pH was performed for the AEA microspheres with 8.9% w/w trimebutine loading. Figure 3 shows the release profiles at pH 1.2, 5, 6 and 6.8. Under acidic conditions (pH 1.2), trimebutine was immediately released from the microspheres, and the drug was almost completely dissolved in the medium in 10 min. A similar fast release pattern was observed at pH 5.0. On the other hand, at pH 6 and 6.8, the pH of the oral cavity, about 2% of the drug was released, mostly within the first minute. The large difference between the release profiles at pH 5.0 and 6.0 can be explained on the basis of the pH-dependent dissolution characteristics of AEA. Since the pH inside the oral cavity is between 6 and 7 (pH 6.3–7.2 for 11 volunteers in the present study), the AEA microspheres would not dissolve in the oral cavity, but would dissolve immediately in the stomach.

Scanning electron micrography revealed that the surface of the AEA microspheres was smooth, with no visible pores or cracking (data not shown). There were no differences among microspheres just before and 60 min after the dissolution test at pH 6.8 and 37°C. This means that the AEA matrix was not eroded or dissolved in the buffer medium at pH 6.8.

A gustatory sensation test was performed to examine the taste-masking effect of the microsphere preparation and the results are shown in Figure 4. The bitterness score increased with increasing concentrations of trimebutine. At the highest concentration tested (500 mg mL^{-1}, almost a 1 mM solution, the molecular weight of trimebutine being 503.5), the bitterness score was found to be 5.73 ± 0.36. In contrast, the bitterness score for the AEA microspheres containing 100 mg trimebutine was 0.80 ± 0.39.

We can calculate that if between 1 and 2% (1–2 mg) of the administered trimebutine were dissolved in the oral cavity (in a small volume of saliva plus the 50 mL of water taken with the AEA microspheres) in the initial phase of administration, this would give rise to an estimated trimebutine concentration in the oral cavity of 20–40 mg mL^{-1}. On the basis of the trimebutine concentration–bitterness score curve, a bitterness score of 0.80 corresponds to a trimebutine concentration of 22.1 mg mL^{-1}. The exact concentration was calculated as follows:

$$0.80 = 1.1558x^2 - 1.12x + 0.2223,\ x = \log C$$

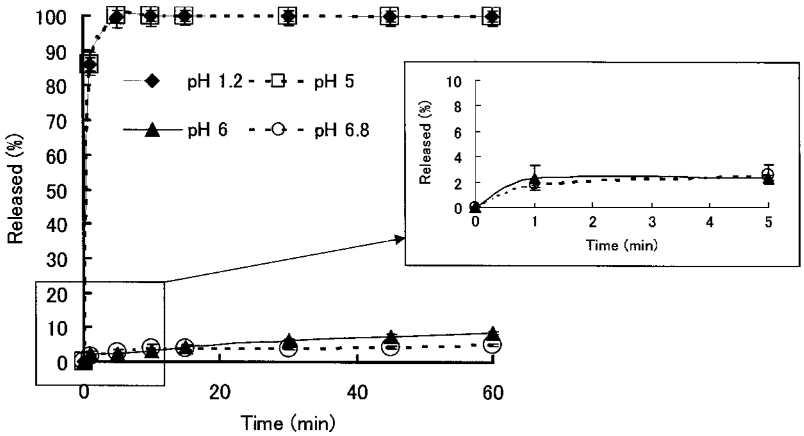

Figure 3 Release profiles of trimebutine from polyvinylacetal diethylaminoacetate microspheres in buffers at various pH. Error bars represent s.e. (n = 4).

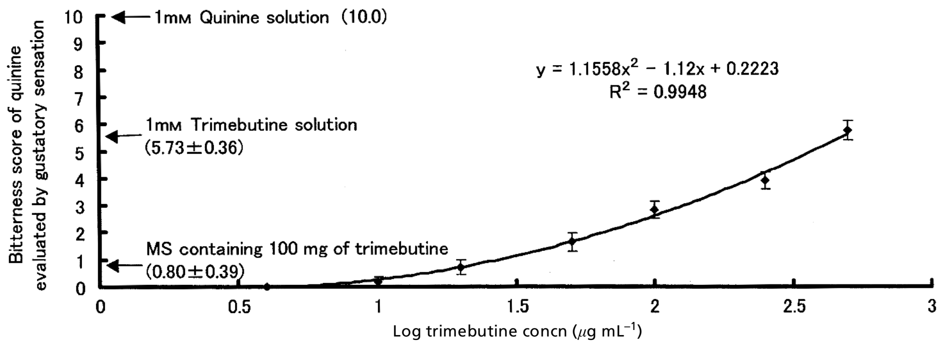

Figure 4 Bitterness score for various concentrations of trimebutine solution or polyvinylacetal diethylaminoacetate microspheres containing 100 mg of trimebutine as evaluated by a gustatory sensation test in human volunteers.

where C is the trimebutine concentration. The obtained x value was 1.354. Therefore C was calculated to be $10^{1.354}$ = 22.1.

This confirms our prediction that only about 1.1% of trimebutine would be released from the AEA microspheres in the oral cavity. Thus, the taste of trimebutine was successfully masked by encapsulation in AEA microspheres in the present study. Since the clinical dose of trimebutine was 50–100 mg, about 500 mg or 1.0 g of AEA microspheres would be administered orally if actually used in patients.

Preparation and characterization of AEA microspheres loaded with basic drugs

AEA microspheres containing other basic drugs (chlorpheniramine maleate, lidocaine hydrochloride, desipramine hydrochloride, amitriptyline hydrochloride, imipramine hydrochloride and promethazine hydrochloride) were also prepared. The volume of the internal aqueous phase and the theoretical drug loading were fixed at 100 mL and 10% w/w, respectively.

Table 1 Solubility at pH 10 and obtained loading efficiencies for salts of basic drugs.

Drug	Solubility ($mL\ mg^{-1}$)	Loading efficiency (%)
Trimebutine (maleate)	108.1±2.2	88.9±1.7
Promethazine hydrochloride	73.5±1.9	83.4±2.0
Amitriptyline hydrochloride	60.0±3.3	90.4±2.4
Imipramine hydrochloride	26.0±0.54	86.8±2.6
Desipramine hydrochloride	2.72±0.14	57.2±0.6
Chlorpheniramine maleate	1.46±0.08	14.0±0.6
Lidocaine hydrochloride	0.162±0.013	5.3±0.4

Data are mean±s.e.m.

Table 1 shows loading efficiencies of salts of basic drugs and their solubility (mg mL^{-1}) in pH 10 buffer as an external aqueous phase. Drugs with large solubility such as lidocaine hydrochloride and chlorpheniramine maleate showed poor loading efficiencies (5.3 and 14%, respectively). On the

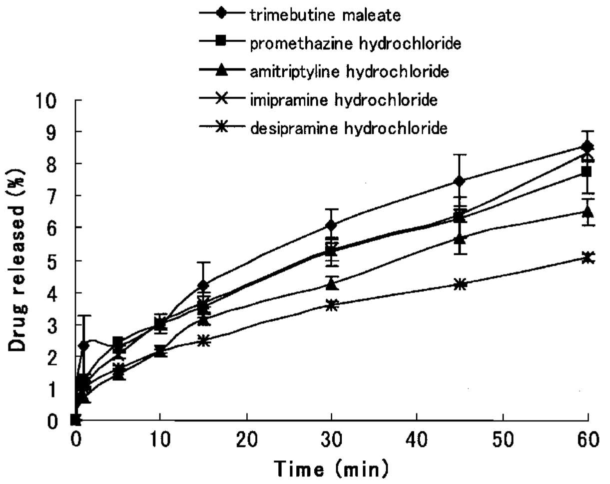

Figure 5 Release profile of hydrochloride salts of basic drugs from polyvinylacetal diethylaminoacetate microspheres in water (pH 6.0). Error bars represent s.e. (n = 3).

other hand, the hydrochloride salts of promethazine, amitriptyline and imipramine, which have low solubility, show comparatively high loading efficiencies (greater than 80%). The loading efficiencies seemed to be ranked in almost inverse proportion with the solubility of the drugs in the external aqueous phase. A solubility of 10 mg mL^{-1} (1% w/v) in the external aqueous phase seems to be the critical solubility for obtaining a loading efficiency above 80% for AEA microspheres.

Dissolution tests in water (pH 6) were carried out on AEA microspheres containing all the studied drugs, except lidocaine and chlorpheniramine. The results are shown in Figure 5. Drug solubility (mg dL^{-1}) in the external aqueous phase was ranked as follows: desipramine > imipramine > amitriptyline > promethazine > trimebutine, while the ranking of the initial phase release rate was: trimebutine > imipramine = promethazine > amitriptyline > trimebutine, even though the percentage release at 1 min was almost 2% or less and not very different among five types of drugs.

At pH 6, the pH of the oral cavity, the amount of drug released within the first 10 min was very limited (less than 9% for all drugs). It is therefore predicted that taste can be masked by encapsulation of drugs in AEA microspheres, even for some basic drugs. The combination of acid soluble polymer and acid salts of basic drugs may therefore be useful as a taste-masking formulation. Recently, Al-Omran et al (2002) reported successful taste masking by microencapsulation using polyethylene glycol as a plasticizer. The use of excipients with hydrophobicity (e.g. wax) might further enhance the taste-masking effect.

In conclusion, the taste of trimebutine was adequately masked by AEA microspheres in human volunteers. The other hydrochloride salts of basic drugs with low solubility were well encapsulated in AEA microspheres and the possibility of taste-masking was demonstrated. These microspheres may be useful for oral administration as fine granules. Another possibility might be tableting of the prepared AEA microsphere, which could enable further taste-masking or sustained-release properties.

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