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MICONAZOLE CHEWING GUM AS A DRUG DELIVERY SYSTEM APPLICATION OF SOLID DISPERSION TECHNIQUE AND LECITHIN

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ABSTRACT

The release of the antifungal drug miconazole from chewing gum was evaluated both in vitro and in vivo. It was proved that the addition of lecithin and the application of a miconazole polyethyleneglycol 6000 solid dispersion increased the release of miconazole from chewing gum. The in vitro results correlated well with the in vivo results. 6 healthy volunteers obtained therapeutically active concentrations of miconazole in saliva when they chewed gum. In the microbiological experiments performed, lecithin did not antagonize the anti-Candida albicans effect of miconazole at pH 7.2.

INTRODUCTION

Chewing gum could constitute a valuable delivery system for drugs intended to act in the oral cavity. Such drugs often have low water/saliva solubility, and

unfortunately they are released very slowly from chewing gum during mastication (1).

In this study miconazole was used. Miconazole is an antifungal drug widely used against oral and gastrointestinal mycoses. Miconazole has low water solubility (2). This means that the drug release from chewing gum is small if the drug is incorporated in chewing gum by a conventional method (3).

It has earlier been shown that the in vitro release of miconazole from chewing gum can be increased through the application of a solid dispersion technique (3).

In this study the in vivo release of miconazole polyethyleneglycol solid dispersion from chewing gum was compared with the release in vitro. In addition a miconazole release promoting effect of lecithin was investigated in vitro and in vivo. The in vitro experiments were performed on a mastication device described in (4) and 6 healthy volunteers participated in the in vivo experiments.

It was reported that lecithin can antagonize the antifungal effect of miconazole (5). The efficacy of miconazole in combination with lecithin against a strain of Candida albicans was therefore examined in vitro. The concentrations of miconazole and lecithin found in the in vivo experiments with chewing gum were used to evaluate the efficacy of the combination.

MATERIALS AND METHODS

Candida albicans PF 1402/88 was originally isolated from a patient with candidiasis and was subcultured at Statens Seruminstitut (DK) before it was delivered to this laboratory. The MIC value was 6.25 µg/ml. Miconazole of pharmaceutical grade was a generous gift from Janssenpharma A/S (DK). Lecithin of food grade was delivered

by Fertin Laboratories A/S (DK). L-α lecithin from egg yolk ~ 98% phosphatidylcholine was purchased from Fluka Chemie AG (Switzerland). Polyethyleneglycol 6000 (PEG 6000) of pharmaceutical grade was purchased from Mecobenzon A/S (DK). Bacto yeast extract, bacto peptone and yeast morphology agar was from Difco (USA) and D(+) glucose monohydrate for biochemistry and microbiology was from Merck (FRG). The other chemicals and reagents were of analytical grade.

Solid dispersion preparation

A solid dispersion containing 20% miconazole and 80% PEG 6000 was prepared by a melt procedure.

Miconazole and PEG 6000 were mixed in a mortar and the mixture was sieved through a 300 µm mesh sieve. The mixture was placed in a beaker and allowed to melt in an oil bath placed on a heating plate. The temperature of the oil bath was 85°C. The mixture was stirred and a homogeneous liquid was obtained. It was kept in the oil bath for 10 min. Then it was poured onto ice-cooled aluminium plates and kept on the plates for 2 hours. The product was placed in an excicator for 48 hours. The solid mass was pulverized in a mortar and put through a 300 µm mesh sieve. The solid dispersions were stored in excicators.

Chewing gum manufacturing

The chewing gum was manufactured at Fertin Laboratories A/S (DK) of common gum ingredients and with the use of a conventional mixer.

Each piece of the four different chewing gum formulations was planned to contain: (1) 50 mg pure miconazole, (2) 50 mg pure miconazole intimately mixed with 50 mg lecithin before the manufacture of the chewing gum, (3) 250 mg of the 20% miconazole 80% PEG 6000 solid

dispersion, and (4) 250 mg of the 20% miconazole 80% PEG 6000 solid dispersion intimately mixed with 50 mg lecithin before the manufacture of the chewing gum.

The chewing gum weighed about 880 mg a piece.

In vitro release of miconazole from chewing gum

The in vitro release experiments were performed on the mastication device described in (4). The release tests were carried out in 0.05 M phosphate buffer pH 7.4 The experiments were performed in the same manner as described in (3), i.e. the 10 ml dissolution medium was replaced every 2 min. and the mastication lasted 30 min. To the collected samples an equivalent volume of methanol was added. Miconazole is fairly soluble in 50% methanol. The concentration of miconazole in the mixture was measured by the reversed phase HPLC method, also described in (3).

In vivo release of miconazole and lecithin from chewing gum

Informed consent was obtained from six healthy persons, age 26 - 49. None of the persons had dentures. The local committee of ethics approved the study.

Miconazole

The voluneteers fasted for 2½ hours, i.e. half an hour prior to and 2 hours after taking the chewing gum. Each volunteer chewed one piece of the four formulations for 30 min. each. In addition, one and two pieces of the chewing gum containing both miconazole:PEG 6000 solid dispersion and lecithin were chewed for 120 min. The chewing gum pieces were taken in a randomized order with at least 48 hours between the chewings.

Saliva samples were collected just before the chewing gum was taken and again 2, 10, 30, 60, 120 and

300 min after. The samples were deproteinized by the additon of acetonitrile and by subsequent centrifugation as in (6). The HPLC equipment used to estimate the concentration of miconazole in the saliva samples was described in (3). The eluent and the method of standard curve construction was as described in (6) with minor modifications.

Lecithin

One of the volunteers twice chewed a piece containing both miconazole as a solid dispersion and lecithin for 60 min. Saliva samples were collected in the periods 10-20 min and 50-60 min. 1.5 ml saliva was mixed with 1.5 ml methanol in a test tube on a whirlmixer for 30 sec. 3.0 ml chloroform was added, and the solution was treated on a whirlmixer for 90 sec. The emulsion was separated by centrifugation at 2500 rpm for 15 min. The procedure is similar to the one described in (7). The chloroform layer was transferred with a pasteur pipette into a flask and the chloroform was evaporated on a rotary evaporator at 65°C. The residue was dissolved in 200 µl chloroform. Thin layer chromatography was used to measure the concentration of lecithin in the saliva. 10 µl of the chloroform solution was applied to precoated TLC plates (Silica Gel 60 without fluorescent indicator), Merck (FRG).

The TLC solvent was chloroform-methanol-7M aq. ammonia (230:90:15, v/v/v) (8).

After development the plates were air-dried. Two different reagent dyes were used. The plates were sprayed with either 3.5% molybdatophosphoric acid, Merck (FRG), in ethanol, or rhodamine B 0.1%, Merck (FRG), in ethanol. The plates with molybdatophosphoric acid were heated at 120°C for half an hour (9, 10), while the plates with rhodamine B were heated at 100°C for 15 min (10). The

main spot, Rf 0.30 was used to evaluate the concentration of lecithin in saliva by visual comparison with standards. The identity of the main spot was verified by chromatographing L-α lecithin from egg yolk ~ 98% phosphatidylcholine, Rf 0.30.

Standards were prepared by spiking saliva with small volumes of a concentrated stock solution of lecithin in water (1 mg/ml). The standards were handled as mentioned above. The saliva used for the standards was collected from one volunteer who chewed placebo chewing gum not containing lecithin.

Determination of miconazole in chewing gum

The procedure of miconazole extraction from the chewing gum using n-heptan and methanol was the same as in (3) and the concentration of miconazole in the extract was after appropriate dilution determined by the HPLC reversed phase method described in (3).

Microbiological experiments

The experiments were carried out at 37°C and cultures were grown either in GPY broth consisting of 4% glucose, 1% peptone, 0.5% yeast extract and 94.5% water (11), pH 5.3 or in the above-mentioned broth adjusted to pH 7.2 with di-sodium hydrogenphosphate buffer 0.5M, broth:buffer, 9:1. Sterile flasks containing broth were inoculated with a freshly prepared suspension of Candida albicans in 0.9% sodium chloride solution. The aim was to obtain an initial cell concentration in the range of 10^5 and $9 \cdot 10^5$ cells/ml. The flasks were placed on a shaker.

At the pH 5.3 experiments miconazole was added to the broth as a solution in dimethyl sulfoxide and lecithin was dissolved in 0.9% sodium chloride before the addition. All the flasks used in the pH 5.3 exper-

Table 1

Formulation	content of miconazole ± rsd% n = 5
pure miconazole	51.1 ± 1.5
pure miconazole + lecithin	49.3 ± 1.1
miconazole PEG 6000 sd	49.3 ± 0.9
miconazole PEG 6000 sd + lecithin	46.9 ± 0.9

iments contained the same concentrations of dimethyl sulfoxide (1%) and 0.9% sodium chloride solution (2%).

To the pH 7.2 broth miconazole was added as a suspension in 0.9% sodium chloride, and lecithin was added as mentioned above.

The two substances were added simultaneously to cultures at zero time. Samples were taken during a 24 hour experimental period. The fungal viability was estimated by plating 0.1 ml of appropriate dilutions (in 0.9% sodium chloride) of the samples on agar plates. The composition of the agar was: 35.0 g bacto yeast morphology agar, 1.5 g di-ammonium sulphate, 0.43 g sodium dihydrogenphosphate dihydrate, 0.92 g di-potassium hydrogenphosphate and water up to one litre, pH 5.9.

The agar plates were incubated at 35°C for 48 hours before colony counting.

The concentrations of miconazole and lecithin used in the microbiological experiments were similar to the average saliva concentrations found earlier in the study during chewing gum mastication.

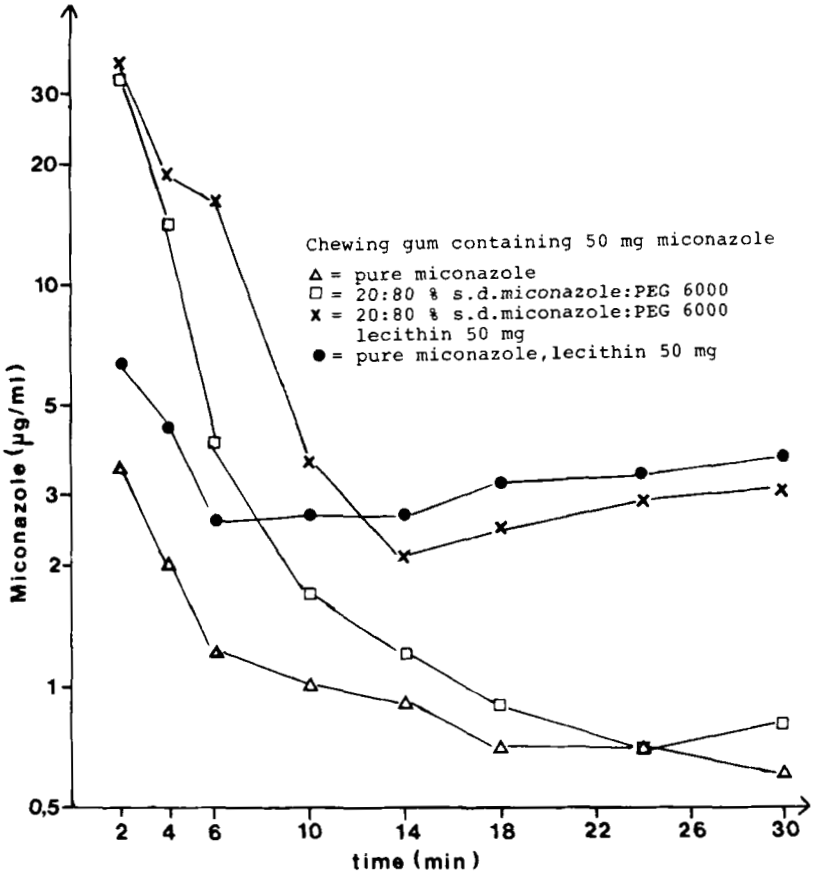

FIGURE 1
Release of miconazole particles from chewing gum in phosphate buffer pH=7.4.Semilogarithmic plot.

RESULTS AND DISCUSSION

The contents of miconazole in the four chewing gum formulations used in this experiment are presented in table 1.

The results of the chewing experiments in vitro and in vivo are depicted in figure 1 and figure 2 respectively. None of the zero time saliva samples contained miconazole.

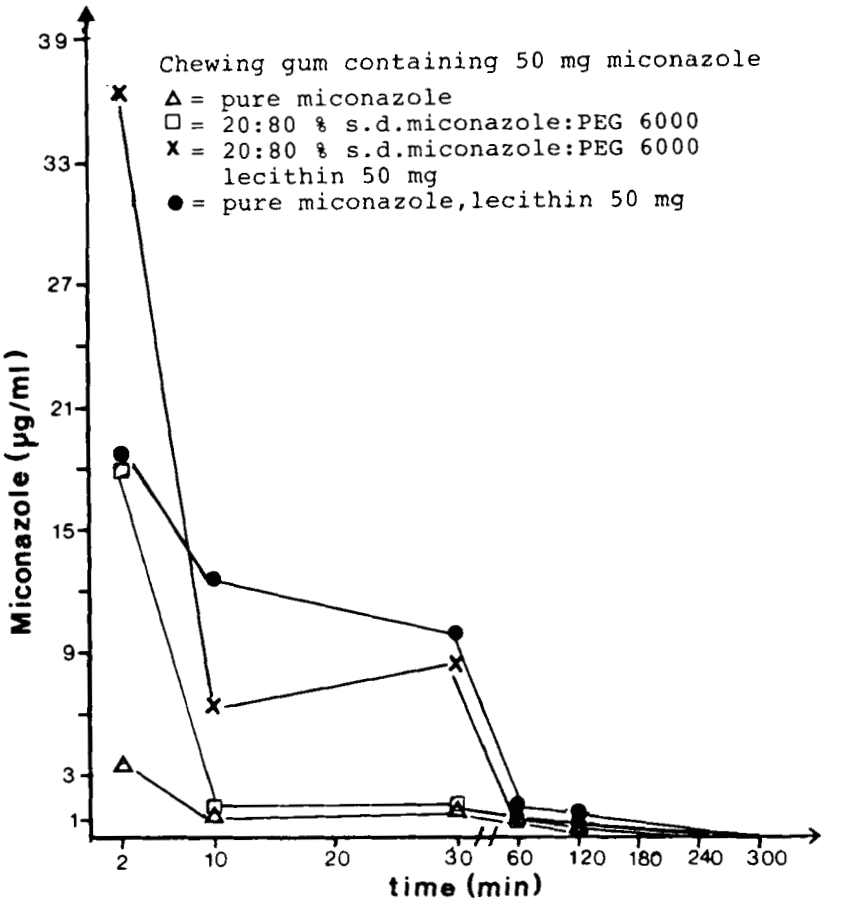

FIGURE 2
Average salivary miconazole concentrations of six volunteers as a function of time. Chewing time 30 min.

A comparison between the figures shows that the in vitro results correlate well with the in vivo results. It is obvious that the formulation of miconazole as a PEG 6000 solid dispersion increases the salivary concentration of the drug during the very first minutes of chewing. From the figures it will also be seen that lecithin has a miconazole release promoting effect.

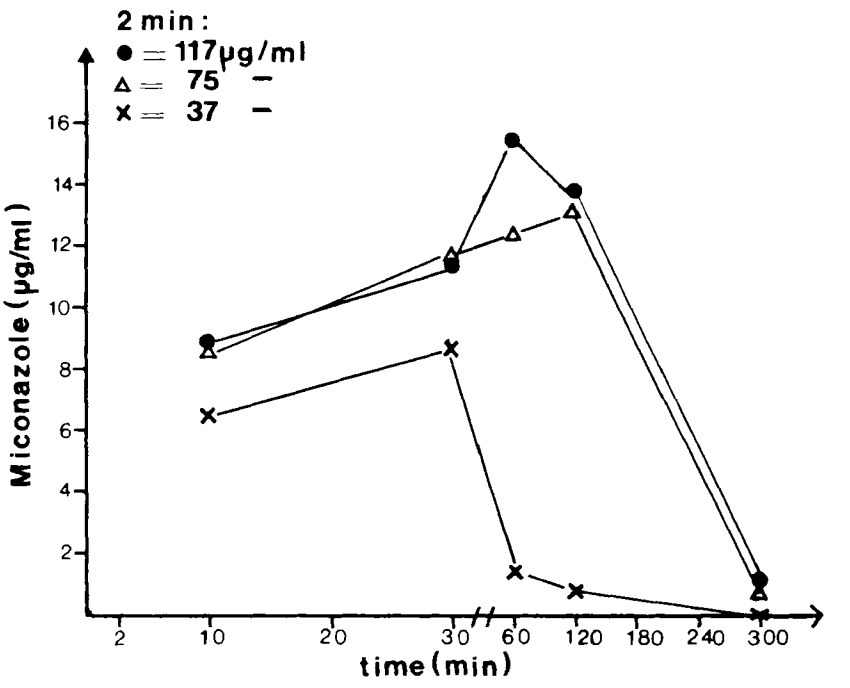

FIGURE 3

Average salivary miconazole concentrations of six volunteers as a function of time.
× = chewing time 30 min,1 piece of chew.gum
Δ = chewing time 120 min,1 piece of chew.gum
● = chewing time 120 min,2 pieces of chew.gum
1 piece of chewing gum contained 50 mg miconazole as 20:80 % s.d.miconazole:PEG 6000 and lecithin 50 mg.

Fortunately, the effect of lecithin lasts longer than that of the solid dispersion. According to figure 3 lecithin is able to increase the salivary concentration significantly even after two hours of mastication. The effect of lecithin is preserved when miconazole is present as a PEG solid dispersion. It was presumed in (3) that the release promoting effect of the solid dispersion may be due to the fact that miconazole is kept

in a hydrophilic environment when the solid dispersion and the chewing gum ingredients are mixed. It is presumed that the effect of lecithin is either due to the ability of lecithin to soften the chewing gum or to its ability to form liposomes in aqueous media (12). It is assumed that formation of liposomes increases the release of miconazole due to the increased solubility of the drug in saliva. It has in fact been shown that an addition of lecithin increases the solubility of miconazole in phosphate buffer 0.05M pH = 7.4, data not published. The saliva concentrations of miconazole when chewing two pieces of chewing gum are similar to the concentrations obtained when one piece is chewed, figure 3.

The explanation is perhaps that the surface area of two chewed pieces is not much bigger than the area of one chewed piece.

It is surprising that detectable and therapeutic concentrations of miconazole (13) were present in saliva 3 hours after the volunteers had removed the chewing gum from the mouth, figure 3. The detection limit of the HPLC method used to determine the concentration of miconazole in saliva was 0.4 µg/ml. The persistence of miconazole in the mouth after dosing with an oral gel formulation of miconazole was reported in (6). The concentrations of miconazole in saliva obtained with chewing gum can compete with the concentrations after dosing with an oral gel.

As to the concentration of lecithin in saliva- when lecithin containing chewing gum was chewed - the experiment showed that the concentration was in the range 30-50 µg/ml in both collection periods, 10-20 min and 50-60 min. The main spot on the TLC plates, Rf = 0.30, corresponding to phosphatidylcholine was used to

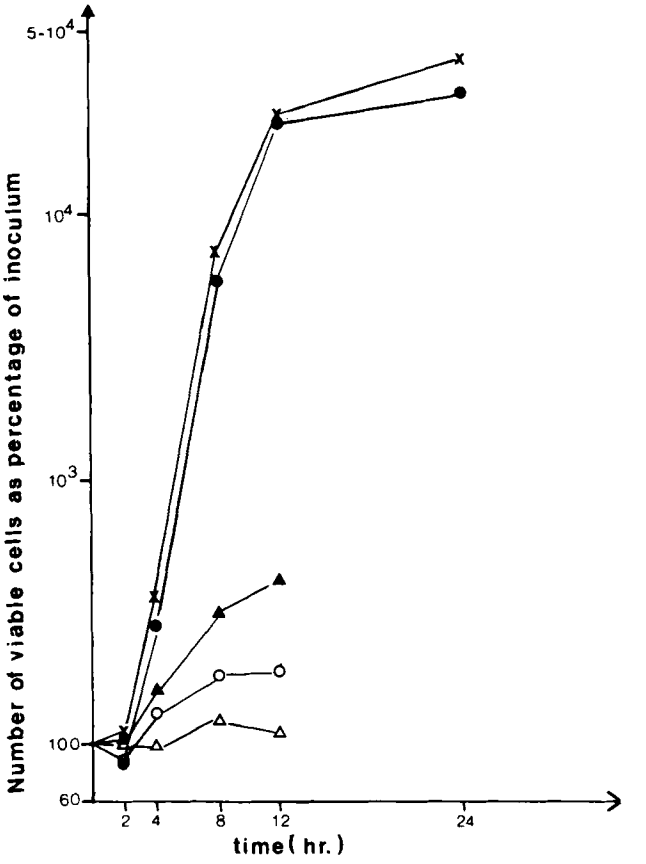

FIGURE 4

Time-dependent changes of the viability of C.albicans cultures in nutrient broth, as influenced by miconazole and lecithin, alone and in combination at pH=5.3. To C.albicans were added at zero time:
X no addition, ● lecithin (50 μg/ml),
▲ miconazole (2 μg/ml), △ miconazole (8 μg/ml) and
○ miconazole (8 μg/ml) plus lecithin (50 μg/ml).

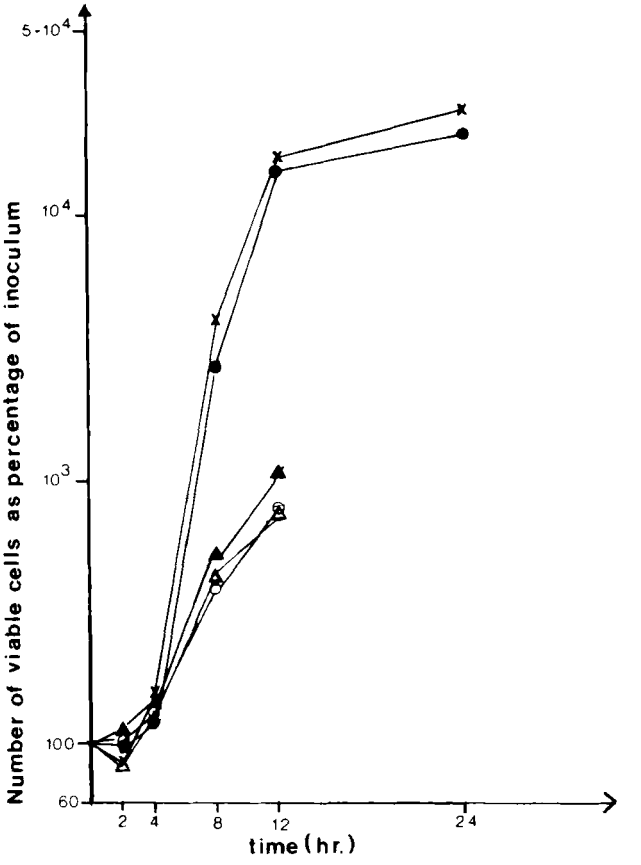

FIGURE 5
Time-dependent changes of the viability of C.albicans cultures in nutrient broth, as influenced by miconazole and lecithin, alone and in combination at pH=7.2. To C.albicans were added at zero time:
X no addition, ● lecithin (50 μg/ml),
▲ miconazole (2 μg/ml), △ miconazole (8 μg/ml) and
O miconazole (8 μg/ml) plus lecithin (50 μg/ml).

estimate the concentration of lecithin. Both dyeing reagents, molybdatophosphoric acid and rhodamine B gave the concentration range of lecithin mentioned above.

The effect of lecithin on anti-Candida albicans activity of miconazole was investigated using the concentrations of lecithin and miconazole found in the previous in vivo experiments. Lecithin 50 µg/ml and miconazole 8 µg/ml were added separately and in combination to Candida albicans cultures. This corresponds to the saliva concentrations obtained with chewing gum containing lecithin and a PEG 6000 solid dispersion of miconazole. In addition, miconazole 2 µg/ml was added to cultures, corresponding to the saliva concentration of miconazole obtained with chewing gum containing pure miconazole. Cultures containing neither lecithin nor miconazole were also incluced.

The growth studies at pH 5.3 and pH 7.2 are presented in figure 4 and figure 5. The curves represent the averages of two growth tests. It is obvious that lecithin has no antimicrobial activity. The ability of lecithin to reduce the antifungal action of miconazole, reported in (5) was confirmed at pH 5.3. The previous experiment (5) was performed at pH 4.5. The antagonistic action of lecithin is not significant at pH 7.2 according to figure 5. The pH of saliva is about 7.2. It is worth noting that the solubility of miconazole in the pH 5.3 and pH 7.2 growth medium is 14 µg/ml and less than 1 µg/ml respectively, data not published. This means that miconazole was partially present as a suspension in the pH 7.2 medium.

New formulations of miconazole chewing gum are currently under investigation in order to achieve higher saliva concentrations of miconazole.

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