

Mastic gum aided amoxicillin trihydrate gastro retentive mucoadhesive microspheres: *In vivo* evaluation

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Abstract

The primary objective of the study is to test gastro retentive mucoadhesive Amoxicillin trihydrate (ATH) microspheres for various *in vivo* properties. Several metrics were used to assess the improved ATH Mastic gum mucoadhesive microspheres (AMMM) *in vitro*. *In vivo* testing was conducted using formula-8 (AMMM-8), as suggested by prior study results by Sowjanya *et al.*, 2022. Four healthy rabbits of both sexes were used to assess the availability of plasma medicines. Pharmacokinetic parameters were measured in plasma samples. The study revealed that *in vivo* animal studies in rabbits showed good drug levels of ATH. After a single dose of formulation AMMM-8 (44.28mg/kg), the symmetrical mean C_{max} (25.69±0.11 µg/ml) t_{max} (6h), $AUC_{(0-10h)}$ (185.1±1.28 µg.h/ml), $AUC_{(0-\infty)}$ (269.1±4.68 µg.h/ml), and AUMC (840.42±2.23 µg.h/ml) were better than ATH. According to the findings, ATH reached systemic circulation faster than the pure medication and had acceptable kinetic values.

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Introduction

The gastro retentive microsphere is one of the attractive segments in delivering stomach-specific drugs additionally they can be easily prepared and administered (Kousar *et al.*, 2022).

An oral product containing amoxicillin trihydrate (ATH) is semi-synthetic amino penicillin with broad-spectrum bactericidal activity (Limousy *et al.*, 2017). ATH is a white or almost white crystalline powder. Based on its physico-mechanical and biopharmaceutical properties, and the pH partition theory, ATH is well absorbed when given orally. In the trihydrate form, amoxicillin is soluble in water, resulting in slight hygroscopic behavior.

Mucoadhesive systems' effectiveness is greatly influenced by the properties of a polymer (Prabaharan and Gong, 2008).

The convenience of taking medicine by mouth is preferred by many patients (Andrews *et al.*, 2009). The bioadhesive drive, which is rare and expensive, has been made with a variety of polymers (Hoffman, 2013). Rather than developing synthetic polymers, In the current study, natural polymer was used that can aid mucoadhesion. The authors used mastic gum (MG) in the study of ATH mucoadhesive microspheres (AMMM). MG has been shown to have a healing effect on stomach/peptic ulcers (Miyamoto *et al.*, 2014) and is effective against *H. pylori* (Dabos *et al.*, 2010; Dimas *et al.*, 2012; Miyamoto *et al.*, 2014). The objective of AMMM is systemic availability at a constant state (Table I). Precision liberation systems are a fantastic choice for quick-acting medications and those that need continuous medication because they are simple to use.

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One variable at a time is the main focus of conventional research techniques because it is the most manageable. It is statistically impossible to look at all of these variables at once. Because of their interdependence, the results will be unreliable. The core component of multivariate analysis is the design of experiments (DOE) (Abdul *et al.*, 2020). Partial factors are covered by a treaty in DOE. The DOE's goals include screening and optimization. Every potential combination of the elements is included in a Factorial Design (FD). In FD, a high level is either (+1) or "low" (-1); these two levels are referred to as FD (Shravani *et al.*, 2020). The results of the study were based on the evaluation of AMMM response to discharge ATH using design expert software.

Among all the above formulations, AMMM-8 possesses all the characteristics of an ideal gastro retentive mucoadhesive microsphere including ATH release at 10h (90.4±2.3%), mucoadhesive strength (23.3±0.2 g), drug entrapment efficiency (86.3±1.5%), mucoadhesion time (14.7±0.9h), particle size (42.9±0.1µm) and swelling index (60.3±0.2%), as described by Soujanya *et al.* (Soujanya and Hindustan, 2022). So, AMMM-8 was subjected to *in vivo* trials.

Materials and methods

Materials

Merck chemicals provided acetonitrile and o-phosphoric acid, as well as triple glass distilled water (HPLC grade) and analytical grade chemicals. Krishna Rabbit Farms in Bengaluru provided the New Zealand Wistar rabbits for the *in vivo* study. Animal Ethical Guidelines for Laboratory Investigations were followed during the *in vivo* study.

The Institutional Animal Ethics Committee (RIPA/2022/COL-05R) for experimenting. The institute was ratified by CPCSEA (792/ac/05/CPCSEA/008/2022) for performing animal experiments.

In vivo Study

The *in vitro* studies on numerous evaluation parameters were attained as chosen by Sharma and Co-workers (2015) (Sharma *et al.*, 2015). The authors selected formula-5 (AMMM-8) for *in vivo* study (Prakash *et al.*, 2008).

In vivo bioavailability study

By using a parallel design, 4 white New Zealand rabbits of any gender (weighing 2-2.5 kg) were haphazardly

parted into two groups of identical size. The task was decided upon to match the pharmacokinetics of ATH selected AMMM-8) having 1000 mg of ATH and 100 mg of mastic gum (MG) and 75 mg of Carbopol 934P. Food was removed 10±1 h previous to the *in vivo* research, and water was provided ad libitum. Rabbits in Group-A were given AMMM-8 orally, while rabbits in Group-B were given pure ATH suspension in water. At 0, 0.5, 1, 2, 4, 6, and 10 h following the therapy, blood samples (2 ml) were collected into heparinized tubes. Centrifugation at 4,000 rpm for 15 min separated the plasma from the heparinized whole blood. Plasma samples were proximately moved to Eppendorf tubes after being separated and stored at -20°C until analysis. HPLC analysis was utilized to determine the drug's plasma concentration, and the following steps were used for HPLC analysis (Purohit *et al.*, 2021; Purohit *et al.*, 2020).

Estimation of ATH in serum samples

ATH in the serum samples was estimated according to the High-Performance Liquid Chromatographic (HPLC) process (Purohit *et al.*, 2020).

Preparation of standard solution and plotting of correction curves

Nagae (Nagae *et al.*, 2007) established the procedure, which was followed with minor changes. 1000 mg ATH (for stock solution A) was properly weighed and moved to a 100 ml volumetric flask, where it was dissolved with the use of sonication in the mobile phase, and the final volume was created using the mobile phase. Working standard solutions were made from these stock solutions using appropriate dilution with mobile phase to obtain concentrations of 15-90 g of ATH (Pushparaj *et al.*, 2018).

Preparation of the spiked plasma sample

This was performed by using a standard procedures (Megoulas *et al.*, 2005; Yu *et al.*, 2005). 250µl of rabbit plasma, 50µL of internal standard, and 10 µL of ATH were pipetted into a 10ml centrifuge tube, and to this 2ml of Acetonitrile was included. 10µL of the supernatant layer was poised (after centrifugation at 3200 rpm for 10min), inserted into HPLC, and chromatogram was obtained (Narendran *et al.*, 2019).

Process development

The mobile phase was a 60:40 (v/v) mixture of freshly made buffer 0.1 percent Acetonitrile and water o-Phosphoric acid, which proved to be an excellent separation mixture. The flow rates were then measured at 0.4, 0.8, 1.0, 1.2, and 1.5 ml/min. Because it provides a superior resolution of the peaks, 1.0 ml/min was chosen for the discovery of ATH at set chromatographic conditions and the detection of 247 nm, with no native nose composite eluting at ATH retention times (Atici *et al.*, 2017; Beg *et al.*, 2012).

Determination of various pharmacokinetic parameters

Several pharmacokinetic parameters were calculated from the time vs. serum ATH data, including peak concentration (C_{max}), time at which drug peaks (t_{max}), the area under the curve (AUC), elimination rate constant K_{el} , biological half-life ($t_{1/2}$), percent absorbed at various times, and absorption rate constant (K_a). Correction curves were used to determine the peak serum level (C_{max}) and the time at which the peak was reached (t_{max}). On a semi-logarithmic graph paper, the serum level and time values were plotted. During the elimination phase, the K_{el} was determined using the slope of the linear line (the best fit linear regression line for the points in the elimination phase was haggard by the process of fewest squares). The constant $t_{1/2}$ was derived using the equation $t_{1/2} = 0.693/K_{el}$. Percent absorbed at different times, and K_a was estimated using the Wagner and Nelson equation. Using the trapezoidal rule, the AUC was determined. Using the following eqs. 1 and 2, the remaining area from 10 h to time was determined (Rocha *et al.*, 2019; Sartini *et al.*, 2022; Soares *et al.*, 2021).

In vivo investigation protocol

To Calculate Animal Equivalent Dose (AED) from Human Dose by eq.3 was employed.

Animal equivalent dose calculations were made using the aforementioned equation and a 70 kg average human weight. A (1000 mg). ATH (44.28 mg) per kg body weight of the animal is the calculated Animal Equivalent Dose (AED) (Nair and Jacob, 2016; Shin *et al.*, 2010)

Treatment of animals

Healthy rabbits of both sexes were starved for 24 h. ATH and AMMM-8 were given at a dose of 44.28 mg/kg. Each

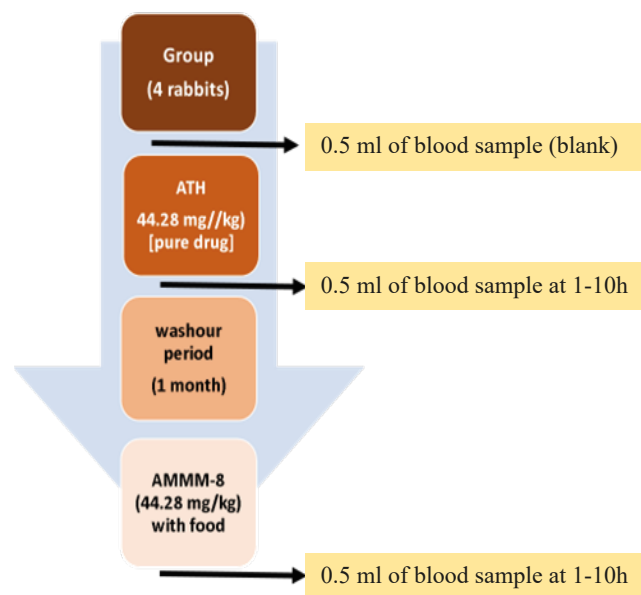


Fig. 1. Treatment and sampling intervals for in vivo studies

product was tested a total of four times ($n = 4$). A crossover study was conducted in conjunction with the *in vivo* trials (fig. 1) (Abdul *et al.*, 2011; Alkharfy *et al.*, 2015).

Blood samples (0.5ml) were taken from rabbits' marginal ear veins, allowed to clot, then centrifuged at 5000 rpm (to separate plasma) and deposited in dry tubes. Before the test, all of the samples were kept in the refrigerator. HPLC was used to determine the quantity of ATH. The plot of time vs. plasma concentration was used to determine peak concentration (C_{max}), time at which peak concentration occurred (t_{max}), Area under the Curve (AUC), elimination rate constant (K_{el}), half-life ($t_{1/2}$), percent absorbed at various periods, and K_a .

Results and discussion

The chromatogram of the plasma sample was taken at 1, 3, 6, and 10th h indicating the presence of ACH in the plasma (fig 2 and table II). The retention time was good at 1st h (4.38 min) with upsurge peak area of 70488 with an asymmetric factor of 1.39.

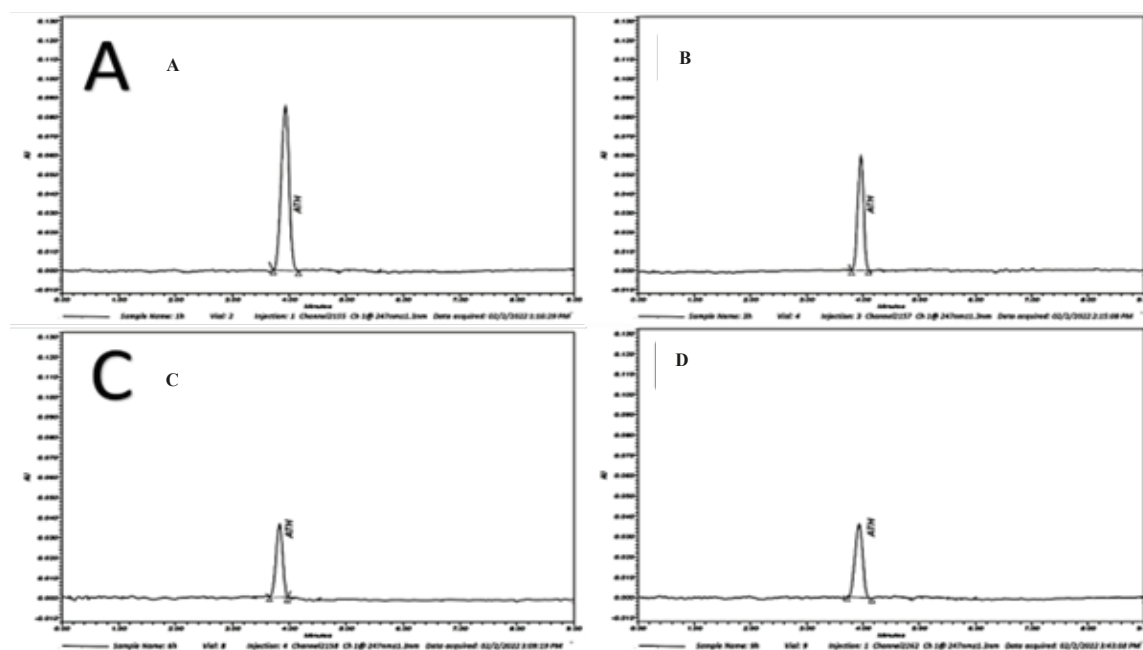
The HPLC chromatogram shows a linearity of 2-12 μ g/ml for all the chromatograms. The LOD and LOQ were more at 1st h of the plasma sample with 0.34 μ g/ml and 1.17 μ g/ml (Table III).

Table I. Composition of the AMMM

| Components | Formulations | | | | | | | | |
|-----------------------------|--------------|--------|--------|--------|--------|--------|--------|--------|--------|
| | AMMM-1 | AMMM-2 | AMMM-3 | AMMM-4 | AMMM-5 | AMMM-6 | AMMM-7 | AMMM-8 | AMMM-9 |
| Amoxicillin trihydrate (mg) | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 | 1000 |
| Ethyl Cellulose (mg) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| MG (mg) | 50 | 50 | 50 | 75 | 75 | 75 | 100 | 100 | 100 |
| Carbomer 934P (mg) | 50 | 75 | 100 | 50 | 75 | 100 | 50 | 75 | 100 |
| Dichloromethane (ml) | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| Span 80 (ml) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Glutaraldehyde (ml) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Liquidparaffin (ml) | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 | 200 |

Table II. Description of HPLC graph of the ATH

| Time (h) | Retention Time (min) | Peak Area (n = 3) | Asymmetric factor |
|-----------------|----------------------|-------------------|-------------------|
| 1 st | 4.38 | 70488 | 1.39 |
| 3 rd | 4.36 | 67922 | 1.37 |
| 6 th | 4.33 | 51999 | 1.22 |
| 9 th | 4.29 | 43905 | 1.18 |

**Fig. 2. The chromatogram of plasma sample at 1st h (A), 3rd h (B), 6th h (C), and 10th h (D)**

The concentration of ATH in plasma with AMMM-8 after oral administration was represented in Tables IV, V and figure 3.

ATH levels in rabbits were shown to be adequate *in vivo* animal experiments. *In vivo* tests in rabbits were performed on the best formulation (among AMMM-1 to AMMM-9), namely AMMM-8. The symmetrical mean C_{max} values of formulation AMMM-8 (25.69 ± 0.11 g/ml of

ATH) after a single dosage of formulation AMMM-8 (ATH: 44.28mg/kg) were greater than those of pure medicines. The AMMM-8's T_{max} values were discovered to be 6 h. The AUC (0-10h) was found to be 185.1 g.h/ml, whereas the $(AUC)_{0-\infty}$ was found to be 269.1 g.h/ml. 840.42 g.h/ml were found to be the AUMC values. Other pharmacokinetic parameters measured in plasma following oral delivery using AMMM-8 (Table IV)

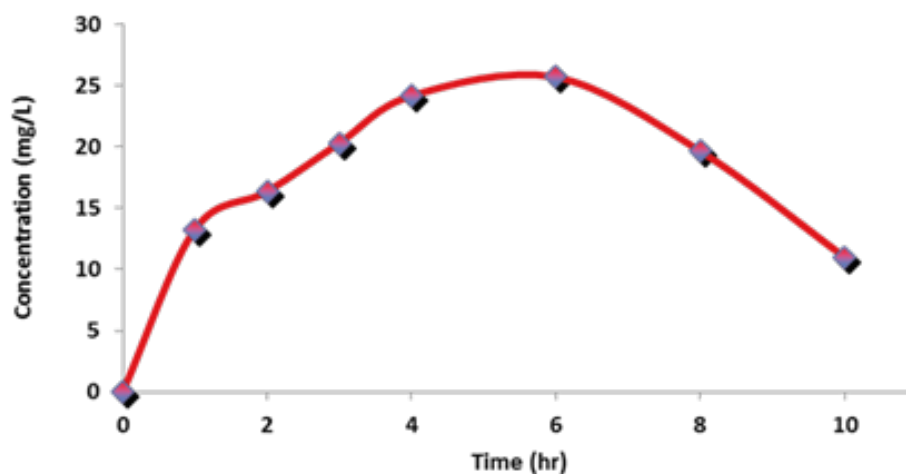


Fig. 3. Plasma concentration of ATH (AMMM-8) in rabbits (p.o)

Table III. Statistical data of the HPLC chromatogram

| Parameters | ATH | | | |
|--|--------|--------|--------|--------|
| | 1h | 3h | 6h | 9h |
| Linearity ($\mu\text{g/ml}$) | 2 – 12 | 2 – 12 | 2 – 12 | 2 – 12 |
| Correlation coefficient | 0.9998 | 0.9996 | 0.9995 | 0.9994 |
| Slope | 5128.7 | 4985.7 | 4858.1 | 4101.3 |
| Intercept | 712.24 | 699.37 | 685.49 | 646.36 |
| Limit of Detection ($\mu\text{g/ml}$) | 0.34 | 0.25 | 0.20 | 0.17 |
| Limit of Quantification ($\mu\text{g/ml}$) | 1.17 | 1.40 | 1.10 | 1.06 |

Table IV. The concentration of ATH in plasma from AMMM-8

| Time (hr) | Conc (mg/L) | ln(Cp) | Δ AUC | AUC (mg.hr/L) | Δ AUC | AUC (mg.hr/L) | Conc.t | AUMC |
|-----------|-------------|--------|--------------|---------------|--------------|---------------|--------|--------|
| 0 | 0 | 0.00 | 0 | | 0.0 | 0 | 0 | |
| 1 | 13.26 | 2.58 | 6.6 | 6.6 | 5.10 | 5.10 | 13.26 | 6.63 |
| 2 | 16.35 | 2.79 | 14.8 | 21.4 | 14.8 | 19.90 | 32.70 | 36.24 |
| 3 | 20.31 | 3.01 | 18.3 | 39.8 | 18.3 | 38.10 | 60.93 | 79.51 |
| 4 | 24.16 | 3.18 | 22.2 | 62.0 | 22.2 | 60.30 | 96.64 | 139.71 |
| 6 | 25.69 | 3.25 | 49.9 | 111.9 | 49.8 | 110.2 | 154.14 | 347.42 |
| 8 | 19.66 | 2.98 | 45.4 | 157.2 | 45.1 | 155.2 | 157.28 | 465.56 |
| 10 | 11.02 | 2.40 | 30.7 | 187.9 | 29.9 | 185.1 | 110.20 | 424.76 |
| Inf | 0.0 | | 84.0 | 271.9 | 84.0 | 269.1 | | 840.42 |

Table V. Pharmacokinetic parameters estimated with AMMM-8 in plasma when administered orally

| Pharmacokinetic parameter | Drug data (AMMM -8) |
|--|---------------------|
| C_{max} (μ g/ml) | 25.69 \pm 0.11 |
| T_{max} (h) | 6.00 \pm 0.00 |
| K_{el} (h^{-1}) | 0.131 \pm 0.06 |
| $T^{1/2}$ (h) | 3.547 \pm 0.08 |
| (AUC) ₀₋₁₀ (μ g.h/ml) | 185.1 \pm 1.28 |
| (AUC) _{0-∞} (μ g.h/ml) | 269.1 \pm 4.68 |
| K_a (h^{-1}) | 3.09 \pm 0.02 |
| AUMC (μ g.h/ml) | 840.42 \pm 3.25 |
| MRT (h^{-1}) | 10.0 \pm 0.00 |
| F | 0.7301 \pm 0.02 |
| P- Value | 0.4095 \pm 0.01 |

Values in mean \pm SD; trials made (n=3)

Conclusion

A combination of Amoxicillin trihydrate (ATH) with mastic gum was prepared as mucoadhesive microspheres. The best design among prepared AMMM-8 was subjected to *in vivo* studies in rabbits. The appraisal of ATH was urbanized by RP-HPLC. *In vivo* animal trials in rabbits revealed good levels of ATH in serum compared to pure ATH. The pharmacokinetic

parameters, viz., C_{max} and AUC were enriched related to pure ATH drug. The ATH release from the AMMM was localized from the stomach and appreciable than the pure drug.

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Declaration of Competing Interest

The author declared no conflict of interests.

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