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FORMULATION AND EVALUATIONOF AMOXYCILLIN: THREE-LAYER GUAR GUM MATRIX TABLET

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ABSTRACT: The objective of the study is to design gastro retentive drug delivery systems for Amoxycillin trihydrate. It was prepared with the objective to obtain site-specific drug delivery for the stomach and to extend its duration of action. The sustained release of amoxicillin is desired because of its short biological half-life. The preparation was carried out by using guar gum as a carrier in the form of a three-layer matrix tablet. Amoxycillin trihydrate was chosen as a model drug because of its site-specificity. Matrix tablets containing either 30 % (M) of guar gum were prepared by wet granulation technique using starch paste as a binder. Three-layer matrix tablets of Amoxycillin were prepared by compressing on both sides of guar gum matrix tablet granules of Amoxycillin M with either 50 (TLM) of guar gum granules as release retardant layers. Both the matrix and three-layer matrix tablets were evaluated for weight variation, hardness, friability, buoyancy and *in-vitro* dissolution studies. Optimized formulation of amoxicillin was found to have increased gastric residence prolonging the release of drug with 74% (matrix tablet) and 81% (three layer matrix tablet) of drug release in 8 hours by diffusion. The mechanism of drug release was found to be diffusion and followed combination of zero order and first order kinetics. Hence gastro retentive drug delivery system of Amoxycillin trihydrate is a promising approach as it can lead to decrease in the frequency of administration and ultimately lead to better patient compliance.

INTRODUCTION: Amoxicillin is a semi-synthetic, orally absorbed, broad spectrum antibiotic. It is widely used in a standard eradication treatment of gastric *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent [1].

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As conventional drug delivery systems do not remain in the stomach for prolonged periods, they are unable to deliver the amoxicillin to the site of infection in effective concentrations. Therefore, it is necessary to design drug delivery systems that not only all aviate the short comings of conventional delivery vehicles but also deliver amoxicillin to the infected cell lines. Some researchers had prepared and reported new amoxicillin formulations, such as floating tablets[2, 3] mucoadhesive tablets[3, 5] and mucoadhesive microspheres[3, 5], which were able to reside in stomach for an extended period for more effective *H.*

pylori eradication. Amongst the described formulations, the floating tablet is preferred for better and less variable gastric retention, but it has a limitation of incorporation of high dose of the drug. The drug with high dose like amoxicillin can be easily incorporated in liquid *in-situ* gelling formulation that upon oral administration can float for a prolonged period of time in the stomach [5]. However, the use of tablet has hardly been reported, so it is interesting to study the release pattern of amoxicillin in the tablet formulations. The purpose of this work was to prepare new sustained release tablets of amoxicillin by using guar gum as a rate-controlling polymer.

MATERIALS AND METHODS: Amoxycillin trihydrate was obtained as gift sample from Zydus Cadila, Ahmedabad, India. Guar Gum, High Media Labs., Mumbai, HPMC, Starch, Magnesium Stearate, Hydrochloric Acid from Rankem, New Delhi, Talc from Jiangsu Huaxi International, India. All other chemicals were ofanalytical grade.

Preparation of Amoxycillin Matrix Tablets [6]: Matrix tablets of Amoxycillin were prepared by wet granulation method. A mixture of talc and magnesium stearate (2:1 ratio) was used as lubricant and HPMC was used as diluents. Guar gum was included in the formulation in various proportions.

Three matrix formulations were prepared with 30 % of guar gum and were coded as M. The composition of formulations used in the study containing 200 mg of Amoxycillin in each case is shown in Table 1. In all the formulations, guar gum was sieved (< 250µm) separately and mixed with Amoxycillin (< 150µm) and HPMC (< 250µm).

The powder mix was granulated with 10 % w/w starch paste in the mortar and pastel. The wet mass was passed through a mesh (# 44) and the granules were dried at 50°C for 2 h in a tray drier. The dried granules were passed through a mesh (# 44) and these granules were lubricated with a mixture of talc and magnesium stearate (2:1) using a cone blender.

The lubricated granules were compressed at a compression force of 4500–5500 kg using 10 mm round, flat and plain punches on a single station tableting machine. Matrix tablets of each composition were tested for their drug content and release characteristics with a suitable number of tablets for each test.

The hardness of the matrix tablets was determined by using Monsanto Hardness Tester.

Preparation of Three Layer Matrix Tablets [6]**:** Three-layer matrix tablets were prepared by compressing 50 mg of granules containing 87 % of guar gum on both sides of matrix granules containing 30 % of guar gum (coded as TLM) shown in **table 2**. The preparation of three-layer matrix tablets involved the following steps:

1. Preparation of granules for the matrix tablets.
2. Preparation of guar gum granules for layering.
3. Compression of a layer of either 50 mg of guar gum granules on both sides of the matrix tablet granules.

Three-layer matrix tablets (TLM) containing 50 mg of guar gum granules as release controlling layer on both sides of 30 % matrix granules was prepared as follows. Granules of the matrix formulation containing 30 % of guar gum (M) were prepared as described above.

Guar gum granules containing 87 % of guar gum for layering were prepared by wet granulation method using 10 % w/w of starch as paste. The guar gum and starch paste was mixed well and the resulting wet mass was passed through a mesh (1680 µm) and the granules were dried at 50°C for 2 h in a tray drier.

The dried granules were passed through a mesh (1190 µm) and these granules were lubricated with a mixture of talc and magnesium stearate (2:1) using a cone blender. The composition of guar gum granules used in the study for layering in three-layer matrix tablets is given in Table 3.4. Initially the volume of the die cavity (10 mm round, flat and plain) was adjusted equivalent to the weight of three-layer matrix tablets (550 mg).

Then preweighed amount of guar gum granules equivalent to bottom layer (50 mg) was taken and placed in die cavity and slightly compressed for uniform spreading. The upper punch was lifted up, and granules of the matrix formulation (M) were placed over the bottom layer of guar gum granules in the die cavity and again slightly compressed for uniform spreading.

The remaining volume of the die cavity was filled with the preweighed amount of guar gum granules equivalent to top layer (50 mg) and compressed with a maximum force of compression on single station tableting machine to obtain three-layer matrix tablets. Thus, the top and bottom layers of the three-layer matrix tablet consisted of release retardant guar gum and the middle layer consisted of guar gum matrix layer along with Amoxycillin.

Evaluation of Tablet:

Evaluation of Granules: Evaluation was performed to assess the physicochemical properties and release characteristics of the developed formulations.

Pre-compression Evaluation Parameters:

Bulk Density (D_b) [7]: It is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder (passed through standard sieve # 20) into a measuring cylinder and the initial volume was noted. This initial volume is called the bulk volume. From this, the bulk density was calculated according to the formula mentioned below. It is expressed in g/cc and is given by:

$$D_b = m/V_o$$

Where, m = Weight of Granules, V_o= bulk Volume of Granules.

Tapped Density [7]: It is the ratio of total mass of powder to the tapped volume of powder. The volume was measured by tapping the powder for 500 times. Then the tapping was done for 750 times and the tapped volume was noted (the difference between these two volumes should be less than 2%). If it is more than 2%, tapping is continued for 1250 times and tapped volume was noted. It is expressed in g/cc and is given by:

$$D_t = m/V_i$$

Where, m= mass of the powder, V_i = tapped Volume of the powder.

Flow Properties (Angle of Repose (θ)) [7]: This is the maximum angle possible between the surface of a pile of powder or granules and the horizontal plane. The angle of repose of granules was determined by funnel method. The funnel was fixed at a particular height (2.5 cm) on a burette stand. The powder sample was passed through the funnel until it forms a heap. Further adding of granules was stopped as soon as the heap touches the tip of the funnel. A circle was drawn across it without disturbing the pile. The radius and height of the heap was noted down. The same procedure was repeated for three times and the average value was taken. The angle of repose was calculated by using equation.

$$\text{Tan } \theta = h/r,$$

$$\theta = \tan^{-1}(h/r)$$

Where, θ = Angle of repose**,** h = Height of the heap/pile**,** r = Radius of the heap/pile

Measurement of Powder Compressibility:

Carr's Consolidation Index (% Compressibility) [7]: The flowability of powder can be evaluated by comparing the loose Bulk density (LBD) and Tapped bulk density (TBD) of powder and the rate at which it packed down. Compressibility index of the granules was determined by the Carr's compressibility index:

$$CI\ (\%) = \frac{TBD - LBD}{TBD} \times 100$$

Hausner's Ratio [7]: It is measurement of frictional resistance of the drug. The Ideal range should be 1.2 – 1.5, it was determined by the ratio of tapped density and bulk density.

$$HR = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Post Compression Parameters:

Tablet Dimensions: Thickness and diameter were measured using calibrated Vernier calipers. Five tablets of each formulation were picked randomly and thickness and diameter was measured individually.

Hardness [8]: Hardness indicates the ability of a tablet to withstand mechanical shocks while handling. The hardness of the tablets was determined using Monsanto hardness tester. It is expressed in kg/cm^2. Five tablets were randomly picked and hardness of the tablets was determined.

Friability Test [8]: The friability of tablets was determined by using Roche friabilator. It is expressed in percentage (%). Ten tablets were initially weighed (W_1) and transferred into friabilator. The friabilator was operated at 25 rpm for 4 minutes or run up to 100 revolutions. The tablets were weighed again (W). The % friability was then calculated by-

$$\% \text{ of Friability} = \frac{1 - W_1}{W} \times 100$$

Where: W_1 = initial weight of tablets (undusted tablets), W = final weight of tablets (dusted tablets)

Uniformity of Weight (Weight Variation Test) [9]: This is an important in-process quality control test to be checked frequently (every half an hour). Corrections were made during the compression of tablets. Any variation in the weight of tablet (for any reason) leads to either under medication or overdose. So, every tablet in each batch should have a uniform weight. 20 tablets were weighed individually. Average weight was calculated from the total weight of all tablets. The individual weights were compared with the average weight. The percentage difference in the weight variation should be within the permissible limits (± 3%). The percent deviation was calculated using the following formula:

$$\% \text{ Deviation} = \frac{\text{Average weight} - \text{Individual weight}}{\text{Average weight}} \times 100$$

In all the formulations, the tablet weight is 1045 mg to 1145 mg, hence ± 3% maximum difference allowed.

Buoyancy/Floating Test: The time between introduction of dosage form and its buoyancy on the simulated gastric fluid and the time during which the dosage form remain buoyant were measured. The time taken for dosage form to emerge on surface of medium called Floating Lag Time (FLT) or Buoyancy Lag Time (BLT) and total duration of time by which dosage form remain buoyant is called Total Floating Time (TFT). The tablets were placed in a 100mL beaker containing 0.1N HCl [10].

In-vitro Dissolution Study: Measurement of in-vitro drug release was carried by using USP Dissolution apparatus-II. Dilution method was used to maintain the different pH conditions in the dissolution studies. 5 ml of the solution was added into the 900ml of buffer solution of pH 1.2which added into the dissolution flask and maintained the temperature at 37.5°C with 50 rpm. At different interval withdrawn the 5 ml aliquots and equal amount of fresh buffer medium was added into it after each sampling. This process continues for 8 hr and after completion of process the collected sample was analyzed through UV spectrophotometer at 272 nm [7].

Curve fitting analysis: The mechanism of Amoxycillin released from the matrix system was studied by fitting the dissolution data obtained to following equations [7].

a) Zero order equation.

b) First order equation.

c) Higuchi square root equation.

RESULTS AND DISCUSSIONS:

Preparation of Amoxycillin Matrix Tablets: Amoxycillin trihydrate three-layer matrix tablets with 50 mg of guar gum as release retardant layer were formulated and prepared (**Table 1**).

TABLE 1: COMPOSITION OF AMOXYCILLIN TRIHYDRATE MATRIX TABLETS CONTAINING 30 % (M) OF GUAR GUM

S. No.	Ingredients	Quantity (mg) present in matrix tablet (M)
1.	Amoxycillin trihydrate	200
2.	Guar gum	150
3.	Starch as paste	50
4.	HPMC	35
5.	Talc	10
6.	Magnesium stearate	5
7.	**Weight of guar gum granules**	**450**

TABLE 2: COMPOSITION OF GUAR GUM GRANULES FOR THREE-LAYER MATRIX TABLETS OF AMOXYCILLIN TRIHYDRATE.

S. No.	Ingredients	Quantity (mg) present in guar gum release retardant layer formulation (TLM)
1.	Guar gum	43.5
2.	Starch as paste	5
3.	Talc	1.0
4.	Magnesium stearate	0.5
5.	**Weight of guar gum granules**	**50**

Guar gum granules containing 87% of guar gum were prepared by wet granulation technique using starch paste as binder. Thus, the three-layer guar gum matrix tablets of Amoxycillin trihydrate contained a middle layer of guar gum and Amoxycillin with only guar gum in the top and bottom layers (Table 2).

Evaluation of Tablet:

Pre-compression evaluation: Bulk density for single layer granules was found to be between 0.447 – 0.575 g/ml. Tapped density was found to be between 0.524 – 0.680 g/ml. Angle of repose of granules was found to be between 24 °30' – 29°40' which were well within the specified limit of 20° - 30° and the type of flow is good. Carr's index for granules was found to be in the range of 12.8 – 16.3. All the granules are well within the specification limit. Hausner's ratio for matrix tablet was found to be between 1.139 – 1.194. With this the granules were found to be free flowing material and showed suitability to be compressed as tablets of expected weight. **Table 3** shows the granular properties of selected formulation i.e. matrix tablet (single layer).

TABLE 3: FLOW PROPERTIES OF GRANULES PREPARED BY DIFFERENT TECHNIQUES (MATRIX TABLET)

S. No.	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of Repose (θ)	Compressibility Index	Hausner's Ratio
1.	0.575	0.657	24 ° 30'	15.4	1.139
2.	0.561	0.680	26 ° 77'	14.2	1.182
3.	0.469	0.524	27 ° 80'	14.8	1.184
4.	0.447	0.565	25 ° 28'	16.3	1.194
5.	0.468	0.529	29 ° 40'	12.8	1.161
Mean	**0.504±0.026**	**0.591±0.032**	**26°71'±0.90**	**14.70±0.58**	**1.172±0.009**

Bulk density for layer 3 granules was found to be between 0.581 – 0.621 g/ml. Tapped density of granules was found to be between 0.495 – 0.584 g/ml. Angle of repose of granules was found to be between 22°09' – 24°40' which were well within the specified limit of 20° - 30° and the type of flow is good. Carr's index for layer 3 was found to be in the range of 15.3 – 16.6. All the granules are well within the specification limit. Hausner's ratio for layer 3 was found to be between 1.115 – 1.211. With this the granules were found to be free flowing material and showed suitability to be compressed as tablets of expected weight. **Table 4** shows the granular properties of selected formulation i.e. matrix tablet (single layer).

TABLE 4: FLOW PROPERTIES OF GRANULES PREPARED BY DIFFERENT TECHNIQUES (THREE LAYER MATRIX TABLET):

S. No.	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Angle of Repose (θ)	Compressibility Index	Hausner's Ratio
1.	0.591	0.510	24 °40'	15.4	1.155
2.	0.581	0.584	23 °55'	15.3	1.211
3.	0.621	0.575	23 °91'	15.9	1.115
4.	0.610	0.551	23 °60'	15.8	1.141
5.	0.607	0.495	22 °09'	16.6	1.133
Mean	**0.602±0.007**	**0.543±0.017**	**23°51'±0.221**	**15.80±0.230**	**1.151±0.016**

Post Compression Parameters:

Tablet Dimensions: Thickness and diameter were measured using calibrated Vernier callipers. Five tablets of each formulation were picked randomly and thickness and diameter was measured individually (**table 5**).

TABLE 5: DIMENSIONS OF THE TABLET:

S. No.	Diameter (mm) of Matrix Tablet	Diameter (mm) of Three Layer Matrix Tablet
1.	3.80	4.30
2.	3.71	4.35
3.	3.78	4.65
4.	3.76	3.67
5.	3.70	4.28
Mean	**3.75±0.019**	**4.25±0.159**

The diameter of the single layer matrix tablet was between 3.70 – 3.80 mm and for three layer matrix tablet was3.67 – 4.65 mm.

Hardness: The hardness of the tablets was determined using Monsanto hardness tester. It is expressed in kg/cm^2 (**table 6**).

TABLE 6: HARDNESS OF MATRIX TABLETS:

S. No.	Hardness (kg/cm^2) Matrix Tablet	Hardness (Kg/cm^2) Three Layer Matrix Tablet
1.	3.8	3.0
2.	3.9	2.9
3.	4.0	2.8
4.	4.2	3.2
5.	4.1	3.1
Mean	**4.0±0.070**	**3.0±0.070**

The measured hardness of single layer matrix tablets between 3.8 - 4.2 kg/cm^2 and three layer matrix tablet between 2.8 - 3.1 kg/cm^2. This ensures good handling characteristics of the tablets.

Friability Test: The friability of tablets was determined by using Roche friabilator. It is expressed in percentage (%) (**table 7**).

TABLE 7: FRIABILITY OF MATRIX TABLET:

S. No.	% Friability of Matrix Tablet	% Friability of Three Layer Matrix Tablet
1.	0.96	0.91
2.	0.72	0.92
3.	0.91	0.95
4.	0.86	0.89
5.	0.82	0.90
Mean	**0.854**	**0.914**

The % friability was less than 1% in all the formulations ensuring that the tablets were mechanically stable.

Uniformity of Weight (Weight Variation Test): Average weight was calculated from the total weight of all tablets. The individual weights were compared with the average weight (**table 8**).

TABLE 8: UNIFORMITY OF WEIGHT (WEIGHT VARIATION TEST) FOR MATRIX TABLET

Batch	Weight variation of tablet Weight of Tablets (mg)
Single layer tablet	502±1.809
Three layer tablet	550±0.507

The percentage of both single and three layer matrix tablet found to be in under the permissible limits (± 3%) (**table 9**).

TABLE 9: FLOATING TIME OF TABLET

Batch	FLT (Sec)	TFT (hrs)
Single layer tablet	40	>12
Three layer tablet	45	>12

The drug content of matrix tablets varied from 74.53 to 82.76 %and for three layer matrix tablet 81.20 to 92.35 % shows the post compressional properties of selected formulation. The buoyancy of the

formulation was found to be more than 20 hours with good matrix integrity.

***In-vitro* Dissolution Study:** *In-vitro* release studies were carried out using USP type II (paddle) dissolution test apparatus. 900ml of 0.1N HCl was filled in dissolution vessel and the temperature of the medium were set at 37°C ± 0.5°Cand rotational speed of 50 rpm 5ml of sample was withdrawn at predetermined time intervals for 6 hours and same volume of fresh medium was replaced. The samples were analyzed for drug content against 0.1N HCl as a blank at max 272 nm using U.V. spectrophotometer. The dissolution profiles for the nine formulations are shown in the below **table 10 and 11**.

TABLE 10: DISSOLUTION PROFILE FOR MATRIX TABLET

Sampling Time (hr)	Absorbance	Conc. (µg/ml)	Amount Release	% Amount Release	% Cumulative Amount Release
0	0.00	00	00	00	00
0.5	0.021	0.30	5.4	2.16	2.16
1	0.030	0.75	13.5	5.4	5.43
2	0.040	1.136	20.45	8.18	8.255
3	0.080	2.96	53.18	21.276	21.389
4	0.12	4.78	85.90	34.36	34.656
5	0.16	6.60	118.636	47.45	47.928
6	0.19	7.95	143.16	57.272	57.932
7	0.21	8.86	159.545	63.81	64.603
8	**0.24**	**10.227**	**184.09**	**73.65**	**74.536**

TABLE 11: DISSOLUTION PROFILE FOR THREE LAYER MATRIX TABLET

Sampling time (hr)	Absorbance	Conc. (µg/ml)	Amount Release	% Amount Release	% Cumulative Amount Release
0	0.00	00	00	00	00
0.5	0.023	0.36	6.5	2.6	2.6
1	0.034	0.87	15.5	6.2	6.236
2	0.045	1.36	24.54	9.81	9.897
3	0.086	3.23	58.04	23.23	23.366
4	0.14	5.681	102.27	40.909	41.232
5	0.19	7.954	143.18	57.272	57.84
6	0.20	8.84	158.54	63.4	64.195
7	0.23	10.225	184.0	73.59	74.384
8	**0.26**	**11.136**	**200.45**	**80.18**	**81.202**

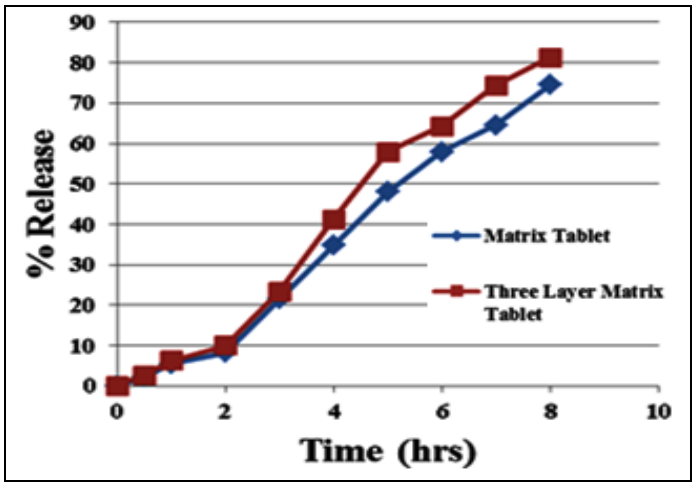

FIGURE 1: *IN-VITRO* DRUG RELEASE PROFILE OF AMOXYCILLIN MATRIX TABLETS USING GUAR GUM

In-vitro release studies were carried out in simulated gastric fluid. Release was observed after a lag period of 15 minutes after which steady and constant release of the drug was observed for a period of 8 h leaving behind the buoyant mass of tablet matrix.

The data obtained was fit in to Zero order, first order and Higuchi's model in order to determine the mechanism of release. Higuchi's model showed a regression coefficient of 0.876 for selected single layer tablet and 0.880 for three layer matrix tablet. The first order kinetics as the regression analysis showed a lower coefficient value of 0.877 for matrix tablet and 0.864 for three matrix tablet. Data plotted as cumulative percent drug release versus time, has shown regression coefficient of 0.985 for matrix tablet and 0.980 for three layer matrix tablet, which suggest that release follows zero order kinetic.

CONCLUSION: From the study, it can be concluded that gastroretentive drug delivery system of Amoxycillin could be successfully prepare dosing guar gum as a polymer. The tablet formulations of

amoxicillin may be an advantageous alternative for oral sustained release formulations and can be helpful for the treatment of peptic ulcer.

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