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RESEARCH ARTICLE

# Formulation and Evaluation of Sustained Release Linezolid Tablet using Natural Antibacterial Polymer - *Aegle marmelos*

**Shrey Patel and Rupal Jani***

Department of Pharmaceutics, Parul Institute of Pharmacy and Research, Parul University, Waghodia, Vadodara - 391760, Gujarat, India; rupal.jani@paruluniversity.ac.in

## Abstract

The objective of the current research study was to formulate and evaluate a sustained-release linezolid tablet using the natural antibacterial polymer A*egle marmelos.* The natural gum of *Aegle marmelos* is becoming increasingly used in pharmaceutical formulations as a beneficial medication with excipients. Natural-based plant materials are biocompatible, free of side effects, biodegradable, and economic. Therefore, in order to maintain the drug releases from the matrix system, *Aegle marmelos* fruit gum as a natural polymer and HPMC grade (K100M) as a synthetic polymer were used in the formulation of the linezolid matrix tablet. The formulation of sustained-release matrix tablets included the wet granulation technique. The formulated matrix tablets were evaluated in terms of weight variation, hardness, diameter, physical appearance, friability, thickness, and *in vitro* drug release. Each formulation's matrix tablet passed the required physical assessment tests. The formulation analyses of the tablets' dissolution showed sustained releases of drugs for up to 10–12 hours. Additionally, several polymer combinations and fillers were used to improve drug release factors using the $3^2$ factorial design approach, drug release kinetics were optimized, and the antibacterial study was evaluated.

**Keywords:** *Aegle marmelos,* Fruit Gum, HPMC K100M, Linezolid, Sustained Release

## 1. Introduction

One of the most widely used methods of medication administration is the oral route of drug delivery due to its convenience for patient compliance, administration, cost-effectiveness, self-medication, and flexible design of the dosage form. More than ninety percent (90%) of today's formulations are taken orally[1]. It demonstrates that the formulation's greatest popularity, availability in all countries, simplicity of production, dosage accuracy, ease of administration, and improved stability have drawn the majority of the researcher's focus in this area. These medications act as a reservoir, releasing medication continuously over a sufficient period of time to keep the plasma drug concentration at a therapeutically acceptable level[2]. Sustained-release tablets offer patients comfort while also increasing compliance with cost-effective illness treatment. The two primary categories of sustained release tablets - active drug dissolution and dissolved drugs - that are used to categorise and control drug release are further clarified by the processes. These mechanisms could function singly or in succession[3]. Linezolid (Figure 1) is regarded as the first member of the oxazolidinone antibiotic family. Its treatment involves simple infections of the skin and skin structure. Impetiginous lesions, simple abscesses and cellulitis furuncles are bacterial infections of the skin and adjacent tissues that are often caused by *Streptococcus pyogenes* and *Staphylococcus aureus*[4].

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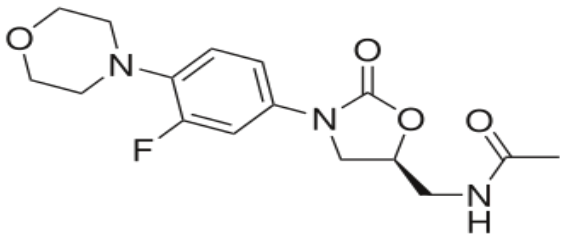


**Figure 1.** Structure of linezolid.

The substance is a synthetic antibiotic that binds to rRNA to prevent bacteria from synthesizing proteins[5]. Additionally, it stops the initiation complex during protein synthesis, which can shorten the formed peptide chains and slow down the pace at which translation elongation occurs[6]. Linezolid promotes diabetic foot infections, limb-threatening infections, pneumonia, and bloodstream infections, and is effective for the symptomatic treatment of skin structure bacterial infection. Rapid heartbeat and extreme sickness. Backaches, burning or soreness, frequent urination, trouble peeing, and all of the above[7]. Due to its rapid plasma elimination and high water solubility, linezolid is a useful model medication for creating controlled-release dosage forms. Linezolid is classified as a Class I drug by the BCS and exhibits high solubility and permeability[8] as well as 100% oral bioavailability. Linezolid mostly bounds to serum albumin, accounting for 31% of its total binding. A hydroxyethyl glycine metabolite, and an aminoethoxyacetic acid metabolite, both of which are formed by morpholine ring oxidation, are the two inactive metabolites that linezolid mostly breaks down into. Most likely formed by non-enzymatic processes, hydroxyethyl glycine is the more abundant of the two metabolites[6]. Linezolid does not appear to be susceptible to metabolism via the CYP450 enzyme system, nor does it appear to substantially inhibit or stimulate these enzymes, even though the precise enzymes involved in linezolid's bio-transformation are unknown. However, the reversible, non-selective inhibitor of monoamine oxidase enzymes is linezolid[5].

Natural gum is now being used more often in pharmaceutical formulations as a useful medicinal excipient. Natural plant-based substances are more cost-effective, free of side effects, biodegradable, renewable, biocompatible, processed in an environmentally responsible way, as well as better tolerated by patients. *Aegle marmelos* fruit gum (Rutaceae family) is frequently known as bael[9]. Its plant part is used as a fruit, which is oval, or oblong in shape, 50-20 cm in diameter, the taste is sweet, the odour is aromatic and minute oil glands dot, hard woody shell of the fruit surface, as well as colour, is grey-green until completely ripe when they turn yellow[10].

In one study, the release retardant properties of *Aegle marmelos* fruit gum were investigated in manufactured sustained-release formulations. The sustained-release method of the matrix tablets was shown to be enhanced for up to 10–12 hours[11]. According to release rate characteristics, swelling behavior, and an *in vitro* dissolution investigation, to create sustained release matrix tablets, the fruit gum from *Aegle marmelos* is an appropriate matrix-forming ingredient. According to the kinetics of the chosen formulation, which followed zero order, *Aegle marmelos* gum is an outstanding matrix-forming polymer that has been shown to delay the release of pharmaceuticals from the formulations[12]. Wet granulation, which gives improved formulation efficacy, was employed in this research to formulate the matrix tablets. The composition of linezolid matrix tablets includes the addition of a natural polymer, *Aegle marmelos* fruit gum, as well as synthetic hydroxypropyl methylcellulose, a hydrophilic polymer, in grades with varying viscosities and concentrations[13]. The study's goal was to develop a novel formulation of prolonged-release linezolid tablets and assess their effectiveness using *Aegle marmelos*, a naturally occurring anti-bacterial polymer.

## 2. Material and Method

### 2.1 Materials

The fresh *Aegle marmelos* fruit was collected from Gujarat, India. Linezolid, Hydroxypropyl Methylcellulose (HPMC), calcium phosphate dibasic, talc, and magnesium stearate were obtained from Alembic Pharmaceuticals Pvt. Ltd., Vadodara, as gift samples. Acetone (99.5%) and AR-grade chemicals, respectively. The experiment also included the use of distilled water.

### 2.2 Methodology

#### *2.2.1 Aegle marmelos Fruit Extraction*

Ripe bael fruits were taken from the *A. marmelos* tree and used to make bael fruit gum (Figure 2). The hard-wooded, spherical fruits were cut into two halves. The pulp and seeds separating the fruit's exterior wall, coupled with the amber-coloured viscous, extremely sticky, gummy material, transparent, were designated as the desirable

**Figure 2.** *Aegle marmelos* fruit.

section. This gum and the seeds were collected in a beaker filled with a glacial acetic acid solution that was 2% (v/v). The solution (slurry) was heated in a water bath for 40-45 minutes with stirring, then it was allowed to cool. To avoid particles, the slurry was filtered through muslin fabric. After adding acetone, the filtering slurry produced the gum, which then precipitated. The precipitates were crushed into a light brown, fine powder after being baked in an oven at 50°C[14].

### 2.2.2 Characterization of Aegle marmelos Gum

#### 2.2.2.1 Taxonomical Classification

The collected *Aegle marmelos* fruit was classified for its family, genus, class, kingdom, species, and order.

### 2.2.3 Physical Characterization

The collected gum was assessed for its physical properties including solubility, odour, appearance, smell, percentage yield, the percentage loss of drying, and moisture content. All values were assessed.

#### 2.2.3.1 Preliminary Phytochemical Characterization

##### 2.2.3.1.1 Detection of Carbohydrates

Molisch's test: 1 ml of the extract, 2 drops of Molisch's reagent, and 2 ml of pure ($H_2SO_4$) sulfuric acid were carefully added along with the side of the test tube. At the intersection, a violet ring that formed suggested the presence of carbohydrates.

##### 2.2.3.1.2 Detection of Protein

Millon's test: To mix 1-2 ml of sample, add 1 ml of Million's reagent, if required, heat the solution till it boils and observe the pink to brick red precipitate.

##### 2.2.3.1.3 Detection of Alkaloids

Wagner's test: Take 1-3 ml of filtrate sample with a few drops of Wagner's reagent to give a reddish brown ppt that is formed.

Dragendroff's test: Take 2-3 ml of the filtrate sample and add a few drops of Dragendroff's reagent. An orange-brown precipitate is formed.

##### 2.2.3.1.4 Detection of Tannins

Ferric chloride test: The sample should first be dissolved in water and ethanol, and then drops of a diluted ferric chloride solution ($FeCl_3$) should be added. Phenols are present when the sample develops a red, green, purple, or blue coloration.

##### 2.2.3.1.5 Detection of Gums

Ruthenium test: Observe a little volume of dried gum powder under a microscope after mounting it on the slide with ruthenium red solution. If a pink coloration appears, mucilage is present[15].

##### 2.2.3.1.6 Detection of Chloride

Add a few drops of the 10% $AGNO_3$ solution to the 3 ml of $HNO_3$-prepared test solution. $AgCl_2$ is noticed as white dilutable ppts in nitrate solution[16].

##### 2.2.3.1.7 Detection of Sulphate

Fill the 5 ml filter with a few drops of the 5% $BACl_2$ solution. $BASO_4$ appears as a white crystalline ppt. that is insoluble in HCL. Lead acetate with the reagent results in a white ppt that is soluble in NAOH[15].

#### 2.2.3.2 Flow Properties

The flow parameters of the dried *Aegle marmelos* fruit gum, including the angle of repose, tapped density, bulk density, Carr's index, and Hausner's ratio, were assessed.

##### 2.2.3.2.1 Determination of Swelling Index

The volume in milliliters filled by 1 gm of medication, including any adherent mucilage, is known as the swelling index, after 4 hours of swelling in an aqueous liquid. 1 gm of gum from each sample was allowed to swell for 4 hours with a small quantity of water, and then 1 ml of ethanol was added to each cylinder. After a few minutes, water was added to the measurement cylinders to bring the volume up to 25 ml. This was vigorously shaken once every ten minutes for an hour and then left standing for 24 hours. An occupied volume was calculated. The average of 3 measurements was used to determine of the swelling index[17].

Swelling Index % (SI) = V2/V1

V1 = Initial Volume in ml

V2 = Final Volume in ml

#### 2.2.3.2.2 Angle of Repose

It is a useful tool for calculating frictional force in loose powder. This is the greatest angle that may be formed between a pile of powder or grains' surface and the horizontal plane[18].

$$\tan\theta = h/r$$

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

Where, θ is the angle of repose,

h is the height of the pile,

r is the radius of the base of the pile.

Each sample's 5 g of powder was poured onto the graph paper using a funnel at a set height. Measurements were taken of the mounds' heights. A pencil was used to outline the piles' perimeters. The large and small squares that were present inside the produced circles were used to compute the areas of the circles, and the parameter 'r' that was determined from the area of the circle was then used to calculate the angle of repose.

#### 2.2.3.2.3 Bulk Density (Db)

Each 50 g of gum was weighed precisely before being placed into a graduated measuring cylinder. The cylinders were fastened to the bulk density device, and the powder's volume was recorded. The initial volume is the bulk volume. The following formula is used to calculate the bulk density using this information.

Db = M/Vb

Where M = Mass of powder,

Vb = Bulk volume of the powder.

#### 2.2.3.2.4 Tapped Density (TD)

Following the bulk volume notation, the same number of samples (each weighing 50 g) were placed into graduated measurement cylinders. The powder was tapped 100 times in a bulk-density device after that. The completed volumes were recorded. It is computed using the formula below[19].

Dt = M/Vt

Where M = Mass of powder,

Vt = Volume of the powder.

#### 2.2.3.2.5 Carr's Index

Compressibility, which is used to determine how smoothly a powder flows, may be calculated using the formula below,

Carr's index = Dt – Db/Dt × 100

Where, Dt = Tapped density of the powder,

Db = Bulk density of the powder.

#### 2.2.3.2.6 Hausner Ratio

The Hausner ratio is a proximate indicator of powder flow ease. The formula used to compute it is as follows[18].

Hausner ratio = Dt/Db

Where Dt = Tapped density.

Db = Bulk density.

### 2.2.4 Method of Preparing the Calibration Curve

In a dry container, 100mg of pure medication (Linezolid) that had been precisely weighed was consumed. The medication was put in a pristine 100 mL volumetric flask. Using the solvent 0.1N HCl and the phosphate buffer solution pH 6.8, the volume is properly made up to 100 ml, and after that, it receives a few slow knocks. Let the 0.1N HCl and pH 6.8 buffer solution thoroughly dissolve the tablet. This solution contains 1000 g/mL of solutions. The stock solution was formed by pipetting 10 ml of the aforementioned solution into 100 ml of the flask and filling the remaining volume with 0.1N HCl and buffer solution pH 6.8. This formed the stock solution, which had a concentration of 100 mg/ml[20].

#### 2.2.4.1 Working Standard Solution

In each case, transfer 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, and 1.8 ml of the solution to a separate 10 ml volumetric flask. The volume is precisely made up of 10 ml. Different concentrations of the stock solution were collected, including 2 g/ml, 4 g/ml, 6 g/ml, 8 g/ml, 10 g/ml, 12 g/ml, 16 g/ml, and 18 g/ml.

#### 2.2.4.2 Procedure of Standard Plot

Using a UV-visible spectrophotometer, different concentrations were created by properly diluting working standard solutions. The data was then plotted using the typical absorbance vs. concentration chart.

### 2.2.5 Fourier Transforms Infrared Spectroscopic Studies (FTIR)

To explore the interaction and compatibility between excipients and drugs, FTIR spectra for both the drug and the excipients will be recorded using an FTIR spectrophotometer and KBr pellets. 4000-400 $cm^{-1}$ on the spectrometer. The KBr will be taken and maintained in a hot-air oven for 2 hours to remove moisture content before being used in the KBr press to press pellets for the FTIR research. The medication pellets and chosen formulation excipients will next be prepared using the aforementioned dried KBr. It should be documented how the infrared experiments for the medicine and powder mixture turned out[21].

### 2.2.6 Preparation of Linezolid Tablet

The Linezolid sustained-release matrix tablets were created using the wet granulation method. Both natural polymers and artificial polymers were employed in varying percentages of 20%, 30%, 40%, and 50%. A total of 9 formulations were created, utilizing linezolid at a constant dose of 300 mg together with various excipient doses. *Aegle marmelos* gum and Hydroxypropyl Methylcellulose (HPMC) are the polymers utilized in formulations (Table 1).

### 2.2.7 Post-compression Evaluation

#### 2.2.7.1 Physical Appearance

The tablets are visually observed for changes in color, capping, lamination and chipping.

#### 2.2.7.2 Tablet Thickness

The thickness of the tablets was measured using the Vernier calliper. The average value was determined, and (5) five pills are needed. Tablet variations might lead to issues with counting and packing. Tablet thickness needs to be kept within 5% of a defined value[22].

#### 2.2.7.3 Hardness

Using a Monsanto hardness tester, the tablets' hardness was measured. The unit of measurement is $kg/cm^2$. 10 pills are chosen at random, and their hardness is measured. The tablet passed the test for hardness test and was found to be resistant to mechanical shocks[23].

#### 2.2.7.4 Weight Variation

Choosing 20 tablets at random and weighing them individually to check for weight variation[24].

#### 2.2.7.5 Friability

Twenty pills were randomly chosen and weighed from each batch. The Friabilator is used to test the friability of tablets for 100 rotations. For 4 minutes, the friability is run at 25 (revolution per minute) rpm. The tablets are thrown from a height of six inches on each revolution while being subjected to a plastic chamber that combines the effects of abrasion and shock. The pills were taken out, cleaned, and weighed once more. It is stated as a (%) percentage. Tablets with less than 1% friability are deemed acceptable[25].

$$\%\text{Friability}=\frac{(\text{initial weight})-(\text{final weight})}{(\text{initial weight})}\times 100$$

**Table 1.** Formulation of linezolid sustained release matrix tablets

| Components | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| Linezolid | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 | 300 |
| *Aegle marmelos fruit gum* | 70 | 80 | 70 | 80 | 70 | 80 | 70 | 75 | 75 |
| HPMC K100M | 10 | 10 | 20 | 20 | 15 | 15 | 10 | 20 | 15 |
| PVP K30 | 25 | 15 | 15 | 5 | 20 | 10 | 20 | 10 | 15 |
| Dicalcium phosphate | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Magnesium stearate | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Talc | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Isopropyl alcohol | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s | q.s |
| Total | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 | 430 |

#### 2.2.7.6 In Vitro Dissolution Study

*In vitro* dissolution of tablet formulations was studied using a rotating paddle (USP technique II). The linezolid dissolving medium would comprise phosphate buffers with a pH of 1.2 and 6.8. Given that the medicine is only moderately soluble at low pH levels, this pH value was selected to produce the highest volume of drug release from the tablets. The dissolving medium temperature was kept at 37± 0.5 °C, with the spinning speed set at 75 rpm. Using filter paper, 5 ml samples are collected at intervals of 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 10.0, 12.0, 18.0, and 24 h. Then, using a spectrophotometric instrument with a 251 nm wavelength, all samples were examined[26].

### 2.2.8 Factorial Design

$3^2$ Full Factorial Design was employed, using the amount of *Aegle marmelos* gum in mg and the amount of HPMC K100M in mg as independent variables. Hardness (kg/cm$^2$) and % cumulative drug release at 10-12 hrs were dependent variables. The responses were assessed using an interactive and polynomial statistical model (Tables 2 and 3).

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_{12} + \beta_{22} X_{22} + \varepsilon$$

Where Y is the dependent variable, X1 and X2 are the independent variables[26].

**Table 2.** $3^2$ Full factorial design layout

| $3^2$ Full Factorial Design Layout | | | | |
|---|---|---|---|---|
| Batch No. | Independent Variable | | | |
| | Coded value | Amount of *Aegle marmelos* gum (mg) | Coded value | Amount of HPMC K100M (mg) |
| F1 | -1 | 70 | -1 | 10 |
| F2 | -1 | 70 | 0 | 15 |
| F3 | -1 | 70 | 1 | 20 |
| F4 | 0 | 75 | -1 | 10 |
| F5 | 0 | 75 | 0 | 15 |
| F6 | 0 | 75 | 1 | 20 |
| F7 | 1 | 80 | -1 | 10 |
| F8 | 1 | 80 | 0 | 15 |
| F9 | 1 | 80 | 1 | 20 |

**Table 3.** Independent and dependent variables of formulation

| | Level | Amount of polymer *Aegle marmelos* (X1) | Amount of polymer HPMC K100M (X2) |
|---|---|---|---|
| Independent Variable | Low | 70 | 10 |
| | Medium | 75 | 15 |
| | High | 80 | 20 |
| Dependent Variable | Hardness (kg/cm$^2$) | | |
| | % Cumulative Drug Release at 10-12 Hrs | | |

### 2.2.9 Kinetic Modelling of in vitro Drug Release

#### 2.2.9.1 Zero Order Kinetics

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation (1):

$$Q_t = Q_0 + K_0 t \quad ---(1)$$

Where Qt is the amount of drug dissolved in time t,

$Q_0$ is the initial amount of drug in the solution (most times, $Q_0 = 0$) and

$K_0$ is the zero-order release constant expressed in units of concentration/time.

To study the release kinetics, data obtained from *in vitro* drug release studies was plotted as the cumulative amount of drug released versus time.

#### 2.2.9.2 First-Order Kinetics

This model describes the absorption and/or elimination of some drugs, although it is difficult to this mechanism on a theoretical basis on the release of the drug which followed first-order kinetics can be expressed by the equation (2):

$$Log\ C = \log C_0 - \frac{Kt}{2.303} \quad ---(2)$$

Where $C_0$ is the initial concentration of the drug,

k is the first-order rate constant, and t is the time.

The data obtained are plotted as a log cumulative percentage of drug remaining vs. time, which would yield a straight line with a slope of -K/2.303[27].

#### 2.2.9.3 Higuchi Model

Accordingly, the model expression is given by the equation (3):

$$f_t = Q = A\sqrt{D\,(2C - C_s)\,C_s t} \quad ---(3)$$

Where Q is the amount of drug released in time t per unit area A,

C is the drug initial concentration,

Cs is the drug solubility in the matrix media and

D is the diffusivity of the drug molecules (diffusion coefficient) in the matrix substance. The data obtained were plotted as cumulative percentage drug release versus square root of time[28].

#### 2.2.9.4 Korsmeyer-Peppas Model

To find out the mechanism of drug release, the first 60% of drug release data were fitted in the Korsmeyer-Peppas mode of the equation (4):

$$\frac{M_t}{M_\infty} = Kt^n \quad ---(4)$$

Where Mt/M∞ is a fraction of the drug released at time t,

k is the release rate constant and

n is the release exponent.

The n value is used to characterize different releases for cylindrical-shaped matrices[28].

### 2.2.10 Antibacterial Study (Zone of Inhibition)

Mueller–Hinton agar plates were used for the test. After pouring approximately 20 ml of molten agar media into sterile Petri dishes, the agar plates were allowed to solidify under a laminar flow hood. It had been demonstrated with $10^5$ organisms/ml suspensions of each strain of Methicillin-Resistant *Staphylococcus aureus* (MRSA). The inoculum of these cultures was spread on the surface of the agar plates. Plates were allowed to air dry for 10 minutes. After creating a zone in each plate, pouring the drug on it, and placing it in the incubator at 37.8°C for 24 hours, the zones of inhibition (diameter in mm) are measured on the agar surface with a scale[29].

## 3. Results and Discussion

### 3.1 Gum Characterization

#### 3.1.1 Taxonomical Classification

It is based on the taxonomy of *Aegle marmelos*, which is classified as belonging to the Kingdom of Plantae, Order of Sapindales, and family Rutaceae. The detailed classifications were shown in Table 4. Characterization and flow properties are shown in Tables 5, 6 and 7.

**Table 4.** Taxonomical classification of *Aegle marmelos* gum

| | |
|---|---|
| Kingdom | Plantae |
| Subfamily | Aurantioideae |
| Order | Sapindales |
| Family | Rutaceae |
| Genus | *A. marmelos* |
| Species | Aegle species |
| Tribe | Clauseneae |

**Table 5.** Physical characterization of *Aegle marmelos* fruit gum

| Parameters | Observation of gum |
|---|---|
| Color | Yellowish brown |
| Odour | Characteristic |
| Taste | Characteristic |
| Nature | Amorphous |

**Table 6.** Phytochemical characterization of *Aegle marmelos* fruit gum

| Test | Observed gum |
|---|---|
| Carbohydrates | Present |
| Glycoside | Absent |
| Tannins | Absent |
| Saponins | Absent |
| Protein | Present |
| Gum | Present |
| Polysaccharide's | Present |

**Table 7.** Flow properties of *Aegle marmelos* fruit gum

| Parameter | Observed gum |
|---|---|
| pH (1% w/v solution) | 6.4 |
| Swelling index | 16.8% |
| Bluk density | 0.47 ± 0.01 (g/cm$^3$) |
| Tapped density | 0.56 ± 0. 01 |
| Hausner's ratio | 1.19 ± 0. 006 |
| Carr's index | 16.23 ± 0. 44 |
| Angle of repose | 28.09 ± 0.70 |

The physicochemical properties and micromeritics properties of gums were performed as per the procedure. It was observed that gum was acidic in nature but they are near neutral. Information on the pH of polymers is an important parameter in determining their appropriateness

in formulations, while the stability and physiological action of most preparations are influenced by pH. The pH of the 1% w/v test solution of gum in water was 6.4. The neutral pH suggested that when it was used in uncoated tablets, it may be less irritating to the GI tract. It may find beneficial application in the formulation of acidic, basic and neutral drugs. *Aegle marmelos* (16.8)% gum has a good swelling index. The gum had a high swelling index, suggesting that the gums may perform well as matrixing agent. Tapped density and bulk density of gum were found to be 0.47 ± 0.01 and 0.56 ± 0.01, respectively, and results of Hausner's ratio, angle of repose, and Carr's index showed passable flow properties of gum powder.

### 3.1.2 FTIR Spectroscopy Study (Figure 3, Table 8)

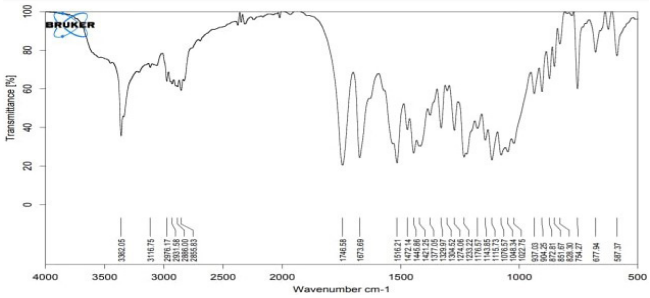



**Figure 3.** FTIR spectra of *Aegle marmelos* gum.

**Table 8.** Wave number of *Aegle marmelos* gum

| Sr. No. | Functional group present | Wave numbers ($cm^{-1}$) of gum |
|---|---|---|
| 1 | OH – Stretching | 3362.05 |
| 2 | C-H Stretching | 2931.58 |
| 3 | C=O Stretching | 1746.86 |
| 4 | C-O Stretching | 1673.69 |
| 5 | C-C Deformation | 1421.25 |
| 6 | Secondary OH | 1022.75 |

### 3.1.3 Calibration curve of Linezolid (Figure 4, Table 9)

**Table 9.** Absorption of the drug in 0.1 N HCL

| Sr. No. | Concentration (ug/ml) | Absorbance |
|---|---|---|
| 1 | 2 | 0.108±0.0012 |
| 2 | 4 | 0.235±0.0012 |
| 3 | 6 | 0.336±0.0012 |
| 4 | 8 | 0.465±0.0012 |
| 5 | 10 | 0.567±0.0008 |
| 6 | 12 | 0.693±0.0012 |
| 7 | 14 | 0.792±0.0012 |
| 8 | 16 | 0.923±0.0012 |

Calibration curve,
Correlation Coefficient ($R^2$) = 0.999
Where y = Absorbance X = Concentration (ug/ml)

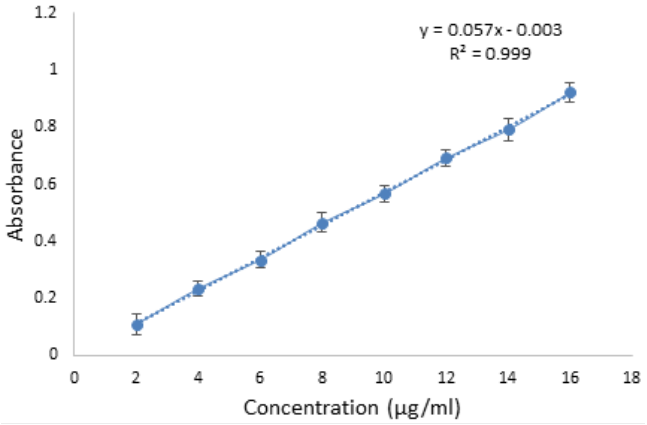



**Figure 4.** Calibration curve of linezolid in 0.1 N HCL.

**Table 10.** Absorption of the drug in phosphate buffer 6.8

| Sr. No. | Concentration (ug/ml) | Absorbance |
|---|---|---|
| 1 | 2 | 0.309±0.0012 |
| 2 | 4 | 0.385±0.0016 |
| 3 | 6 | 0.466±0.0012 |
| 4 | 8 | 0.537±0.0008 |
| 5 | 10 | 0.616±0.0016 |
| 6 | 12 | 0.686±0.0016 |
| 7 | 14 | 0.777±0.0012 |
| 8 | 16 | 0.905±0.0012 |
| 9 | 18 | 0.984±0.0012 |

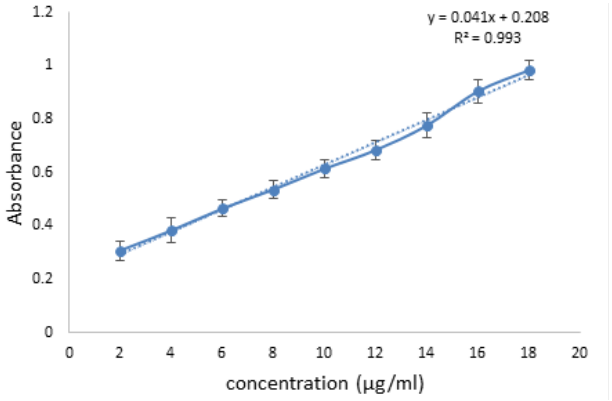



**Figure 5.** Linezolid in phosphate buffer 6.8.

The regression coefficient value was found to be 0.999 and the line equation was y = 0.057x + 0.00 for HCL and y = 0.041x + 0.208, $R^2$ = 0.993 for a buffer, $R^2$ value of drug showed linearity of the standard curve (Figure 5, Table 10).

### 3.1.4 *Factorial Design Formulations of Linezolid Sustained Release Matrix Tablet (Figure 6, Tables 11, 12 and 13)*

**Table 11.** Results of powder blends of factorial design tablet

| Batch No. | Angle of Repose (°) | Bulk Density (g/ml) | Tapped Density (g/ml) | Carr's Index (%) | Hausner Ratio |
|---|---|---|---|---|---|
| F1 | 28.15±0.04 | 0.394±0.02 | 0.465±0.03 | 15.26±0.03 | 1.18±0.02 |
| F2 | 29.23±0.02 | 0.395±0.04 | 0.471±0.03 | 16.13±0.02 | 1.19±0.02 |
| F3 | 30.65±0.03 | 0.356±0.03 | 0.468±0.02 | 23.93±0.03 | 1.31±0.03 |
| F4 | 30.44±0.04 | 0.384±0.02 | 0.472±0.03 | 18.64±.0.02 | 1.22±0.02 |
| F5 | 34.9±0.03 | 0.378±0.03 | 0.571±0.02 | 33.80±0.03 | 1.51±0.03 |
| F6 | 36.75±0.02 | 0.394±0.02 | 0.471±0.02 | 16.34±0.02 | 1.19±0.04 |
| F7 | 29.85±0.04 | 0.395±0.03 | 0.471±0.03 | 16.34±0.03 | 1.19±0.03 |
| F8 | 30.65±0.03 | 0.384±0.02 | 0.472±0.02 | 18.64±0.02 | 1.22±0.03 |
| F9 | 30.86±0.02 | 0.355±0.04 | 0.448±0.03 | 20.75±0.03 | 1.26±0.02 |

(Where n = 3, Mean ± SD)

**Table 12.** Results of post-compression of factorial design tablet

| Batch no. | Weight variation | Friability (%) | Thickness (mm) | Hardness (kg/cm$^3$) | Drug content |
|---|---|---|---|---|---|
| F1 | 429±0.01 | 0.464±0.04 | 3.5±0.04 | 5.3±0.02 | 91.02 |
| F2 | 427±0.05 | 0.465±0.05 | 3.6±0.03 | 7.2±0.03 | 96.65 |
| F3 | 429±0.02 | 0.466±0.06 | 3.4±0.02 | 5.8±0.02 | 93.21 |
| F4 | 429±0.02 | 0.464±0.05 | 3.7±0.03 | 8.0±0.03 | 98.52 |
| F5 | 422±0.01 | 0.463±0.06 | 3.6±0.04 | 5.5±0.02 | 92.86 |
| F6 | 429±0.02 | 0.465±0.04 | 3.5±0.06 | 7.7±0.03 | 97.86 |
| F7 | 431±0.01 | 0.465±0.05 | 3.6±0.04 | 6.0±0.03 | 95.01 |
| F8 | 428±0.02 | 0.463±0.05 | 3.8±0.05 | 6.9±002 | 96.11 |
| F9 | 431±0.04 | 0.464±0.04 | 3.6±0.03 | 6.2±0.02 | 95.65 |

**Table 13.** Results of *in vitro* dissolution study of factorial design batch F1-F9

| Time (Hours) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | **0** | 0 | 0 | 0 | 0 | 0 |
| 1 | 7.95 | 8.06 | 8.18 | **7.92** | 7.96 | 7.59 | 8.03 | 9.45 | 8.02 |
| 2 | 19.82 | 18.75 | 20.95 | **19.82** | 17.78 | 20.36 | 18.75 | 22.75 | 31.75 |
| 3 | 22.97 | 22.09 | 27.45 | **22.97** | 31.32 | 32.02 | 35.39 | 26.54 | 39.48 |
| 4 | 33.56 | 36.55 | 35.69 | **33.58** | 39.49 | 42.45 | 36.52 | 36.25 | 48.36 |
| 5 | 40.35 | 39.54 | 39.46 | **40.58** | 48.35 | 49.88 | 45.62 | 46.15 | 56.45 |
| 6 | 47.45 | 46.48 | 46.83 | **47.45** | 56.45 | 58.45 | 55.38 | 50.66 | 67.78 |
| 7 | 53.65 | 52.68 | 58.45 | **53.65** | 67.88 | 69.55 | 56.55 | 58.23 | 73.26 |
| 8 | 65.26 | 66.49 | 63.68 | **65.26** | 73.28 | 75.95 | 67.96 | 68.36 | 78.76 |

(*Continued*)

**Table 13.** (Continued)

| Time (Hours) | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|---|---|---|---|
| 9 | 72.36 | 70.05 | 71.45 | **72.36** | 78.85 | 81.16 | 74.56 | 75.06 | 83.85 |
| 10 | 79.55 | 73.65 | 76.69 | **79.55** | 83.85 | 85.16 | 83.25 | 80.23 | 87.48 |
| 11 | 86.45 | 85.36 | 83.68 | **86.45** | 87.48 | 89.26 | 87.45 | 87.65 | 91.82 |
| 12 | 90.22 | 97.12 | 92.54 | **98.29** | 91.25 | 92.76 | 93.56 | 96.25 | 94.23 |

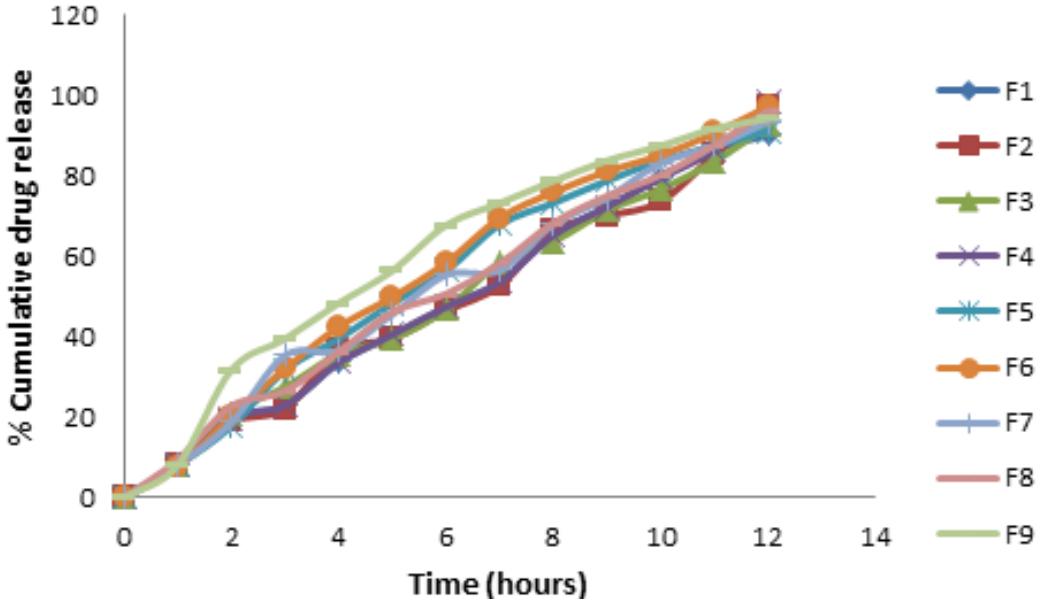



**Figure 6.** Drug release profile of batches F1-F9.

### *3.1.5 $3^2$-factorial Design (Figures 7 and 8)*

- Response 1: Hardness

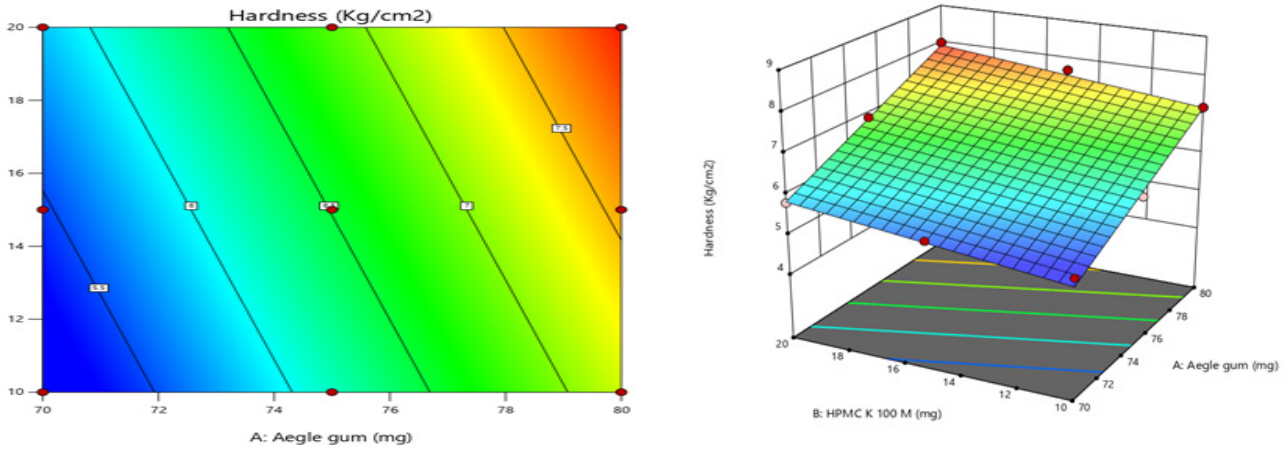



**Figure 7.** Contour plot for the effect of concentration of gum (X1) and concentration of polymer (X2) on the hardness.

- Response 2 - % CDR

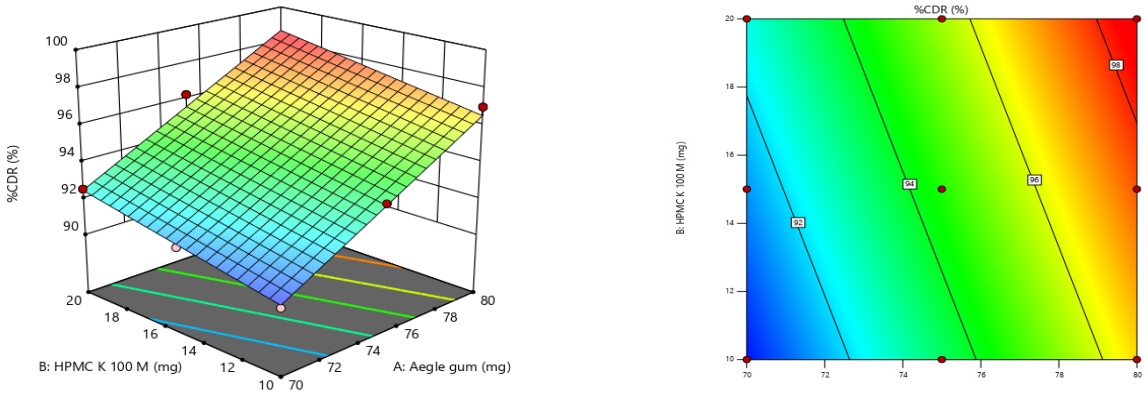



**Figure 8.** Contour plot for effect of gum (X1) and amount of HPMC K100M (X2) on % cumulative drug release at 10-12 hrs.

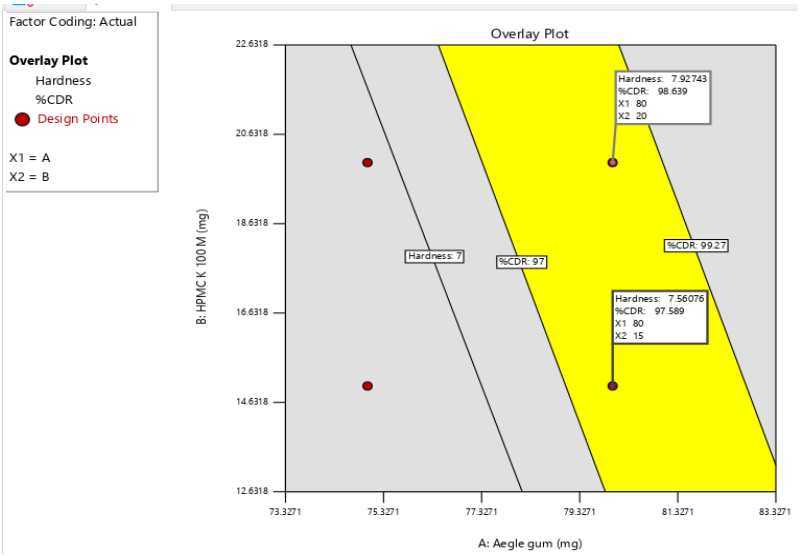



**Figure 9.** Overlay plot showing the combined effect of *Aegle marmelos* gum (X1) and polymer (X2).

Discussion: The overlay plot revealed that the area of interest is in the "yellow zone" of Figure 9. Formulation having a concentration of *Aegle marmelos* gum (X1) (80) and an amount of polymer (X2) (20 mg) with a hardness of 8.0 kg/cm$^2$, and in the experimental area of the overlay plot, the %CDR is 97.29% showed the greater acceptability than other checkpoint batches. It was therefore chosen as batches OF1 and OF2 (Table 14).

Discussion: The checkpoint batch findings showed no significant difference between the two results. As a result, in certain variable ranges, the equation obtained for specific responses is validated. The similarity between observed and expected response values was used to measure the robustness of prediction and these statistics show the validity of the produced model. Batch F4 was selected as the optimized formulation (Figure 10).

**Table 14.** Results of checkpoint batch OF1 and OF2

| Batch Code | Parameters | Predicted Value | Experimental Value |
|---|---|---|---|
| OF1 | Hardness | 7.92 | 8.01±0.03 |
| | % cumulative drug release up to 12 hrs. | 98.63 | 98.12±0.87 |
| OF2 | Hardness | 7.56 | 7.35±0.52 |
| | % cumulative drug release up to 12 hrs | 97.58 | 96.98±0.42 |

**Figure 10.** Tablets of Optimized formulation batch F4.

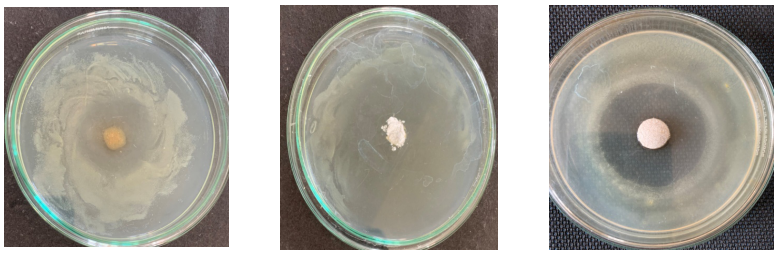
### 3.1.6 Kinetic Modelling of In Vitro Drug Release (Table 15)

**Table 15.** Release kinetics of linezolid from sustained release matrix tablets

| Formulation | Zero-order kinetics | First-order kinetics | Higuchi kinetics | Korsmeyer–Peppas kinetics | |
|---|---|---|---|---|---|
| | $R^2$ | $R^2$ | $R^2$ | $R^2$ | n |
| F1 | 0.9961 | 0.7975 | 0.9782 | 0.8363 | 1.30 |
| F2 | 0.9921 | 0.8323 | 0.9663 | 0.8366 | 1.13 |
| F3 | 0.9939 | 0.8065 | 0.9739 | 0.7835 | 1.18 |
| F4 | 0.9972 | 0.7960 | 0.9696 | 0.8425 | 1.15 |
| F5 | 0.9948 | 0.7787 | 0.9819 | 0.6835 | 1.21 |
| F6 | 0.9705 | 0.8978 | 0.9949 | 0.7635 | 1.23 |
| F7 | 0.9861 | 0.7888 | 0.9875 | 0.7585 | 1.29 |
| F8 | 0.9949 | 0.7877 | 0.9838 | 0.5862 | 1.35 |
| F9 | 0.9412 | 0.7960 | 0.9874 | 0.8030 | 1.37 |

The mechanism of drug release from the sustained-release matrix tablet, formulations were subjected to modelling and drug release kinetic studies such as zero-order, first-order, the Higuchi model, and the Korsmeyer–Peppas model. The value of the Regression coefficient ($R^2$) from the drug release kinetic model was tabulated. The result of the modelling study revealed that the release of drug from matrix tablets of *Aegle marmelos* gum contained tablets fitted best into zero-order kinetics with maximum values of $R^2 = 0.9972$ and a n value of 1.15. It was revealed that the mechanism of drug release was a super case-II transport, indicating the drug release rate does not change over time and the release is attributed to zero-order.

### 3.1.7 Antibacterial Study (Figure 11, Table 16)



**(a)** **(b)** **(C)**

**Figure 11.** Zone of inhibition **(a)** Linezolid, **(b)** AMG, **(c)** Formulation.

**Table 16. Zone of inhibition of different sample**

| Sr. No. | Sample | Zone of inhibition (mm) |
|---|---|---|
| 1 | API | 32 |
| 2 | Herbal polymer | 28 |
| 3 | Optimized formulation batch F4 | 38 |

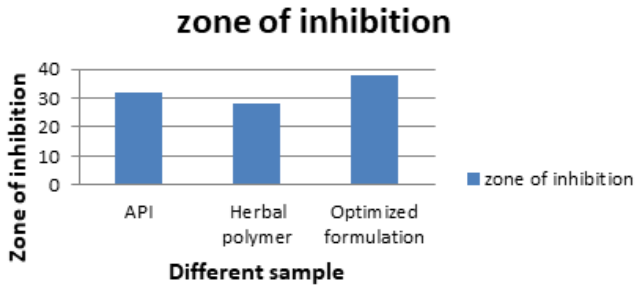



**Figure 12.** Zone of inhibition of different samples.

Discussion: Regarding the antibacterial activity against methicillin-resistant *S. aureus*, it was observed that there was no significant difference in the zone of inhibition between pure drug, herbal polymer, and optimised formulation, which reveals that the optimised formulation of drug and *Aegle marmelos* gum maintains the antibacterial activity (Figure 12).

## 4. Conclusion

In this research work, the novel linezolid matrix tablets were formulated using a wet granulation technique with improved tablet hardness, thickness, friability, dissolution release profile, and the $3^2$-factorial designs (batch F4) selected for the optimization of the formulation. The *in vitro* drug release shows the extended release of the drug up to 10–12 hours, The release kinetics revealed that the formulations followed the zero order kinetics. The antimicrobial study showed that *Aegle marmelos* had a synergistic effect on the culture of Methicillin-Resistant *Staphylococcus aureus* (MRSA) and showed a zone of inhibition in the range of 28–38 mm in diameter. Thus, the gum acted as a hydrophilic polymer that can be employed in formulating successful matrix sustained release tablets.

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