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RESEARCH ARTICLE
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STABILITY STUDIES OF NOVEL ANTIBACTERIAL CAPSULE AGAINST MULTI DRUG RESISTANCE GRAM NEGATIVE BACTERIA

Anum Tariq[1], Mirza Tasawer Baig[1*], Samina Sheikh[1], Anum Tariq[2] ,Anwar Ejaz Beg[3], Uzma Shahid[4], Farhan Ahmed[5], Minahil Baig[6], Muhammad Aurangzeb Khan[7]

[1]Department of Pharmacy Practice, Ziauddin University, Karachi Pakistan
[2]Jinnah Sindh Medical University, Karachi Pakistan
[3]Department of Pharmaceutics, Ziauddin University, Karachi Pakistan
[4]Department of Pharmacology, Ziauddin University, Karachi Pakistan
[6]Federal Medical College (PIMS), SZABMU, Islamabad Pakistan
[7]Shaheed Benazir Bhutto Dewan University, Karachi Pakistan

Corresponding Author: Prof. Dr. Mirza Tasawer Baig,
*Email: tasawer.baig@zu.edu.pk

ABSTRACT
Capsules are solid dosage forms which help to mask the bitter taste of drug and are quite save to engulf so they are common to be used. This study deals with the formulation and characterization of novel antibacterial capsules containing salicylic acid, citric acid and sodium citrate effective against multidrug gram-negative organism i.e, Klebsiella pneumoniae, E. coli, Acinetobacter baumannii and Pseudomonas aeruginosa. Using different ratios five capsules were formulated and tested for dissolution and disintegration studies, drug content and weight variation. Out of 5 formulations only F5 qualify all evaluation parameters. Stability testing was also performed as per ICQH guideline. The uniformity of weight test results showed that all capsule size "0" were within the acceptable weight range i.e 480 ± 2.7mg. F5 capsules recorded the lowest disintegration time of 6.8 ± 0.2 while over 85% of active ingredients were released within 30 minutes. Stability studies were also conducted, all measured parameters remained within pharmacopeial acceptance criteria, indicating that the formulation sustained acceptable stability under the tested conditions.

Key Words: Salicylic Acid, multidrug gram-negative bacteria, Capsule, antibacterial

INTRODUCTION
Antimicrobial resistance (AMR) is considered to be one of the most critical public health encounters of the 21st century. In 2021, approximately 4·71 million deaths were directly linked with bacterial AMR, with low-income and middle-income countries unduly affected (GBD 2021). In particular, MDR strains of Enterobacteriaceae (especially Klebsiella pneumoniae and Escherichia coli), Acinetobacter baumannii and Pseudomonas aeruginosa, have emerged as predominantly serious threat, In United States, Multidrug resistant (MDR) strains of these organisms/microbes have been reported from hospitals throughout the country and are not limited to a small subset of hospitals (Cerceo et al., 2016).
Recently, bacteria have developed resistance to many commonly used antimicrobial agents rendering them useless. How ever, in addition to research to find novel antimicrobials, harnessing

old medicine/drugs for their antimicrobial or appurtenant properties is an exciting venture. Salicylic Acid (SA) have been studied extensively and their effect/outcomes on the antimicrobial profiles of pathogenic microbes is of great attention (Sykes et al., 2024).

Previous research into combination therapies has focused largely on conventional antibiotic pairings, but the incorporation of generally recognized as safe (GRAS) excipients with intrinsic antimicrobial activity offers a promising new avenue. Formulation into oral capsules provides a systemic route for addressing infections in the gut and urinary tract, where pH manipulation can alter microbial viability (Shrestha et al., 2019).

Salicylic acid|(SA) was first recognized in willow (genus Salix) bark, which has been Utilized since ancient times to lessen pain and diminish fevers due to its antipyretic, analgesic, and anti-inflammatory properties (Macario et al., 2024). SA traditionally used in dermatological applications, has demonstrated broad-spectrum antimicrobial activity. when combined with agents like sodium citrate and citric acid, the formulation may benefit from enhanced antimicrobial synergy, potentially affecting bacterial pH tolerance and membrane stability (El-Soudany et al., 2024). Oral formulations of such synergistic agents remain underexplored in the fight against MDR infections.

Various researches also shows that citric acid can significantly lower the terminating effect of bactericidal antibiotics after has been exposed to bacterial organisms, and it also shows a dose dependency with an elevating concentration. Citric acid, which is an organic acid with bacterial killing ability, has a mechanism which is anti bacterial and mainly involves destroying the cell membrane of bacteria and lowering the pH to hault bacterial growth (Li et al., 2023)

The patient friendly form of dosage that is quite acceptable by patients are capsules. The more favourable things of capsule forms include capsules can masked the bitter taste and completely hide the odour of the core medicinal material, these are very easy to engulf, are usually temperature resistant and shows stability in storage, it can be packed with alone or combine with medicinal contents in granular form , and the processing can be very quick and quite easier as it does not required a lot of extra ingredients/auxiliaries like tablets (Masfria et al., 2023).

The definition of stability testing is described as a complicated process due to various numbers of different steps which has profound effect on the stability of a pharmaceutical drug/ product. The number of factors is important which include stability of the active ingredient(s); complex reactions between ingredients which are active and excipients, various categories of dosage form, manufacturing process that can be used, container/closure device system used for packing and various environmental factors like light, moisture and heat or cold conditions at a time of shipment, handling and storage. Further more, disintegration steps like hydrolysis or racemization, oxidation and reduction, which plays a significant role in stabilization of a pharmaceutical drug ingredient, it also depends on different factor like pH, radiation, concentration of reactants catalysts etc., as well as the basic materials that can be used and the time between manufacture and usage of the product (Bajaj et al., 2012).

This study proposes a novel anti bacterial oral capsule formulation containing salicylic acid, sodium citrate, and citric acid. The capsules so formulated will be further evaluated for the quality control test and also performed stability studies.

METHODOLOGY

Material/Reagents used: The basic components that are used in this study were pro analytical quality materials. Sigma-Aldrich is the base component from which Salicylic Acid were obtained while Sodium citrate and Citric Acid from Merck. Microcrystalline Cellulose obtained from FMC Biopolymer's. Lubricant such as talc purchased from local supplier while Magnesium stearate was purchased from BASF, and size 0 empty gelatine capsule shells were obtained from “Capsugel”.

Preparation of Capsule: All ingredients were sieved using a #60 mesh. Salicylic acid, sodium citrate, and citric acid were dry-blended with microcrystalline cellulose. Lubricants (magnesium stearate and talc) were added and blended gently. The final blend was filled into size 0 hard gelatine capsules (~480 mg). Capsules were stored in HDPE bottles at 25°C and 40°C for further evaluation.

Analysis of Capsules. The capsules were estimated for their appearance, weight variation, disintegration time and dissolution.

Weight Variation**:** For weight variation, twenty capsules were randomly selected from each batch. The capsules were weighed using a digital balance, and the average weight was calculated. To determine the weight variation, the individual capsule's weights were compared to the calculated average weight (Uddin et al., 2015).

Disintegration: The test for disintegration was done by introducing one capsule into each basket tube, then one disc was placed into each tube, and the system was run. The medium used for disintegration was distilled water kept at 37±0.5°C. At the end of the time limit stated as the "capsule disintegration time", the capsule is declared destroyed if no more capsules are left on the tube (Osei-Asare et al., 2021).

Dissolution Test: The study was carried out using type-2 paddle type USP apparatus. The set condition was 900ml of 6.8 pH phosphate buffer, at 50 rpm, 37^{o}c for 30 minutes, 5 mL samples were withdrawn and immediately replaced with an equal volume of fresh, pre-warmed dissolution medium to maintain sink conditions and constant volume. The withdrawn samples were suitably diluted prior to further analysis (USP2020).

Assay: Salicylic acid content was determined using a validated High-Performance Liquid Chromatography (HPLC) method. Citrate and citric acid content were quantified using a UV-Visible spectrophotometric method by measuring the absorbance at approximately 209–210 nm.

Stability studies This study was conducted in accordance with the International Committee on Harmonization guidelines (ICH QIA (R2), 2003). Formulation placed at room temperature for 6 months at 25°C ± 2°C and 60% RH ± 5% and at accelerated states 40°C ± 2°C and 75% RH ± 5%. The placed samples were collected at 0, 1, 2, 3 and 6 months. Assessment was done on the basis of various test including physical appearance, assay, dissolution test and microbial load.

RESULTS

An oral antibacterial capsule was developed containing salicylic acid, citric acid, and sodium citrate in different ratios. All capsule batches (F1–F5) were opaque white, uniform, and free from cracks or powder leakage. Composition of Five batches which were optimized as shown in Table 1. F5 was selected as the final optimized batch due to superior dissolution and assay results.

Table 1: Composition of(F1-F5) Capsules

Batch	Salicylic Acid (mg)	Citric Acid (mg)	Sodium Citrate (mg)	Microcrystalline Cellulose (mg)	Total (mg)
F1	200	50	100	130	480
F2	180	60	120	120	480
F3	160	70	140	110	480
F4	150	80	150	100	480
F5 (Final)	140	80	160	100	480

Table 2: Quality Assessment of F5 Capsule

Parameter	Observed Values
Capsule fill weight	480 ± 2.7
Disintegration time	6.8 ± 0.2
Dissolution (30 min)	91.7% ± 0.6
Assay (SA/SC/CA)	98.7% / 97.5% / 96.8%
Microbial growth	Nil

Figure 1: Disintegration Profile of F5 capsule at different interval

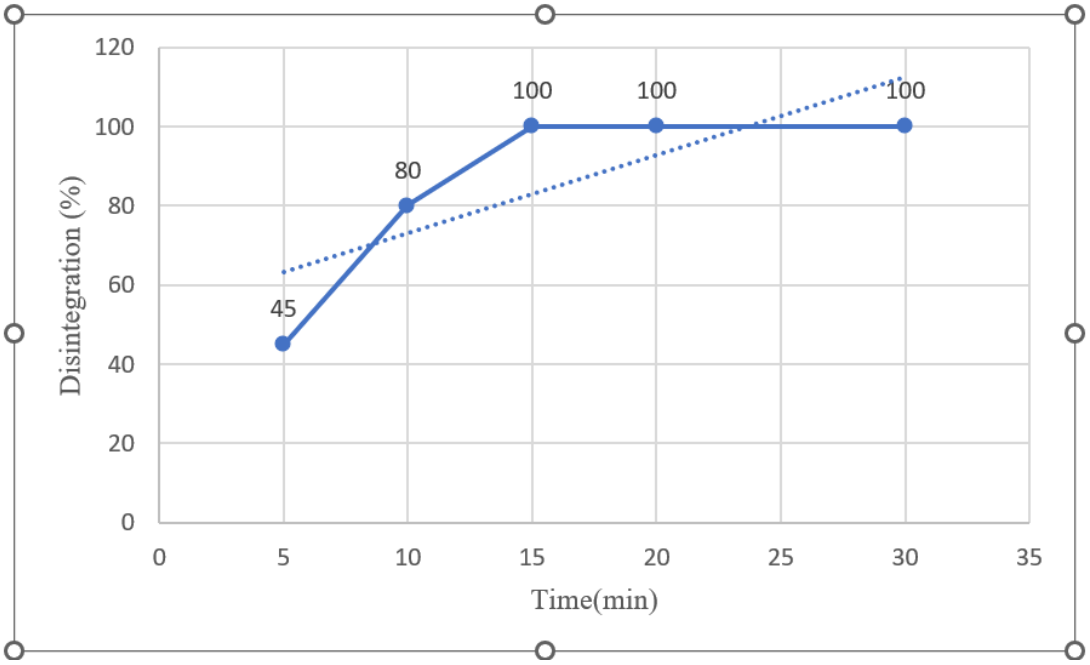

Figure 2: Dissolution Profile before vs Accelerated Stability

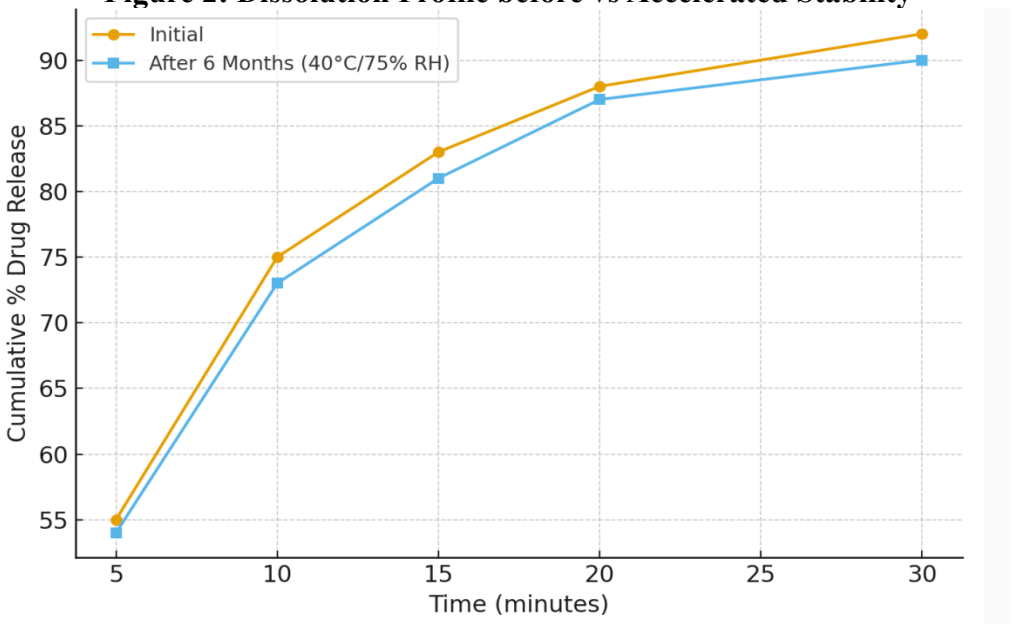

Table 3: Stability at 25°C/60% RH

Month	Appearance	SA (%)	SC (%)	CA (%)	Dissolution (%)	Microbial Load
0	Intact	98.7	97.5	96.8	91.2	<10 CFU/g
1	No change	98.2	96.9	96.0	90.4	<10 CFU/g
2	No change	97.3	96.1	95.3	89.1	<10 CFU/g
3	Slight fade	96.5	95.2	94.2	87.8	<10 CFU/g
6	Slight discoloration	94.8	94.1	93.0	85.5	<50 CFU/g

Table 4: Accelerated Stability at 40°C/75% RH

Month	Appearance	SA (%)	SC (%)	CA (%)	Dissolution (%)	Microbial Load
0	Intact	98.7	97.5	96.8	91.2	<10 CFU/g
1	No change	97.5	96.1	95.4	89.8	<10 CFU/g
2	Slight haze	96.2	95.0	94.2	87.3	<50 CFU/g
3	Faded	94.9	93.4	92.5	84.9	<100 CFU/g
6	Discolored	92.3	90.7	89.8	81.2	<150 CFU/g

DISCUSSION

Oral dosage forms remain the most convenient route for systemic delivery (Kute et al., 2023). Capsule formulations allow precise dosing, improved stability, and ease of administration. The results of this study demonstrate that the formulated oral capsule containing salicylic acid, sodium citrate, and citric acid is physically and chemically stable against multidrug-resistant Gram-negative pathogens.

Preparation of Capsule

Table 1 depicted five different capsule formulations how ever, only F5 selected for further analysis.

Capsule evaluation parameters

Weight variation

As discussed above table 2, The uniformity of weight test results showed that all capsule size “0” were within the acceptable weight range i.e 480 ± 2.7mg. This indicates uniform filling of powder blend during capsule filling. Not more than two of the individual weights deviated from the average weight by more than 5% and none deviated by more than twice that percentage, which provided good weight uniformity as shown in Table 5.

Table 5: USP limits for weight variation for Capsule (Thakur et al., 2019)

Less than 130mg	±10.5%
130mg-324mg	±7.5%
324mg or more	±5%

Disintegration test

Disintegration is the first step in the bioavailability cascade (Berardi et al., 2021). It provides critical It offers critical safety potential related to drug bioavailability in the body without requiring the use of in vivo techniques. Formulated capsules which fail the disintegration test may not release the drug on time to provide the desired therapeutic effect.

The disintegration time for hard gelatine capsules is set at not more than 900 s (15 min) (Stegemann et al., 2014). F5 capsules recorded the lowest disintegration time of 6.8 ± 0.2min. However, Figure 1 depicted the disintegration of F5 capsule at different interval i.e 5, 10 15, 20, 30 min which is found concordant with Pharmacopoeia standards which requires a maximum of 15 min for capsule products to disintegrate using hard gelatine capsules.

Dissolution study

Drug absorption and physiological availability based on the drug substance on the drug's substance being dissolved at the absorption site. Dissolution test determines the rate and extent to which drug dissolution occur from the capsule dosage forms. This test serves as a quality control assessment tool to validate that various medication/drug product batches have comparable/similar drug release properties and that a specific batch of capsules dissolves similarly to the batch that was firstly determined to be clinically effective (Aliyu et al., 2020).

The results of the dissolution studies on the F5 capsule were shown revealed that over 85% of active ingredients were released within 30 minutes, indicating rapid onset of action. This rapid dissolution is essential for antimicrobial therapies, especially for gastrointestinal or systemic infections, where time to action correlates with clinical outcomes (Gao et al.,2020). Dissolutions profile of F5 capsule at different interval 5, 10 15, 20, 30 min before and after accelerated stability graphically illustrated in Fig 2

Stability Studies

Stability of pharmaceutical dosage forms is a critical determinant of product quality, efficacy, and patient safety. In this study stability testing were conducted for 6 months under controlled conditions (25°C / 60% RH) and accelerated condition (40°C and 75% RH). Physical appearance, dissolution assay and microbial load were recorded at 0, 1,2,3, and 6 months illustrated in table 3 and 4.

The oral formulations developed in this study exhibited minimal physicochemical changes under both real-time and accelerated conditions as per ICH Q1A(R2) guidelines. The assay of active components remained within 95–105% of label claim, confirming excellent chemical integrity. While Dissolution profiles indicated more than 85% drug release within 30 minutes, complying with USP standards. These findings collectively indicate that the acid-buffer system effectively stabilizes the active constituents, offering a pharmaceutically robust formulation suitable for scale-up and industrial production, like wise the minimal microbial load throughout the storage period confirms the protective effect of proper capsule sealing and the inherent antimicrobial nature of the active ingredients and excipients.

CONCLUSION

Five different capsule formulations (F1-F5) were developed. The results showed that formulations F5 were comply pharmaceutical parameters. These Oral capsules having antibacterial activity against multidrug resistance gram negative bacteria. The introduction of these capsules will aid to combat the infections caused by multidrug gram-negative organism. The findings also demonstrate that weak organic acids, when formulated with appropriate buffering agents, can provide broad-spectrum antimicrobial effects with excellent physical and chemical stability. Overall, the data strongly support the stability and synergistic antibacterial potential of the combination in capsule form, warranting further in vivo studies to evaluate pharmacokinetics and therapeutic efficacy.

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