

INTRODUCTION

Antimicrobial resistance (AMR) has become a growing concern in healthcare, posing significant challenges in treating and preventing surgical site infections (SSIs) specially those associated with invasive fixation strategies followed by bone fracture (Fig 1). Limited treatment options, an increased risk of SSIs, reduced effectiveness of prophylactic antibiotics, and inadequate infection control measures are some of the difficulties posed by AMR. Biomaterials, such as bioactive glass, have shown great potential in preventing and treating SSIs through various mechanisms, such as providing a physical barrier against bacteria, releasing antimicrobial agents, or promoting tissue healing. Bioactive glass is a unique type of glass that can interact with biological tissues, promoting tissue regeneration and preventing bacterial adhesion and biofilm formation. This property makes bioactive glass an attractive material for use in medical devices, including drug delivery systems. Recent studies have shown that bioactive glass-based delivery systems for targeted antibiotic delivery are effective in preventing and treating SSIs. The combination of bioactive glass with antibiotics is an area of active research, and it has potential benefits for the treatment of infections arising during bone repair and surgeries.

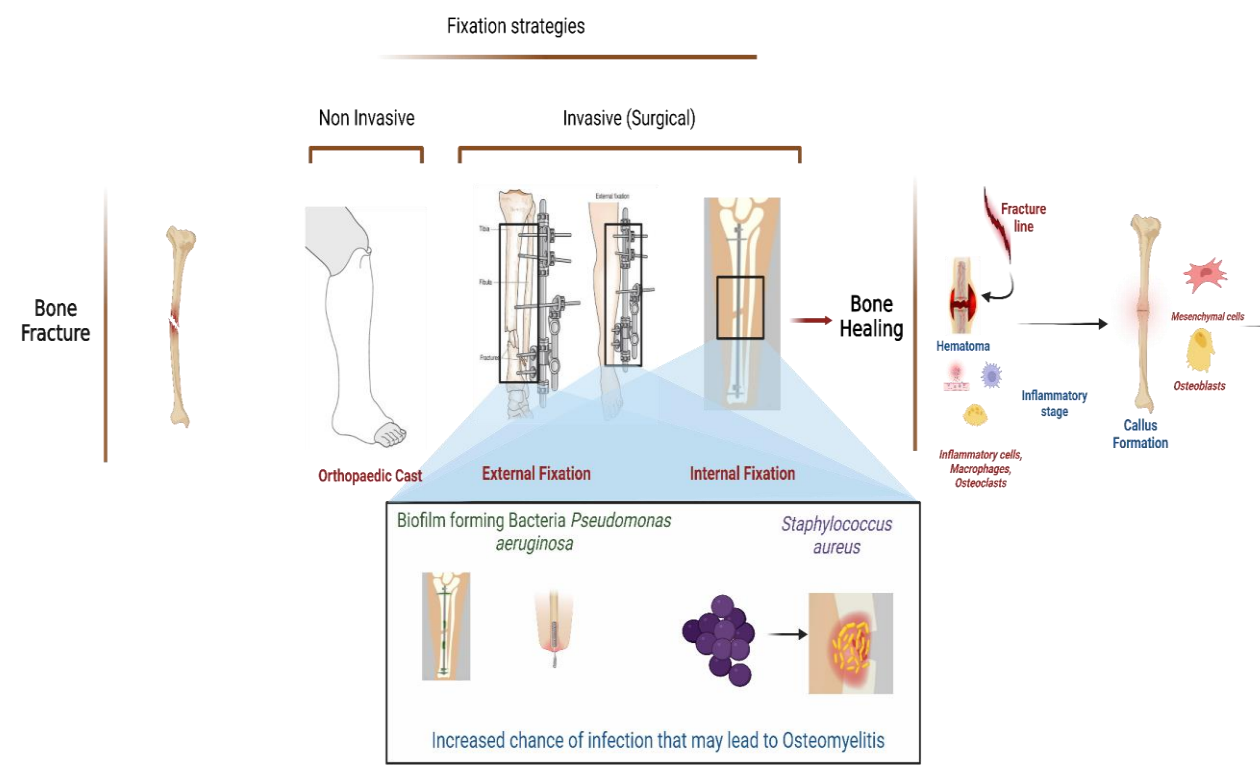


Figure 1: Fixation strategies and bone healing stages followed by fracture

The objective of this research endeavor is to develop an innovative method for targeted administration of antibiotics by utilizing Bioglass as a carrier for the drugs and employing carbohydrates as agents for controlling the release of the drugs.

METHODOLOGY

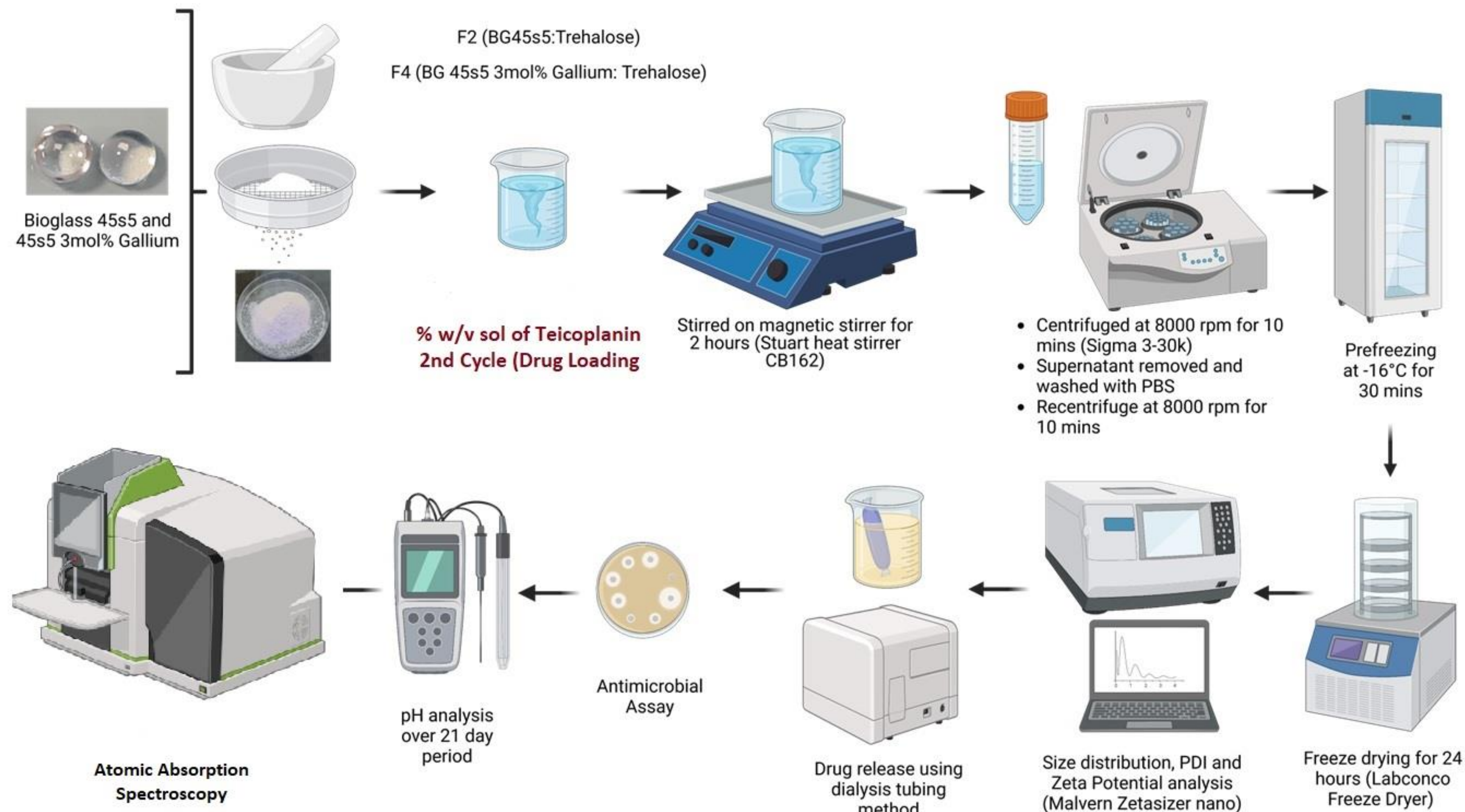


Figure 2: Method of Preparation of Bioglass particles, loading of drug and analysis and characterisation procedures.

Table 1: Formulation codes

Formulation Code	Bioglass	Carbohydrate	Proportion of Carbohydrate	Antibiotic	Remarks
F1	45s5	Sucrose	1:1(d), 1:2(h), 1:3(l)	Teicoplanin (0.5%w/v, 1%w/v, 1.5%w/v)	Formulation not Progressed after Initial optimization
F2	45s5	Trehalose			Formulation progressed successfully
F3	45s53mol%Gallium	Sucrose			Formulation not Progressed after Initial optimization
F4	45s53mol%Gallium	Trehalose			Formulation progressed successfully

RESULTS

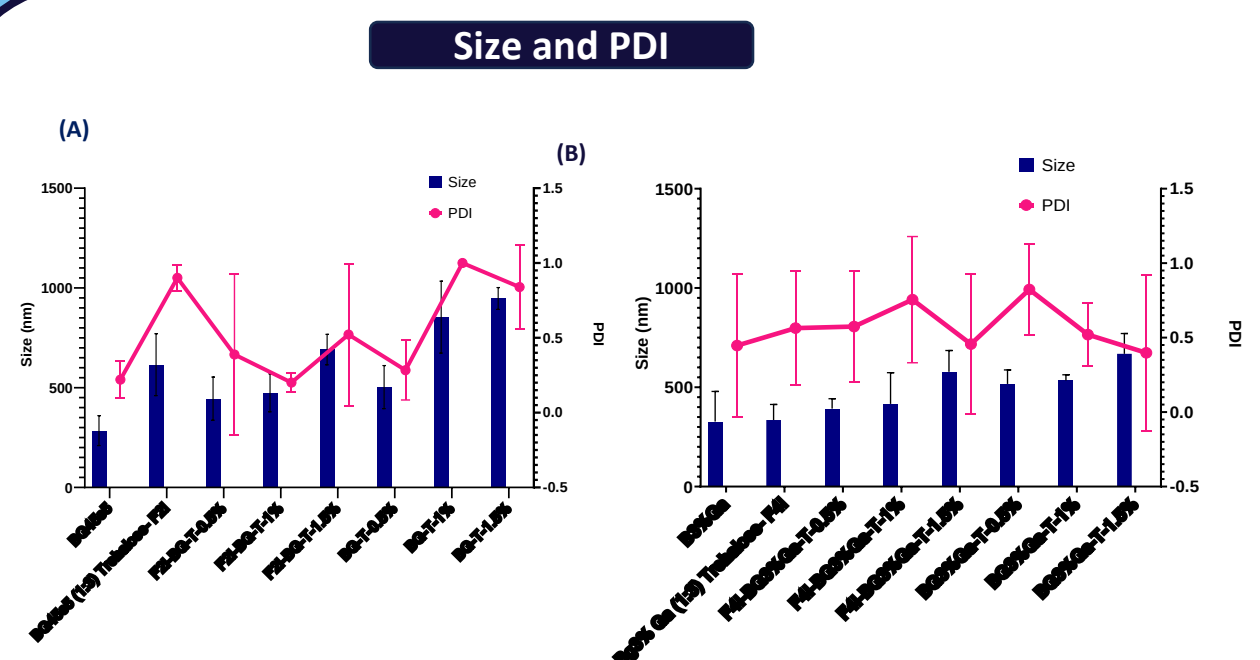


Figure 3: Size analysis of formulations F2l (A) and F4l (B) n=3

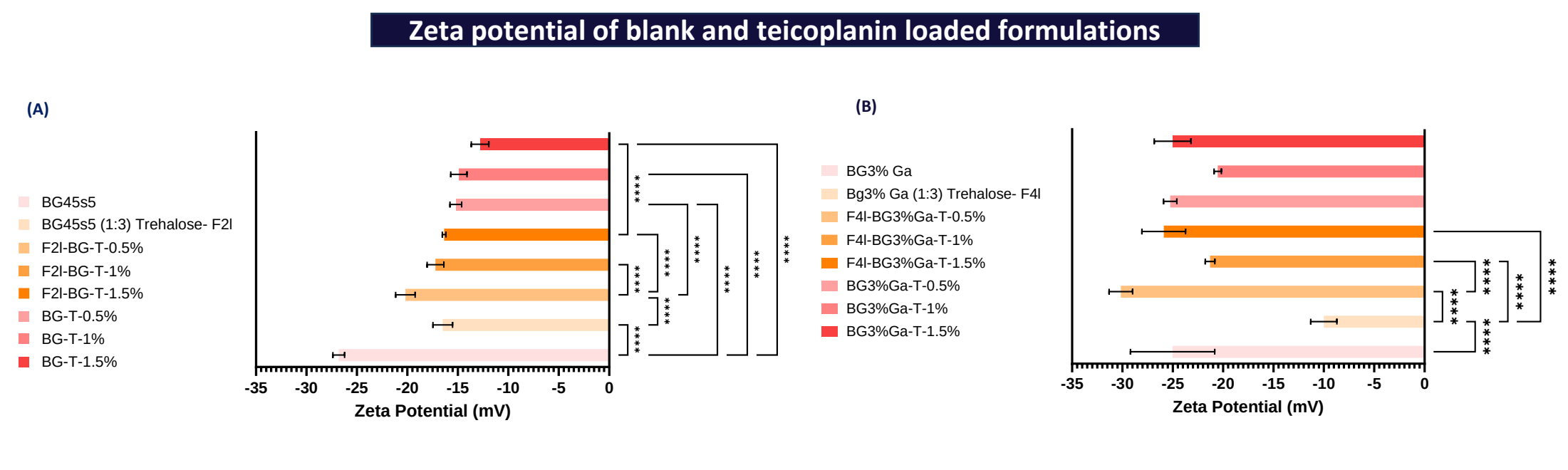


Figure 4: Zeta potential of Bioglass 45s5, blank F2l, teicoplanin loaded BG45s5 and F2l formulations [A] and BG3%Ga, blank F4l, loaded BG3%Ga and F4l formulations [B] (n=5).

It was observed that the size of the particles in formulations composed of Bioglass 45s5 and Bioglass 45s5 3% gallium tend to increase with the increase in loading concentration of the teicoplanin (Fig 3). The DLS measurements showed that the negativity of zeta potential of the teicoplanin loaded formulations in comparison to Bioglass 45s5 and Bioglass 45s5 3% Ga decreased significantly ($p < 0.0001$), whereas a significant rise in negativity was observed when the blank formulations were loaded with different concentrations of teicoplanin ($p < 0.0001$) (Fig 4).

Formulation pH stability in SBF over two weeks

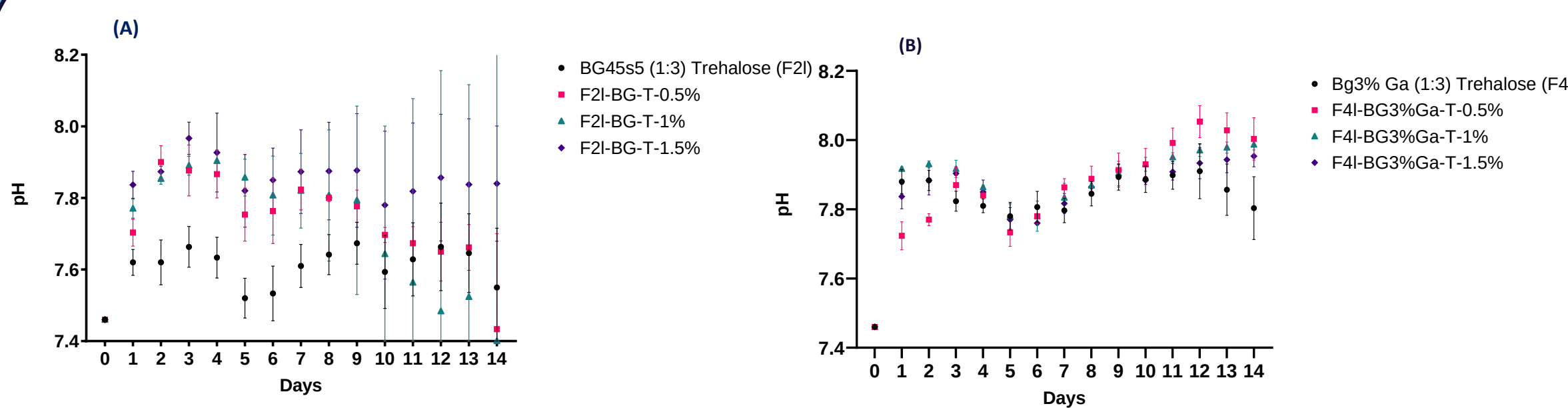


Figure 5: pH measurements over a period of 14 days in SBF of formulations F2l (A), and F4l (b)

Upon analysis it was observed that the pH tend to increase with time when the blank and loaded formulations were immersed in SBF and incubated at 37°C. The pH elevation takes place due to an ion exchange between Ca^{+} and Na^{+} ions within the glass and H_3O^{+} protons present in the medium. Because finer powders are utilized, which resulted in a larger exposed surface area for dissolution, the media exhibit higher pH levels.

The Bioactive glass undergo chemical reactions when immersed in simulated body fluid (SBF), and it occurs on the glass surface by leaching, dissolution and precipitation. The first reaction on the glass surface involves cation replacement (Ca^{+} , Na^{+} , K^{+}) with H^{+} or H_3O^{+} from the solution, leading to an increase in solution pH. Leaching generates an alkaline microenvironment as $-Si-O-Si-$ bonds break, mainly due to hydroxyl ions. Dissolution at the glass surface produces silanol ($Si-OH$) groups at the interface with the solution. Condensation and re-polymerization of these hydrated silica groups lead to the formation of a SiO_2 -rich layer (silica gel) on the surface. This process is aided by Ca^{+} and PO_4^{3-} ion migration to the surface within the SiO_2 -rich layer, creating an amorphous CaP layer above it. This amorphous CaP layer expands through the incorporation of soluble CaP from the supersaturated solution. The crystallization of the amorphous CaP layer occurs through the incorporation of OH^{-} , CO_3^{2-} or F^{-} anions from the solution. This process leads to the formation of hydroxyapatite (HCA) or apatite layers, with the potential substitution of fluoride ions (F-HCA). These reactions are likely similar in nature and contribute to the crystallization of a calcium-deficient apatite layer. Given that the composition of apatite crystals aligns with the inorganic mineral phase of bone, these reaction steps are commonly employed for characterizing biomaterials that foster interfacial tissue bonding. In an optimal scenario, the reaction progresses until the bioactive glasses are fully replaced by new tissue.

Table 2: In vitro release of calcium from Bioglass 45s5 and BG3%Ga, blank F2l and F4l formulations, and teicoplanin loaded F2l and f4l formulations using atomic absorption spectroscopy (n=3).

Formulations	Calcium release ($\mu g/ml$)	Formulations	Calcium release ($\mu g/ml$)
BG45s5	693.3 $\pm$ 117.6	BG3% Ga	816.9 $\pm$ 163.2
BG45s5 (1:3) Trehalose-F2l	978.4 $\pm$ 24.6	Bg3% Ga (1:3) Trehalose- F4l	1158.1 $\pm$ 68.7
F2l-BG-T-0.5%	1020.3 $\pm$ 170.7	F4l-BG3%Ga-T-0.5%	1135.9 $\pm$ 119
F2l-BG-T-1%	1070.1 $\pm$ 73.7	F4l-BG3%Ga-T-1%	1086.6 $\pm$ 57.9
F2l-BG-T-1.5%	1136.7 $\pm$ 86.9	F4l-BG3%Ga-T-1.5%	1002.8 $\pm$ 78.3
BG-T-0.5%	813.5 $\pm$ 47.7	BG3%Ga-T-0.5%	940.7 $\pm$ 97.5
BG-T-1%	819.8 $\pm$ 68	BG3%Ga-T-1%	997.5 $\pm$ 114.3
BG-T-1.5%	798.8 $\pm$ 35	BG3%Ga-T-1.5%	1001.3 $\pm$ 11.4

In vitro drug release of teicoplanin loaded formulations at pH 7.4

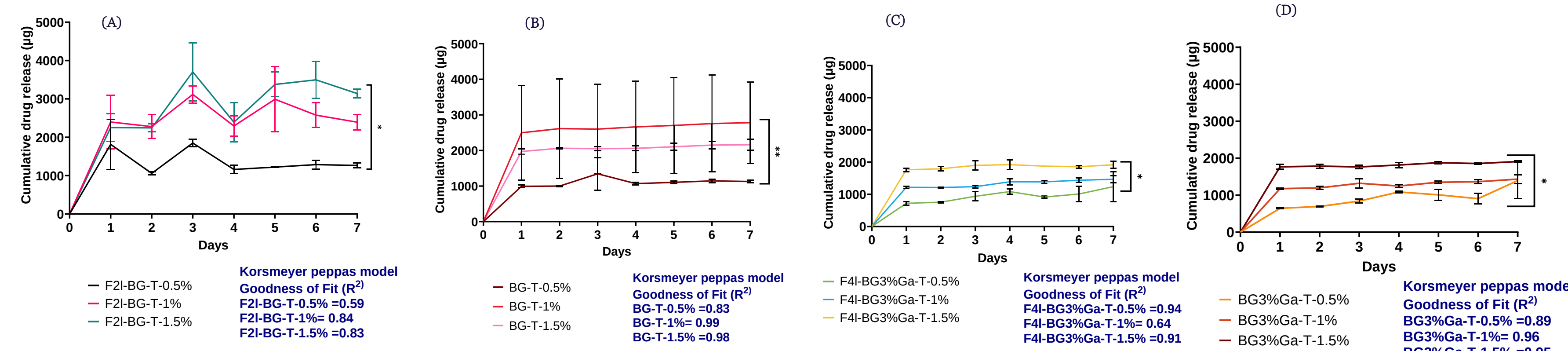


Figure 6: Cumulative percentage release of Teicoplanin from the formulations F2l (A), Bioglass 45s5 (B), formulations F4l (C) and BG3%Ga (D).

In vitro evaluation of antimicrobial activity of teicoplanin loaded formulations against selected pathogens

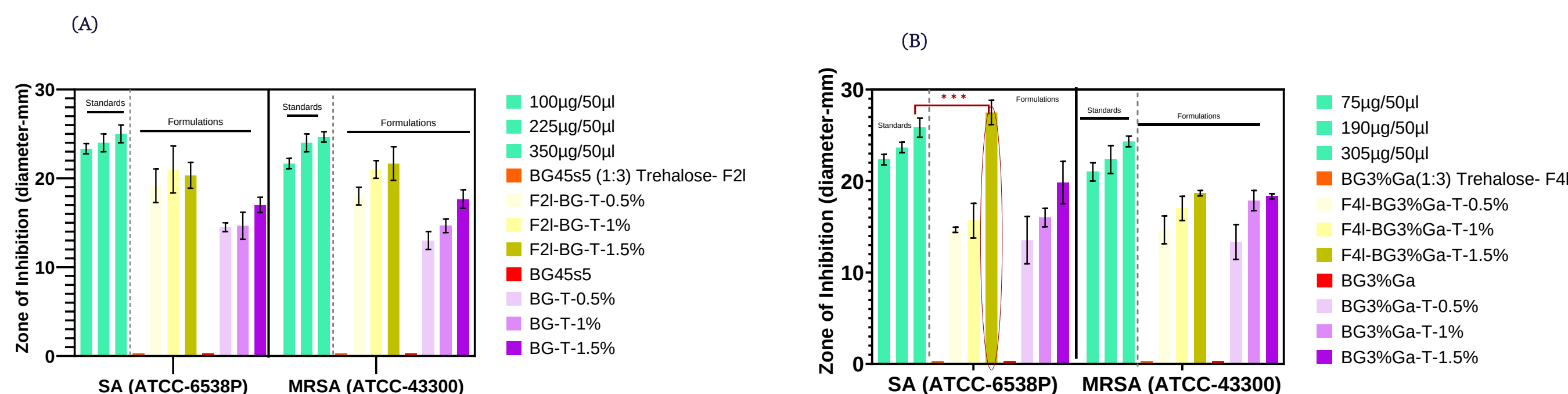


Figure 7: Zone of inhibition assay of Bioglass 45s5, BG45s5 formulations, F2l Formulations (A) and BG3%Ga, BG3%Ga formulations and F4l Formulations loaded with 0.5% w/v, 1% w/v and 1.5% w/v teicoplanin against Staphylococcus aureus and Methicillin resistant Staphylococcus aureus strains (n=3).

CONCLUSION

In summary, this study emphasizes the promise of Bioglass as a valuable biomaterial with unique qualities for enhancing bone healing. Our formulations have effectively achieved controlled release of teicoplanin for up to 7 days, and potentially even longer. Additionally, the results highlight the release of calcium and the formation of hydroxyapatite on the Bioglass surface. The inclusion of trehalose in the formulation offers a dual advantage by controlling drug release and reducing inflammation, thereby enhancing its potential in promoting bone healing and infection prevention for fractures.

ACKNOWLEDGMENT

Special thanks to Prof. Richard Martin for providing the Bioglass and my supervisors for their invaluable support throughout the project.



- In vitro antimicrobial tests of the selected formulations using OD measurements and cell viability assays
- X-ray diffraction (XRD) to analyse the hydroxyapatite formation
- BET specific surface area testing

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