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In-vitro release of FITC-dextran from biocompatible hydrogels fabricated for inflammatory biomarker capture and release in periodontal disease diagnostics

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Introduction

The detection of inflammatory markers in periodontal disease (PD) is essential for diagnosing and managing the condition [1]. Hydrogel scaffolds have been identified as a promising solution for the minimally invasive capture and release of biomarkers due to their biocompatibility, sustained release capacity and suitable porosity [2]. The process of releasing macromolecules from hydrogels can occur through diffusion, which is influenced by the hydrogel properties and the size of the molecules being released. In PD the targeted agents are typically inflammatory markers, growth factors, epigenetic markers and antibiotics. This work aims to fabricate hydrogels for PD diagnostics and study the *in vitro* release of FITC-dextran, a model macromolecule from hydrogels intended for inflammatory marker uptake within the periodontal pocket and subsequent release.

Methods

Hydrogel formulation process

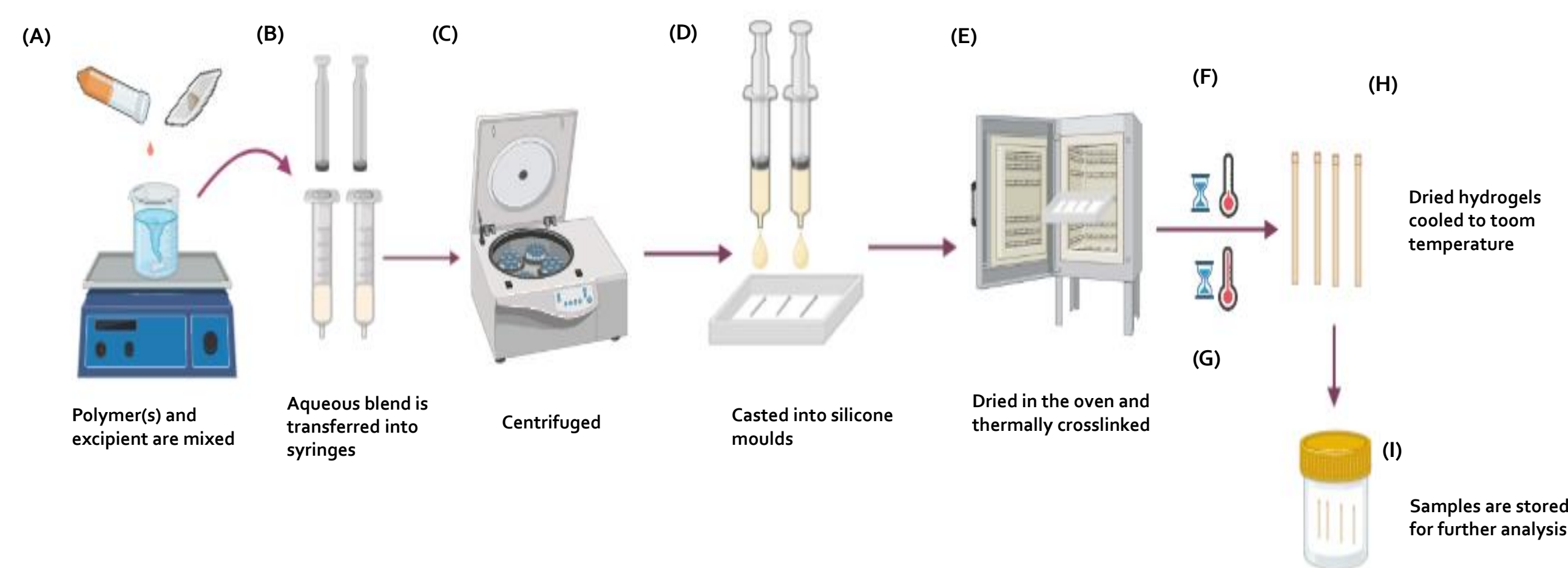


Figure 1 : Schematic of prototype hydrogel device fabrication using silicone moulds. (A) Stock polymer blend and or powdered polymers are mixed with crosslinkers to form aqueous (B) The aqueous blend is transferred into syringe barrels secured with cling film (C) The syringe barrels are centrifuged (D) Aqueous blend is then dispensed into silicone moulds (E) The cast blend is dried in the oven (F) 25°C for 48 h and then (G) thermally crosslinked at 80°C for 3 or 24 hours (H) Prototype formulations are then cooled and (I) stored for further tests

A total of 41 hydrogel formulations were fabricated. Based on several physicochemical tests and in particular swelling and mechanical tests, 11 formulations were selected for further studies. These formulations are **F1, F2, F3, F4, F9, F10, F13, F20, F39, F40, F41**. With the exception of F13 and F20 which contain polyvinyl alcohol, all formulations contained methyl vinyl ether co-maleic acid.

Mechanical test, swelling profile, and uptake and release of FITC-dextran process

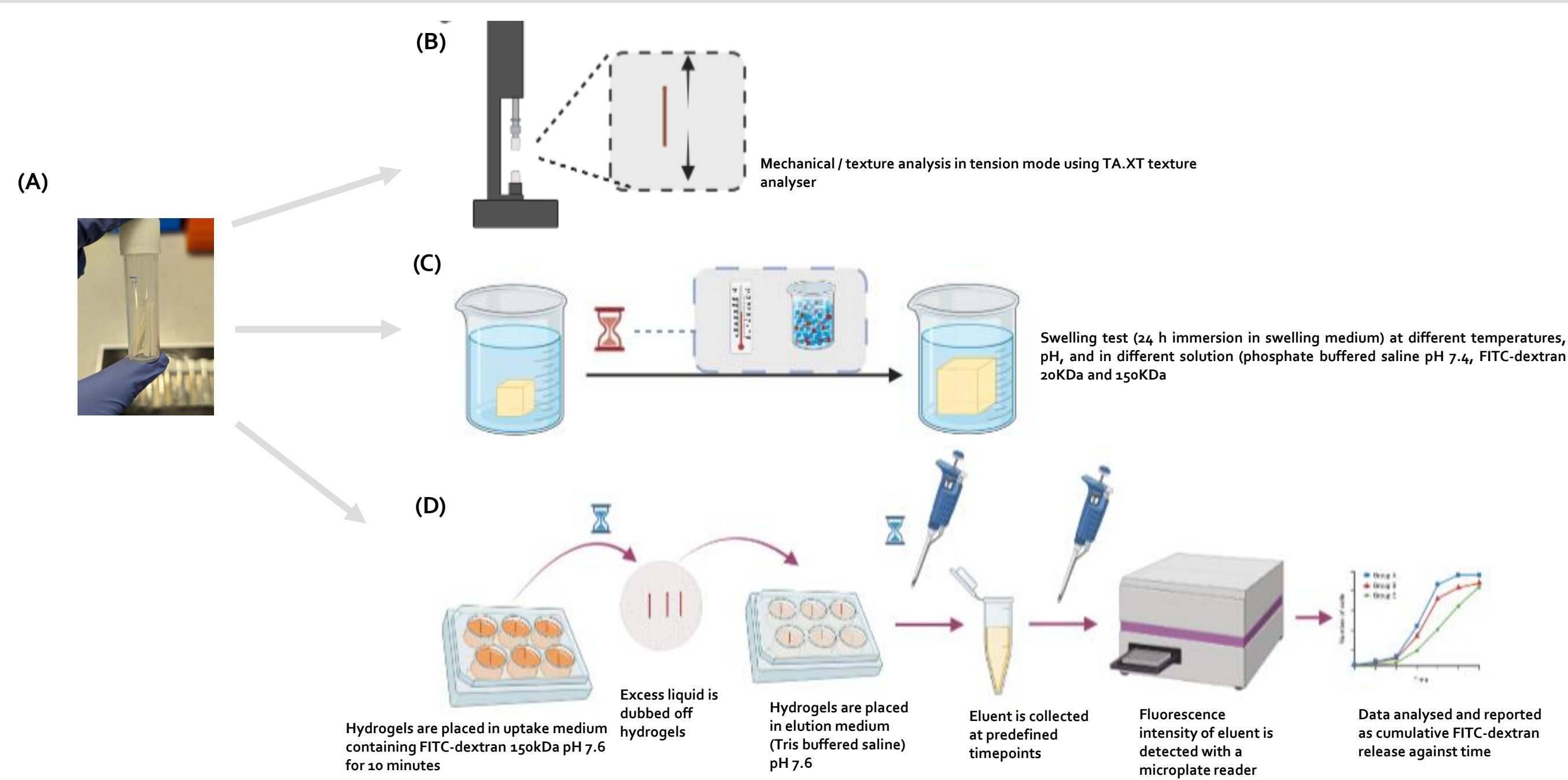


Figure 2 : Illustration of three main tests on prototype hydrogel biofluid sampling device (A) Freshly prepared hydrogel formulations (B) Texture analysis of the hydrogels set up in a tension mode. (C) The swelling properties of the hydrogel samples at varied temperatures, pH values, and in different solutions (FITC-DEXTRAN 20kDa and 150kDa both) at pH 7.6, and phosphate buffered saline pH 7.4 (D) Uptake of FITC-dextran 150kDa in tris buffered saline pH 7.6 at room temperature. All tests were performed in a minimum of triplicate determinations.

Conclusion

- In this study, the selection criteria for hydrogel formulations for FITC-dextran release was established based on swelling profiles and texture analysis.
- Hydrogels exhibited a rapid release of FITC-dextran in the first hour followed by a sustained release over 24 h with 72% of samples showing erratic release after the first hour.
- Formulations F1, F3, and F9 which contained the same amount of Na₂CO₃ exhibited an initial burst and subsequent sustained release pattern. Hence will be considered for biological marker loading and release. Na₂CO₃ could be contributing significantly to stable FITC-dextran release pattern.
- Three formulations F1, F3 and F9 (figure 5 (A)) which contained Na₂CO₃ could be promising prototypes to capture and release relevant biomarkers in PD diagnosis.

References

- [1] Kc, S., Wang, X.Z. and Gallagher, J.E., 2020. Diagnostic sensitivity and specificity of host-derived salivary biomarkers in periodontal disease amongst adults: systematic review. *Journal of clinical periodontology*, 47(3), pp.289-308.
- [2] Ali, A., Hussain, M.A., Haseeb, M.T., Bukhari, S.N.A., Tabassum, T., Farid-ul-Haq, M. and Sheikh, F.A., 2022. A pH-responsive, biocompatible, and non-toxic citric acid cross-linked polysaccharide-based hydrogel from *Salvia spinosa* L. offering zero-order drug release. *Journal of Drug Delivery Science and Technology*, 69, p.103144.

Results

All samples are responsive to pH, different solutions, and temperature. Na₂CO₃ increases stimuli responsiveness

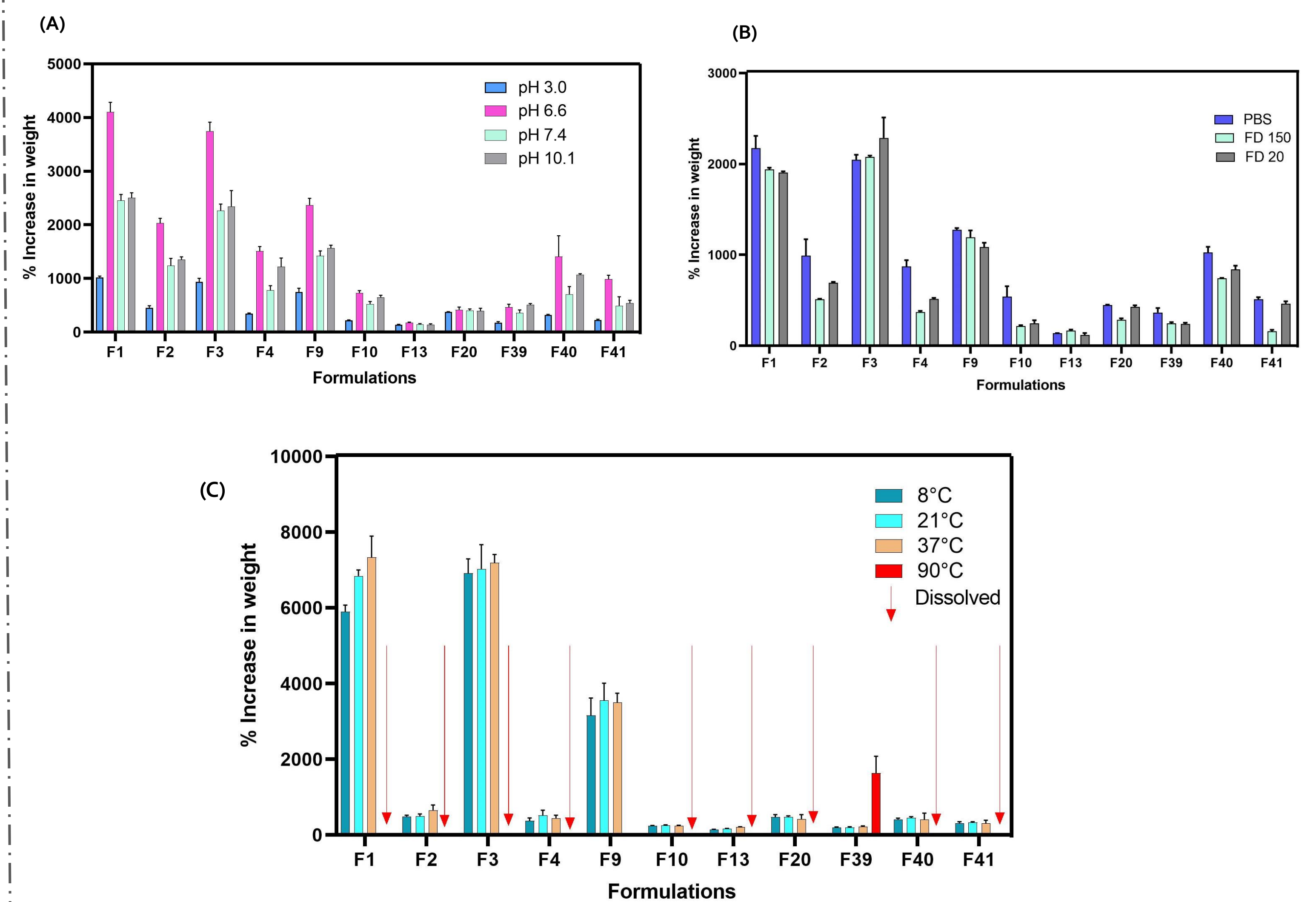


Figure 3 : A plot of the percentage increase in weight of hydrogels after a 24 h immersion (A) in buffer solutions of varied pH 3.0, 6.6, 7.4, and 10.1 (B) in different solutions PBS (pH=7.4), FITC-dextran 20 and 150kDa solutions (pH=7.6) and (C) at varied temperatures 8, 21, 37, and 90°C (n=6 ± SD).

Texture analysis : Polyvinyl alcohol - containing formulations are the most ductile. Na₂CO₃ reduces the ductility of samples

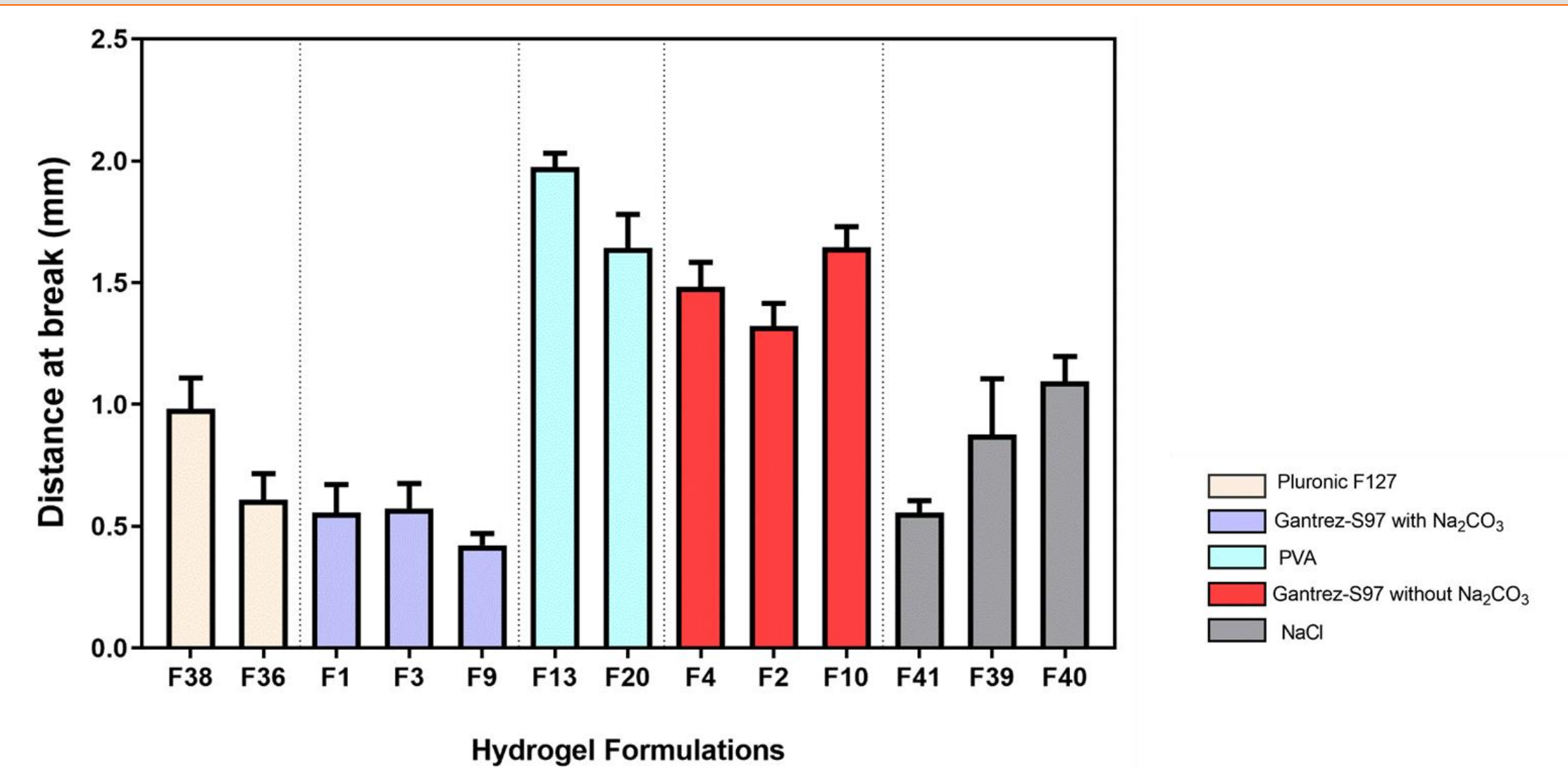


Figure 4 : A graph illustrating the longest distance travelled by an applied force to break the hydrogel formulations during texture analysis with a micro stable system TA.XT texture analyser. (n = 5 ± SD). Data are analysed separately as groups with either Welch's t-test (for two in a group) or one-way ANOVA followed by a Tukey post hoc test. Formulations are significantly different from each member of its group if p < 0.05.

Cumulative FITC-dextran release profile shows burst release in first hour. Na₂CO₃ reduces erratic release

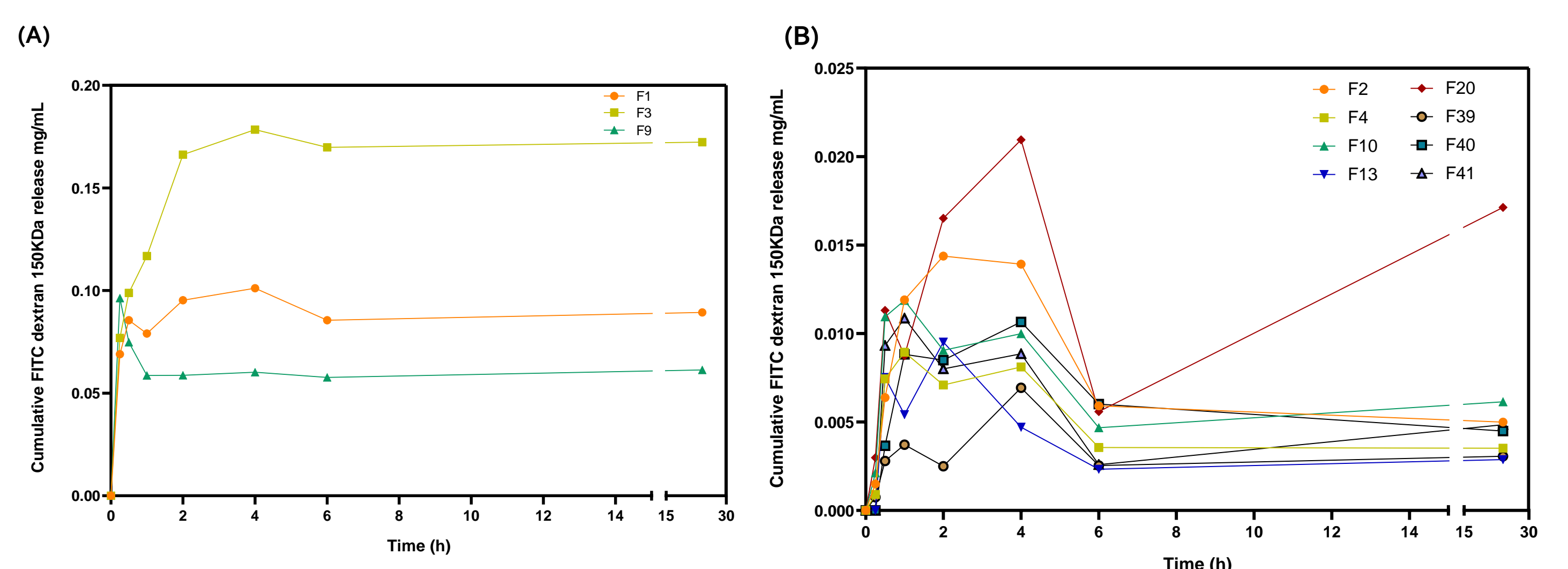


Figure 5 : Cumulative FITC-dextran 150kDa released from hydrogel samples at time points 0.25, 0.5, 1, 2, 4, 6, and 24 h (A) release pattern for samples F1, F3, and F9 (B) release pattern for samples F2, F4, F10, F13, F20, F39, F40 and F41. n = 5 ± SD

Acknowledgement

