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Enhancement of the solubility of 5-fluorouracil through encapsulation within β-cyclodextrin to control fibroblast growth in glaucoma surgery

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5-Fluorouracil (5-FU) is an antimetabolite agent used in chemotherapy and wound healing management, with low solubility and bioavailability. To overcome these challenges, this study explores the encapsulation of 5-FU within β-cyclodextrin (β-CD). The inclusion complex was prepared using the solubilization process, and its stability was evaluated under a variety of light, temperature, and pH environments. The complex was integrated into a drug delivery system using a layer-by-layer (LBL) technique, and the 5-FU release kinetics in a phosphate-buffered saline solution (PBS) were monitored using ultraviolet-visible spectroscopy (UV-Vis). Atomic force microscopy (AFM), UV-Vis spectroscopy, thin-layer chromatography (TLC) and differential scanning calorimetry (DSC) were used to improve the conditions for encapsulating 5-FU in the β-CD cavity and to study the stability of the inclusion complex under different light, temperature and pH conditions. The results show that encapsulation promotes the solubility of the drug, with increased absorbance intensity at a 1 : 1 molar ratio in a basic solution. The β-CD:5-FU complex was perfectly incorporated into a drug delivery system with controlled drug release over time.

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1. Introduction

5-FU is one of the most commonly used cancer therapies, with effective results in a variety of solid tumors, including colorectal and breast malignancies. It belongs to the antimetabolite drug class that disrupts normal DNA activity by blocking the nucleotide-synthesizing enzyme thymidylate synthase.[1–3] It has also been utilized in ophthalmic eye drops to treat ocular surface squamous neoplasia, as well as in soaked sponges administered locally to prevent tissue repair and scarring in past trabeculectomy blebs.[4] The use of this drug in such diverse applications highlights its versatility and potential benefits in various medical fields. Ongoing research continues to explore additional therapeutic uses and optimal delivery methods to maximize its effectiveness while minimizing side effects.

Because of its low affinity for cell membranes and low bioavailability, higher doses are needed for the desirable effect, increasing the toxicity and side effects such as local pain, burning, and itching. To control these issues, many efforts have been devoted to creating a complex with other molecules that act as carriers.[5] One approach was developed by Wang *et al.*, who constructed a system with 5-FU complexed with copper cross-linked polyethyleneimine encapsulated in liposomes as a carrier for tumor cells. The authors evaluated the efficiency of this complex in tumor tissue by microdialysis and concluded that this complex improved tumor exposure to the drug and potentially reduced tumor growth compared to the free drug solution.[6]

Cyclodextrins (CDs) are cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic inner cavity. The most prevalent cyclodextrins are α-CDs (6 glucose units); β-CDs (7 glucose units); and γ-CDs (8 glucose units), which have the largest inner cavity. CDs can produce stable hydrophobic holes that can trap "guest" molecules. This ability to encapsulate "guest" molecules makes CDs valuable in various applications, including drug delivery systems, food enhancement, cosmetics, and agricultural industries, where they can help in controlling the release of flavors, fragrances, and active ingredients. Their unique structure allows for increased solubility and stability of the encapsulated compounds, leading to improved efficacy in these fields. This enhancement is particularly valuable in pharmaceutical applications, where increasing the solubility of active ingredients can lead to more effective drug formulations.[7–10]

Nguyen *et al.* used β-CD/alginate nanoparticles to safely deliver 5-FU for anticancer treatment.[11] β-CD's unusual shape, with a hydrophobic cavity and wide inner diameter, makes it

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ideal for improving pharmacological characteristics. Thermodynamically, the formation of β-CD:drug complexes is ruled by several factors, such as the inclusion of the hydrophobic part of the "guest" molecule inside the β-CD cavity, dehydration of the "guest" molecule, interactions through hydrogen bonds, conformational transformation of β-CD after complexation, and the release of water molecules from the inside of the β-CD cavity.[12] During the host–guest complexation, the water molecules that are inside the β-CD cavity are partially replaced by part of the hydrophobic guest molecule.[13] In aqueous solution, water molecules occupy the hydrophobic β-CD cavity, which is readily replaced by molecules with a lower polarity than water. The interaction of a guest molecule with β-CD is characterized by electrostatic forces, hydrogen bonds, charge transfers, hydrophobicity, and van der Waals forces.[12] These interactions collectively contribute to the stability and specificity of the host–guest complex, allowing for the selective inclusion of various guest molecules based on their chemical properties. Consequently, β-CD has become a valuable tool in fields such as drug delivery, where it enhances the solubility and bioavailability of hydrophobic compounds, like 5-FU.[14]

Previous research has found that β-CD can form a compound with brimonidine and can be used in drug delivery nanostructured films to treat glaucoma.[15] This study demonstrates the complexation of 5-FU in a β-CD cavity, including its physicochemical characteristics and enhanced solubility as an antimetabolite. The complex's formation was investigated for use in a drug delivery system with time-controlled drug release. This application was investigated in a simple film made up of four bilayers of a hydrosoluble polymer (PBAE) and the encapsulated drug (β-CD:5-FU), followed by a study of drug release kinetics to a comparable physiological fluid (PBS) at 37 °C. These layer-by-layer (LBL) films enable precise control over drug release kinetics, making them ideal for sustained and localized delivery of anti-glaucoma agents. By incorporating therapeutic complexes such as 5-FU with β-CD, the system enhances drug stability, solubility, and bioavailability. When embedded in LBL coatings, this complex allows for a gradual and targeted release of 5-FU, an antimitotic agent used to prevent post-surgical fibrosis, directly at the implantation site. This localized approach reduces the need for repeated topical or systematic administration, minimizes side effects, and significantly improves treatment adherence and efficacy.

2. Experimental section

2.1 Materials

Sigma-Aldrich supplied hydrated β-CD and 5-FU. All additional reagents used in the buffer solutions were analytical reagent grade. The buffer solutions at various pH levels were prepared according to the instructions.

2.2 Synthesis of the β-CD/5-FU complex in different buffer solutions

To achieve the best conditions for the encapsulation, different buffer solutions were used in a pH range of 4.3–9.8. They were prepared using the respective amounts of acetate-acetic acid (pH = 4.3), phosphate-buffered solution (pH = 7.1), and sodium carbonate (pH = 9.8). The pH of the solutions was controlled by a calibrated pH meter. The respective amounts of hydrated β-CD and 5-FU in a 1 : 1 molar ratio were added to a round flask and stirred for 24 hours at room temperature.

2.3. Physicochemical characterization of β-CD/5-FU encapsulation

2.3.1. Differential scanning calorimetry (DSC). Thermal changes in the molecules before and after encapsulation were analyzed by a DSC instrument (DSC, Mettler Instrument). The samples were weighed (2-5 mg) and sealed in an aluminum capsule. A rate of 10 °C min^{-1} and a flow rate of 50 ml min^{-1} within the range of 30–300 °C were used.

2.3.2. Ultra-violet visible (UV-vis) spectroscopy. UV-Vis spectra were studied to determine how hydrated β-CD improves 5-FU solubility. The 5-FU concentration remained steady as the hydrated β-CD concentration increased. The experiments were carried out using a JASCO V-730 spectrophotometer at room temperature.

2.3.3. Thin-layer chromatography (TLC). A drop of each solution was applied to a 4 × 7 cm silica plate, and a mixture of ethyl acetate and water (98 : 2) was used as the eluent. The experiments were performed at room temperature (23 ± 2 °C), and the TLC ascendent method was applied. The migration distance was 6 cm from the start line. The results were analyzed under UV light.

2.3.4. Atomic force microscopy (AFM). The drop-casting approach was used to create a thin film of encapsulation solutions on a highly oriented pyrolytic graphite (HOPG) substrate, and the topography was examined using AFM in non-contact mode with APPNano silicon probes. A NanoObserver from Concept Scientific Instruments (CSI) was employed, and the images were processed and analyzed using Gwyddion software.

2.4. Stoichiometry studies

The stoichiometry of encapsulation was established using the Job method, which involved mixing unbuffered solutions of 5-FU and β-CD at different molar ratios:

$$R = \frac{5\mathrm{FU}}{5\mathrm{FU}\cdot\beta\mathrm{CD}}$$

Fig. 7 shows the Job plot $\Delta A.R$ against R, where ΔA represents the absorbance difference between free 5-FU and hydrated β-CD, and R is the molar ratio. At $R = 0{,}5$, the curve was regarded to be its maximum.

2.5 Stability of β-CD:5-FU complex

Inclusion complexes at a concentration of 0,3 mg ml^{-1} (as measured by the amount of hydrated β-CD in solution) were prepared in the buffer solutions (pH = 4.3, 7.1, and 9.8). The effects of light, temperature, and pH on the stability of inclusion complexes were studied in the following manner: aqueous solutions were exposed to natural light, UV light (365 nm), and

darkness for 105 hours. Absorbance was measured at 265.50 nm to determine the stability of the complexes under various light conditions.

The complex solutions were immersed in water baths at various temperatures (20–100 °C) for a certain time. The samples were then removed, cooled to room temperature, and their absorbance at 265.50 nm was measured to test the thermal stability of the complexes.

2.6. Drug delivery system

A drug delivery film with the complex β-CD:5-FU at pH 7.1 and a hydrosoluble polymer (poly(β-amino ester) was prepared using the LBL assembly.

The layers were adsorbed on a quartz substrate that was pretreated with UV-oxygen plasma in a vacuum chamber (Plasma Cleaner PDC-002-CE, Harrick Plasma) to improve the hydrophilicity of the surface.

The immersion time in each solution was determined in previous work[15] and applied in the present study.

The drug delivery film was prepared by successive immersion in PBAE and encapsulation solution, followed by cleaning in a sodium acetate solution to remove the physiosorbed particles and drying with nitrogen gas flow between layer solutions. Immersion in the two-layer solutions formed a bilayer PBAE/β-CD:5-FU. The methods were repeated four times to create four bilayers of PBAE/β-CD:5-FU. UV-Vis spectroscopy was used to monitor the adsorbed layers, as shown in Fig. 15.

2.6.1. Drug release kinetics. To study the 5-FU release kinetics, the film was immersed in a PBS solution with parameters similar to biological fluids in terms of pH (pH = 7.4) and salt concentration at 37 °C. After each time point, 2 ml of the PBS solution was transferred to an Eppendorf to be measured, and the same amount (2 ml) of new solution was added. The solutions' UV-Vis spectra were recorded at each time point.

3. Results and discussion

3.1 Best conditions for the complexation of β-CD/5-FU

As reported by Di Donato *et al.*,[16] β-CD and 5-FU can have distinct interactions according to the solution conditions. To confirm this fact and to find the ideal conditions for the hydrated β-CD:5-FU complex, spectroscopic and microscopy methods were used at different pH values, 4.3, 7.1, and 9.8.

3.2 DSC characterization

Thermal analysis methods like DSC are frequently used to study the inclusion of guest molecules into the β-CD cavity. H. E. Grandeli *et al.* demonstrated the complexation of naproxen in β-CD using DSC with the presence of a new exothermic energy peak. When naproxen melts, cyclodextrin and naproxen interact to generate an exothermic complex, as evidenced by this new peak.[17] Rossel *et al.* wanted to enhance the bioavailability of acyclovir, an antiviral drug, by complexation in the β-CD cavity.

The authors confirmed the inclusion of acyclovir in the β-CD cavity using DSC analysis. The results showed that the characteristic peak disappeared in the melting process, and this was evidence of the formation of a complex between acyclovir and β-CD.[18] The drug partially replaces the water molecules inside the β-CD cavity during the complexation to form β-CD/drug, which significantly alters the mobility of water molecules and lowers or even eliminates the drug's melting point following encapsulation, and DSC has the ability to observe this effect.[12,13,17,19]

DSC was used to study the changes that occurred with the complexation. A first heating was performed from 30 °C to 150 °C, and the findings in Fig. 1 indicate that the dehydration of the water molecules corresponds to the typical endothermic peak for β-CD at 90 °C. This characteristic peak varies across all complexes on comparing it to the temperature curves at each pH. The pH 4.3 thermal curve (c) has an endothermic peak at 60 °C, which is indicative of low-energy water molecules.

Water molecules outside the β-CD cavity (free in solution) have weak bonds; therefore, they are the first to be released with temperature. The peaks at approximately 100 °C are attributed to the firmly bonded water molecules within the β-CD cavity. The thermal curve at pH 7.1 (d) shows a drop in the melting point of water molecules, which is a sign of complex formation. The drug replaced the water molecules in the β-CD cavity, allowing the water molecules to move freely. These results are consistent with the conclusions made by Li *et al.*[19] in the study of the inclusion complex of trimethoprim with β-CD. The thermal curve at pH 9.8 presented two peaks at 100 °C and 130 °C, corresponding to water release. The characteristic endothermic peak of β-CD shifted to a higher temperature, and this effect can be explained by the fact that the released water molecules from the β-CD cavity were replaced by the drug molecule, with the energy of the remaining water molecules in the cavity changing to higher values. This explains the presence of the two endothermic peaks, which correspond to the different water molecules outside the cavity with the lowest energy and inside the β-CD cavity with higher energy and higher

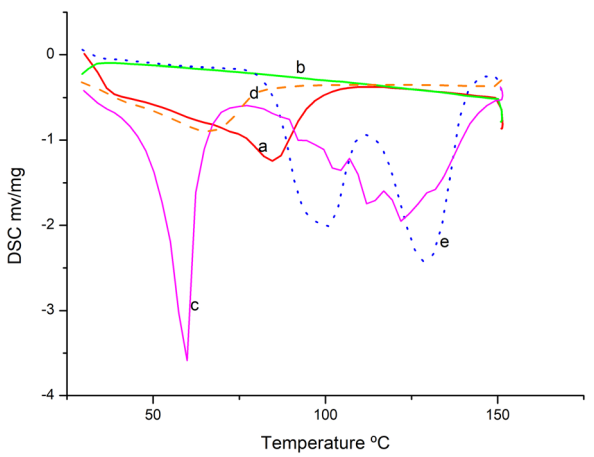

Fig. 1 DSC first heating (30 to 150 °C) for (a) β-CD, (b) 5-FU, (c) β-CD:5-FU at pH 4.3, (d) β-CD:5-FU at pH 7.1, and (e) β-CD:5-FU at pH 9.8. All curves show the dehydration of water molecules outside the β-CD cavity.

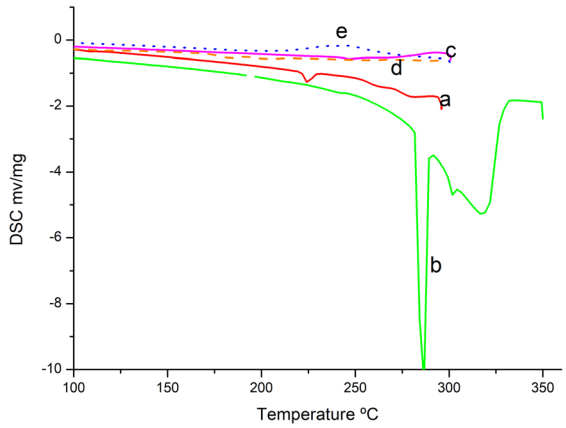

Fig. 2 DSC second heating (30–300 °C) for (a) β-CD showing the characteristic endothermic peak at 280 °C, (b)5-FU representing the characteristic endothermic peak at 225 °C, (c) β-CD:5-FU at pH 4.3; (d) β-CD:5-FU at pH 7.1 and (e) β-CD:5-FU at pH 9.8. Curves (c)–(e) show no endothermic peaks.

melting temperature, because they are strongly retained. This confirms the inclusion of the drug inside the β-CD cavity.

A second heating was performed from 30 °C to 300 °C (Fig. 2 and 3), and the heating curve of β-CD (a) presents its characteristic endothermic peak from the melting point at 225 °C. The results for 5-FU (b) show the characteristic melting point at 280 °C and the degradation peak at 300 °C. These two peaks for β-CD and 5-FU do not appear on the DSC of the complexes, revealing the inclusion of 5-FU into the β-CD cavity.

However, the DSC at pH 9.8 shows a weak exothermic peak at 245 °C, and this effect can be explained by the transitions into the anhydrous β-CD structure from the crystalline to amorphous state, indicating that inside the β-CD only the drug molecule remains, and all the water molecules were released from its cavity. The result is the formation of a stable complex. The absence of the characteristic endothermic peak of 5-FU at 280 °C is another piece of evidence of the inclusion of 5-FU in the β-CD cavity, which is in concordance with the findings of Li *et al.*[14]

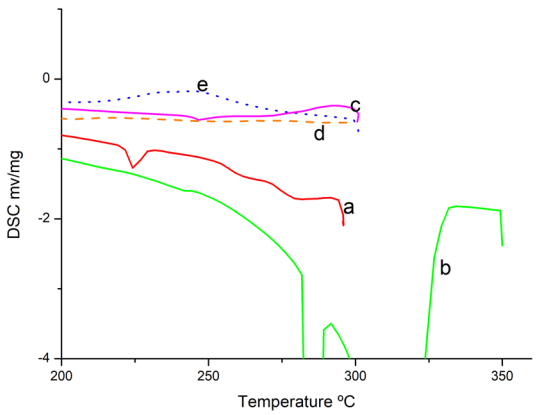

Fig. 3 DSC second heating (30–300 °C) for (a) β-CD, (b) 5-FU, (c) β-CD:5-FU at pH 4.3, (d) β-CD:5-FU at pH 7.1, and (e) β-CD:5-FU at pH 9.8.

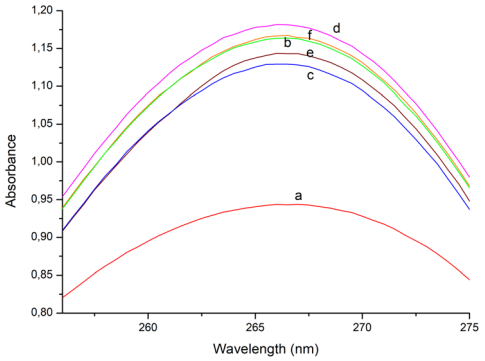

Fig. 4 Absorption spectra of 5-FU with varied concentrations of β-CD at pH 7.1; $T = 25$ °C. Concentrations in (a) to (f) are as follows: 0; 3.0×10^{-6}; 4.0×10^{-6}; 5.0×10^{-6}; 6.0×10^{-6} and 7.0×10^{-6} M. This shows the increase in the solubility of 5-FU in the presence of β-CD.

3.3 UV-Vis spectroscopy characterization

The inclusion of drug molecules into the β-CD cavity is accompanied by changes in the drug's properties, like its solubility, thermal properties, and absorbance in the UV-Vis spectrum.[19,20,21]

The complexation was investigated using UV-Vis spectroscopy on complex solutions in various buffer solutions with pH 4.3, 7.1, and 9.8. All buffer solutions had a constant 5-FU concentration of 1.75×10^{-4} M and β-CD values of 0; 3.0×10^{-6}; 4.0×10^{-6}; 5.0×10^{-6}; 6.0×10^{-6}, and 7.0×10^{-6} M. Because β-CD does not absorb. in the UV-Vis region, it can only be detected using 5-FU's absorption spectra. Fig. 4 and 5 show the absorption spectra of 5-FU with varying β-CD concentrations at pH 7.1 and 9.8. The study found that β-CD raises the absorbance intensity of 5-FU, indicating that it boosts its solubility. At pH 7.1, the absorbance intensities of 5-FU alone and 5-FU encapsulated in β-CD differ significantly.

Because of problems with spectral resolution, the absorption spectra could not be recorded at the above mentioned concentrations in the 4.3 buffer solution; therefore, the investigation was conducted using other varied concentrations, and the

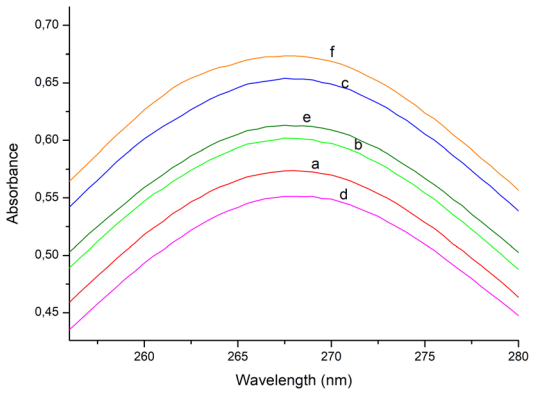

Fig. 5 Absorption spectra of 5-FU with various concentrations of β-CD at pH 9.8; $T = 25$ °C. Concentrations in (a) to (f) are as follows: 0; 3.0×10^{-6}; 4.0×10^{-6}; 5.0×10^{-6}; 6.0×10^{-6} and 7.0×10^{-6} M. This shows the increase in the solubility of 5-FU in the presence of β-CD.

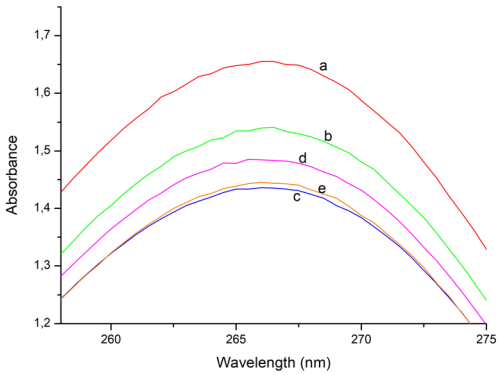

Fig. 6 Absorption spectra of 5-FU with various concentrations of β-CD at pH 4.3; $T = 25$ °C. Concentrations in (a) to (e) are as follows: 0; 8.3×10^{-8}; 1.1×10^{-7}; 1.4×10^{-7}; 1.7×10^{-7} M. The solubility of 5-FU does not increase in the presence of β-CD. These are not good conditions for the encapsulation.

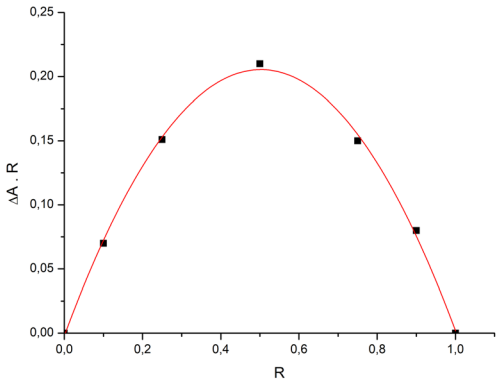

Fig. 7 Job plot for the determination of the complex 5-FU/β-CD stoichiometry. $R = 0{,}5$; therefore, the stoichiometry for the encapsulation is 1 : 1 (5-FU: β-CD).

results are shown in Fig. 6. The results reveal a decrease in absorbance intensity between unbound and encapsulated drugs.

3.4 Stoichiometry studies

The stoichiometry for the encapsulation was determined by the Job method. According to the Job method, when we have two compounds in equilibrium, A + B → AB, and the concentrations of A and B are constant and the proportions of A and B are varied, the curvature of the absorbance gives the relative stoichiometry of A and B.[22]

To achieve the stoichiometry for the encapsulation, unbuffered solutions of 5-FU and β-CD with the same concentrations were mixed at different molar ratios:

$$R = \frac{5\mathrm{FU}}{5\mathrm{FU} \cdot \beta\mathrm{CD}}$$

The Job plot $\Delta A.R$ against R is represented in Fig. 7, where ΔA is the absorbance difference between the free 5-FU and β-CD, and R is the molar ratio.

The maximum of the curve $\Delta A.R$ against R gives the stoichiometry of the complex. In this case, the maximum R is 0.5, which means that the stoichiometry is 1 : 1 (β-CD:5-FU).

4 TLC characterization

In TLC, it is possible to characterize different compounds based on their different interactions with the sorbent (plate) and the mobile phase. If 5-FU is encapsulated in the β-CD cavity, it will change the behavior of both compounds with the sorbent, as is demonstrated in Fig. 8.[23,24]

The results for the β-CD and the β-CD:5-FU complex at pH 9.8 showed the same behavior; the molecules were retained at the start line. However, in solutions with pH 4.3 and 7.1, the complexes showed the typical migration of the free molecules, indicating that they are in solution. The same results were found by Sbârcea *et al.*,[23] and it can be assumed that 5-FU was encapsulated in the β-CD cavity at pH 9.8.

4.1 Stability of β-CD:5-FU complex

4.1.1 Effect of light. Fig. 9, 10, and 11 depict the exposure of the β-CD:5-FU complex solutions to natural light, dark, and UV light for certain time periods.

The results reveal that the complex buffer solutions 4.3 and 7.1 remained stable under the three types of light for 57 hours. The 9.8 buffer solution remained stable under natural light, dark, and UV light for the entire 105-hours experiment.

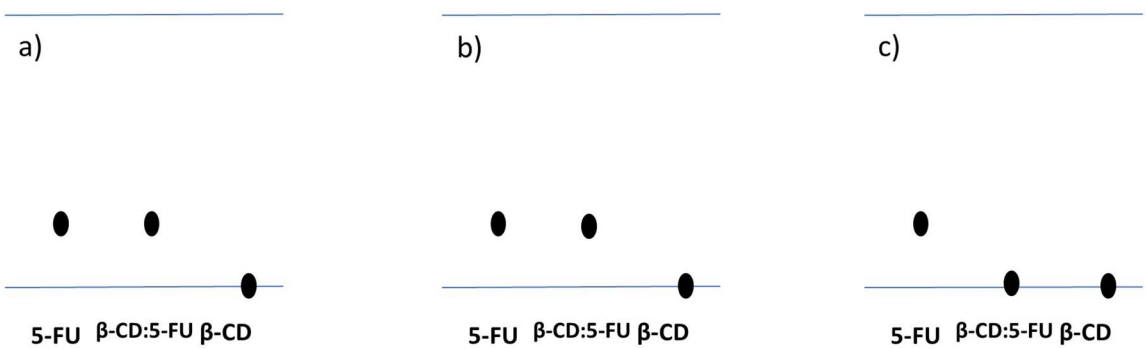

Fig. 8 TLC for 5-FU; 5-FU: β-CD; β-CD: (a) pH 4.3, (b) 7.1, and (c) 9.8 with the eluent ethyl acetate : water (98 : 2). It shows the encapsulation of 5-FU in the β-CD cavity at pH 9.8.

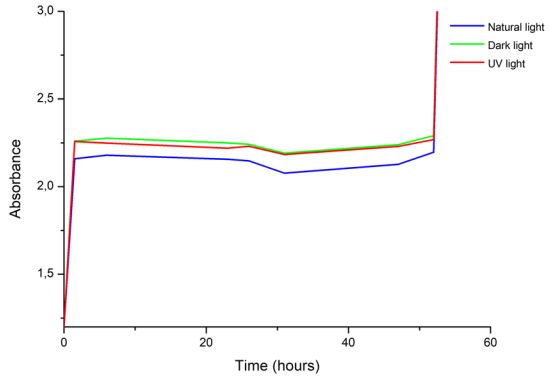

Fig. 9 Evaluation of the complex β-CD:5-FU at pH 4.3 under natural light, dark, and UV light over time. The complex remained stable for 57 hours.

4.1.2 Effect of temperature. Temperature can affect inclusion stability, as shown in Fig. 12, where the 4.3 and 7.1 buffer solutions do not remain stable above 60 °C. The 9.8 buffer solution protects the complex and does not change as the temperature increases. This verifies DSC observations that 5-FU occupies the whole β-CD cavity, generating an amorphous state with strong interactions between the two molecules.

4.1.3 AFM characterization. A thin film of the encapsulation solution was made by the drop-casting method on an HOPG substrate, and the topography was analyzed through AFM. AFM is a powerful technique used to characterize nanomaterials based on their size, morphology, surface texture, and roughness.

The samples were analyzed using the non-contact mode, and images of topography, amplitude, and phase were recorded and are presented in Fig. 13 and 14 for pH 4.3 and pH 9.8, respectively.

According to the AFM images, the complex has a more uniform and compact layer at pH 9.8, and the molecules are arranged in an orderly manner along the layer. This feature makes it possible to create a reliable and effective nanostructured film for drug delivery.

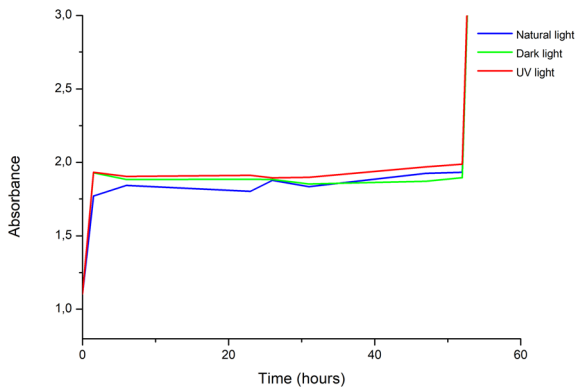

Fig. 10 Evaluation of the complex β-CD:5-FU at pH 7.1 under natural light, dark, and UV light over time. The complex remained stable for 57 hours.

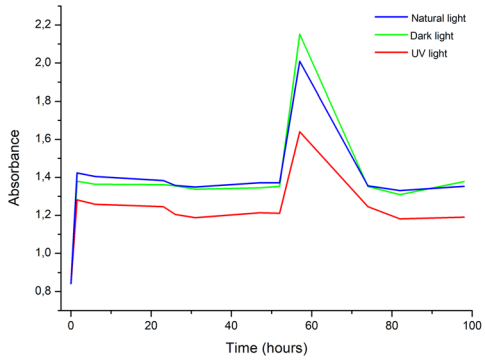

Fig. 11 Evaluation of the complex β-CD:5-FU at pH 9.8 under natural light, dark, and UV light over time. The complex remained stable during the 105-hours experiment.

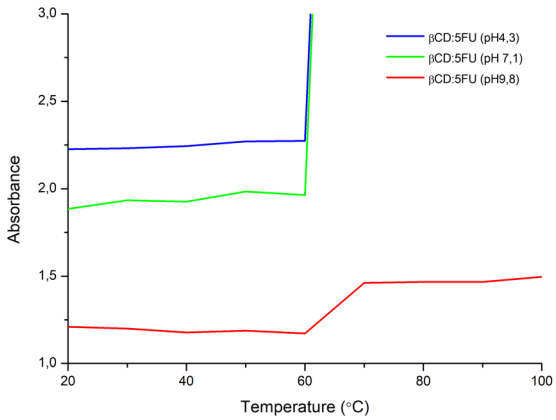

Fig. 12 Evaluation of the complex β-CD:5-FU at pH 4.3, 7.1, and 9.8 with increasing temperature. At pH 4.3 and 7.1, the complex remained stable until 60 °C. At pH 9.8, the complex solution was stable from 20 °C to 100 °C.

4.1.4 Drug delivery system. A nanostructured film composed of 4 bilayers of PBAE/β-CD:5-FU was prepared using the LBL technique. The adsorbed layers were monitored by UV-Vis, and the results presented in Fig. 15 show a linear increase in absorbance intensity with the addition of each layer.

4.1.5 Drug release kinetics. A UV-Vis spectrophotometer was used to monitor drug release into the PBS solution at 37 °C, and the solution spectra at each time point were applied to the previously determined 5-FU calibration curve in PBS.

Fig. 16 depicts the cumulative release profile of 5-FU over time from the LBL film. Initially, there was a sharp increase in 5-FU concentration during the first few minutes, indicating a burst release, which is characteristic of the drug located near or at the surface of the film. Following this burst, the release rate became more gradual, suggesting a sustained release phase where the drug diffuses more slowly from deeper layers within the system. This controlled release continued over approximately two hours, after which the curve plateaued, indicating that most of the drug had been released and the system approached saturation or exhaustion of the active compound. This biphasic release pattern, with the initial rapid release

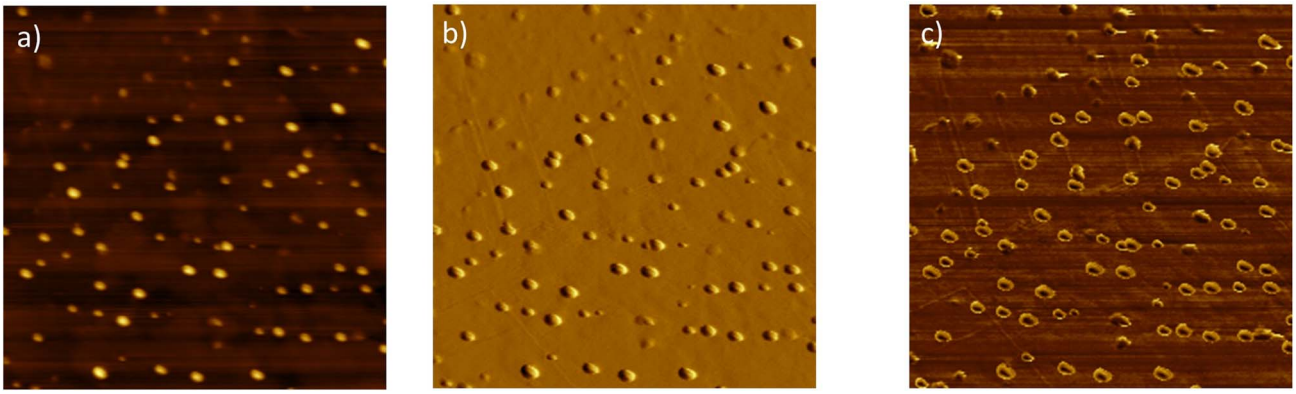

Fig. 13 The topography (a), amplitude (b), and phase (c) images of β-CD:5-FU at pH 4.3 on a HOPG substrate with dimensions of 2.0 μm. The poor deposition of β-CD:5-FU molecules on the HOPG surface is shown.

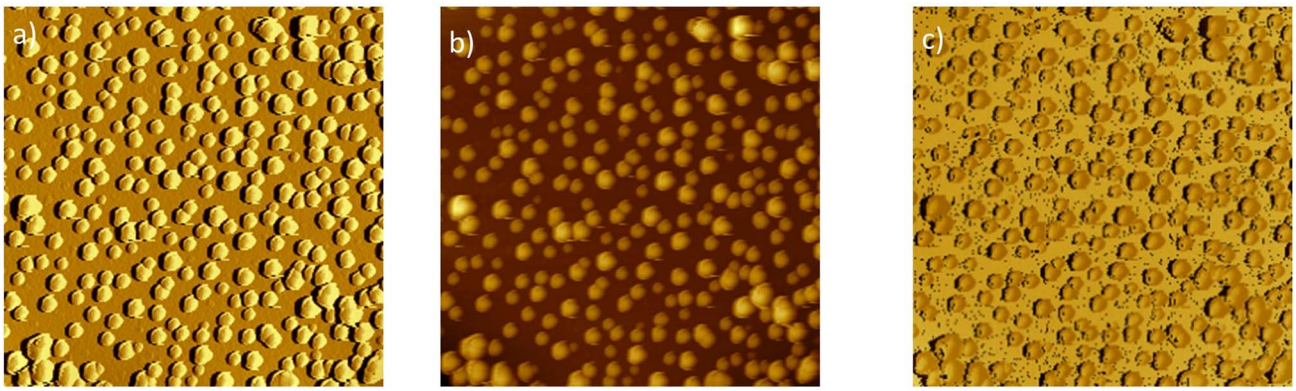

Fig. 14 The topography (a), amplitude (b), phase (c) images of β-CD:5-FU at pH 9.8 in a HOPG substrate with dimensions of 2.0 μm, showing a uniform and compact layer.

followed by a slower sustained delivery, also provides strong evidence for the successful encapsulation of 5-FU within β-CD prior to its incorporation into the LBL matrix. The inclusion of 5-FU in the CD cavity enhances its stability and modulates its release kinetics. The sustained release phase observed in the profile strongly suggests that the drug is not simply adsorbed or loosely bound but is instead retained within the structured host–guest complex, gradually diffusing out of the film over time. The initial burst may reflect a small amount of unencapsulated or surface-exposed drug, while the slower phase corresponds to the controlled release of the encapsulated fraction. This validates the functional integration of the β-CD:5-FU complex into the delivery system and highlights its potential

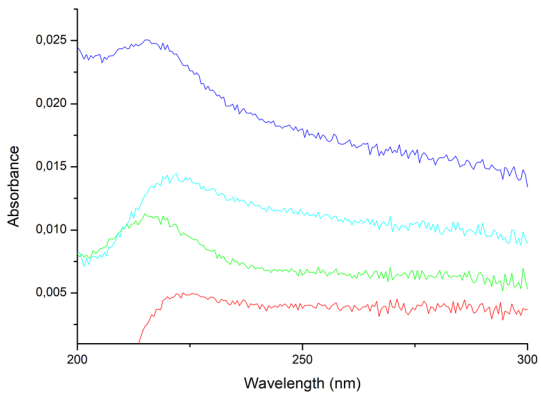

Fig. 15 Evaluation of the adsorbed bilayers of PBAE/β-CD:5-FU on a quartz substrate. The layers were linearly adsorbed on the substrate.

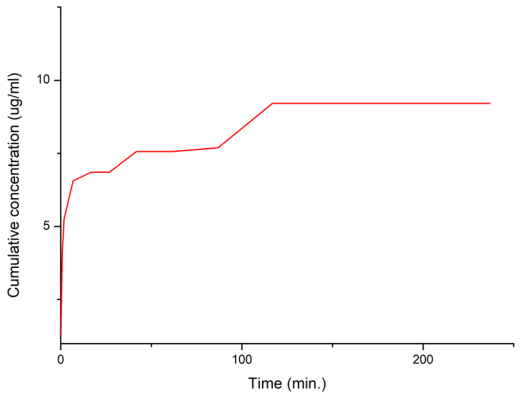

Fig. 16 Monitoring of the 5-FU release kinetics over time. The 5-FU concentration is released in a regulated manner into the PBS solution.

for localized, long-acting anti-fibrotic therapy following glaucoma surgery.

4.1.6 Discussion. To investigate the effectiveness of encapsulating 5-FU in the β-CD cavity, solutions with different pH values were tested. DSC was employed to detect temperature variations in compounds during complexation, particularly those containing water molecules. Encapsulation was confirmed by a mild exothermic peak at 245 °C, indicating a transition from crystalline to amorphous β-CD structures. The drug molecule remained inside the β-CD, whereas all water molecules left the cavity. Fig. 3 shows the absence of 5-FU's endothermic peak at 280 °C, indicating its incorporation into the β-CD cavity at pH 9.8.

To improve 5-FU solubility, UV-Vis analysis was performed on several solutions. Encapsulating 5-FU in the β-CD cavity leads to higher solubility and absorbance intensity. Fig. 4 and 5 show that the addition of β-CD causes an increase in absorbance intensity in the complex solution at 7.1 and 9.8 pH, although not in a linear fashion. These findings, confirmed by literature,[25] suggest that an effective inclusion complex with β-CD improves drug solubility.

The 1 : 1 stoichiometry in aqueous solution was determined using a Job plot.

Fig. 14 demonstrates that pH 9.8 is optimal for encapsulating 5-FU in β-CD due to its complex solution and ability to create a compact and uniform layer using the AFM technique.

The study examined how temperature and light conditions affect the stability of the β-CD:5-FU complex in the different buffer solutions. The results indicate that the 9.8 buffer complex remains stable under natural light, dark, and UV light over time and at different temperatures (from 20 °C to 100 °C).

The β-CD:5-FU complex was tested in a drug delivery system with 4 bilayers of PBAE/β-CD:5-FU at pH 7.1. Adsorption of each bilayer occurred in a regulated and linear way. Fig. 16 depicts the 5-FU release kinetics, which reveal a regulated release of the drug; it took 110 minutes for the entire film to be released into the PBS solution.

5 Conclusions

5-FU is a well-established antimetabolite that is widely used in chemotherapy and wound healing. However, its clinical applications are limited by poor aqueous solubility and low bioavailability, thus making it essential to improve its physicochemical properties. One effective strategy to enhance solubility while reducing toxicity is encapsulation within carrier molecules, such as CDs.

In this study, a combination of analytical techniques, including pH monitoring, AFM, UV-Vis spectroscopy, DSC, and TLC, was employed to determine the optimal conditions for incorporating 5-FU into β-CD. The stability of the resulting β-CD:5-FU complex was further evaluated under light and thermal stress. The results demonstrated strong and stable inclusion complex formation, with the most favorable interaction occurring at pH 9.8. This encapsulation significantly improved the solubility of 5-FU and enabled the formation of a homogeneous and compact film on HOPG surfaces.

At physiological pH (7.1), the β-CD:5-FU complex was successfully integrated into a 4-bilayer nanostructured film composed of PBAE and β-CD:5-FU, deposited on a quartz substrate. The resulting LBL film demonstrated controlled drug release kinetics, with sustained delivery over a period of 110 minutes. These findings confirm that β-CD encapsulation not only stabilizes 5-FU but also enables its integration into functional nanostructured coatings with therapeutic potential for localized and prolonged drug delivery.

Author contributions

M. M., Q. F. and G. A. S. wrote the article; M. M. was involved in the conceptualization and methodology of the article; M. M., G. A. S., J. T. F. and Q. F. were involved in the review and editing of the article; G. A. S., J. T. F. and Q. F. were involved in the supervision of the work. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Data availability

The data supporting the article "Enhancement of the solubility of 5-fluorouracil through encapsulation within β-cyclodextrin to control fibroblast growth in glaucoma surgery" have been included as part of the article, in the experimental and results section.

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References

1 D. B. Longley, D. P. Harkin and P. G. Johnston, 5-Fluorouracil: Mechanisms of action and clinical strategies, *Nat. Rev. Cancer*, 2003, **3**(5), 330–338, DOI: **10.1038/nrc1074**.

2 D. L. Melnikova, Z. F. Badrieva, M. A. Kostin, C. Maller, M. Stas, A. Buczek, M. A. Broda, T. Kupka, A. M. Kelterer, P. M. Tolstoy and V. D. Skirda, On complex formation between 5-fluorouracil and β-cyclodextrin in solution and in the solid state: Ir markers and detection of short-lived complexes by diffusion nmr, *Molecules*, 2020, **25**(23), 5706, DOI: **10.3390/molecules25235706**.

3 D. B. Longley, D. P. Harkin and P. G. Johnston, 5-Fluorouracil: Mechanisms of action and clinical strategies, *Nature*, 2003, 3, DOI: 10.1038/nrc1074.
4 M. G. Joag, A. Sise, J. C. Murillo, I. O. Sayed-Ahmed, O. Ibrahim, J. R. Wong, C. Mercado, A. Galor and C. L. Karp, Topical 5-Fluorouracil 1% as Primary Treatment for Ocular Surface Squamous Neoplasia, *Ophthalmology*, 2016, **123**(7), 1442–1448, DOI: 10.1016/j.ophtha.2016.02.034.
5 A. M. T. D. P. V. Cabral, A. C. G. Fernandes, N. A. M. Joaquim, F. Veiga, S. P. C. Sofio, I. Paiva, M. A. Esteso, M. M. Rodrigo, A. J. M. Valente and A. C. F. Ribeiro, Complexation of 5-Fluorouracil with β-Cyclodextrin and Sodium dodecyl Sulfate: A useful tool for encapsulating and removing this polluting drug, *Toxics*, 2022, **10**(6), 300, DOI: 10.3390/toxics10060300.
6 W. Wang, P. Joyce, K. Bremmell, R. Milne and C. A. Prestidge, Liposomal 5-Fluorouracil Polymer Complexes Facilitate Tumor-Specific Delivery: Pharmaco-Distribution Kinetics Using, *Pharmaceutics*, 2022, **14**(2), 221, DOI: 10.3390/pharmaceutics14020221.
7 G. Raffaini, S. Elli, M. Catauro and A. D'Angelo, Different drug mobilities in hydrophobic cavities of host-guest complexes between β-cyclodextrin and 5-Fluorouracil at different stoichiometries: A molecular dynamics study in water, *Int. J. Mol. Sci.*, 2004, **25**(11), 5888, DOI: 10.3390/ijms25115888.
8 W. Gu and Y. Liu, Characterization and stability of beta-acids/hydroxypropyl-β-cyclodextrin inclusion complex, *J. Mol. Struct.*, 2020, **1201**, 127159, DOI: 10.1016/j.molstruc.2019.127159.
9 P. Saokham, C. Muankaew, P. Jansook and T. Loftsson, Solubility of cyclodextrins and drug/cyclodextrin complexes, *Molecules*, 2018, **23**(5), 1161, DOI: 10.3390/molecules23051161.
10 A. Cid-Samamed, J. Rakmai, J. C. Mejuto, J. Simal-Gandara and G. Astray, Cyclodextrins inclusion complex: Preparation methods, analytical techniques and food industry applications, *Food Chem.*, 2022, **384**, 132467, DOI: 10.1016/j.foodchem.2022.132467.
11 C. H. Nguyen, K. S. Banh, C. H. Dang, C. H. Nguyen and T. D. Nguyen, β-cyclodextrin/alginate nanoparticles encapsulated 5-fluorouracil as an effective and safe anticancer drug delivery system, *Arabian J. Chem.*, 2022, **15**(6), 103814, DOI: 10.1016/j.arabjc.2022.103814.
12 L. Garcia-Rio, J. C. Mejuto, P. Rodrigez-Dafonte and R. W. Hall, The role of water release from the cyclodextrin cavity in the complexation of benzoyl chlorides by dimethyl-β-cyclodextrin, *Tetrahedron*, 2010, **66**(13), 2529–2537, DOI: 10.1016/j.tet.2009.12.005.
13 N. G. Hadaruga, G. N. Bandur, I. David and D. I. Hadaruga, A review on thermal analyses of cyclodextrins and cyclodextrin complexes, *Environ. Chem. Lett.*, 2019, **18**, 349–373, DOI: 10.1007/s10311-018-0806-8.
14 C. Di Donato, M. Lavorgna, R. Fattorusso, C. Isernia, M. Isidori, G. Malgieri, C. Piscitelli, C. Russo, L. Russo and R. Iacovino, Alpha- and Beta-Cyclodextrin inclusion complexes with 5-Fluorouracil: Characterization and cytotoxic activity evaluation, *Molecules*, 2016, (21), 1644, DOI: 10.3390/molecules21121644.
15 M. Machado, G. A. Silva, D. B. Bitoque, J. Ferreira, L. A. Pinto, J. Morgado and Q. Ferreira, Self-Assembled Multilayer Films for Time-Controlled Ocular Drug Delivery, *ACS Appl. Bio Mater.*, 2019, **2**(10), 4173–4180, DOI: 10.1021/acsabm.9b00417.
16 C. Di Donato, M. Lavorgna, R. Fattorusso, C. Isernia, M. Isidori, G. Malgieri, C. Piscitelli, C. Russo, L. Russo and R. Iacovino, "Alpha- and Beta-cyclodextrin Inclusion Complexes with 5-Fluorouracil: Characterization and Cytotoxic Activity Evaluation", *Molecules*, 2016, **21**, 1644, DOI: 10.3390/molecules21121644.
17 H. E. Grandelli, B. Stickle, A. Whittington and E. Kiran, Inclusion complex formation of β-cyclodextrin and Naproxen: A study on exothermic complex formation by differential scanning calorimetry, *J. Inclusion Phenom. Macrocyclic Chem.*, 2013, **77**(1–4), 269–277, DOI: 10.1007/s10847-012-0241-6.
18 C. Rossel, P. Von, J. Sepúlveda Carreño, M. Rodriguez-Baeza and J. B. &Alderete, Inclusion complex of the antiviral drug acyclovir with cyclodextrin in aqueous solution and in solid phase, *Quim. Nova*, 2000, **23**(6), 749–752, DOI: 10.1590/S0100-40422000000600007.
19 N. Li, Y. H. Zhang, Ya N. Wu, X. Li Xiong and Ya H. Zhang, Inclusion Complex of trimethoprim with β-cyclodextrin, *J. Pharm. Biomed. Anal.*, 2005, **39**(3–4), 824–829, DOI: 10.1016/j.jpba.2005.05.011.
20 K. P. Sambasevam, S. Mohamad, N. M. Sarih and N. A. Ismail, Synthesis and characterization of the inclusion complex of β-cyclodextrin and azomethine, *Int. J. Mol. Sci.*, 2013, **14**(2), 3671–3682, DOI: 10.3390/ijms14023671.
21 H. you Wang, J. Han, X. G. Feng and Y. L. Pang, Study on inclusion complex formation between tropaeolin and β-cyclodextrin by spectrophotometry and Infrared spectroscopy, *Spectrochim. Acta, Part A*, 2006, **65**(1), 100–105, DOI: 10.1016/j.saa.2005.09.034.
22 J. S. Renny, L. L. Tomasevich, E. H. Tallmadge and D. B. Collum, Method of continuous variations: Applications of Job Plots to the study of Molecular Associations in Organometallic Chemistry, *Angew. Chem., Int. Ed.*, 2013, **52**(46), 11998–12013, DOI: 10.1002/anie.201304157.
23 L. Sbârcea, L. Udrescu, L. Dragan, C. Trandafirescu, Z. Szabadai and M. Bojita, *Thin-layer Chromatography Analysis for Cyclodextrins Inclusion Complexes of Fosinopril and Zofenopril*, Farmacia, 2010, pp. 478–484.
24 M. Santiago and S. Strobel, Thin Layer Chromatography, *Methods Enzymol.*, 2013, **533**, 303–324, DOI: 10.1016/B978-0-012-420067-8.00024-6.
25 B. Ewert de Oliveira, J. Amorim, O. Henrique, L. Lauro, R. A. Rezende, N. C. Mestnik, E. Bagatin and G. Leonardi, Ricci; 5-Fluorouracil, innovative drug delivery systems to enhance bioavailability for topical use, *J. Drug Delivery Sci. Technol.*, 2020, **61**, 102155, DOI: 10.1016/j.ddst.2020.102155.