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Kinetics of Fluorescein-Starch and Cholesterol Interactions: A Colorimetric Study Using Exponential Decay Modelling

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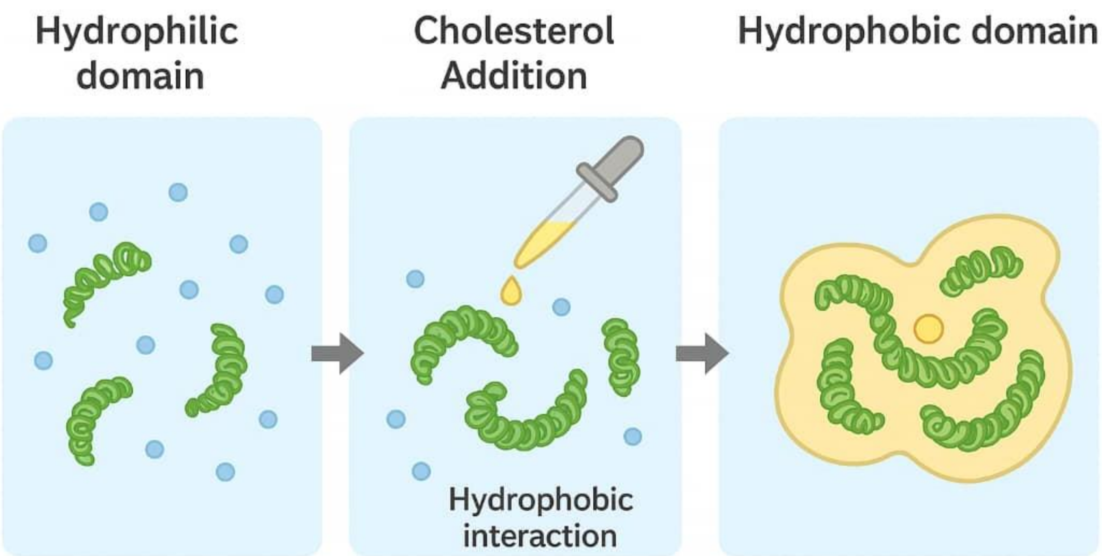

Abstract: The molecular interaction between fluorescein-labeled starch and cholesterol was investigated using a colorimetric approach by monitoring absorbance at 490 nm. Kinetic profiles were recorded for starch–cholesterol blends containing varying cholesterol concentrations, and experimental data were fitted to exponential decay models using GraphPad Prism software. According to the analysis, the interaction kinetics are adequately described by both one-phase decay and plateau followed by one-phase decay models, with higher cholesterol content resulting in longer interaction lifetimes and more robust hydrophobic associations within the starch matrix. At larger concentrations, viscosity and steric hindrance probably play a part, but other elements like probe aggregation or local microviscosity changes might also be involved. These results highlight the usefulness of colorimetric kinetics as a quantitative method for examining interactions between cholesterol and biopolymers that are pertinent to food, medicinal, and nutraceutical applications.

Keywords: Molecular interaction, Fluorescein-starch, Cholesterol, Kinetics, Colorimetry

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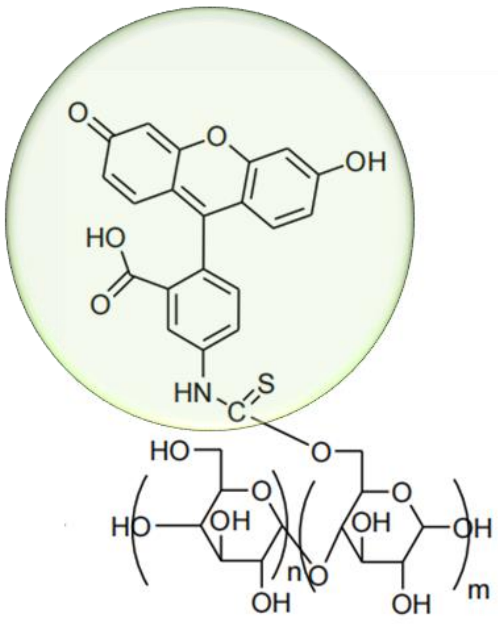
Introduction

For the advancement of industries like drug delivery, food science, food packaging, wastewater treatment, industrial waste removal, and biosensor development, it is crucial to understand the molecular interactions between biopolymers and lipophilic substances [1–15]. Because of its biocompatibility, non-toxicity, and structurally modifiable nature, starch (a naturally occurring, extensively researched polysaccharide) has gathered significant attention among these systems [12,15-20]. Its interactions with cholesterol, a significant hydrophobic component of biological membranes, provide information about how to build functional materials, especially those that are intended to modulate or absorb lipids [21].

In order to facilitate colorimetric or fluorescent monitoring of molecular behavior, fluorescein and its derivatives (a hydrophilic and pH-sensitive dye) are frequently attached to biopolymers such as starch [22]. A detectable probe that can show physicochemical alterations in the polymer matrix during interactions with hydrophobic compounds like cholesterol is produced when fluorescein is coupled with starch. By measuring absorbance at particular wavelengths (490 nm being especially important for fluorescein-based systems) these changes can be quantitatively tracked [22]. The chemical structure of fluorescein 5(6)-isothiocyanate-coupled starch (FITC–S) is depicted in Scheme 1. An isothiocyanate linker covalently bonded fluorescein to the starch backbone in this structure, creating a thiourea bond between the dye and the hydroxyl groups of starch. The spectroscopic monitoring of molecular interactions was made possible by the use of the fluorescein moiety, which is well-known for its potent optical qualities. The carrier matrix was starch. This change produced a biopolymer that was spectroscopically labeled, which made it possible to use spectroscopic methods to track interactions like hydrophobic binding.

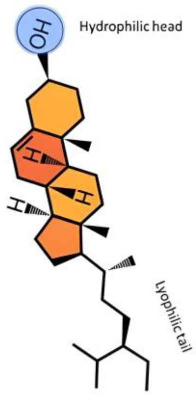


Scheme 1. Fluorescein 5(6)-isothiocyanate-starch (FITC-S).

Cholesterol, a key lipid molecule ubiquitous in animal cells, has long been recognized for its dual roles in human health and disease. First identified as a solid component in gallstones by François Poulletier de La Salle in 1769 [23], cholesterol has since been established as an essential structural and functional component of biological membranes. Unlike plant cells, which possess rigid cell walls, human cell membranes rely on cholesterol to maintain fluidity, stability, and organization. Embedded within the lipid bilayer, cholesterol influences membrane dynamics and serves as a biosynthetic precursor for steroid hormones, bile acids, and vitamin D [23]. Structurally, cholesterol comprises a rigid tetracyclic steroid nucleus, a polar hydroxyl group at position C3, and a nonpolar hydrocarbon tail at C17. This amphipathic nature allows cholesterol to interact with both hydrophilic and hydrophobic domains of biological macromolecules and membranes. Its structural and physicochemical properties are shown in Scheme 2.

While cholesterol is vital for numerous physiological processes, imbalances in its levels are closely linked to pathological conditions such as atherosclerosis and hypolipidemia. Over the past three decades, the detrimental role of cholesterol in the progression of atherosclerosis has been well documented [23]. Clinically, managing cholesterol levels is central to preventing and treating cardiovascular diseases, as elevated LDL and reduced HDL cholesterol are critical risk factors [24]. LDL is primarily responsible for transporting cholesterol from the liver to peripheral tissues, whereas HDL facilitates reverse cholesterol transport, returning excess cholesterol to the liver for excretion or steroidogenesis a process mediated by the enzyme lecithin cholesterol acyltransferase (LCAT), which esterifies cholesterol on HDL particles [25].

Given its amphiphilic character, cholesterol is also prone to form molecular interactions with natural polymers such as potato starch [26]. Investigating such interactions provides insights into potential cholesterol-sequestering agents for biomedical and nutraceutical applications[27]. In particular, capacity of starch to form inclusion complexes or interact via hydrogen bonding and hydrophobic interactions may offer a promising strategy for modulating cholesterol bioavailability or facilitating its removal from the gastrointestinal tract[28].



Scheme 2. Cholesterol structure.

Fluorescein-labeled starch could interact with cholesterol through hydrophobic associations and possible inclusion complex formation, leading to reduced dye accessibility in aqueous media and a measurable decline in absorbance. These interactions, which intensify with higher cholesterol concentrations, follow distinct kinetic patterns that could be accurately described by exponential decay models.

The primary aim of this work is to investigate the molecular interactions between fluorescein-labeled starch and cholesterol using colorimetric measurements.The study intends to evaluate the effects of varying cholesterol concentrations on the kinetics of this interaction in order to objectively quantify the generated data using exponential decay functions. Important kinetic parameters, including rate constants and plateau values, are obtained in an effort to provide a mechanistic understanding of the interaction dynamics. The study also examines the formation of hydrophobic domains within the starch matrix as a function of cholesterol level to elucidate the ways in which lipid–polysaccharide interactions affect the optical properties of hydrophilic fluorescent probes.

Results and discussion

Since fluorescein is a hydrophilic chromophore, whose optical signal can be affected by its local environment, specifically polarity and solvent accessibility [22], the kinetics of the interaction between fluorescein-labeled starch and cholesterol were assessed by tracking changes in absorbance at 490 nm over time. It is anticipated that the addition of cholesterol, a hydrophobic biomolecule, to the starch matrix will modify these local conditions because of hydrophobic domains formation. The kinetic plot (Figure 1) showed a dual pattern with a mono-exponential decrease after an initial plateau phase at a cholesterol concentration of 0.1 mg/mL. With a stable absorbance of around 1.7 a.u. and a plateau that lasted for about 50 seconds, the interaction appears to have been delayed, most likely because it takes time for cholesterol molecules to move and engage with accessible starch chains. This delay pattern, which is frequently seen in heterogeneous molecular systems, can be explained by diffusion restrictions and molecular rearrangement before any notable complexation [4]. A decrease in absorbance to about 1.45 a.u. was noted once contact started, suggesting the formation of non-polar domains that protect fluorescein residues from the aqueous phase and hence lower their absorbance. The plateau followed by one-phase decay model provided a good match to the kinetic data for this concentration (Table 1), yielding a life-time (τ) of 72.15 s, a half-life of 50.01 s, and a rate constant (k) of 0.01386 s^{-1}. The dual pattern nature of the process is supported by the moderate R^2 value of 0.8035. The very mild interaction speed indicated by these numbers might be a sign of the early phases of hydrophobic association, when there is not yet enough cholesterol present to saturate all of the accessible starch binding sites. Its worthy to mention that two-phase decay model did not fit the interaction of 0.1 mg/mL of cholesterol with FITC-S solution, this is resolved by the low R^2 value of 0.6829.

The response of system changed as the cholesterol level rose to 0.5 mg/mL (Figure 2). With no discernible plateau, the best-fitting model changed to a single-phase exponential decay. This implies that the diffusion limitation observed at lower concentrations is overcome at this concentration, where cholesterol is more easily accessible and reacts nearly instantly upon mixing. With a half-life of 192.8 s and a rate constant of 0.003594 s^{-1} (Table 1), the decay was noticeably slower, suggesting a slower overall decrease in absorbance. As more cholesterol is added, the solution may become more viscous or experience steric hindrance, which can impair starch chain mobility and slow down interaction dynamics [3]. Even though these mechanisms provide a compelling explanation, other factors like probe aggregation or local microviscosity changes could potentially account for the observed behavior [29, 30].

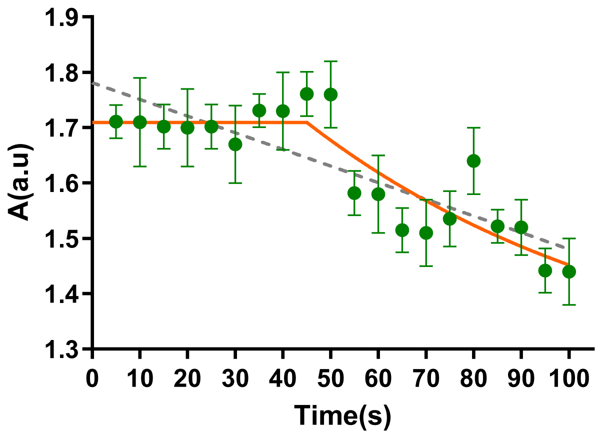


Fig. 1. Kinetic plot for fluorescein-starch blending with cholesterol determined by monitoring the variation in absorbance values at 490 nm. (0.1 mg/mL of cholesterol in 10 wt% of fluorescein-starch aqueous solution at 25 ºC and pH 7, ● experimental data, I error bar, ▬ plateau followed by one phase decay model, and - - - two-phase decay model).

Remarkably, the dual pattern behavior was recovered when the cholesterol concentration was raised to 0.75 mg/mL (Figure 3). After a brief plateau, absorbance rapidly decreased, with a shorter half-life (39.12 s) and a greater rate constant (0.01772 s^{-1}) than for earlier concentrations (Table 1). The excellent match is confirmed by the high R^2 value (0.9429). This behavior points to a concentration threshold over which cholesterol begins to form localized clusters or microdomains that effectively bind to starch, causing fluorescein to be shielded more quickly and effectively. Findings in polysaccharide-lipid interactions, where micelle-like structures and molecular crowding can occur and significantly change physicochemical properties, are in line with this [2, 5]. The interaction once more displayed a straightforward one-phase decline pattern at the maximum measured concentration of 1.0 mg/mL (Figure 4). The half-life (129.1 s) and rate constant (0.005368 s^{-1}) suggested a moderately paced interaction, and the R^2 value (0.9286) showed a strong model fit (Table 1). This non-linear trend across concentrations suggests that the dynamic creation of hydrophobic pockets, starch accessibility, and cholesterol concentration interact in a complicated way. Excess cholesterol may cluster or self-associate at very high concentrations, decreasing its effective interaction with starch. This behavior has been shown in systems similar to these where lipophilic substances show saturation kinetics [1].

The calculated kinetic parameters (Table 1) clearly show that the cholesterol concentration has no monotonic effect on the interaction profile. Even though early increases in cholesterol enhance interaction through domain development, increases in cholesterol can alter the rate and extent of contact by creating steric constraints or generating cholesterol aggregation. These patterns demonstrate that cholesterol serves as a binding partner, probe aggregator, local microviscosity changer, and a structural modifier of the polymeric matrix in these systems. Mechanistically, hydrophobic rearrangements that reduce exposure of fluorescein to the aqueous phase account for the absorbance decrease. Such microenvironmental alterations have been well documented in studies of biopolymer interactions and fluorescence quenching, where ambient polarity is essential for modulating optical signals [7]. This confirms the efficacy of fluorescein-starch as a probe, offering a simple yet efficient way to investigate molecular behavior at the interface between lipids and polymers. Furthermore, the kinetic study explains how cholesterol alters the local environment within starch matrices. Depending on its content, cholesterol can either speed up or slow down initial contact due to aggregation or viscosity. These findings are consistent with the larger literature on polysaccharide-lipid systems, which is influenced by the balance of hydrophobic contacts, microphase structure, and steric accessibility [6, 21, 23]. Understanding these dynamics is crucial for applications like food packaging, biosensors, and medication delivery, where lipid-polymer interactions determine functionality.

Table 1. Absorbance-based determination of interaction lifetime and associated parameters for starch–cholesterol complexes at varying cholesterol concentrations (25 °C, pH 7).

Cholesterol content (mg/mL),	Mathematical fit	K (s^{-1})	$t_{1/2}$ (s)	τ (s)	R^2
0.10	Plateau followed by one phase decay	0.01386±0.00020	50.01±0.1	72.15±0.4	0.8035(0.6829*)
0.50	One phase decay	0.003594±0.00004	192.8±0.4	278.2±0.1	0.9189(0.9189*)
0.75	Plateau followed by one phase decay	0.017720±0.00002	39.12±0.2	56.44±0.3	0.9429(0.9200*)
1.00	One phase decay	0.005368±0.00005	129.1±0.3	186.3±0.2	0.9286(0.9286*)

*R^2 values generated by two phase decay mathematical model.

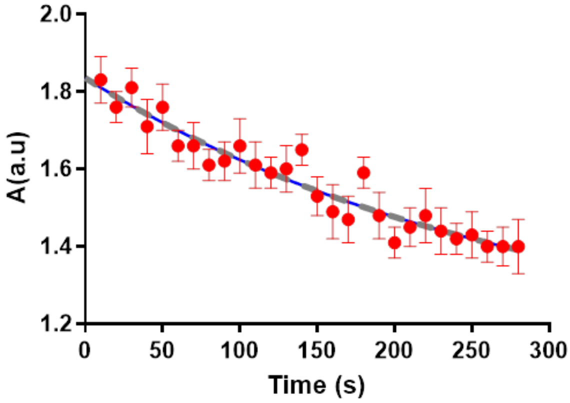


Fig. 2. Kinetic plot for fluorescein-starch blending with cholesterol determined by monitoring the variation in absorbance values at 490 nm. (0.5 mg/mL of cholesterol in 10 wt% of fluorescein-starch aqueous solution at 25 ºC and pH 7, • experimental data, I error bar, ▬ one phase decay model, and - - - two-phase decay model).

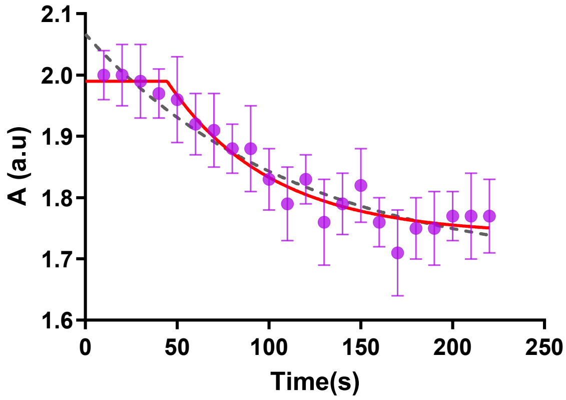


Fig. 3. Kinetic plot for fluorescein-starch blending with cholesterol determined by monitoring the variation in absorbance values at 490 nm. (0.75 mg/mL of cholesterol in 10 wt% of fluorescein-starch aqueous solution at 25 ºC and pH 7, • experimental data, I error bar, ▬ plateau followed by one phase decay model, and - - - two-phase decay model).

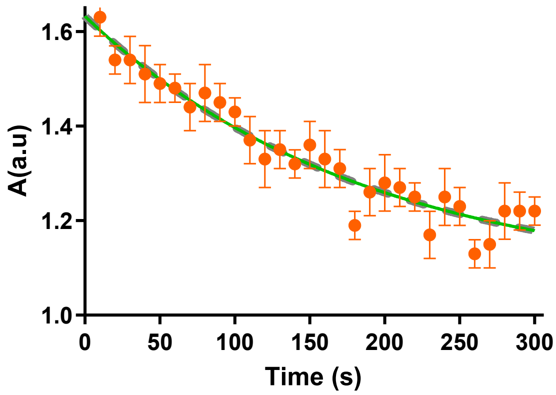


Fig. 4. Kinetic plot for fluorescein-starch blending with cholesterol determined by monitoring the variation in absorbance values at 490 nm. (1 mg/mL of cholesterol in 10 wt% of fluorescein-starch aqueous solution at 25 ºC and pH 7, • experimental data, I error bar, ▬ one phase decay model, and - - - two-phase decay model).

Material and methods

All materials in this project have been used without further purifications unless was mentioned.

Materials

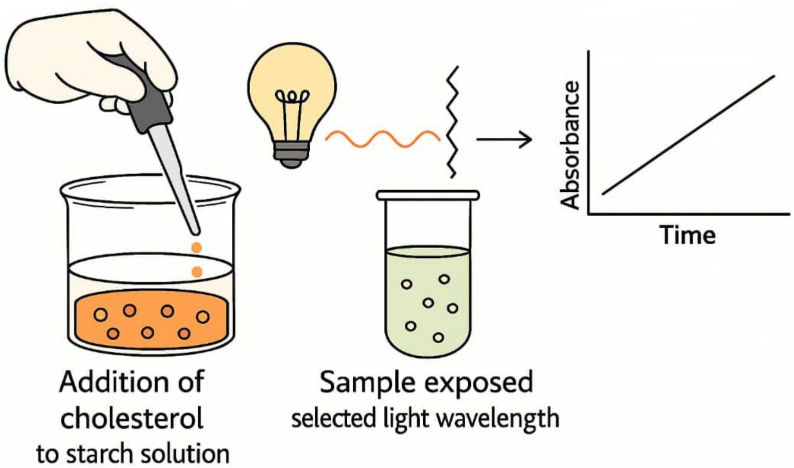
Starch-Fluorescein 5(6)-isothiocyanate (form our previous work [22]). Cholesterol (≥99% purity; Sigma-Aldrich). Dimethyl sulfoxide (DMSO) (Sigma-Aldrich). Distilled water (Research Laboratory). Ethanol (Sigma-Aldrich).

Preparation of Cholesterol Solutions

Cholesterol was dissolved in a minimal volume of DMSO to prepare stock solutions (10 mg/mL). Working solutions of varying concentrations (0.1 mg/mL to 1.0 mg/mL) were prepared by dilution in water containing 1% ethanol to ensure miscibility.

Interaction Kinetics Procedure

Equal volumes of fluorescein-starch solution (10 wt%) and cholesterol solutions were mixed in quartz cuvettes. The final volume was adjusted to 3 mL. Immediately upon mixing, the absorbance at 490 nm was measured over a period of time at regular intervals (10 seconds) using a digital colorimeter (Biocheom, Libra S2). Control samples containing fluorescein-starch without cholesterol were included for baseline comparison. The stemps of colrimetric invistgations are showed in Scheme 3.



Scheme 3. Principle of colorimetry.

Data Analysis and Kinetic Modeling

Raw absorbance data were exported and analyzed using GraphPad Prism software. Non-linear regression models were applied to fit the data: Plateau followed by one phase decay and one phase decay models. Goodness of fit was assessed using R^2 values and residual plots. Kinetic parameters such as rate constants (k), amplitude, and half-life ($t_{1/2}$) were extracted. All experiments were performed in triplicate. Figure 5 depicts one phase decay and its parameters.

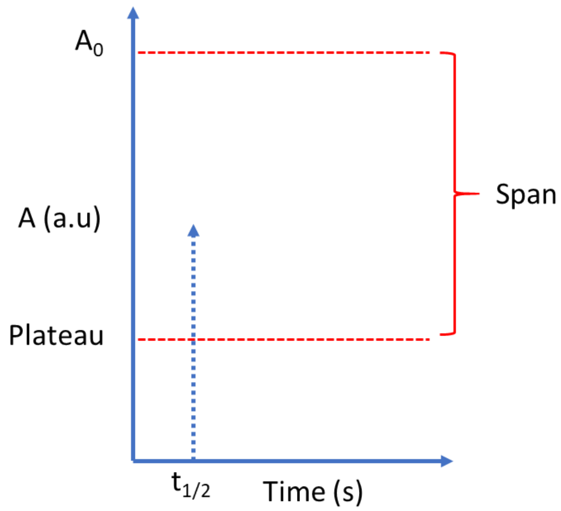


Fig. 5. The one phase decay curve.

The one-phase decay equation describes how a quantity decreases exponentially over time. It's often used in situations like radioactive decay, drug elimination, or the dissociation of a ligand from a receptor. The model is:

$$A = (A_0 - Plateau) * e^{(-Kt)} + Plateau \qquad (1)$$

The plateau followed by a one-phase decay curve is represented by the following model:

$$A = IF\left(t<t_0\,, A_0, Plateau + (A_0 - Plateau) * e^{(-K*(t-t_0))}\right) + Plateau \qquad (2)$$

This equation models a situation where a value (A) remains relatively stable (plateau) up to a certain time (t_0), then decays exponentially to a new plateau.

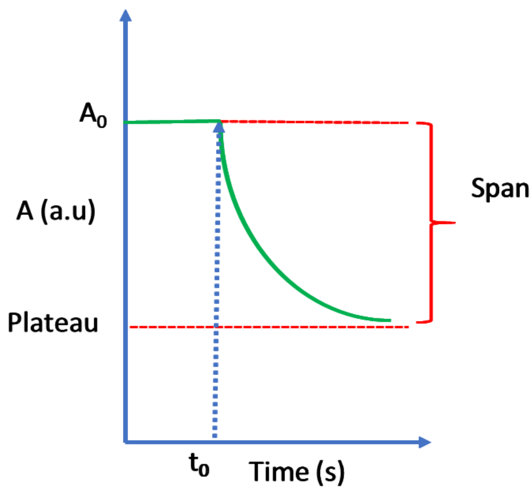


Fig. 6. The plateau followed by a one-phase decay curve.

Rationale for Model Selection

The physicochemical environment of system and data fitting were taken into consideration when selecting the one-phase decay and plateau followed by one-phase decay models. Specifically, the experimental data at 0.1 mg/mL of cholesterol in 10 weight percent fluorescein–starch aqueous solution, where the signal obviously followed a single exponential trend, was not well fitted by the two-phase decay model. At higher cholesterol concentrations (0.5, 0.75, and 1 mg/mL), two-phase decay offered a decent statistical fit; however, its interpretation was not practically acceptable in this situation. The notion of two simultaneous decay processes is not physically plausible because the mixture of cholesterol and fluorescein-starch generates a homogeneous aqueous solution rather than a system with different kinetic subpopulations. Because of this, the one-phase decay model provides a more meaningful description of the dynamics of the system and a firmer mechanistic foundation. Similarly, the plateau followed by one-phase decay model accurately described the observed behavior without needless complication in situations when an initial stabilization was visible. Together, these models delivered the most parsimonious and physically reliable framework for evaluating the experimental data.

$$A = \left(Plateau + Span\ Fast * e^{(-Kfast*(t))}\right) + \left(Span\ Slow * e^{(-KSlow*(t))}\right) \qquad (3)$$

Where, Kfast and Kslow are the two rate constants, expressed in reciprocal of the time units.

Conclusions

With concentration-dependent patterns indicating the creation of hydrophobic domains and, at higher levels, viscosity and steric effects that influence interaction rates, this work demonstrates that the interaction between fluorescein-starch and cholesterol is well represented by exponential decay models. Although these mechanisms offer a convincing explanation, the observed behavior could also be explained by other factors such probe aggregation or local microviscosity changes. All things considered, the results demonstrate the value of colorimetric kinetics in clarifying lipid–biopolymer interactions and their possible uses in pharmaceutical, food, and biomaterial systems.

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