



Interaction of Curcumin with Poly Lactic-Co-Glycolic Acid and Poly Diallyldimethylammonium Chloride By Fluorescence Spectroscopy

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Abstract

Poly Lactic-Co-Glycolic Acid (PLGA) and Poly Diallyldimethylammonium Chloride (PDDA) are widely being used for drug delivery and curcumin is being studied as potential drug molecule for its anti-oxidant, anti-inflammatory and anti-cancer activities. The interaction between PLGA, PDDA and curcumin was investigated by fluorescence spectroscopy. The modified Stern–Volmer equation was used to estimate the value of the binding constant K_a and the van't Hoff equation was used to estimate the corresponding thermodynamic parameters (ΔH° , ΔS° , and ΔG°). The obtained results showed that the binding constant between PLGA and Curcumin is due to the formation of hydrogen bonds and van der Waals forces. However, PDDA interacts with curcumin through hydrophobic interactions. Moreover, zeta potential measurements were obtained for these polymers and the surface charge was compared in presence and absence of the negatively charged curcumin molecules. It was found that the results obtained by zeta potential measurements are in agreement with those obtained by fluorescence spectroscopy. It is also found that binding of curcumin with PDDA is further encouraged in the presence of PLGA.

Keywords Curcumin · PDDA · PLGA · Fluorescence spectroscopy · Zeta potential · Binding constant

Introduction

The scientific world has been constantly working in an attempt to make better, more effective, less toxic, and long lasting effect for therapeutic drugs [1]. As a drug, curcumin gained popularity and got labelled as ‘next generation multipurpose drug’ due to its multiple roles, such as antioxidant, anti-inflammatory, anticancer, antidiabetic, antiangiogenic and antimicrobial activities [2–6]. Supramolecular structures featuring the relationship of two singular molecular variety formed by weak intermolecular interactions are an attractive group of arranged matter. The interactions that govern the self-assembly range from electrostatic to $\pi-\pi$ or hydrogen bonding [7, 8]. Supramolecular structures of two or more compounds that self-assemble into spheres, rods, or sheets have been reported [7]. Polymers have played a vital role in the progression of drug delivery technology by providing controlled release of hydrophilic and hydrophobic therapeutic agents [9, 10]. The conjugation of a drug with a

polymer forms ‘polymeric prodrug’. Such conjugation is considered as a fast growing and an effective tool to improve the usage of drugs in therapeutic applications [11, 12]. The concept of conjugation of drugs with natural and synthetic polymers was introduced 60 years ago using polyvinylpyrrolidone (PVP) polymer and dipeptide spacer conjugated to mescaline drug [13].

Poly-diallyldimethylammonium chloride (PDDA) is a water-soluble cationic polymer [14]. It is synthesized by copolymerization and it forms linear homo-polymer with quaternary ammonium groups on the rings involved in the backbone of the polymer chain [15, 16]. The presence of these groups in its chemical structure displays exceptional antimicrobial activity [17]. This polymer has been used as a flocculent or coagulant in water treatment in order to remove organic and mineral contaminants [14]. Also it has been used as a composite for biosensors [15, 18].

Furthermore, Poly lactic-co-glycolic acid (PLGA) is a copolymer synthesized through a random ring opening copolymerization of the cyclic dimers of the lactic and glycolic acids [19]. PLGA belongs to the family of the FDA approved biodegradable polymers [20]. PLGA is utilized as a biomaterial because of its biocompatibility and biodegradability [19].

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In supramolecular chemistry, typically the first query that a researcher might need to ask is: “how strong is this complex or interaction?”. Binding constants are a distinct case of equilibrium constants. It helps to extent the bonding affinity amongst two or more molecules at equilibrium. In supramolecular chemistry, binding constants for either host–guest complexation or host–host aggregation (e.g., dimerization) are typically the topic of concern. The determination of binding constants is an essential step in understanding and describing molecular interactions [21]. The binding constant can be measured using specialized techniques as the analytical methods such as solubility methods, potentiometric, and mass spectrometry were also used for this purpose [22]. In addition, other techniques were investigated as NMR [23], UV–vis [24], fluorescence [25], and calorimetric titrations [26]. It is possibly safe to say that above 90% of all experimentally calculated binding constants in supramolecular chemistry are recently determined using the last fore cited techniques. Thus, nowadays researchers are focusing on the efficiency of fluorescence spectroscopy technique for the determination of binding constant. In fact, this technique has arisen as a common method because it is quick, very sensitive, and easy to perform [27].

In fact, PLGA polymer and PDDA polymer are widely used for the preparation of curcumin nanocapsules. The encapsulation of curcumin into the core of polymer boost its usage especially in drug delivery. However, the interaction between curcumin and PLGA polymer along with the binding sites is not well established. For this reason, in this study, fluorescence spectroscopy was utilized in order to study for the first time and compare the interaction between curcumin and both polymers PLGA and PDDA respectively. Also, thermodynamic parameters, binding constants, binding sites and the type of present interaction forces were explored.

Materials and Methods

Materials

Curcumin, Poly(D,L-Lactide-co-glycolide) ester terminated (Mw 50,000–75,000), poly-diallyl dimethyl ammonium chloride 20 wt. % in H₂O and chloroform were purchased from Sigma Aldrich. All the chemicals were used as obtained without any further purification steps.

Fluorescence Studies

Fluorescence studies were conducted by a Jobin–Yvon–Horiba fluorometer (model No: FL3-22). Emission and excitation slits were set at 5 nm and the excitation wavelength of 425 nm. The fluorometer was equipped with a 100 W Xenon lamp and an

R-928 detector functioning at 950 V. The emission spectra of samples in the absence and presence of various concentrations of PLGA and PDDA were recorded over a wavelength range 440–700 nm at three different temperatures (25, 35, and 45 °C). The temperature was controlled by a thermostat which is coupled to the fluorometer sample holder. An external thermometer was used in order to fix the sample temperature. The width of the used cuvette was 1 cm.

Sample Preparation

In order to study the interaction of curcumin with PLGA in solution, fluorescence measurements for 11 samples of different PLGA concentrations in the range of 0–0.290 g/L were conducted. Briefly, PLGA was first dissolved in chloroform then evaporated using rotatory evaporator. Later on, double distilled water was added and the sample was sonicated for final use.

Likewise, to study the interaction of curcumin with PDDA in solution, 9 samples with different PDDA concentrations in the range of 0–5.6 µg/L were prepared. Curcumin's concentration was maintained constant at 2 µM in all samples.

To check the emission intensity of PLGA and PDDA alone, a sample containing 290 µg/L of PLGA and 3.5 µg/L of PDDA were prepared respectively.

Results and Discussion

The interaction of curcumin with PLGA and PDDA was estimated in order to find the binding constant for PLGA and PDDA.

Spectral Properties of Curcumin

Initially, when dissolving curcumin in methanol ($C = 2 \mu\text{M}$), a broad characteristic UV–visible absorption peak is obtained at around 300–500 nm with maximum absorption band at $\lambda_{\text{abs}} = 425 \text{ nm}$ (See Fig. 1A). Therefore when exciting at $\lambda_{\text{ex}} = 425 \text{ nm}$, the emission wavelength is found to be around $\lambda_{\text{em}} = 544 \text{ nm}$ (See Fig. 1B). In fact, the presence of the electronic dipole allowed $\pi\text{-}\pi^*$ type excitation of its extended conjugation system, and therefore resulting in the maximum absorption at 425 nm. Subsequently, an electrostatic interaction between polar solvent (methanol) and polar chromophores in curcumin molecule will take place. Henceforth, methanol tends to stabilize the bonding electronic ground states and the π^* excited states. Consequently, the $n\text{-}\pi^*$ transition move to higher energy and $\pi\text{-}\pi^*$ transition move to lower energy. Thus, the $\pi\text{-}\pi^*$ and $n\text{-}\pi^*$ absorptions of curcumin move close to each other [28].

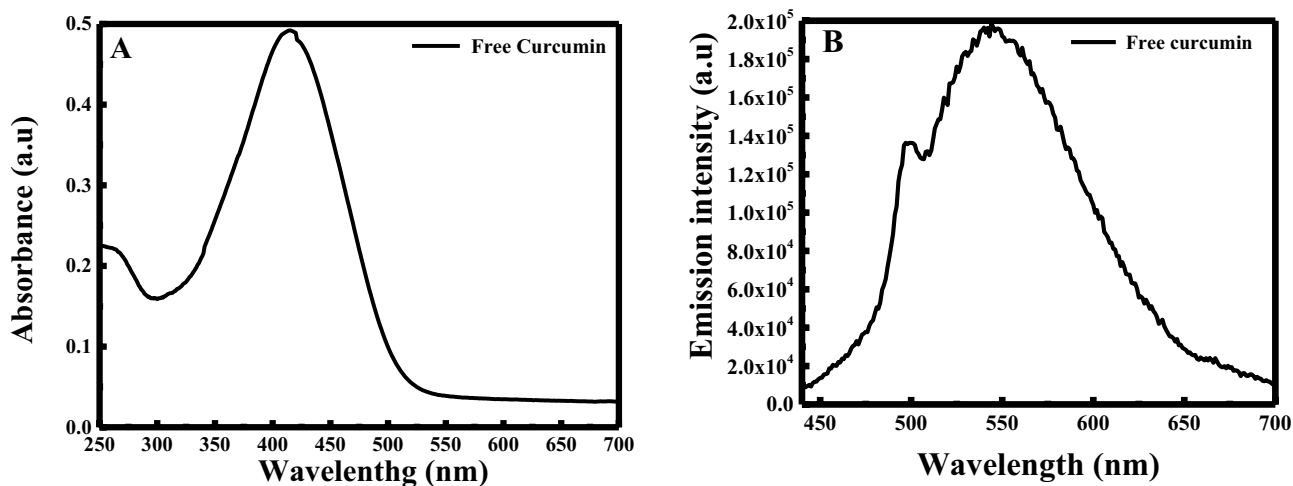


Fig. 1 A Uv-Visible and B Fluorescence emission intensity of pure curcumin ($C=2 \mu\text{M}$) in methanol

Binding Constant of PLGA

Curcumin's concentration was fixed at $2 \mu\text{M}$, while the concentration of PLGA was increased from 0 to 0.29 g/L. It was found that the emission intensity of curcumin is proportional to the increase of PLGA's concentration, where the emission intensity increases within the increase of PLGA concentration (See Fig. 2A). The enhancement of the intensity was also accompanied with a blue shift from $\sim 542 \text{ nm}$ to $\sim 507 \text{ nm}$. This blue shift is due to the fact that curcumin experiences a more nonpolar environment in PLGA, meaning that Curcumin is being incorporated in the hydrophobic core of the polymers.

To find the binding constant and thermodynamic parameters of the interaction of PDDA with curcumin, the plot of $\log [(F-F_0)/F_0]$ vs the logarithm of polymer concentration was established according to the modified Stern–Volmer equation:

$$\log(F-F_0)/F_0 = \log K_a + n \log[P] \quad (1)$$

where n is the number of sites and K_a is the binding constant [29].

Based on the curve obtained in Fig. 2B, the binding constant (K_a) value of curcumin with PLGA was found to be equal to 119.89 L/g. Hence, the linear fit equation was set as $y = 1.7557x + 2.0788$. These results showed that curcumin possessed two binding site to PLGA polymer at ambient temperature.

To have a better understanding of the thermodynamics of the reaction between Curcumin and PLGA, the binding constant was evaluated at three different temperatures; 298.15 °K, 308.15 °K, and 318.15 °K. Thereby, the standard enthalpy change (ΔH°) and the entropy change (ΔS°) were

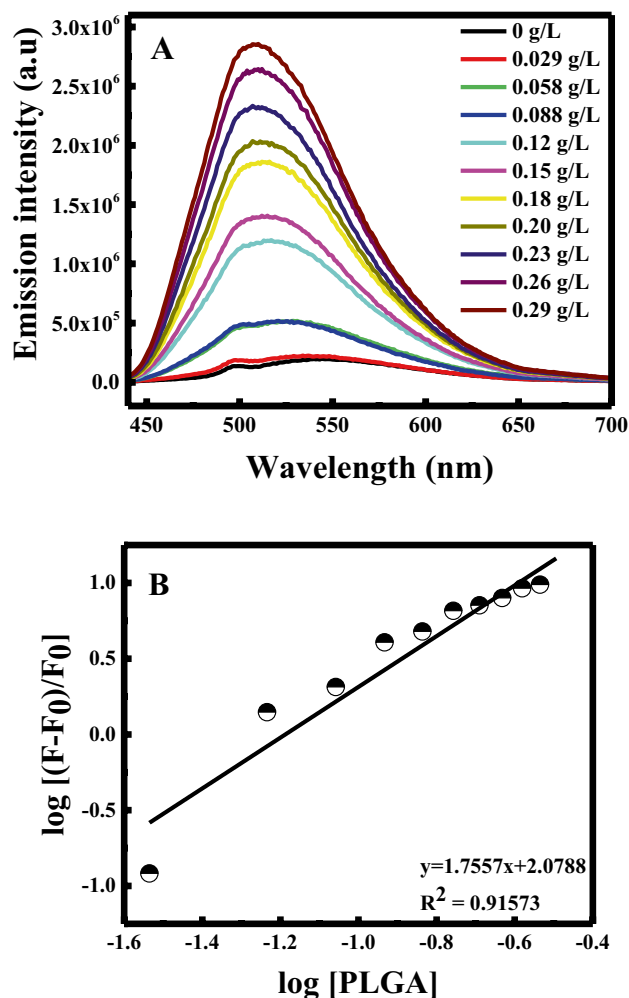


Fig. 2 A Fluorescence emission spectra of curcumin at different concentrations of PLGA excited at $\lambda=425 \text{ nm}$ and B Modified Stern–Volmer plot for PLGA

determined by using the van't Hoff equation (Eq. 2) and the standard free energy change (ΔG°) was estimated using the thermodynamics equation (Eq. 3) [1].

$$\text{Log}(K_a) = \Delta S^\circ/R - \Delta H^\circ/RT \quad (2)$$

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (3)$$

As shown in Fig. 3A, the emission intensity of curcumin increases within the increase of PLGA concentration, thus decreases with the increase of the temperature. This variation is due to the change of the PLGA environment while the temperature increases. Modified Stern–Volmer plot for PLGA at 3 temperatures and van't Hoff plot were depicted in Fig. 3B and C. The thermodynamics parameters (ΔH° , ΔS° and ΔG°) were calculated and tabulated in Table 1.

Generally, based on the values of standard enthalpy changes (ΔH°) and standard entropy changes (ΔS°), there are three different types of interaction between drug and biomolecules that can exist [1, 30]:

- If both ΔH° and ΔS° are positive, then hydrophobic interaction exists.
- If both ΔH° and ΔS° are negative, then van der Waals interactions and hydrogen bonds occur.
- If ΔH° negative and ΔS° positive, then electrostatic interactions are present.

Henceforth, the negative ΔH° and negative ΔS° calculated, confirm the presence of van der Waals interaction and hydrogen bonding with curcumin. Moreover, the negative value of ΔG° indicates that the interaction of PLGA with curcumin was due to a spontaneous process.

Binding Constant of PDDA

Similarly, the binding constant of PDDA was conducted by varying its concentration from 0–55.47 $\mu\text{g/L}$. As observed in Fig. 4A the increase in PDDA concentration, boost the intensity of curcumin with a blue shift from ~ 550 nm till ~ 499 nm. Hence, these results were identical to the results obtain with PLGA polymer, where curcumin experiences also a more nonpolar environment in PDDA micelles, indicating that curcumin is being incorporated in the hydrophobic core of the PDDA polymer.

The binding constant (K_a) was calculated after analyzing the binding curve in Fig. 4B. K_a value of curcumin with PDDA was found to be equal to 30.38 L/g. Interestingly, the main difference between PLGA and PDDA polymer is that curcumin possessed one binding site to PDDA based on the linear equation $y = 0.6879x + 1.4826$.

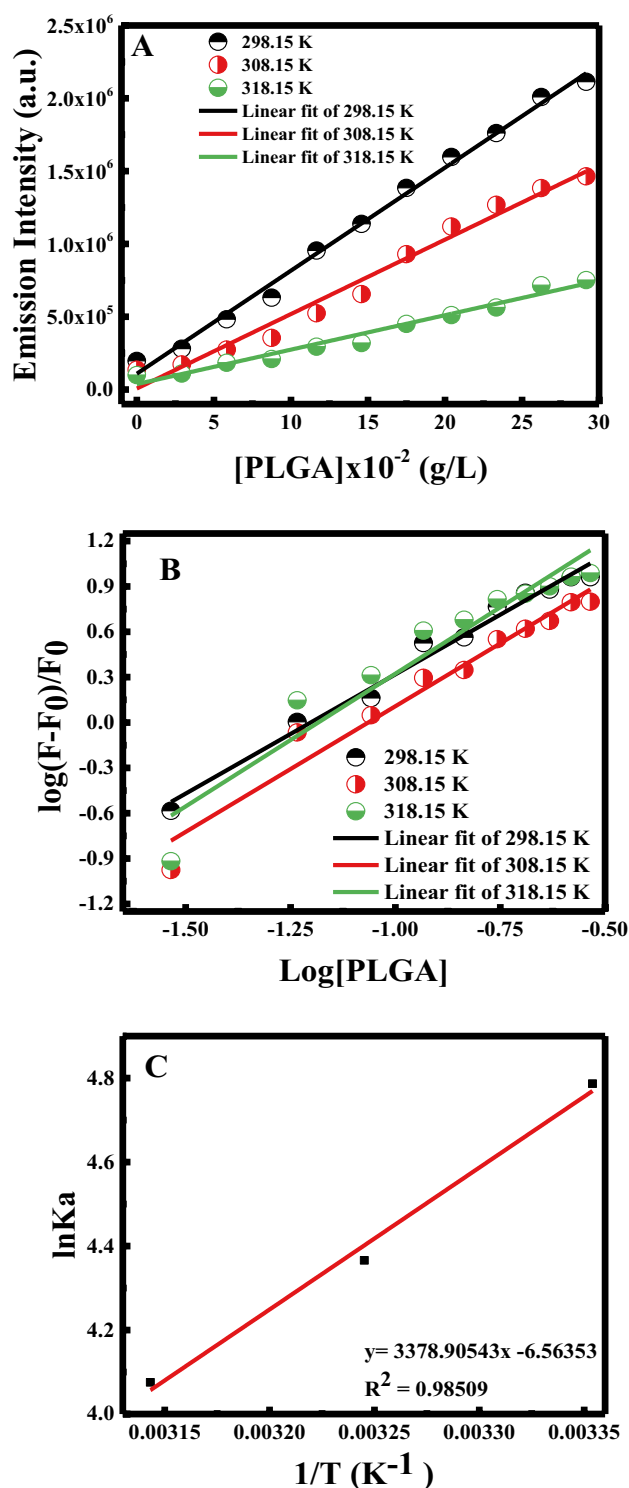


Fig. 3 A Effect of the temperature on the emission intensity of curcumin in the presence of different PLGA concentration; B Modified Stern–Volmer plot for PLGA at 3 temperatures and (C) van't Hoff plot

Table 1 Binding and thermodynamic parameters for curcumin-PLGA interaction at different temperatures

| Temperature (°K) | n | K _a (L/g) | ΔS (J.mol ⁻¹ .K ⁻¹) | ΔG (KJ.mol ⁻¹) | ΔH (KJ.mol ⁻¹) |
|------------------|------|----------------------|--|----------------------------|----------------------------|
| 298.15 | 1.76 | 119.67 | -54.57 | -11.82 | -28.10 |
| 308.15 | 1.57 | 78.72 | | -11.28 | |
| 318.15 | 1.66 | 58.75 | | -10.73 | |

Afterwards, the binding constant was evaluated at three different temperatures; 298.15 °K, 308.15 °K, and 318.15 °K. As shown in Fig. 5A, the emission intensity decreases with the increase of the temperature. This can be due to the increase of the PDDA viscosity, inhibiting the entrapment of curcumin into the micelles. Furthermore, Modified Stern–Volmer plot for PDDA at 3 temperatures and van't Hoff plot were presented in Fig. 5B and C respectively. The thermodynamics parameters (ΔH° , ΔS° and ΔG°) were calculated and supplied in Table 2.

Based on the calculated values, the positive ΔH° and positive ΔS° values suggest that the dominant interaction between Curcumin and PDDA is hydrophobic interaction. Besides, the negative value of ΔG° indicates that the interaction of PDDA with curcumin was based on a spontaneous process.

Binding Constant of PDDA in the Presence of PLGA

To study the effect of PLGA on the interaction of curcumin with PDDA, 9 samples were prepared where PDDA concentration was increased in the range of 6.9 g/L to 56 g/L, PLGA and curcumin concentrations were fixed at 20 $\mu\text{g/L}$

and 2 μM respectively. As can be seen in Fig. 6A the emission intensity of curcumin increased around ~ fourfold in the presence of PLGA. In fact, in the absence of PLGA the emission intensity increases by ~ sevenfold. Yet, the presence of PLGA decreased the emission intensity by ~ threefold. This diminution in the emission intensity can be due to the fact that more particles are present in the solution and thus more collisions are generated inducing radiation-less decay. These radiation-less decay cause a loss of energy as heat that in turn lead to the decrease in the fluorescence intensity. After plotting the modified Stern Volmer plot at 3 different temperatures (See Fig. 6B), the binding constants were calculated and formulated in Table 3. Comparing K_a values in the presence of PLGA values to K_a values in absence of PLGA, we can see that the binding affinity has increased in presence of PLGA. This is because PLGA is forcing curcumin in water phase and pushing it toward the PDDA polymer.

Zeta Potential Measurements

To further understand the interaction between Curcumin, PLGA and PDDA, zeta potential measurement was performed

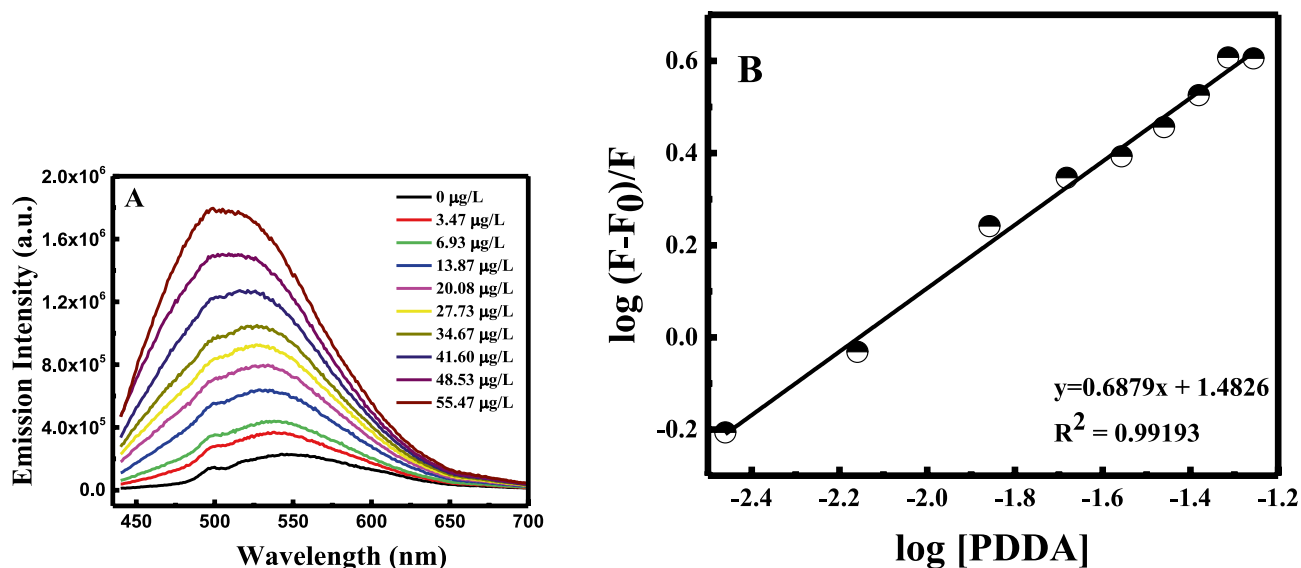


Fig. 4 A Fluorescence emission spectra of curcumin at different concentrations of PDDA excited at $\lambda = 425$ nm and B Modified Stern–Volmer plot for PDDA

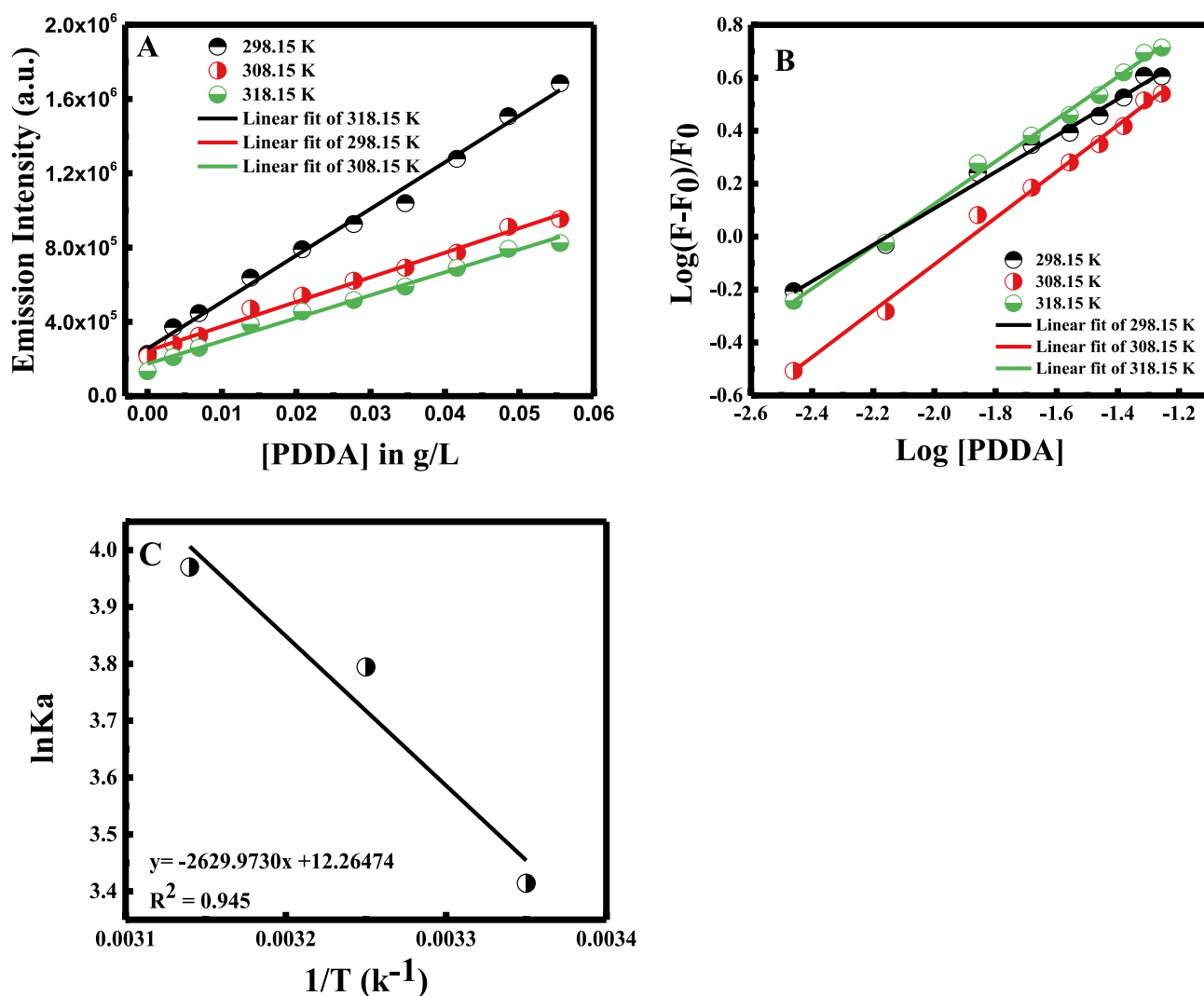


Fig. 5 **A** Effect of the temperature on the emission intensity of curcumin in the presence of different PDDA concentration; **B** Modified Stern-Volmer plot for PDDA at 3 temperatures and **C** van't Hoff plot

using Particulate systems, NanoPlus Zeta Potential/Nano Particle analyzer. By measuring the zeta-potential, it is possible to probe a characteristic colloidal property in a mixture of particles, making it a useful technique to elucidate their behavior [30]. The zeta-potential is generated between the

interfacial double layer of the dispersed particle versus the continuous phase away from the interface [31]. Thus, the biomaterial's zeta-potential reveals the electric surface properties. Zeta potential was obtained in the first place for curcumin, PLGA, and PDDA alone. Then, it was also measured

Table 2 Binding and thermodynamic parameters for curcumin-PDDA interaction at different temperatures

| Temperature (°K) | n | Ka (L/g) | ΔS (J.mol ⁻¹ .K ⁻¹) | ΔG (KJ.mol ⁻¹) | ΔH (KJ.mol ⁻¹) |
|------------------|------|----------|--|------------------------------------|------------------------------------|
| 298.15 | 0.69 | 30.39 | 101.97 | -8.54 | 21.87 |
| 308.15 | 0.88 | 44.45 | | -9.56 | |
| 318.15 | 0.80 | 52.98 | | -10.58 | |

Fig. 6 **A** Fluorescence intensity of curcumin versus concentration of PDDA in presence of PLGA at different temperatures and **B** Modified Stern–Volmer plot for PDDA in presence of PLGA at 3 temperatures

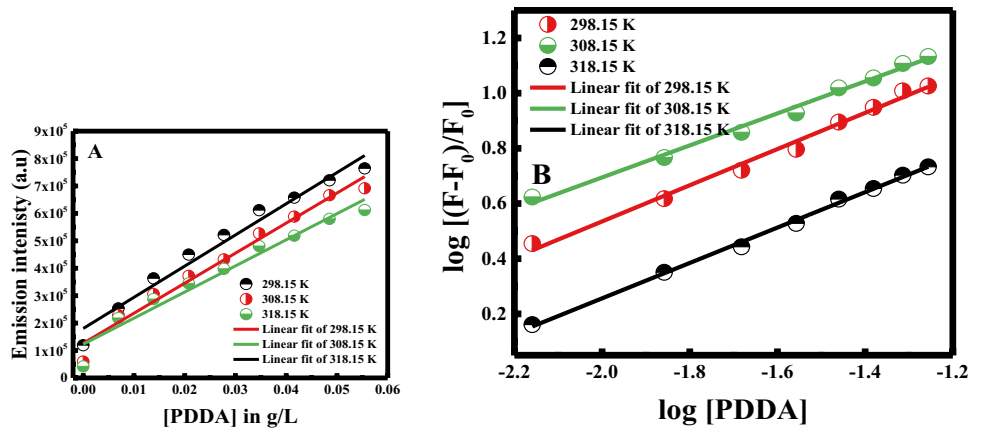


Table 3 Comparison of K_a values for the interaction of PDDA in presence and absence of PLGA

| Temperature (°K) | K_a in presence of PLGA (g/L) | K_a in absence of PLGA (g/L) |
|------------------|---------------------------------|--------------------------------|
| 298.15 | 34.59 | 30.39 |
| 308.15 | 70.28 | 44.45 |
| 318.15 | 71.58 | 52.98 |

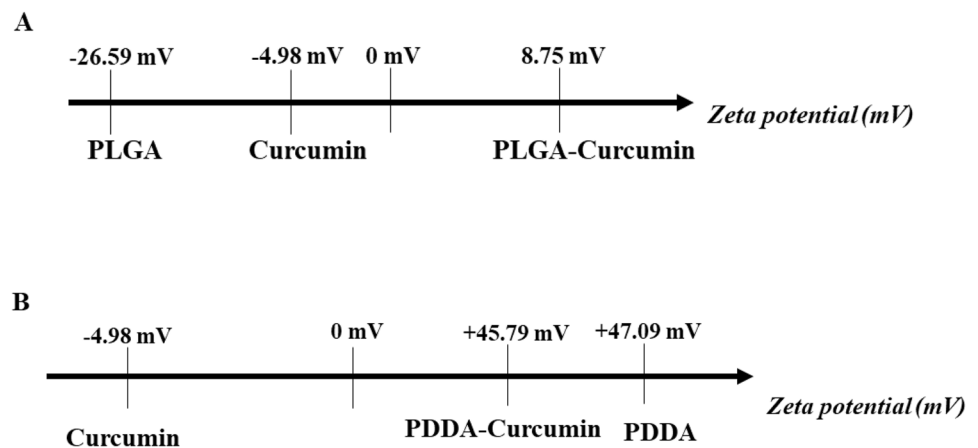
for the mixture of curcumin-PLGA, curcumin-PDDA, and curcumin-PLGA-PDDA. PLGA and Curcumin exhibit a negative surface charge equal to -26.59 mV and -4.98 mV respectively. Interestingly, upon mixing these two together a positive zeta potential was obtained. Considering the negative

charge of both compounds, we would expect an increase in the total negative charge. Thus, a positive surface charge was obtained confirming the formation of hydrogen bond upon binding curcumin with PLGA molecules (See Fig. 7A).

Similar results was obtained by Meesaragandla et al., where the mixture of the negatively charge humane serum albumin with the negatively charged PEG-NH₂ coated NPs results in an increase in the overall positive potential. Such unexpected behavior was related to the formation of hydrogen bonding between these molecules [32].

With respect to PDDA polymer which is positively charged (+47.09 mV), a decrease in its charge was obtained upon mixing it with curcumin (See Fig. 7B). This decrease in the surface charge confirms the hydrophobic interaction between these molecules.

Fig. 7 zeta potential values for Curcumin, PLGA, and PDDA and their mixture



Conclusion

In summary, different sample with varying PLGA and PDDA concentration were prepared in presence of fixed curcumin concentration. The emission spectra were obtained for these samples at three different temperatures (298.15, 308.15, and 318.15 °K) at 425 nm excitation wavelength. Starting from these data the Modified Stern–Volmer plot was obtained at room temperature and the binding constant of PLGA and PDDA with Curcumin was estimated to be 119.67 and 30.39 g/L respectively. Thermodynamic parameters were calculated using Van't Hoff equation. Based on the obtained values, it was concluded that curcumin binds to PLGA through hydrogen bonding and van der waals interaction, while PDDA interacts with curcumin through hydrophobic interactions. Moreover, binding of curcumin with PDDA is further encouraged in the presence of PLGA.

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Author Contribution Hanine Zakaria: Methodology, Formal analysis, Investigation, Experiment, Validation, Writing- Original draft preparation; Riham El Kurdi: Formal analysis, Experiment, Analysis, Writing-Reviewing and Editing; Digambara Patra: Conceptualization, Supervision, Resources, Project administration, Writing- Reviewing and Editing.

Availability of Data and Material Not applicable.

Code Availability Not applicable as we used standar software from the instrument.

Declarations

Ethics Approval We declare that we have not violated any ethical responsibilities. No Human Participants and/or Animals were used in this research.

Consent to Participate This is not applicable as we have not used any human participants.

Consent for Publication This is not applicable as we have not used any human participants.

Conflict of Interest The authors have no relevant financial or non-financial interests to disclose.

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