POLIMERY

Stabilization of poloxamine and poloxamer block copolymer complexes in chlorinated organic solvents – mathematical modelling

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Abstract: To develop a mathematical model describing the behavior of chromophore complexes, the main factors influencing the stability of poloxamine and poloxamer complexes were determined. Gel permeation chromatography, ultraviolet absorbance measurements and FT-IR were used for the analysis. It was found that the studied complexes in chlorinated organic solvents are unstable in the presence of oxygen, air, or water. The developed mathematical model allows for more effective planning and execution of experiments due to the possibility of predicting UV absorption based on the distribution of complexes in chlorinated solvents.

Keywords: poloxamines, poloxamers, block copolymers, ammonium ferrothiocyanate, colorimetric analysis, mathematical modeling.

Stabilizacja kompleksów kopolimerów blokowych poloksaminy i poloksameru w chlorowanych rozpuszczalnikach organicznych – modelowanie matematyczne

Streszczenie: W celu opracowania modelu matematycznego opisującego zachowanie kompleksów chromoforowych określono główne czynniki wpływające na stabilność kompleksów poloksamin i poloksamerowych. Do analizy zastosowano chromatografię żelową, pomiary absorbancji w ultrafiolecie i metodę FT-IR. Stwierdzono, że badane kompleksy w chlorowanych rozpuszczalnikach organicznych są niestabilne w obecności tlenu, powietrza lub wody. Opracowany model matematyczny pozwala na efektywniejsze planowanie i wykonywanie eksperymentów ze względu na możliwość przewidywania absorpcji UV na podstawie rozkładu kompleksów w chlorowanych rozpuszczalnikach.

Słowa kluczowe: poloksoaminy, poloksamery, kopolimery blokowe, ferrotiocyjanian amonu, analiza kolorymetryczna, modelowanie matematyczne.

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Poloxamines and poloxamers, trademarked as Pluronic[®] and Tetronic[®], respectively, are biodegradable amphiphilic triblock liquid copolymer non-ionic surfactants [1]. They exhibit solubility in both polar and non-polar organic solvents, making them versatile materials widely employed across pharmaceutical, nanoparticle surface engineering, biomedical, and cosmetic industries [2]. Comprising an ABA-type triblock copolymer structure (Fig. 1), each chain consists of a hydrophilic polyethylene oxide (PEO) block pictured in Fig 1a, followed by a hydrophobic polypropylene oxide (PPO) segment, visible in Fig. 1b, and again terminating with a hydrophilic PEO block, with this sequence repeated "n" times [3].

In aqueous media, the PEO and PPO units self-assemble into micellar structures, forming polymeric micelles [4]. These micelles interact with nanomaterial surfaces via their hydrophobic PPO core, while their hydrophilic PEO side arms extend outward, contributing to particle suspension stability through steric stabilization mechanisms involving enthalpic and entropic factors [5]. The conformation, performance, and strength of copolymer adsorption onto nanoparticle surfaces are influenced by factors such as the ratio and size of PPO and PEO segments, surface charge, hydrogen bonding, and constituent groups on the nanoparticle surface [6].

Accurate quantification of these copolymers is crucial for nanoparticle surface engineering but is often challenging due to the lack of precise and sensitive analytical methods [7]. Existing methods are deemed unreliable and impractical, often requiring costly instrumentation like size exclusion chromatography and large sample volumes [8]. A previously reported method demonstrated sensitivity in detecting low concentrations of polyethylene glycol (PEG), a component shared with poloxamers and poloxamines [9]. However, its complexity rendered it unstable and non-reproducible [8]. To address this, a Reagent Cover Technique (RCT) was introduced,



where: polyethylene oxide (PEO): HO(CH₂-CH₂-O)_a polypropylene oxide (PPO): (CH(CH₃)-CH₂-O)_b

Fig. 1. Schematic drawing of a) poloxamine, b) poloxamer copolymers involving the addition of reagents at top extracted organic phases during UV measurements to stabilize the complex. This technique proved effective in yielding consistent and reproducible results across various poloxamines, poloxamers, and chlorinated organic solvents [10]. The current study seeks to delve deeper into the instability of complexes within chlorinated organic phases, elugidate the structural formula of the extracted pink chromophore complex in the organic layer, and develop methods for stabilizing said chromophore complex.

Utilizing enthalpic and entropic variables in steric stabilization processes to promote particle suspension stability [5]. The size and ratio of PPO and PEO segments, surface charge, hydrogen bonding, and component groups on the nanoparticle surface are some of the parameters that affect the conformation, efficacy, and strength of copolymer adsorption onto nanoparticle surfaces [6]. For nanoparticle surface engineering, accurate quantification of these copolymers is essential, but it is frequently difficult since sensitive and accurate analytical techniques are lacking [7]. Current techniques are considered unreliable and unfeasible, frequently necessitating huge sample volumes and expensive instruments such as size exclusion chromatography [8]. A technique that was previously published showed sensitivity in identifying tiny levels of polyethylene glycol (PEG), a substance that is also present in poloxamers and poloxamines [9]. But its intricacy made it, non-reproducible and unstable [9, 10]. To solve this, the Reagent Cover Technique (RCT) was developed, which entails stabilizing the complex by adding reagent on top of extracted organic phases during UV measurements. This method worked well for producing repeatable and consistent results with different poloxamines, poloxamers, and organic solvents that had been chlorinated [10]. The present work aims to clarify the structural formula of the retrieved pink chromophore complex in the organic layer, investigate the instability of complexes within chlorinated organic phases in greater detail, and devise strategies for stabilizing the chromophore complex.

EXPERIMENTAL PART

Materials

Poloxamine and poloxamer grades from BASF Pharma (Bishop, TX, USA) and their characteristics are listed in Table 1. Purified clinical grade poloxamer 188 (FlocorTM) was supplied by CytRx Corporation (Los Angeles, CA, USA). Ammonium thiocyanate, anhydrous ferric chloride, cis 1,2-dichloroethylene (98%), and chloroform (99%) were purchased from Sigma-Aldrich (Merck, Darmstadt, Germany). Analytical solvents were used as received.

Molecular weight measurements

The molecular weights of poloxamines and poloxamers were measured using gel permeation chromatography

(GPC). Before testing, the copolymer was diluted in 2% w/v dimethylformamide and filtered through a 0.2 μ m polyamide membrane. A 10.0 μ m sample solution was injected using RAPRA technology (Shropshire, UK) and passed through a 30 cm column. The refractive index of the eluent was measured for monitoring. The equivalent molecular weights of PEG/PEO were used to express the results, which were obtained after calibrating the instrument using PEG and PEO standards.

Preparation of ammonium ferrothiocyanate reagent

The ammonium ferrothiocyanate reagent was prepared by dissolving 400 mmol of ammonium thiocyanate and 100 mmol of anhydrous ferric chloride in 1 L of distilled water, and the mixture was thoroughly and kept in the dark.

Two-phase test procedure

A series of glass vials (15 mL) were used, each containing 5 mL of ammonium ferrothiocyanate solution and 5 mL of the appropriate organic solvent (chloroform). Samples (5 mL) containing known amounts of poloxamine and poloxamer dissolved in distilled water were added to the two-phase system. The vials were shaken vigorously for 5 min using a vortex mixer. The samples were then centrifuged for 3 min at 16,000 rpm at room temperature. The lower pink organic layers were then extracted for UV measurements using RCT.

UV measurements

For UV measurements, a 1-mL quartz cuvette with a path length of 1 cm was used. The surface of extracted samples was covered with 100 μ L of RCT, and UV absorbance was measured at 510 nm using a Helios β unicum spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI, USA).

FT-IR measurements

Infrared spectra of the aqueous reagent and organic phase were recorded on a Nicolet Avatar 320 FT-IR spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) using a Golden Gate attenuated total reflectance (ATR) accessory with a ZnSe window at 22°C.

RESULTS AND DISCUSSION

Molecular weight determination

The different grades of poloxamines and poloxamers employed in our study are naturally polydisperse, which means they are made up of molecules with different sizes, according to the GPC data presented in Table 1. Understanding the behavior and effectiveness of these copolymers in pharmaceutical formulations depends critically on their polydispersity. Additionally, this investigation revealed a large difference between the providers' theoretical molecular weight estimates and the actual ones measured.

Source of purple, pink color developed in the organic phase

Aqueous solutions of ammonium ferrothiocyanate contain a metal complex $[Fe(SCN)_n]^{3-n}$, where the central ferric iron complexes with thiocyanate are renowned to give an intensely red-colored chromophore compound in an aqueous medium and form a reagent [9]. When the reactants (Fe³⁺ and Cl⁻) came into contact, they exchanged ions or partners. This is sometimes referred to as a switch-partner reaction. The iron ions now bind with the thiocyanate ions instead of chloride ions, giving the reagent a distinctive intense dark red color, as shown in Fig 2, the top layer.

The Fe electron configuration in the reagent used was Fe^{3+} , and partial hydrolysis occurred inside the aqueous reagent; however, the normal composition remained unaffected, that is, $[Fe(H2O)_{6-x-y}(OH)_xCl_y]^{(3-x-y)}$. The weights of Fe^{2+} and Fe^{3+} are almost identical, but their ionic radii vary in size, so that both Fe^{2+} and Fe^{3+} exhibits significantly different hydration behaviors owing to their charge density differences [11]. The thiocyanate ion should completely lack color when combined with Fe^{2+} , while creating red when combined with Fe^{3+} , confirming the reagent red color caused by the Fe^{3+} thiocyanate complex, as illustrated in the following Equation 1:

$$6 \operatorname{SCN}^{-} + \operatorname{Fe}^{3+} \to [\operatorname{Fe}(\operatorname{SCN})_6]^{3-} \tag{1}$$

T a b l e 1. Physicochemical properties of different grades of copolymers

Copolymer	$M_{w'}$ g/mol		Doludioporaitu	PEO unito	DDO unito
	Measured	Theoretical	Polydispersity	PEO units	rro units
Poloxamine 908	22455	25000	1.687	4 × 119	4×17
Poloxamine 904	3193	6700	1.460	4×15	4×17
Poloxamine 901	2070	4700	1.182	4×4	4×17
Poloxamer 407	11930	12600	1.3185	2 × 98	1×68
Poloxamer 402	2210	2500	1.343	2 × 11	1×68
Poloxamer 188	7361	8400	1.274	2 × 75	1×30
Purified Poloxamer 188	8566	8400	1.040	2 × 75	1×30



Fig. 2. PPO[PEO-[Fe(SCN)_n]³⁻ⁿ] complex visible as pink color of the lower organic layer

The presence of a surfactant in the reagent is essential for the formation of a new complex, PPO[PEO-[Fe(SCN),]³⁻ⁿ], that is soluble in the organic phase. This is presumed to be the driving force for the development of a pink color in the chloroform layer. All the different poloxamine and poloxamer grades used in this study had similar outcomes: when partitioning into the organic phase, they produced a pink color complex and exhibited instability upon exposure to air or water, including purified (clinical) grade of poloxamer 188 (FlocorTM). This indicates that the block copolymer molecules are either identical with low polydispersity or not identical with high polydispersity (a combination of several species with varying degrees of polymerization with different molecular weights) and are all partitioned into the organic phase in a manner that is quite similar.

Poloxamines and poloxamers show the ability to form reverse-type architecture micelles with hydrophobic heads oriented towards the exterior, which prevents them from forming normal micelles in aqueous media. PPO-PEO-PPO block copolymer surfactants are considered to create closed association aggregates, such as spherical/discoidal shaped micelles or random networks with irregular reverse micellar networks, which are centered by iron ion complexes that are partitioned into chloroform [12].

The intrinsic structure for the reverse micelle is driven by the reduction in free energy that results from the hydrophilic blocks being surrounded by organic medium, the entropic variety in the arranged PPO in chlorinated layer, will provide a chance for development of a cross-connected 3D structure, enough to reach the critical volume concentration which allows saturation of the organic layer [10]. Adding more surfactants did not result in the formation of additional reverse micelles. Surplus surfactants dissolve as individual molecules in the medium.

The precise chemical structure of the cross-connected 3D chromophore metal complex formed in the chlorinated organic layer is not yet clear. However, based on their ability to correlate the performance and characteristics of these copolymers using a wide range of molecular weights and EO/PO ratios, molecular geometry, the electronic structure of specific copolymers, and the identification of certain functional groups, it was possible to speculate that PEO chains can be used as a crosslinking agent with ferrothiocyanate to form a network of PEO chains through electrostatic interactions between the negatively charged SCN⁻ group and the positively charged Fe³⁺ ions in the presence of PEO units, while PPO extended outside. This will lead to a self-assembly of amphiphilic 3D coordinated chromophore complex, which partitioned into chlorinated organic layer; the proposed structure formula for the complex formed is shown in Figure 3.

Before the bleaching process, the extracted pink color of the organic phase was stable, as shown in Figure 4a. The chromophore complex formed becomes unstable in the organic phase and starts to decompose immediately when it meets aerial or aqueous media, eventually producing a colorless chloroform phase, as shown in Figures 4b-d. The hydrolysis process is spontaneous, and the decomposition products of the extracted chromophore



Fig. 3. Proposed chemical structure for the extracted chromophore complex







Fig. 4. Differences in the stability of freshly prepared chromophore complexes: a) chromophore stable for 24 h in RCT, b) chromophore complex extracted with distilled water, c) chromophore complex extracted with distilled water after 10 min, d) chromophore complex extracted with distilled water after 6 h



Fig. 5. IR spectra of poloxamine extracts in chloroform

complex vary depending on media exposure. The aqueous media causes bleach and formation of yellow solid particles at the interface, as shown in Figure 4d, whereas air exposure causes bleach without the formation of any particles at the interface, clearly indicating two different pathways of hydrolysis. It is unknown what exactly constitutes this yellow material. However, the hydrolysis of the chromophore complex in the organic phase produces several (chloro) [13] or sulfur [14]-complex species, such as hydrated *ferric chloride* $FeCl_3 \cdot xH_2O$, with distinctive yellow crystalline solid particles, related to its reaction with water, and hydrous ferrous sulfate, (FeSO₄ \cdot xH₂O) which is a light yellow powder or granule that is easily oxidized in air. These two hydrous inorganic compounds are moderately water-soluble, so the yellow particles at the interface could be fully or partially composed of these hydrous compounds along with iron(II) oxide, iron(III) oxyhydroxide $Fe(OH)_{\gamma}$ and/or trans- $[Fe(H_2O)_4Cl_2]^+Cl^-$ 2H₂O, which has a low solubility, as shown in Fig. 4d.

FT-IR analysis of poloxamines block copolymers

The IR spectra of freshly prepared purple-pink extracted chloroform phase obtained from 200 µg/mL of poloxamine 908, presented in Figure 5, shows strong peaks at 2100 and 800 cm¹ due to stretching of the SCNgroup. The same sample was allowed to decolorize, and IR was measured again. The distinctive feature of this spectrum is the complete disappearance of the thiocyanate peaks from the colorless extracted chloroform. This indicates that the SCN⁻ group plays an essential role in the complex formation and development of a purple-pink color in the organic phase. In addition, an extra peak at 1021 cm⁻¹ appeared in the spectrum owing to the formation of SO₄ which is a product of complex degradation. This was tentatively assigned to v S=O of sulfate ions. Sulfate has been detected as a degradation product of aqueous [Fe $(SCN)_n$]³⁻ⁿ[11].

Thiocyanate ions are known to be reducing agents [15], therefore the SCN⁻ functional group in the ferric thio-

cyanate chromophore complex is unstable in organic media once exposed to air or water, causing hydrolysis and bleaching by reducing ferric ions (red-blood) to ferrous ions (colorless) by thiocyanate ions and diminution of the purple-pink color of the medium [16]. The change in the oxidation of Fe⁺³ to Fe⁺² by SCN⁻ is a reversible process [17], and the equilibrium between the two forms of iron ions can be shifted by changing the conditions of the environment, that is, water or oxygen.

Stabilization of the divided complex

As the organic layer was extracted from the two-phase system, there was a persistent loss of purple, pink color, and this continuous reduction in UV absorbance readings made it impossible to achieve a consistent and reproducible reading. Therefore, the first step in the development of this test was to stabilize the complex in the organic phase and generate reliable and reproducible measurements, therefore RCT technique was adopted.

A new approach carried out by this study and managed to stabilize the extracted purple pink color organic phase, by keeping it under nitrogen gas for 18 h, no decomposition occurred, and the chlorinated organic phase maintained its pink color and was confirmed by the UV scanning peak at 510, which indicated that water, air, and/or oxygen gas was the main source of destabilization for this complex, and nitrogen gas replacement prevented an unstable complex from hydrolysis. This finding is in line with the stability of UV reading obtained by an earlier report used in nanoparticle engineering after covering the extracted chromophore complex with a reagent [18].

The RCT was employed to cover the top of the extracted purple pink organic phase, in order to protect the Fe³⁺ ion from the oxidation process, as iron metal can change its Fe³⁺ oxidation state $[Fe(H_2O)_6]^{3+}$, which is a strongly acidic medium, and strong enough oxidizing agents to oxidize sulfide ions to sulfate in the presence of oxygen, causing the detachment of the SCN⁻ group from the complex and liberating the chains of the copolymers in the organic phase. This finding confirmed that the addition of fresh reagent without any copolymer to the completely bleached organic layer restored the pink color, indicating the importance of the SCN⁻ group. This pattern of complex instability and restoration of the bleached extract was observed for all copolymers in various chlorinated organic solvents.

Mathematical models

The rates and kinetics for the hydrolysis of chromophore complex in chloroform phase, is affected by the environment, especially in the presence of the covering aqueous reagent layer, the complex was stable with a loss of not more than 0.5% in UV absorbance over 18 hours, while the absence of RCT, the UV absorbance measurements decreased by 10% within the first hour and 80%

after 10 h. The concentration of depleted Fe³⁺ ions was identified as a third variable affecting these outcomes. Based on these observations and additional data from the depleted figure shown in [10]. This research developed a mathematical model to describe the decomposition kinetics of the chromophore complex. The experimental data collected over a number of time periods served as the basis for the model. To ascertain the rate law and the kinetics of the decomposition, the Fe ion concentration was monitored at various intervals. The creation of a mathematical model in this article carefully considers the following points in consideration that the model equations should only include prominent features. Data from experiments must be used to support the idea. It is impossible to apply a single mathematical theory to every complicated disintegration system since mathematical models of complex instability in the organic phase is not common. The decomposition kinetics of a chromophore complex in a chloroform layer was investigated using zero-order and first-order reaction models. Experimental data, consisting of reactant concentrations measured at various time intervals, were analyzed to determine the decomposition order and rate constants. For the zeroorder model, the integrated rate law $[A]=[A]_0-kt$ yielded a rate constant of $k\approx 0.00783$ M/s. The first-order model, using the integrated rate law $[A] = [A]_0 e^{-kt}$, resulted in a rate constant of $k \approx 0.0103 \text{ s}^{-1}$. The final equations are presented in (2) and (3)

Zero-order model equation: $[A] = 1 - 0.00783t$	(2)
First order model equation: $[A] = 1.00e^{-0.0103t}$	(3)

0.7

0.6

The predicted concentrations from both models were compared with experimental values to evaluate their accuracy. The sum of squared residuals (SSR) was calculated for both models, with the zero-order model producing an SSR of 0.0070 and the first-order model yielding an SSR of 0.00019. The significantly lower SSR for the first-order model indicates a better fit to the experimental data, suggesting that the decomposition of the chromophore complex follows first-order kinetics. This analysis provides a robust mathematical framework for understanding the reaction dynamics, facilitating more accurate predictions of reactant behavior in similar systems.

To visualize in three dimensions the relationship, between UV absorbance measurements, depleted Fe³⁺ ion concentration, and the time, a response surface methodology (RSM) as a statistical technique was applied and the regression polynomials were calculated and applied to approximate the response surfaces plot, presented in Figure 6, the UV absorbance changes as the depleted Fe³⁺ ion concentration and time vary, the UV absorbance decreases exponentially over time, with a steeper decrease in the first hour and a slower decrease over the next 10 h until it reaches a minimum at 20% of the initial concentration, while the initial Fe³⁺ ion concentration of 8 mol Fe/mol copolymer, considered to be (100%), at zero time, then starting to deplete and drop to almost 0% after almost 6 h. This response surface plot is a useful tool for understanding, identifying, and interpreting the optimal combination of depleted Fe³⁺ concentration and time to produce a certain level of UV absorbance.

The exponentially decaying of Fe³⁺ ion concentration versus time plot, exhibits first-order behavior, the decay



Fig. 6. Estimated response surface plot illustrating the relationship between UV absorbance measurements, Fe³⁺ depletion concentration, and time

rate for the extracted pink color chloroform layer was attributed to the oxidation changes in the concentration of only one of the reactants, that is iron ion from 3+ to iron 2+, displacing the iron ligand in the octahedral complexes, resulting discoloration for the extracted chloroform phase.

The response surface curve was further employed to capture the complex polynomial mathematical models and is expressed as

- Time (h) "X" is an independent variable,
- %Fe loss "Y" as an independent variable,
- UV absorbance × "Z" as dependent variable

 $Z = f(X, Y) = 1.78 \cdot \exp^{(-0.1X)} \cdot \exp^{(-0.08Y)}$

The equation represents a two-variable function, where the UV absorbance (Z) is determined by the time (X) and the percentage of Fe loss (Y) through an exponential decay relationship. The coefficient values (1.78, -0.1, -0.08) reflect the specific relationship between the variables based on the UV absorbance measurements. This mathematical model was constructed to link independent factors (time and %Fe Loss), and the dependent variable (UV absorbance) was first represented quantitatively. The model accurately depicts how the UV absorbance decays with time and %Fe Loss using exponential deterioration functions. This helps to anticipate UV absorbance values for certain combinations of time and percent Fe Loss and provides greater knowledge of how these factors affect UV absorbance. This model can be used for optimization, allowing researchers to pinpoint the ideal circumstances that result in the desired UV absorbance values. This mathematical model is an expedient source for studying and forecasting UV absorption based on the decomposition of chromosphere complexes in various chlorinated organic solvents using different types of block copolymers.

CONCLUSIONS

The complete chemical structure of the complex formed between poloxamine and poloxamer surfactants and the chromophore [Fe(SCN)]²⁺ has not yet been described. An octahedral molecular geometry has been proposed based on the association of Fe³⁺ with SCN- and PEO. Iron interacts with oxygen and/or moisture in the air and oxidizes immediately, causing complete hydrolysis of the extracted complex in the chlorinated organic phases. However, the products formed by decomposition vary depending on the surrounding environment. Furthermore, the presence of air causes bleaching without any associated compounds on the surface. The presence of an aqueous environment causes the formation of yellow solid particles at the interface, which are Fe³⁺ oxyhydroxide and/or in combination with other insoluble compounds. It was possible to stabilize the extracted chromophore complex in the chlorinated organic layer using nitrogen. It is important to reduce the exposure to oxygen levels in the extracted pink solution, because oxygen interferes with SCN⁻, leading to its complex hydrolysis, which interrupts the decomposition of the iron/iron redox couple and lowers the decomposition rate of the extracted chromophore complex in the organic phase, allowing the extracted chloroform layer to maintain its pink color for up to 18 h. A mathematical model was developed to explain the behavior and stability of chromophore complexes. This model allows more efficient planning and execution of experiments, resulting in significant time and cost savings.

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Authors contribution

O.A. – stands for methodology, investigation, writingoriginal draft and conceptualization; S.A. – stands for validation, supervision, methodology, writing-review, and editing; A.D. – stands for conceptualization, supervision, validation, research techniques and editing; N.J. – participate in writing-original draft, visualization, validation, conceptualization.; N.S. – writing, methodology and mathematical molding investigation; M.A. – validation, conceptualization, visualization; mathematical molding design and writing.

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Conflict of interest

The authors declare no conflict of interest.

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