2019

MECHANISMS AND THERMODYNAMICS OF THE INFLUENCE OF SOLUTION-STATE INTERACTIONS BETWEEN HPMC AND SURFACTANTS ON MIXED ADSORPTION ONTO MODEL NANOPARTICLES

Salin Gupta Patel

University of Kentucky, salingpatel@gmail.com
Author ORCID Identifier:
https://orcid.org/0000-0001-7460-8754
Digital Object Identifier: https://doi.org/10.13023/etd.2019.294

Right click to open a feedback form in a new tab to let us know how this document benefits you.
STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student’s advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student’s thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Salin Gupta Patel, Student

Dr. Eric J. Munson, Major Professor

Dr. David Feola, Director of Graduate Studies
MECHANISMS AND THERMODYNAMICS OF THE INFLUENCE OF SOLUTION-STATE INTERACTIONS BETWEEN HPMC AND SURFACTANTS ON MIXED ADSORPTION ONTO MODEL NANOPARTICLES

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Pharmacy at the University of Kentucky

By
Salin Gupta Patel
Lexington, Kentucky
Co-Directors: Dr. Eric J. Munson, Professor of Pharmaceutical Sciences and Dr. Bradley Anderson, Professor of Pharmaceutical Sciences
Lexington, Kentucky 2019

Copyright © Salin Gupta Patel 2019
https://orcid.org/0000-0001-7460-8754
Nanoparticulate drug delivery systems (NDDS) such as nanocrystals, nanosuspensions, solid-lipid nanoparticles often formulated for the bioavailability enhancement of poorly soluble drug candidates are stabilized by a mixture of excipients including surfactants and polymers. Most literature studies have focused on the interaction of excipients with the NDDS surfaces while ignoring the interaction of excipients in solution and the extent to which the solution-state interactions influence the affinity and capacity of adsorption. Mechanisms by which excipients stabilize NDDS and how this information can be utilized by formulators a priori to make a rational selection of excipients is not known.

The goals of this dissertation work were (a) to determine the energetics of interactions between HPMC and model surfactants and the extent to which these solution-state interactions modulate the adsorption of these excipients onto solid surfaces, (b) to determine and characterize the structures of various aggregate species formed by the interaction between hydroxypropyl methylcellulose (HPMC) and model surfactants (nonionic and ionic) in solution-state, and (c) to extend these quantitative relationships to interpret probable mechanisms of mixed adsorption of excipients onto the model NDDS surface.

A unique approach utilizing fluorescence, solution calorimetry and adsorption isotherms was applied to tease apart the effect of solution state interactions of polymer and surfactant on the extent of simultaneous adsorption of the two excipients on a model surface. The onset of aggregation and changes in aggregate structures were quantified by a fluorescence probe approach with successive addition of surfactant. In the presence of HPMC, the structures of the aggregates formed were much smaller with an aggregation number (N_{agg}) of 34 as compared to micelles (N_{agg} ~ 68) formed in the absence of HPMC. The strength of polymer-surfactant interactions was determined to be a function of ionic strength and hydrophobicity of surfactant. The nature of these structures was characterized using their solubilization power for a hydrophobic probe molecule. This was determined
to be approximately 35% higher in the polymer-surfactant aggregates as compared to micelles alone and was attributed to a significant increase in the number of aggregates formed and the increased hydrophobic microenvironment within these aggregates at a given concentration of surfactant.

The energetics of the adsorption of SDS, HPMC, and SDS-HPMC aggregate onto nanosuspensions of silica, which is the model solid surface were quantified. A strong adsorption enthalpy of 1.25 kJ/mol was determined for SDS adsorption onto silica in the presence of HPMC as compared to the negligible adsorption enthalpy of 0.1 kJ/mol for SDS alone on the silica surface. The solution depletion and HPMC/ELSD methods showed a marked increase in the adsorption of SDS onto silica in the presence of HPMC. However, at high SDS concentrations, a significant decrease in the adsorbed amount of HPMC onto silica was determined. This was further corroborated by the adsorption enthalpy that showed that the silica-HPMC-SDS aggregation process became less endothermic upon addition of SDS. This suggested that the decrease in adsorption of HPMC onto silica at high SDS concentrations was due to competitive adsorption of SDS-HPMC aggregates wherein SDS is displaced/desorbed from silica in the presence of HPMC. At low SDS concentrations, an increase in adsorption of SDS was due to cooperative adsorption wherein SDS is preferentially adsorbed onto silica in the presence of HPMC. This adsorption behavior confirmed the hypothesis that the solution-state interactions between pharmaceutical excipients such as polymers and surfactants would significantly impact the affinity and capacity of adsorption of these excipients on NDDS surfaces.

KEYWORDS: Nanoparticles, drug delivery, surfactant-polymer interactions, thermodynamics of excipient interactions, adsorption of excipients, nanoparticle stability.
MECHANISMS AND THERMODYNAMICS OF THE INFLUENCE OF SOLUTION-STATE INTERACTIONS BETWEEN HPMC AND SURFACTANTS ON MIXED ADSORPTION ONTO MODEL NANOPARTICLES

By

Salin Gupta Patel

Dr. Eric J. Munson
Co-Director of Dissertation

Dr. Bradley Anderson
Co-Director of Dissertation

Dr. David Feola
Director of Graduate Studies

06/10/2019
Date
Dedicated to my parents, my husband, and my son
ACKNOWLEDGMENTS

I would like to begin by expressing my deep gratitude the large group of very generous people who have helped through my tenure of being a graduate student at the University of Kentucky and beyond. It has been a long journey with my doctoral dissertation and would not be possible without all your support.

I would like to begin my thanking my mentor, Dr. Paul Bummer. He was one of the kindest and giving people I know and taught me on not just how to become an independent and good researcher but more importantly how to be a more thoughtful, kind and giving person. His love for science and so many very insightful discussions helped shape the body of this work. I am very grateful to have met Dr. Bummer, from whom I learnt a lot and miss him dearly. To Dr. Eric Munson, I want to thank you for taking me under your wing and encouraging me and inspiring me to move forward and to accepting to serve as my advisor and mentor under this unusual circumstance. I am deeply grateful for your encouragement, your patience, for adopting my research project and focusing on publishing my work. To Dr. Bradley Anderson, I am very grateful for your very thorough and extremely valuable training and guidance. With your help, I was able to elevate this dissertation and training experience to another level. I am also very thankful that you have constantly challenged me and taken such a keen interest in my work. I really appreciate your efforts, your valuable time and insights. I will forever be grateful for your mentorship and patience. I would also like to thank my doctoral committee members, Dr. Markos Leggas, Dr. Zach Hilt, and Dr. Tonglei Li for their support, encouragement and insightful discussions. I would also like Dr. Bing for agreeing to serve as my outside examiner and the Pharmaceutical Sciences graduate program at the University of Kentucky for providing me with this opportunity and overall doctoral training. I would like to acknowledge the
partial financial support from Pfizer Inc., Groton, CT. I would like to thank the University of Kentucky for a Graduate School Fellowship. I cannot thank Catina enough for all her help, guidance and support in fulfilling various requirements of the graduate program. I am very thankful to all my fellow lab members in Dr. Bummer’s Lab including Melissa, Shaoxin, Lin Song, and Abebe for their friendship and making this journey more enjoyable. I would like to thank Gifty and Vivian, from Dr. Knutson’s Lab, for sharing their ITC instrument so generously with me and Michael from Dr. Anderson’s Lab with his help with the lyophilization experiments, Paritosh from Dr. Dziubla’s Lab for sharing the fluorescence spectroscopy equipment with me. Finally, I would like to thank my family for their unconditional love and support, my loving and supportive husband, Dr. Dhaval Patel, my loving son, Dev Patel and my loving and giving parents Arti and Dr. Jagdeo Gupta, my in-laws, Manjula and Dinesh Patel and my sisters, Kavita Patel and Vinita Gupta. I love you all!
# TABLE OF CONTENTS

Acknowledgments........................................................................................................iii

List of Tables..................................................................................................................vii

List of Figures................................................................................................................viii

Chapter 1: Statement of Aims
   Specific Aims..............................................................................................................1

Chapter 2: Introduction..................................................................................................11

Chapter 3: Development of a Robust Method for Simultaneous Quantification of Polymer (HPMC) and Surfactant (Dodecyl β-D-Maltoside) in Nanoparticulate Drug Delivery Systems (NDDS)
   Introduction..............................................................................................................42
   Materials and Methods............................................................................................44
   Results and Discussion.............................................................................................47
   Conclusions..............................................................................................................55

Chapter 4: Thermodynamics of Aggregate Formation between a Non-Ionic Polymer and Ionic Surfactants: an Isothermal Titration Calorimetric Study
   Introduction..............................................................................................................67
   Materials and Methods............................................................................................70
   Results and Discussion.............................................................................................72
   Conclusions..............................................................................................................91

Chapter 5: Exploring Factors Influencing Structural Characteristics of Surfactant-Polymer Aggregates using a Fluorescence Probe Technique
   Introduction..............................................................................................................104
   Materials and Methods............................................................................................107
   Results and Discussion.............................................................................................109
   Conclusions..............................................................................................................121

Chapter 6: Mixed Adsorption of Model Ionic Surfactants with Hydroxypropyl Methylcellulose on a Model Nanoparticle Surface, Colloidal Silica
   Introduction..............................................................................................................134
   Materials and Methods............................................................................................135
   Results and Discussion.............................................................................................139
   Conclusions..............................................................................................................150

Chapter 7: Conclusions .................................................................................................159

Appendices.....................................................................................................................164
   Appendix A. Representative data transformation and statistics (std. dev and CI) to determine critical thermodynamic parameters obtained by processing raw data
directly obtained from isothermal titration calorimetry (peak area) for SDS-HPMC enthalpograms…………………………………………………………164
Appendix B. Abbreviations………………………………………………….165

References……………………………………………………………………167

Vita………………………………………………………………………………195
LIST OF TABLES

Table 3.1: ELSD variables as factors and their levels in full factorial DoE………….57
Table 3.2: Full factorial DoE with ELSD variables and responses for standard solutions containing mixtures of DM and HPMC K-4M…………………58
Table 3.3: Results of fitting response logarithmic model to peak area response data for single standard solutions of DM, HPMC and DM/HPMC in the mixed standards……………………………………………………………………59
Table 3.4: ANOVA results of DoE model and significant terms for responses of DM and HPMC 4M standard solutions…………………………………60
Table 3.5: Summary of retention time, sensitivity, LOD, and LOQ for DM and HPMC standard solutions in optimized design space…………………………..61
Table 4.1: Thermodynamic parameters for SDS micellization…………………….93
Table 4.2: Thermodynamic parameters for DTAB micellization………………….94
Table 4.3: Thermodynamic parameters for SDS-HPMC aggregation……………….95
Table 5.1: Aggregation and Solubilization Characteristics of SDS Micelles at Various Concentrations of NaCl at 25°C……………………………………….123
Table 5.2: Effect of Ionic Strength on Aggregation and Solubilization Characteristics of SDS-HPMC Aggregates at 25°C………………………………….124
LIST OF FIGURES

Figure 2.1: Creation and Stabilization of Nanoparticles Stabilized with Excipients…36
Figure 2.2: Representative organic crystal surface with different functional groups…37
Figure 2.3: Structures of model polymer and surfactants to be used in dissertation studies………………………………………………………………………………………………………………38
Figure 2.4: Schematic of the possible equilibria in NDDS between surfactant, polymer, and model solid in the solution-state………………………………………………………………………………………………………………………………39
Figure 2.5: Schematic of HPLC with ELSD technique for quantitative HPLC analysis of NDDS stabilizers…………………………………………………………………………………………………………………………40
Figure 2.6: Schematic of ITC setup for the power compensation mechanism and raw data for a general case……………………………………………………………………………………………………………………41
Figure 3.1: Structures of a model surfactant and a polymer: (a) dodecyl β-D-maltoside (DM) and (b) hydroxypropyl methylcellulose (HPMC)…………………62
Figure 3.2: Representative SEC-ELSD chromatograms for (a) dodecyl β-D-maltoside (DM), (b) hydroxypropyl methylcellulose (HPMC K-4M), and (c) DM and HPMC K-4M…………………………………………………………………………………………………………63
Figure 3.3: Standard curves for (a) hydroxypropyl methylcellulose (HPMC) and (b) dodecyl β-D-maltoside (DM) in standard solutions containing mixtures of DM and HPMC. Inset in (a) and (b) show sensitivity of the assay for HPMC and DM, respectively……………………………………………………………………………………………………64
Figure 3.4: 3-Dimensional plot of the desirability index for responses (% deviation of slope, accuracy, precision, and sensitivity) with respect to two significant ELSD variables (pressure and temperature) at an instrument gain value of 10………………………………………………………………………………………………………………………………………65
Figure 3.5: 3-Dimensional plot of the desirability index for responses (% deviation of slope, accuracy, precision, and sensitivity) with respect to two significant ELSD variables (pressure and temperature) at an instrument gain value of 12. ..........................66

Figure 4.1: Schematic of isothermal titration calorimetry for exploring the energetics of surfactant micelles and surfactant-HPMC aggregates..........................96

Figure 4.2: Representative calorimetric data transformation to determine critical concentrations: (a) raw data directly obtained from isothermal titration calorimetry (heat flow (µW) vs. time), (b) enthalpograms depicting apparent enthalpy change (ΔH_{app}) as a function of model surfactant (CTAB) concentration, and (c) the first derivative of the curve (b)......................97

Figure 4.3: Apparent enthalpy change (ΔH_{app}) for the titration of the micellar solution of SDS (a) and DTAB (b) in water at 25°C (circles), 32°C (triangles), 40°C (diamonds) and 50°C (squares).........................................................98

Figure 4.4: Plot of (a) apparent enthalpy change (ΔH_{app}) and (b) corrected enthalpy change (ΔH_{corr}) as a function of total SDS concentration in the presence of 0% HPMC K-4M (squares), 0.25% HPMC K-4M (diamonds) and 0.5% HPMC K-4M (triangles) at 25°C.................................................................99

Figure 4.5: Effect of temperature on (a) apparent enthalpy change (ΔH_{app}) and (b) corrected enthalpy change (ΔH_{corr}) as a function of total SDS concentration in the presence of .25% w/w HPMC K-4M at 25°C (squares), 32°C (triangles) and 40°C (crosses for ΔH_{app} and diamonds for ΔH_{corr})........100
Figure 4.6: Plot of (a) apparent enthalpy change ($\Delta H_{app}$) and (b) corrected enthalpy change ($\Delta H_{corr}$) as a function various molecular weight of 0.25% w/w HPMC at 25°C, HPMC K-4M (squares), HPMC K-15 (triangles for $\Delta H_{app}$ and diamonds for $\Delta H_{corr}$) and, HPMC K-100 (circles)..................101

Figure 4.7: Enthalpogram for the effect of NaCl on SDS-HPMC aggregation behavior for 0.25% w/w HPMC at 25°C (a) apparent enthalpy change ($\Delta H_{app}$) and (b) corrected enthalpy change ($\Delta H_{corr}$) at 0 % NaCl, (cross), 0.1% NaCl (squares), 0.3% NaCl (triangles) and 0.6% NaCl (diamonds)..............102

Figure 4.8: Plot of (a) apparent enthalpy change ($\Delta H_{app}$) and (b) corrected enthalpy change ($\Delta H_{corr}$) as a function of total DTAB concentration in the presence of 0% HPMC K-4M (squares) and 0.25% HPMC K-4M (diamonds) at 25°C.................................................................103

Figure 5.1: Representative pyrene fluorescence emission spectra in SDS solution….125

Figure 5.2: Micropolarity index ($I_1/I_3$) for pyrene fluorescence as a function of SDS concentration in SDS-water systems with 0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles) NaCl..............................................126

Figure 5.3: Ratio of excimer to monomer peak intensities ($I_e/I_{mon}$) of pyrene fluorescence as a function of SDS concentration in SDS-water systems with 0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles) NaCl. (Inset: $I_e/I_{mon}$ ratio after maximum peak as a function of SDS conc. at various concentrations of NaCl).........................................................127
Figure 5.4: Micropolarity index ($I_1/I_3$) for pyrene fluorescence as a function of SDS concentration in SDS-water (squares) and HPMC (0.25%)-SDS-water (diamonds) systems. .................................128

Figure 5.5: Micropolarity index ($I_1/I_3$) for pyrene fluorescence as a function of SDS concentration in HPMC (0.25%)-SDS-water systems with 0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles) NaCl. ..................129

Figure 5.6: Ratio of excimer to monomer peak intensities ($I_e/I_{mon}$) of pyrene fluorescence as a function of SDS concentration in SDS-HPMC-water systems with 0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles) NaCl. .................................................................130

Figure 5.7: Pyrene solubilization power of (A) SDS micelles and (B) SDS-HPMC aggregates plotted as a function of SDS concentration at various concentrations of NaCl [0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles)]. .................................................................131

Figure 5.8: Micropolarity index ($I_1/I_3$) for pyrene fluorescence as a function of surfactant concentration in HPMC-DTAB (squares) and SDS-HPMC (diamonds) systems. .................................................................132

Figure 5.9: Micropolarity index ($I_1/I_3$) for pyrene fluorescence as a function of hydrophobicity (chain length) of cationic surfactants in surfactant-HPMC systems. DTAB-HPMC (squares), TTAB-HPMC (triangles) and CTAB-HPMC (diamonds) systems .......................................................133

Figure 6.1: Representative SEC-ELSD chromatograms for HPMC and SDS ..........152

Figure 6.2: Adsorption isotherms of (a) HPMC and (b) DTAB on silica ...............153
Figure 6.3: Effect of ionic strength on the adsorption of SDS on (a) silica and (b) carbon black.................................................................154

Figure 6.4: Mixed adsorption isotherm of SDS on silica surface in the presence of HPMC........................................................................155

Figure 6.5: Plot of corrected enthalpy ($\Delta H_{corr}$) for SDS adsorption on silica as a function of SDS concentration in the presence of 0.5%.................................................156

Figure 6.6: Plot of corrected adsorption enthalpy for DTAB adsorption on silica as a function of DTAB concentration in the presence of (a) no HPMC and (b) 0.5% HPMC K-4M at 32ºC.................................................................157

Figure 6.7: Apparent enthalpy ($\Delta H_{app}$) for DTAB-HPMC interactions as a function of DTAB concentration in the presence of (a) no NaCl at 32º C and (b) 0.1% NaCl at 25º C.................................................................158
Chapter 1

Statement of Aims

It is well known that a significant number of new chemical entities exhibit poor water solubility, resulting in poor oral absorption and the need for bioavailability enhancement [1-3]. Nanoparticle drug delivery systems (NDDS) are one of the several possible routes for bioavailability enhancement of poorly soluble drugs through enhanced dissolution rate, solubility, or both [4, 5]. However, NDDS often encounter varying degrees of thermodynamic instability leading to nanoparticle aggregation [6-8]. This instability is attributed to the extensive surface area generated by either of the two distinct approaches commonly used to produce NDDS: (1) top-down method where larger particles are broken down into nanoparticles through attrition, and (2) bottom-up method where nanoparticles are created through physicochemical reactions. The higher surface area is accompanied by a large positive free energy, and without any effort to dampen the surface energy, the system prefers to move to an equilibrium state of the lowest free energy via aggregation of the smaller particles into larger particles [9, 10]. Physical stabilization of NDDS is challenging, often requiring an optimum combination of surfactants and polymers thus allowing for synergy in the types of interactions between these stabilizers and the drug molecule as a means to enhance the physical stability [11, 12, 13].

Stabilizers are effective in producing physically stable NDDS by the adsorption of polymer, surfactant and polymer-surfactant aggregates onto the surface of NDDS, potentially decreasing the surface energy of nanoparticles [14, 15]. To understand the adsorption behavior of polymers and surfactants, their concentration-dependent interactions and speciation need to be explored. Although the adsorption of polymers and
surfactants (i.e., mixed adsorption) to the surface of nanoparticles have been linked to their beneficial effect on NDDS stability [9, 16], the mixed adsorption process generally involves an interplay of interactions between polymers, surfactants, solvents, and the surface [17]. An in-depth understanding of the mixed adsorption process is essential to select the type and levels of surfactants and polymers to maximize the extent of adsorption of these stabilizing excipients onto nanoparticles.

The adsorption of polymer and surfactant has been extensively studied due to its various industrial application such as cosmetics, petroleum products, pharmaceuticals, and food items [18]. The mixed adsorption of polymers and surfactants is dependent on attractive or repulsive interactions between polymers, surfactants, and surfaces [19]. Mixed systems consisting of ionic and non-ionic surfactants and non-ionic polymers such as polyvinyl pyrrolidone (PVP), polyethylene oxide (PEO), and polystyrene sulfonate as well as their adsorption on oxide surfaces has been studied [20]. The adsorption of polystyrene sulfonates of different molecular weights on hematite was significantly influenced by the presence of electrolytes [21]. While the mixed adsorption of several polymers and surfactants have been studied, the complexity of polymer-surfactant interactions hinders rational selection of the type and levels of polymer and surfactants during the development of pharmaceutical products containing nanoparticles. In general, empirical approaches have been used to develop nanoparticle-based formulations, which highlights the need to mechanistically understand the parameters that govern the mixed adsorption of pharmaceutically relevant polymers and surfactants on model surfaces.

As a pharmaceutical excipient, hydroxypropyl methylcellulose (HPMC) is employed in a wide range of solid and liquid formulations [22]. HPMC, the model non-
ionic polymer used in this work, is a mixed alkyl hydroxyalkyl cellulose ether that is derivatized with hydroxypropyl and methoxyl groups. The chromatographic quantification of HPMC with acceptable baseline separation from other excipients is challenging due to its wide molecular weight distributions and the lack of strong chromophores. There are only a few reports describing quantitative assays for HPMC that are suitable for pharmaceutical products [23-25]. For example, Delker et al. [23] employed the refractive index to detect HPMC in polyethylene glycol. Whelan et al. [24] used an evaporative light scattering detector (ELSD) to quantify HPMC in the presence of ibuprofen. Rashan et al. [25] used a Polymer X RP-1 column along with a gradient elution method that is not typically used with ELSD detectors for the analysis of different grades of HPMC. All methods mentioned above lacked sensitivity, and the elution of HPMC was close to the solvent peak, and therefore could not simultaneously quantify HPMC with other excipients. Methods for the detection of surfactants such as dodecyl-β-D-maltoside (DM) reported in the literature include total organic carbon (TOC) and calorimetry, both of which are also neither fast, accurate nor sensitive [26]. Hence, it is critical to develop methods to simultaneously quantify polymers and surfactants in order to develop a mechanistic understanding of adsorption of these excipients on nanoparticles.

In general, some gaps were identified from the review of previous studies containing HPMC and surfactants: (1) pharmaceutically relevant higher concentrations of HPMC typically utilized in formulation of NDDS (0.25-1 %w/w) [27-29] have not been evaluated, (2) detailed investigation of the factors influencing the structural characteristics of surfactant-polymer aggregates such as ionic strength of solution and surfactant properties (i.e., head group, chain length) on the interaction between ionic surfactants with
HPMC has also not been conducted, (3) the influence of the ionic strength in potentially manipulating the solution-state environment to enhance the interactions between surfactants and HPMC is not well understood, (4) systematic investigation of aggregation number of surfactant-HPMC aggregates using excimerization and modelling of fluorescence data without the use of a fluorescence quenchers and the various assumptions that need to be considered with their use (i.e., solubilization and distribution of quenchers relative to the probe molecule) [30, 31] and, (5) lack of mechanistic understanding of the extent and mechanism of adsorption of pharmaceutically relevant mixed systems (such as HPMC and surfactants) onto nanoparticles.

The overall goal of this project is to develop a mechanistic and thermodynamic understanding of the influence of solution-state interactions between a model polymer, HPMC, and model non-ionic and ionic surfactants upon the mixed adsorption onto model nanoparticles. These studies focused upon (1) developing methods to study the solution-state interactions between HPMC and surfactants, and (2) understanding the extent to which solution-state and solid-state interactions between HPMC, surfactants, and model nanoparticles modulate the mixed adsorption of polymer and surfactant systems onto nanoparticles. The bulk interactions between a non-ionic polymer (HPMC) with ionic surfactants (sodium dodecyl sulfate (SDS) and dodecyltrimethylammonium bromide (DTAB)) and non-ionic surfactant, dodecyl-β-D-maltoside (DM) were explored. It is known that ionic surfactants can bind to nonionic polymers at their hydrophilic sites by electrostatic effects, however nonionic surfactants may also bind to nonionic polymers by hydrophobic interactions, hence the solution conditions such as pH, ionic strength, and properties of polymer and surfactant (i.e., molecular weight, head group), may significantly
impact the strength of these interactions. The influence of bulk interactions on the adsorption process at the surface was explored. As described below, several specific aims were developed to design these studies:

*Aim 1: To develop and validate an HPLC-ELSD method using a multifactorial optimization of critical ELSD parameters for an accurate, robust, and simultaneous quantification of HPMC and model nonionic surfactant, DM and ionic surfactants, SDS and DTAB.*

For a better selection of the polymers and surfactants, it is essential to quantify these stabilizers as a first step towards developing a mechanistic understanding of the interactions between the stabilizers themselves and with drug molecules. A significant gap in the literature continues to exist in understanding the extent to which solution-state and solid-state interactions between HPMC, surfactants, and model nanoparticles modulate the mixed adsorption of polymer and surfactant systems onto silica nanoparticles due to the lack of suitable analytical methods to simultaneously and accurately quantify the concentrations of polymers and surfactants in NDDS. The objective of this study was to develop and validate a robust method to simultaneously quantify a model polymer, HPMC, and model surfactants, DM, SDS and DTAB. For the simultaneous detection of HPMC and DM in NDDS, size exclusion (SEC) based high-pressure liquid chromatography (HPLC) with ELSD was selected since both excipients (DM and HPMC) are non-volatile and lack UV-chromophores [32-34]. SEC has been used to resolve polymers based on differences in molecular size where separation occurs as a result of the pore size of packing material [23]. ELSD is more sensitive and solvent compatible as compared to other universal techniques (i.e., refractive index (RI) and liquid chromatographic mass spectroscopy (LCMS). Some of the limitations of ELSD are low selectivity, the
requirement for a volatile mobile phase, non-linearity, and being destructive to the sample that is analyzed [35]. ELSD has been applied effectively for at least the last two decades to quantify a wide spectrum of natural and synthetic compounds including pharmaceuticals [36-40], biologics [41, 42], and foods and beverages [43, 44]. There are no methods published outlining an SEC-ELSD assay for the simultaneous detection of DM and HPMC. This study utilizes a full factorial design to optimize the impact of SEC and ELSD method variables and their interactions on the precision, accuracy, and sensitivity of the assay as per the Guidance for Industry, ICH-Q2A [45]. This method was applied in subsequent studies to understand the mechanism of nanosuspension stabilization by model surfactants and HPMC.

Aim 2: To determine the energetics of aggregate formation between the model non-ionic polymer, HPMC, and model ionic surfactants, including SDS, hexadecyltrimethylammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DTAB) using ITC.

I. Validate critical concentrations of micellization and aggregation (i.e., CAC & CMC) of HPMC-ionic surfactant interactions in solution-state.

II. Determine critical thermodynamic parameters such as a change in enthalpy of micellization and aggregation of HPMC-ionic surfactant aggregates, and the free energy change of micellization and aggregation.

III. Determine the influence of temperature, ionic strength and molecular weight on the solution-state interactions between HPMC and ionic surfactants

IV. Determine the driving force for the solution-state interactions between HPMC and ionic surfactants.
In order to understand the mixed adsorption isotherms of polymers and surfactants, the bulk solution-state interactions between polymers and surfactants need to be explored. It was hypothesized that hydrophobicity and ionic strength will influence the extent and nature of HPMC-surfactant aggregation behavior in solution. The goal of this study was to determine the effect of various parameters including temperature, ionic strength, and hydrophobicity (i.e., molecular weight of polymer, head-group and chain length of surfactant) on the energetics of surfactant-HPMC aggregation using ITC with a novel data treatment method. Isothermal titration microcalorimetry (ITC), solution depletion techniques and ELSD-SEC were applied for this purpose. ITC was used to directly measure the change in heat flow as various interactions occurred between HPMC and surfactants that were used to quantitatively determine critical thermodynamic parameters of processes such as aggregation of surfactants with HPMC. A novel data treatment was used in conjunction with ITC that increased the accuracy of the measured thermodynamic parameters of surfactant-HPMC aggregation and subsequent interpretation. This treatment involved the identification and appropriate accounting for the concentration-dependent species (i.e., surfactant monomers and micelles, and surfactant-HPMC aggregates) and related enthalpies. Additionally, the influence of ionic strength, HPMC molecular weight, and the type of ionic surfactant head group on the energetics was explored. The understanding from this study was used in subsequent studies to characterize the nature of HPMC-surfactant aggregates (Chapter 5) and their adsorption onto the surface of nanoparticles (Chapter 6).

Aim 3: To study (1) the effect of a model non-ionic polymer, HPMC on the state of aggregation of model anionic and cationic surfactants, and (2) the impact of surfactant
properties (i.e., type of head-group and chain length) and solution properties (i.e., ionic strength) on the structural characteristics (i.e., aggregation number/size and microenvironment) of surfactant-HPMC aggregates.

I. Determine the effect of hydrophobicity and ionic strength on the critical concentration parameters as well as the structure and microenvironment of HPMC-surfactant aggregates in solution using pyrene fluorescence spectroscopy.

II. Use model fitting to determine aggregation numbers (N_{agg}) and examine the microstructures to ascertain the micropolarity within these aggregates.

III. Determine solubilization capacity and solubilization power for pyrene within these aggregates using UV spectrophotometry and correlate structural changes such as N_{agg} to ionic strength and hydrophobicity of the surfactants. Probe the nature of the polymer-surfactant interactions at a molecular level with the goal of determining how interactions will govern molecular structures thus influencing the solubilization of a probe molecule, pyrene, within these structures.

It was hypothesized that the mechanism of interactions between HPMC and surfactants would be concentration dependent and influence the structure of aggregates formed. Smaller aggregates would be formed at low concentrations, and markedly different aggregates would be formed at high concentrations. The fluorescence probe method using pyrene as a hydrophobic probe molecule was used to determine the structural characteristics of the ionic surfactant-HPMC aggregates as pyrene is preferentially solubilized within hydrophobic microdomains (such as micelles and aggregates), which
can result in a change in the intensity of emission (monomer) or excimer (dimer or higher) peaks of pyrene; this change in intensity was used to determine the characteristics of ionic surfactant-HPMC aggregates. The pyrene fluorescence method with the Poisson distribution data analysis was selected to study the properties of micelles and surfactant-HPMC aggregates due to the following advantages: (1) it provides quantitative information on critical concentration parameters, size and aggregation number of micelles and aggregates without the use of fluorescence quenchers, and (2) it provides qualitative information on the microenvironment of micelles and aggregates. Since the properties of SDS micelles have been studied previously by this laboratory using isothermal titration calorimetry and by others using techniques such as NMR and surface tensiometry, the pyrene fluorescence method was first validated by comparing the CMC of SDS determined by the current method as well as the same reported in the literature [46, 47]. Upon validation, the pyrene method was used to determine the critical structural characteristics (CAC and aggregation number), and the microenvironment of ionic surfactant-HPMC aggregates. The understanding gained from this work was applied in subsequent studies exploring the impact of solution-state interactions on the mechanism of adsorption of surfactant-HPMC aggregates onto the surface of nanoparticles (Chapter 6).

Aim 4: To determine the extent to which solution-state interactions between HPMC and SDS modulate their adsorption onto silica and carbon black, model nanoparticle surfaces.

I. Determine the extent of adsorption of HPMC and SDS on their adsorption onto silica and carbon black as a function of SDS concentration by equilibrium solution depletion experiments.
II. Determine the energetics of silica-polymer-surfactant interactions as a function of surfactant concentrations using a combination of ITC and solution depletion experiments.

III. Determine the driving forces of mixed adsorption of HPMC and SDS on a model nanoparticle surface, silica.

It was hypothesized that HPMC and a model ionic surfactant (i.e., sodium dodecyl sulfate) would adsorb onto a model surface, silica, in a cooperative manner at low surfactant concentrations, and in a competitive manner at high surfactant concentrations and this could be modulated by changes in solution-state interactions between surfactant and polymer. Isothermal titration microcalorimetry (ITC), solution depletion techniques and ELSD-SEC were applied for this purpose. ITC was used to directly measure the change in heat flow as various interactions occurred between the HPMC, SDS, and silica. The change in heat flow data were used to quantitatively determine critical thermodynamic parameters associated with aggregation of SDS with HPMC and adsorption of various species formed during aggregation on the solid-liquid interface with silica. Furthermore, the influence of ionic strength on the energetics of SDS-HPMC aggregation and adsorption onto silica were quantitatively determined, and probable mechanisms were discussed.
CHAPTER 2

Introduction

1. Nanoparticulate drug delivery systems (NDDS)

New drug candidates emerging from high throughput screening methods typically employed in drug discovery emphasize the importance of a good fit with the target-receptor geometry. As a result, these candidates generally tend to have higher molecular weight and lipophilicity. Factors such as these contribute towards the poor water solubility of new drug candidates emerging from drug discovery. Bioavailability enhancement for these candidates is therefore needed to compensate for their poor water solubility and to increase in-vivo exposure [1, 2].

Nanoparticulate drug delivery systems (NDDS) include nanocrystals, nanosuspensions, solid state nanoparticles, and other colloidal dispersions. All these delivery systems are designed to enhance the therapeutic potential of drug candidates and could also provide enhanced bioavailability, stability, safety, targetability, and decreased food effect variability [5, 48]. NDDS are now more frequently being used in oral, parenteral, pulmonary and brain delivery, oncology therapy and diagnosis of diseases because of their unique properties such as having a manipulatable particle size distribution, surface properties, and release characteristics [48]. NDDS where the drug remains in either a native crystalline or an amorphous state in the nanometer particle size range are one of the preferred routes for bioavailability enhancement. NDDS can also improve the food effect and dose proportionality problems commonly observed with conventional formulations with larger particle size. The increase dissolution rate is directly proportional large increase in surface area as per the Noyes-Whitney model. Particles in the lower
nanometer size (<50nm) range may also provide an additional advantage in solubility enhancement due to its smaller size and curvature [49]. Although the extensive surface area of NDDS may lead to bioavailability enhancement, it also causes challenges in physical stabilization of these particles. The large surface area is accompanied by an increase in the positive free energy for NDDS and the system thus prefers to move towards the equilibrium state of the lowest Gibbs free energy by aggregation of smaller particles into larger particles. As shown in Figure 2.1, the water molecules in close proximity to the hydrophobic surface of these aggregating nanocrystals are driven away from their surface due to the unfavorable energetics, and in order to overcome this phenomenon they aggregate due to hydrophobic effect [6].

Pharmaceutical excipients such as surfactants and polymers are generally effective in increasing physical stability of NDDS by preventing particle aggregation [50]. A combination of polymer and surfactant could provide a synergistic effect towards the stabilization of nanoparticles through the adsorption of polymer-surfactant aggregates onto the surface of nanoparticles [51-55]. For a rational, a priori selection of excipients during NDDS development, a comprehensive understanding of the role of surfactant-polymers aggregates in stabilizing nanoparticles as well as the factors that influence their structural characteristics is essential.

1.1. Types of NDDS

The various types of NDDS include nanocrystals, nanosuspensions, solid-lipid nanoparticles, and colloidal dispersions such as nanoliposomes, emulsions, micelles, nanotubes, and lipid drug conjugates.
Nanocrystals are submicron particles of pure crystals of drug candidates that are prepared by the top-down and bottom-up approaches described in a later section. This NDDS is particularly useful for BCS class II compounds that exhibit poor solubility and hence have challenges with drug absorption. Nanocrystals, as a result of the massive increase in surface area in the creation of these particles, can lead to a significant increase in dissolution rate or in some cases increase in solubility that directly impact the oral absorption of the drug. The uniformity of the particle size can also help with the minimization or elimination of food effects and pharmacokinetic variability seen with poorly soluble molecules[48]. Nanocrystal surfaces have to be physically stabilized by excipients such as polymers and surfactants because of the propensity of these crystals to aggregate which will eliminate any dissolution rate enhancement advantages [56].

Nanosuspensions are either liquid or solid dispersions of nanocrystals that are used for oral and parenteral drug delivery. Nanosuspensions are designed, prepared, stabilized and provide similar benefits as nanocrystals [14]. The size, shape, and surface characteristics of nanosuspensions determine their suitability for oral, parenteral or pulmonary drug delivery. Solid nanocrystals are obtained when a nanosuspension is converted into a solid-state form by the addition of a carrier that is often a stabilizing excipient such as sucrose, mannitol, dextrose, lactose, microcrystalline cellulose or colloidal silicon dioxide. The liquid dispersion containing a carrier can be spray coated onto a solid substrate, freeze dried or spray dried to obtain a free-flowing powder, which when reconstituted in water or biorelevant media results into stable liquid nanosuspensions [57, 58]. The conversion into a solid dispersion such that NDDS can be formulated as a
tablet, capsule or powder in bottle type dosage form may further improve patient compliance.

1.2. Design & Manufacturing of NDDS

NDDS are designed and manufactured commonly using either a top-down or bottom-up approach. A top-down approach involves milling or shearing down larger crystals in an aqueous dispersion state with media mills or homogenizers that include low shear and high shear mills that are either stationary or planetary. The shear mills for both utilize a milling media such as polystyrene, glass, high-density ceramic beads such as yttria-stabilized zirconia [14]. The particle size reduction is generally related to the milling parameters such as dispersion feed rate, milling media size and load, milling speed, temperature and residence time of the dispersion in the milling chamber [6]. For the high-pressure homogenization process, the dispersion to be milled present in a smaller chamber passes through an orifice into a larger chamber at high pressure. During this process, the orifice size has a significant impact on the particle size reduction as it determines the amount of attrition and cavitation process experienced by the dispersed particles. Similar to the shearing process, type of turbulent flow, temperature and drug load of the dispersion and stabilizing excipients are critical parameters for high-pressure homogenization [4, 5]. The top-down approach provides a homogeneous and monodispersed particle size distribution that is in the nanometer range, and do not require any pre-processing of insoluble drug candidates such as solubilization [5]. It can be easily scaled-up for drug candidates that are insoluble in both organic and aqueous solvents. Some of the limitations of the top-down approach is that it is a high energy process that could lead to physical and chemical stability issues for the final drug product and the milling media used could also
leave trace amounts of contaminants. Both of the limitations can be overcome by monitoring for impurities.

The bottom-up approach consists of an anti-solvent process where an organic solvent containing the dissolved drug candidate is introduced to an anti-solvent (generally aqueous) in a controlled manner so as precipitate the crystalline drug. The direction of flow and flow rate of the solvent and anti-solvent, drug loading in the solvent, properties of the solvent and anti-solvent, and stabilizing excipients are critical parameters in controlling the size and physicochemical properties of the nanocrystals [5]. This approach is considered more economical since it can be scaled-up or manufactured in a continuous type of operation more easily. Optimization of the variables described above however is often challenging with the potential of crystal growth and presence of residual organic solvents that can be considered as toxic [59].

In the top-down or bottom-up approaches described above, stabilizing excipients such as various polymers and surfactants are critical in both achieving the desired particle size reduction as well as maintaining the particle in a nanometer particle size range that have an inherent tendency to aggregate because of the unfavorable energetics associated with the particle size reduction process and the subsequent large increase in surface area.

1.3. Stabilization of NDDS

NDDS often encounter varying degrees of thermodynamic instability due to the extensive surface area, which can lead to nanoparticle aggregation. The higher surface area is accompanied by a large positive free energy, and without any effort to dampen the surface energy, the system tends to move to an equilibrium state of the lowest free energy via aggregation of the smaller particles into larger particles [11, 16, 60]. Another impact
of the higher surface area is the increase in saturation solubility of the nanoparticles that can lead to Ostwald ripening [11]. Increase in saturation solubility can lead to a greater disorder in the crystalline state and higher surface free energy. These stability issues may be controlled using stabilizers such as polymers and surfactants [1, 6].

The adsorption of excipients such as surfactants and polymers onto the surface of NDDS could potentially decrease the surface energy leading to stabilization of the nanoparticles [6, 56]. Optimal stabilization of NDDS occurs when a strong barrier is placed between two aggregating particles. Polymers and surfactants are thought to complement each other by providing both electrostatic and steric mechanisms of stabilization [61-63]. Surfactants and surface-active polymers will decrease the interfacial tension at the solid-liquid interface of the hydrophobic crystal surface thus decreasing the overall free energy of the system. Surfactants can stabilize a crystal surface by decreasing the surface tension by promoting attractive water-surfactant interactions [6]. A two-component system consisting of a polymer and surfactant might promote tighter packing of stabilizers per surface area on nanoparticulate surfaces with polar and non-polar sites [64]. Synergy is also seen when neutral polymers interact with ionic surfactants acting as anchors to the polymer, allowing tighter packing and improved coverage. This two-component system thus provides superior stabilization power compared to the single component system. Steric stabilization (entropic stabilization provided by repulsion caused polymer chain compression) tends to provide stabilization to NDDS that is less sensitive to temperature fluctuations [65-67]. Surface potential is another factor that may be manipulated by the right combination of polymer-surfactant, and the properties of such systems can also be tunable by altering the solution state conditions[68-70].
Organic crystalline drug candidates generally have different exposed chemical
groups and net charges at the different crystal faces (Figure 2.2). It is reasonable to expect
that at different faces (surfaces) of the organic crystals, different types of interactions may
be observed. These interactions may range from electrostatic, hydrophobic, hydrogen
bonding and thus may exhibit preferential interactions with polymers and surfactants based
on the properties of the excipients [14]. The structures of the model surfactant and polymer
are shown in Figure 2.3. Model surfactants and polymers contain hydrophilic and
hydrophobic moieties, which allow these molecules to adsorb onto different types of
surfaces. The rate and extent of adsorption are known to change depending on the type
and properties of the polymer-surfactant-surface system, ionic equilibria of the various
species present in the solution-state and aggregation process of these excipients. Several
equilibria may exist between the monomers of the surfactant and the surface, and free
micelles of the surfactant, polymer, mixed micelle or aggregates (Figure 2.4). These
equilibria and equilibrium constants will govern the adsorption process that may be
independent additive, competitive or cooperative. The present surfactant-polymer system
that will be studied is probably one of the most widely used in the pharmaceutical world
and surprisingly not explored in mechanistic detail.

There are two separate viewpoints described in the literature on interactions
between polymers and surfactants [71]. The first one is the “polymer-centered” approach
where the polymer is considered to possess the sites for surfactant binding and this
interaction between the surfactant binding on the various polymer sites is thought to
constitute the strong perturbation in the bulk containing the surfactant solution that is
simply considered as a reservoir for binding surfactant molecules. This approach ignores
the strong hydrophobicity associated with surfactant molecules that basically acts as the driving force for the formation of the hydrophobic aggregates in an aqueous environment. This tendency of the surfactant molecules to self-associate leads to the second and more widely accepted viewpoint of a “surfactant centered” approach. In this approach the polymer-surfactant interactions are essentially due to the perturbation of the micellization process of the surfactant by the presence of the polymer. In our work we will adopt the “surfactant centered” viewpoint [64].

The stabilization of the NDDS surface often requires an optimal combination of the various species of polymer and surfactant formed in the solution-state [72-74]. Adsorption of two components (i.e., polymer and surfactant) on the solid-liquid interface can be additive, cooperative or competitive and are likely to be influenced by various solution and surface properties. Solution properties such as concentration, pH and ionic strength can be very important in determining the extent of adsorption of solutes on the solid-liquid interface [63, 70]. Polymer and surfactant adsorption have been extensively studied in various industrial application such as cosmetics, petroleum products, pharmaceuticals and food items [75, 76, 77]. Mixed systems consisting of ionic and nonionic surfactants and non-ionic polymers such as polyvinyl pyrrolidone (PVP), polyethylene oxide (PEO), and polystyrene sulfonate as well as their adsorption on oxide surfaces have been studied [78-81].

Aggregate formation between polymers and ionic surfactants is linked with stabilization of nanoparticulate drug delivery systems (NDDS) [8, 16]. Several literature studies have explored the effect of surfactant concentration on polymer-surfactant aggregate formation [72, 82, 83]. In general, there are three critical concentrations: (1)
critical aggregation concentration (CAC) representing the formation of polymer-surfactant aggregates, (2) polymer saturation concentration ($C_{\text{sat}}$) representing the saturation of the available polymer sites where additional binding of surfactant is not favorable, and (3) critical micelle concentration (CMC) where the formation of surfactant micelles is favorable [84]. While the concentration effect has been well understood, the thermodynamics of polymer-surfactant interactions and related aggregate formation need to be further explored in order to predict NDDS stability.

Although certain polymer and surfactant systems have garnered interest in the scientific community, typical polymers and surfactants and the levels of these excipients used in the development of pharmaceutical NDDS have lacked attention. Most of the literature studies on polymer-surfactant aggregation have focused on non-pharmaceutically relevant systems containing polymers such as poly(2-(dimethylamino)ethyl methacrylate [85, 86], ethylene oxide (EO) copolymer [87, 88], polyethylene oxide (PEO) [89-93], and polyacrylamide [94, 95] as well as surfactants such as lithium dodecylsulfate (LiDS) [92, 96], gemini cationic surfactants [97-99], sodium dodecylbenzenesulfonate [19,100], and sodium dodecylsulfonate-dodecylamine hydrochloride [95]. In the case of pharmaceutically relevant systems, the main body of work has focused on excipients such as SDS [101], polyvinylpyrrolidone (PVP) [91, 102, 103], polyethylene oxide (PEO) [103-107], polyethylene glycol (PEG) [102, 108], and HPMC [27, 109].

Nilsson [27] evaluated binding of SDS to HPMC in water using viscometry, equilibrium dialysis, dye solubilization and fluorescence techniques in a dilute range of HPMC (0.05-0.2% w/w). The author reported the critical concentration parameters for binding of SDS to HPMC. Although Nilsson’s work applied only to dilute concentrations
of HPMC and did not provide significant thermodynamic information, it has a major advantage over other work in that SDS monomer concentrations were measured, giving a basic platform for interpretation of data from other studies including microcalorimetry used in this work. Singh et al. [29] studied the HPMC/SDS system by microcalorimetry and reported thermodynamic parameters for the system. However, the data treatment used in these studies did not take into consideration the formation of various species during the calorimetric titration experiment. Another study by Ridell et al. [109] also used microcalorimetry to determine the effect of counterions on the SDS-HPMC aggregation. They reported that the type of counterion significantly changed the critical concentration parameters and the nature of the aggregates. However, the data treatment lacked the appropriate adjustments needed to account for speciation occurring during titration. In the above-mentioned studies, accurately accounting for the enthalpies of dilution of all species could have provided a more accurate determination of the energetics of interactions and interpretation of the enthalpy plots than those reported.

The interactions (i.e., cooperative and competitive) between surfactants and polymers lead to the formation of polymer-surfactant aggregates and the properties of these surfactants and polymers play a significant role in the formation of aggregates [64, 72-74]. These interactions are generally considered as cooperative where the binding of a ligand such as a surfactant molecule at an adsorbate site affects the binding of ligands at other binding sites of the same adsorbate [110]. Alternately, the interactions are considered competitive when a ligand could preferentially displace another molecule from a binding site of the macromolecule [111].
Some authors have reported a cooperative adsorption of sodium dodecyl sulfate (SDS) on polyethylene oxide (PEO) at the critical aggregation concentration (CAC) for this system wherein the formation of PEO-SDS aggregates (or mixed micelles) at concentrations above the CAC were reported. The PEO-SDS aggregates were formed at the interface by SDS adsorption on PEO chains, followed by the formation of free SDS micelles when the SDS concentration reached the critical micellar concentration (CMC) [107].

While Nilsson [27] reported that the interactions between hydroxypropyl methylcellulose (HPMC) and SDS were cooperative in forming HPMC-SDS aggregates by the adsorption of SDS on HPMC, Hammarstrom et al. [112] utilizing nuclear magnetic resonance (NMR) (including the chemical shift and self-diffusion) found that the size and shape of HPMC-SDS clusters did not change in the presence of HPMC within the composition range selected for the analysis and is not in agreement with the results reported by Nilsson [112]. Although the observation that the interactions between anionic surfactants and some polymers such as PEO, HPMC, polyvinyl pyrrolidone (PVP), and ethyl hydroxyethyl cellulose (EHEC) may be cooperative, not much information on the role of the properties of these excipients or their interactions with other cationic surfactants or their mechanisms is available [99].

Although the reported interactions between anionic surfactants and some polymers such as PEO, HPMC, polyvinyl pyrrolidone (PVP), and ethyl hydroxyethyl cellulose (EHEC) may be cooperative, not much information on the role of the properties of these excipients, their interactions with other cationic surfactants, and mechanisms are available [99]. Moreover, while the combinations of surfactant and polymer have been reported to
provide improved stability to NDDS as compared to the utilization of either a surfactant or a polymer alone [6, 113], the mechanism of these observations and the role of the solution-state environment remain unclear. Therefore, despite the above-mentioned studies, formulation scientists generally use an empirical, screening-based approach to select polymers and surfactants for NDDS development. The impact of various formulation parameters such as the nature of surfactant head-group (i.e., charge and size), hydrophobicity or chain length of surfactant, and ionic strength of the solution-state on the formation and structural properties of HPMC-ionic surfactant aggregates and, in turn, the adsorption of these aggregates to the surface of nanoparticles is still not well understood. Additionally, for a better selection of polymers and surfactants and to develop a mechanistic understanding of their stabilizing effect, it is essential to analyze and quantify these stabilizers in nanosuspensions.

An in-depth understanding of the mixed adsorption process is essential to select the type and levels of surfactants and polymers to maximize the extent of adsorption on nanoparticle surfaces [114]. In order to understand the extent of adsorption in the mixed adsorption of polymers and surfactants, bulk solution-state interactions between polymers and surfactants, characterization of surfactant-polymer aggregates formed, analysis and quantification of surfactants and polymer in nanoparticles, and the thermodynamic of these interactions with more accurate models need to be explored.

1.4. Applications of NDDS

NDDS can result in the conversion of poorly soluble drug candidates generally considered to have unacceptable properties into acceptable candidates for drug development by improving their solubility and or rate and extent of dissolution. Particle
sizes of $\leq 50$ nanometers can lead to an increase in saturation solubility that can impact bio-performance profoundly [11, 14]. The increase in dissolution rate is due to the large increase in total surface area of the nanosized particle size and can lead enhanced bio-performance as the more dissolved drug is available at the absorption site. Bioavailability enhancement increases the potential of these drug candidates to move through drug discovery to early and late-stage development that would have been previously difficult to evaluate. Since the top down and bottom-up approaches can be used to reliably produce small quantities of NDDS with desired characteristics at a small bench scale, this approach is especially useful in early-stage drug candidate evaluation.

NDDS formulated for poorly soluble drug candidates can lead to several advantages over conventional oral formulations. NDDS such as nanocrystals keep the drug crystals in their primary crystalline state, so there is a lower risk of phase or form change of the drug candidate during and after processing. This delivery system also allows for high drug loading in the final drug product. Drug loadings up to 90% have been reported thus greatly reducing the footprint of the oral dosage form [57]. This is a huge advantage over amorphous solid dispersion formulations that consist of changing the phase of the drug substance from a crystalline to an amorphous phase and can require large amounts of stabilizers such as polymers and surfactants to maintain the drug in its amorphous state for the shelf life of the drug product and mitigate the high long-term physical stability risk. Megace® ES product for the anorexia and cachexia indication is a good example of a nanocrystal aqueous dispersion formulation overcoming shelf life stability issues without the need for refrigeration [6].
NDDS provides the flexibility of dosing poorly water-soluble compounds through various routes of administration such as oral, ophthalmic, pulmonary, transdermal, and parenteral. For oral delivery, NDDS would provide an increase in dissolution rate, saturation solubility and absorption at the site of action which is the gastrointestinal (GI) tract. This route also provides an additional advantage of higher drug loading and therefore a smaller footprint of the dosage form, lower production cost, and fewer constraints for storage. In cases where the drug candidate has limited GI absorption, metabolism or degradation in GI tract other routes of administration such as pulmonary, transdermal and parenteral may be considered. NDDS can be formulated for parenteral delivery similarly with some adjustments needed for stabilizers that are more suitable for parenteral use (i.e., surfactants and polymers such as polysorbates, Vitamin E polyethylene glycol succinate, PEG, cellulose derivates, and PVP) [58]. Compared to the conventional parenteral formulations where large amounts of solvents or co-solvents, or pH shifts are required to provide the desired solubility, the nanosuspension approach can circumvent these issues by providing the drug crystals in a nanosized particle size range in an aqueous dispersion form. This approach can also be used to provide a sustained release or as a depot by controlling the particle size and or by employing release controlling excipients such as polymers or surfactants. A long-acting parenteral nanoparticle formulation at a particle size of 200 nm was reported for an oncology drug product rilpivirine, a non-nucleoside reverse transcriptase inhibitor [115] and long-acting nanoparticles for palperidone [14] using the intramuscular route were reported in providing improved patient compliance and therapeutic effect. The use of NDDS for oncology drug candidates such as paclitaxel,
camptothecin, etoposide, and pipsulfan were reported to have increased the efficacy and tolerance of these molecules thus improving patient compliance [6].

In some cases, NDDS could also reduce or mitigate the food effect for these classes of poorly soluble candidates administered orally [11, 14]. Reduction in food effect is often the result of the uniformity of the particle size distribution and rapid dissolution of drug particles that is unaffected by the presence or absence of food. Since these particles are extremely small, it was reported that they might be trapped in the intestinal lumen microvilli thus increasing their gastrointestinal retention time in relation to a solution or a larger solid dosage form. Noxafil®, an antifungal compound known to have a significant food effect when formulated as an oral aqueous nanosuspension formulation with particle size with a $d_{50}$~150 nm was reported to successfully eliminate the observed food effect seen with the conventional formulation by increasing site-specific bioavailability at the same dose [6]. Aprepitant® when formulated as a solid-nanoparticulate formulation was reported to overcome a strong food effect seen with a conventional formulation. This was especially critical since the drug was to be commercialized as an anti-emetic and administration with food would not be viable [6, 14].

2. Characterization of NDDS

NDDS such nanocrystals, nanosuspensions, solid nanoparticles are characterized similarly to conventional drug crystals or suspensions to determine particle size, surface area, appearance, physical and chemical stability, solubility, dissolution, re-dispersibility, and bioavailability. The commonly utilized techniques for characterization are laser diffraction for particle size and re-dispersibility, BET for surface area and porosity, microscopy and powder X-ray diffraction for crystallinity and HPLC for testing impurities.
In this work, some novel techniques such as HPLC with ELSD were utilized to quantify the amount of stabilizers adsorbed to understand the mechanism of stabilization for model nanoparticles. BET was used to characterize the surface area and pores of the model nanoparticle surfaces. ITC was used to study aggregate formation between the stabilizers and the energetics of these interactions along with the adsorption of these aggregates on the surface of the model nanoparticle surfaces. A fluorescence probe method was used to investigate structural information of these aggregates. More details on these techniques will be discussed below.

2.1. High-Pressure Liquid Chromatography (HPLC) with Evaporative Light Scattering Detector (ELSD)

All quantitative HPLC analyses of NDDS stabilizers (i.e., surfactants and polymers) that did not possess chromophores were carried out on an HPLC system that consisted of a Waters 2695 Separations Module (Waters Corp., Milford, MA) coupled with a Sedex® 85 low-temperature evaporative light scattering detector (SEDERE, France). Typically used ultra-violet (UV) or photo diode array detectors would not be viable options in the absence of UV-active chromophores on these stabilizing excipients such as SDS, DM, DTAB, and HPMC. Therefore, as these excipients cannot absorb UV light, the Sedex® 85 low-temperature evaporative light scattering detector was used along with high-performance liquid chromatography (HPLC). Any analytes that are comparatively less volatile (i.e., semi-volatile or non-volatile) than the mobile phase can be detected universally. The ELSD system consists of three distinct regions (1) nebulization (2) mobile phase evaporation and, (3) the detection region (Figure 2.5). In the nebulization phase, once the mobile phase containing the sample passes from the HPLC/SEC into the ELSD,
it is combined with nitrogen gas and forced into the nebulizer. This process aerosolizes the analyte droplets as they enter the heated drift tube. The solvent phase is then evaporated in this region, and the size of the droplets decreases based on the evaporation rate until only the sample/analyte particles remain. The analyte particles subsequently enter the detection region consisting of a photomultiplier tube where the amplifier gain becomes an important factor in detecting the analyte accurately. When used in ELSD, solvent gradients can be quite problematic as the response factor may no longer remain constant. As the organic content of the mobile phase increases, the transport efficiency of the nebulizer may increase leading to changes in size and number of droplets carrying the analyte to the detector \[116\]. Nonlinearity of the standard curve is often the result. Hence careful consideration needs to be given to the solvent system as well as the variables of the detector. A significant gap in literature continues to exist in the area of simultaneously and accurately quantifying polymer and surfactant concentrations commonly used for NDDS stabilization due to the lack of suitable analytical methods, narrowing this gap will be one of the aims of this work \[117\].

2.2. Isothermal Titration Calorimeter (ITC)

Isothermal titration calorimetry (ITC) was used in this work to determine the thermodynamics of polymer-surfactant interactions in the solution-state and adsorption of surfactant-polymer aggregates onto model nanoparticle surfaces. TAM III ITC used in this work operates in a power compensation mode principle wherein the temperature of the sample cell is maintained constant using a temperature sensor with a feedback system utilizing a reference cell (Figure 2.6). When an endothermic or exothermic event (i.e., chemical reaction, molecular reorganization, binding, solubilization) occurs during
titration, the power supplied to a heater or cooler to maintain isothermal conditions is directly measured [118]. At the start of an experiment, the reference cell contains the same solution as the sample cell. In a given ITC measurement, the energetics associated with the titration process is directly measured at a constant temperature. For example, if an exothermic event occurred during the titration, the compensation mode (feedback loop) would cool the sample until the temperature of the sample cell was brought to the temperature of the reference cell. The signal (peak) thus obtained from the feedback loop is integrated directly to yield the heat associated (Q) with that event. As shown in Figure 2.6, the heat signal (Q) is directly related to the concentration and volume of the titrant in each injection, which is then normalized with respect to the moles (δₙ) of analyte added to the sample cell. The signal was further analyzed to obtain the apparent enthalpy change using TAM III Lab Assistant Software provided by TA Instruments shown in the equation: 

\[ \Delta H_{\text{app}} = \frac{Q}{\delta_n} \]

The apparent enthalpy change (ΔH_{app}) is plotted against the concentration or amount of titrant.

2.3. Ultraviolet-Visible (UV-Vis) Spectroscopy

UV-Vis spectroscopy measurements were conducted using the Cary-50 Bio UV-Visible spectrophotometer (Varian, Santa Clara, CA). The principle of the equipment is that it measures the intensity of the light as it passes through a sample (I). The intensity of light ratio after it passes through the sample and before it passes through the sample (I₀) is known as the transmittance. The absorbance of the sample is related to the transmittance by the equation below:

\[ A = \log \frac{I}{I_0} \]
A UV spectrophotometer consists of a light source, sample holder, a prism diffractometer that splits the light from the source into various wavelengths on the monochromator grating, and a detector. The light source may consist of a deuterium arc lamp, tungsten filament, xenon arc lamp and light emitting diodes that cover the wavelength of light from the UV to the visible range. The detector consists of a photodiode array and a photomultiplier tube; respectively that allows only a single wavelength of light to enter at a given point of time. These single wavelengths are then scanned to measure the intensity.

The solubilization power was measured in this work by preparing the samples by the addition of an excess amount of pyrene microcrystals to SDS or SDS-HPMC aqueous solutions. The supernatant obtained after sonication were utilized to measure the absorbance intensity. The molar absorptivity value of pyrene solubilized in micelles was used as reported in the literature [119].

2.4. Fluorescence Probe Technique

Steady-state fluorescence measurements in this work were performed using the Varian Cary Eclipse fluorescence spectrophotometer (Varian, Santa Clara, CA). When a sample is irradiated with either ultraviolet, visible or near-IR light, steady-state fluorescence spectroscopy measures the long-term average fluorescence of the sample. The fluorescence spectrum consists a plot of fluorescence intensity vs. wavelength (energy and frequency) at one selected excitation wavelength. The fluorescence intensity measurements include the emission and excitation scans that are used to determine the presence of fluorophores (i.e., pyrene) at the various concentrations. Single photon counting is utilized for the spectral measurements [120]. In this work, the structural
characteristics of surfactant-polymer aggregates and aggregation phenomena were investigated using steady-state fluorescence. All measurements for these studies were carried out at an excitation wavelength of 340 nm, and the emission spectrum was recorded between 340 and 550 nm in a 10 mm path length quartz cuvette. The excitation and emission slit widths of 5 nm and 1.5 nm, respectively, were used. Data was acquired using Cary Eclipse software from Varian (Varian, Santa Clara, CA). The fluorescence probe used in this technique was pyrene, an extremely hydrophobic molecule.

2.5. Equilibrium Dialysis

Equilibrium dialysis is a technique often used to analyze the binding of a low molecular weight ligand such as a surfactant to a high molecular weight macromolecule such as a polymer. Equilibrium dialysis provides stoichiometric data for aggregates or complexes formed, cooperative binding information, as well as the binding affinity [121]. For an equilibrium dialysis experiment, the solutions containing the ligand and the macromolecule are placed in two compartments separated by a semipermeable membrane. The dialysis membrane molecular weight cut off is selected based on the ligand being tested in order to allow passage through the membrane. The ligand redistributes between the two compartments. At equilibrium the free ligand concentration is considered to be equal on both sides of the membrane along with ligand present in the bound form. The total ligand concentration in each compartment is analyzed after equilibrium, the excess ligand present in the compartment with the macromolecule/receptor that could not partition across the membrane is assumed to be the bound ligand concentration. The experiment is conducted for various ligand and macromolecule concentrations and the data thus collected
is evaluated through a Scatchard Plot, that fits the data by least squares regression to obtain the relevant binding or interaction parameters as shown in the equation below [121]:

\[ K_d = \frac{r}{(n-r)(c)} \]

where \( K_d \) is the binding constant, \( r \) is the ratio of the concentrations of the bound ligand to the macromolecule/receptor, \( n \) is the number of binding sites for the ligand on the macromolecule, and \( c \) is the unbound or free ligand concentration.

2.6. Surface Area Measurement by BET Nitrogen Adsorption

The surface area of silica was measured utilizing the Brunauer–Emmett–Teller (BET) method where nitrogen was used as a model adsorbate and the adsorption of nitrogen was measured using a Tristar 3000 (Micromeritics, USA) instrument.

Adsorption of nitrogen is commonly utilized technique to measure pore size, surface area and sometimes particle size of a solid material such as our model NDDS surface, silica [122]. This technique is non-destructive and utilizes sufficient sample amount. For this work, the silica samples (triplicate) were purged with nitrogen for approximately 4 hours and degassed at 120°C prior to analysis. This ensures that the samples are free of any moisture especially in the pores of the material for an accurate measurement.

BET theory is an extension of the Langmuir adsorption theory where in this case the nitrogen molecules are assumed to adsorb in a monolayer manner on free, identical, and limited adsorbent sites followed by the multilayer adsorption where the gas molecules in the monolayers interact with adjoining layers. This can be shown by the linearized BET equation [122].

\[ \frac{P}{v(P_0-P)} = \frac{1}{v_m C} + \frac{C-1}{v_m C} \frac{P_0}{P} \]
where the \( v \) is the amount of gas adsorbed on the surface i.e., \( v_m \) is the adsorption capacity of the material, \( P_0 \) is the ratio of the equilibrium pressure and saturation pressure and linear relationship of the BET equation is applicable in the range of \( 0.05 < \frac{P_0}{P} < 0.35 \). \( c \) is the BET constant.

The data obtained from the experiment is fitted to the linear BET model with \( \frac{P}{v (P_0 - P)} \) on the y axis and \( \frac{P_0}{P} \) as the x-axis to calculate the surface area of the sample. The surface area of the solid material is calculated based on the monolayer adsorption capacity that is calculated from the slope \( \frac{C - 1}{v_m C} \) and intercept \( \frac{1}{v_m C} \) of the linear equation wherein C is obtained from \( \left( \frac{C - 1}{v_m C} + 1 \right) \) and \( V_m \) is obtained from \( 1/\left( \frac{C - 1}{v_m C} + \frac{1}{v_m C} \right) \). Specific surface area (SA; m²/gm) is then calculated using equation for SA below:

\[
SA = \frac{v_m Na}{m \times 22400}.
\]

where \( N \) is the Avogadro constant and \( a \) is the area of a molecule of adsorbate and \( m \) is the mass of the adsorbate being tested.

**2.7. Solution Depletion Method for Adsorption Isotherms**

The solution depletion method was used to determine adsorption isotherms for various adsorbates including SDS, HPMC, and DTAB on silica at 25°C. In the solution depletion method, appropriate amounts of adsorbates were equilibrated with aqueous dispersions of silica in centrifuge tubes using a mechanical shaker. For example, 200 mg of silica was dispersed in 10 mL of HPMC or HPMC-surfactant solution at different concentrations. The equilibration time for the adsorption samples was determined to be 36 hours for all three adsorbates. Upon equilibration, the samples were centrifuged at 5000
RPM for 45 minutes to separate the silica particles, and the adsorbate concentration in the supernatant was measured after equilibration using the SEC-ELSD method developed for the simultaneous detection of HPMC and surfactants. This methodology was used to determine the extent of adsorption of these excipients on the model nanoparticulate surface.

3. **Unmet Need and Next Steps**

A combination of surfactant and polymer have been reported to be useful in stabilizing these nanosized colloidal dispersions, however, at present, there is no molecular-level understanding that relates critical properties of a drug molecule to the type of polymer-surfactant system that may provide optimal stabilization. A lack of mechanistic understanding of the kinetic stabilization process and its relationship to solute-excipient interactions in bulk and on the surface has resulted in scientists being forced to employ a labor-intensive and costly trial and error approaches to formulation development [1-3].

Optimal stabilization of NDDS occurs when a strong barrier is placed between two interacting particles, the addition of polymers and surfactants are thought to complement each other by providing both electrostatic and steric mechanisms of stabilization [20-23]. The adsorption of polymers and surfactants to nanoparticles can be additive, cooperative or competitive and are likely to be influenced by various solution and surface properties. Solution properties such as bulk concentration, pH and ionic strength can be very important for the extent of adsorption of the solutes on the solid-liquid interface [11-13]. Although polymer and surfactant systems have attracted a vast amount of interest in the non-pharmaceutical scientific community, typical polymers and surfactants used in the development of pharmaceutical NDDS have not received sufficient attention [24-30].
Several gaps were identified from the review of literature studies containing HPMC as a stabilizer (1) the higher concentrations of HPMC typically utilized in NDDS (0.25-1 %w/w) [8, 11, 58] have not been evaluated, (2) the influence of HPMC molecular weight on the number of available binding sites constraints has not been evaluated, (3) the effects of ionic strength and surfactant properties (i.e., head group, chain length) on the interaction between surfactants with HPMC have not been investigated, and (4) relatively less sensitive and selective techniques such as tensiometry, fluorescence and viscometry have been used [102, 123, 124]. For a better selection of polymers and surfactants and to develop a mechanistic understanding of their stabilizing effect, it is essential to analyze and quantify these stabilizers in nanosuspensions. A significant gap in the literature continues to exist in this area due to the lack of suitable analytical methods to simultaneously and accurately quantify the levels of polymers and surfactants in nanosuspensions. In studies where more sensitive techniques such as microcalorimetry were used, the calorimetric data treatment could be further optimized. The use of modern isothermal titration calorimetry (ITC) has gained momentum particularly due to its increased sensitivity and selectivity. Using ITC, thermodynamic parameters (i.e., enthalpy, free energy, and entropy) for polymer-surfactant aggregate formation and structural rearrangement information can be obtained from a single experiment [102, 123, 124]. For a better selection of polymers and surfactants, it is essential to develop a better understanding of the mechanisms and thermodynamics of the solution-state interactions between commonly used stabilizers such as HPMC and surfactants upon the adsorption of solid nanoparticles. This increased understanding would serve as a step towards a more
rational *a priori* selection of excipients instead of the trial and error often employed currently.
Water molecules are energetically driven away from the hydrophobic drug crystal surface. Drug crystal surface stabilized by a combination of polymer and surfactant adsorbed.

**Figure 2.1. Creation and Stabilization of Nanoparticles Stabilized with Excipients [4].**
Figure 2.2. Representative organic crystal surface with different functional groups that could promote ionic or steric interactions requiring specificity of surface stabilizers.
Figure 2.3. Structures of model polymer (HPMC) and surfactants to be used in dissertation studies
Figure 2.4. Schematic of the possible equilibria in NDDS between surfactant, polymer, and a model nanoparticulate surface in the solution-state.
Figure 2.5. Schematic of HPLC with ELSD technique for quantitative HPLC analysis of NDDS stabilizers [125].
Figure 2.6. Schematic of an ITC setup for the power compensation mechanism and raw data for a general case of micellization [118].
CHAPTER 3

Development of a Robust Method for Simultaneous Quantification of Polymer (HPMC) and Surfactant (Dodecyl β-D-Maltoside) in Nanoparticulate Drug Delivery Systems (NDDS)\textsuperscript{117}

1. Introduction

Polymers such as hydroxypropyl methylcellulose (HPMC), polyvinyl pyrrolidone (PVP), and hydroxypropyl cellulose (HPC) as well as surfactants such as dodecyl β-D-maltoside (DM) and sodium dodecyl sulfate (SDS) are frequently used in nanoparticulate drug delivery systems (NDDS) as stabilizers to enhance the physical stability of nanosuspensions [126, 127]. The physical stabilization of nanosuspensions is challenging, often requiring an optimum combination of surfactants and polymers as a means to enhance the physical stability of NDDS [12, 13]. For a better selection of polymers and surfactants and to develop a mechanistic understanding of their stabilizing effect, it is essential to analyze and quantify these stabilizers in nanosuspensions. A significant gap in the literature continues to exist in this area due to the lack of suitable analytical methods to simultaneously and accurately quantify the levels of polymers and surfactants in nanosuspensions. The objective of this study was to develop a robust method to simultaneously quantify a model polymer, HPMC, and a model surfactant, DM.

As a pharmaceutical excipient, HPMC is employed in a wide range of solid and liquid formulations [22]. HPMC is a mixed alkyl hydroxyalkyl cellulose ether that is derivatized with hydroxypropyl and methoxyl groups. The chromatographic quantification of HPMC with acceptable baseline separation from other excipients is challenging due to its wide molecular weight distributions and the lack of strong chromophores. There are
only a few reports describing quantitative assays for HPMC that are suitable for pharmaceutical products [23-25]. For example, Delker et al. [23] employed the refractive index to detect HPMC in polyethylene glycol. Whelan et al. [24] used an evaporative light scattering detector (ELSD) to quantify HPMC in the presence of ibuprofen. Rashan et al. [25] used a Polymer X RP-1 column along with a gradient elution method that is not typically used with ELSD detectors for the analysis of different grades of HPMC. All above-mentioned methods lacked sensitivity, and the elution of HPMC was close to the solvent peak, and therefore could not simultaneously quantify HPMC with other excipients.

DM is an alkyl polyglucoside, a derivative of glucose and fatty alcohol manufactured from sugar [128-130]. DM has garnered a considerable amount of interest as a result of its low surface tension, ionic strength tolerance, and environmental compatibility [131-135]. DM was used as a model nonionic surfactant in this study. Methods for the detection of DM reported in the literature include total organic carbon (TOC) and calorimetry, both of which are neither fast, accurate nor sensitive [26].

For the simultaneous detection of HPMC and DM in nanosuspension, size exclusion (SEC) based high-pressure liquid chromatography (HPLC) with ELSD technique was selected since both excipients (DM and HPMC) are non-volatile and lack UV-chromophores [32-34]. SEC has been used to resolve polymers based on differences in molecular size where separation occurs as a result of the pore size of packing material [23]. ELSD is more sensitive and solvent compatible as compared to other universal techniques (i.e., refractive index (RI) and liquid chromatographic mass spectroscopy (LCMS)). Some of the limitations of ELSD are low selectivity, the requirement for a volatile mobile phase, non-linearity, and being destructive to the sample that is analyzed.
ELSD has been applied effectively for at least the last two decades to quantify a wide spectrum of natural and synthetic compounds including pharmaceuticals [34, 36-40], biologics [41, 42], and foods and beverages [43, 44].

To the best of the author’s knowledge, there are no methods published outlining an SEC-ELSD assay for the simultaneous detection of DM and HPMC. This study utilizes a full factorial design to optimize the impact of SEC and ELSD method variables and their interactions on the precision, accuracy, and sensitivity of the assay as per the Guidance for Industry, ICH-Q2A [45]. This method was applied in subsequent studies to understand the mechanism of nanosuspension stabilization by model surfactants and HPMC.

2. Materials and Methods

2.1. Materials

DM (>98%) was obtained from Sigma-Aldrich Inc. (St. Louis, MO). HPMC (Benecel® K-4M) was obtained from Ashland Inc. (Wilmington, DE). The chemical structures of DM and HPMC are shown in Figure 3.1. Colloidal silicon dioxide (Cab-O-Sil® EH-5; specific surface area ~202 m²/gm), a model nanoparticulate surface consisting of non-porous fumed particles, was purchased from Cabot Corp. (MA). Acetonitrile (HPLC grade) was purchased from Fischer Inc. (Fair Lawn, NJ). HPLC grade water (18.2 megohm-cm) obtained from a Milli-Q water purification system (Millipore, Billerica, MA). All other reagents were of pharmaceutical grade and used as received. Nitrogen gas (ultra-pure >99%) was obtained from Scott Gross Company Inc. (Lexington, KY).
2.2. Methods

2.2.1. Chromatographic conditions

All studies in this work were carried out on an HPLC system that consisted of a Waters 2695 Separations Module (Waters Corp., Milford, MA) coupled with a Sedex 85 low-temperature evaporative light scattering detector (SEDERE, France). The signal was acquired and processed with Millennium software (Waters Corp., Milford, MA). A Waters Ultrahydrogel® 120 size exclusion column (5 µm, 300 mm x 7.8 mm) with a pore size of 120Å (Waters Corp., Milford, MA) was used to separate DM and HPMC. The column was equilibrated for two hours under the above conditions prior to the first injection. The column temperature was maintained at 25°C, and the injection volume was fixed at 100 µL. The column was conditioned by a minimum of four consecutive injections of the standard solutions.

The mobile phase of acetonitrile: Milli-Q water (30:70 v/v) with a flow rate of 1 mL/min under isocratic conditions was used. As the organic content of a mobile phase increases, the transport efficiency of nebulizer may increase leading to changes in the size and number of analyte droplets that can result in non-linearity [116]. Hence, several combinations of mobile phases were tested before selecting the isocratic conditions described above.

2.2.2. Sample preparation

Standard solutions were prepared by dissolving known quantities of HPMC and DM in the mobile phase. The samples containing mixtures of DM, HPMC and colloidal silicon dioxide (silica) were diluted with the mobile phase and then centrifuged at 5000 rpm for 1 hour to sediment any undissolved silica. The supernatant obtained from this was
used for analysis. The working range for DM and HPMC standard concentrations was 10-325 µg/mL. Standards and samples were prepared on the day of use.

2.2.3. Design of Experiments (DoE)

A design of experiments was used to optimize the ELSD-based chromatographic assay conditions for robust and simultaneous detection of DM and HPLC. Stat-Ease® software used for the design and analysis of experiments was obtained from Stat-Ease, Inc. (Minneapolis, MN). A two-level full factorial design was chosen to generate response surfaces to select the optimal levels of ELSD variables that are critical for a robust and accurate assay (Table 3.1).

2.2.3.1. DOE FACTORS

ELSD works on the principle of detecting non-volatile particles that scatter light, and it is therefore imperative to fully control the ELSD variables that are critical for the formation of these particles, most notably the optimal drift tube temperature, carrier gas pressure and amplifier gain [35, 36]. Accordingly, the ELSD factors selected in this DoE were (1) drift tube temperature, (2) carrier gas pressure, and (3) amplifier gain. Preliminary screening experiments showed that drift tube temperatures outside of 40-50°C and carrier gas pressures outside of 3-3.2 bar resulted in poor reproducibility and amplifier gain values outside 10-12 resulted in very low signal/noise. These preliminary results defined the relatively narrow ranges for the levels of DoE factors as shown in Table 3.1.

2.2.3.2. DOE RESPONSES

The DoE responses were (1) the deviation of slopes of single-component and two-component/mixed standard curves, and (2) the accuracy and precision of assay (Table 3.2). The deviation of the slope was obtained by subtracting the slopes of two-component/mixed
(DM and HPMC solutions) standard curves from the slopes of single-component (DM or HPMC) standard curves. The accuracy of the assay was determined by calculating the absolute value of the difference between the peak areas of either DM or HPMC from their mixed standards as well as single-component standards at 100 μg/mL, which was expressed as the % of a single-component standard peak area. The precision of assay expressed as percent relative standard deviation (RSD), was determined from the replicate analysis of four independent injections at 300 μg/mL.

3. Results and Discussion

3.1. Chromatograms for Simultaneous Detection of DM and HPMC

The SEC HPLC column with a pore size of 120Å provided optimal resolution of DM and HPMC. The mobile phase and flow rate were adjusted to obtain the sharpest peak for the molecular weight grade of HPMC used in this work. After a series of preliminary chromatographic experiments to optimize resolution, a suitable isocratic mobile phase, flow rate, and injection volume were identified as described in the methods section. The chromatograms of DM, HPMC, and mixed DM-HPMC standards are provided in Figure 3.2. HPMC eluted at 4.9 minutes when present in either a single or a mixed standard solution whereas, DM eluted at 15.9 minutes in a mixed standard solution and 15.7 minutes in a single standard solution. The relative standard deviation (RSD) for the mean retention times of HPMC and DM were 0.35% and 0.37%, respectively (n=16). The resolution (Rs) between HPMC and DM peaks in mixed standards was calculated using Eq. 3.1.

\[
R_s = 2 \frac{(RT_a - RT_b)}{(W_a - W_b)}
\]  

(3.1)

where \(RT_a\) and \(RT_b\) are the retention times and \(W_a\) and \(W_b\) are the widths at baseline of HPMC and DM peaks, respectively. In all mixed standards, \(R_s\) values greater than 1.5
were observed that assured good peak resolution for quantification purposes as specified in the FDA-CDER guidelines [136] and by other authors [137].

Initial method development showed three peaks for HPMC (data not shown). HPMC K-4M used in this study exhibits a wide range of molecular weights wherein the manufacturer reports a weight-average molecular weight range from 20,000-115,000 and a number-average molecular weight of 86,000. This can result in the reported broad or multiple peaks observed by SEC for HPMC [25]. However, through chromatographic manipulations, a sharper peak with a small shoulder was achieved for HPMC and despite this diversity of molecular weights, a relatively symmetric chromatogram was observed (Figures 3.2b and 3.2c). The small shoulder in the HPMC peak observed in the chromatogram is likely indicative of a low molecular weight HPMC fraction resolved by the column. Additionally, a sharp and symmetric peak was obtained for DM in both mixed and single standards with SEC.

3.2. Standard Curves for DM and HPMC

The signal intensity of the ELSD detector has been related to the concentration of an analyte according to Eq. 3.2.

\[
\text{Signal Intensity} = \alpha [\text{Analyte}]^{\beta}
\]  

(3.2)

The parameters \( \alpha \) and \( \beta \) are directly influenced by factors such as the size of the particles, nature and volatility of the analyte, nebulizer gas flow rate, mobile phase flow rate and temperature of the drift tube. Some authors have employed a linear model similar to Beer’s Law; however, the concentration range in such cases is typically quite narrow [39, 138, 139]. Logarithmic models have been successfully employed to fit ELSD response data
over a much wider range of analyte concentration [37, 140, 141]. A logarithmic transformation of Eq. 3.2 is described by Eq. 3.3:

$$\log(\text{Signal Intensity}) = \beta \log[\text{Analyte}] + \log\alpha$$

(3.3)

where $\beta$ is the slope and $\log\alpha$ is the y-intercept, respectively. The areas under the chromatographic peaks were collected for both DM and HPMC in the 10-325 µg/mL concentration range and fit to Eq. 3.3. A standard curve was constructed for each solute in the mixed and single component DM and HPMC samples (Figure 3.3).

### 3.3. Influence of DoE variables on assay responses

DoE responses including a deviation of the standard curve slope, accuracy, and precision of the chromatographic assay were employed to evaluate the impact of ELSD variables on the development of a simultaneous and robust detection method for DM and HPMC (Table 3.3). The responses (Table 3.3) and statistical analyses for each variable are discussed in greater detail below.

Half-normal probability plots displaying the effects of individual factors and their interactions were used to identify and select them for the subsequent building and analysis of DoE models for each response. For all responses, a similar stepwise regression routine was employed to fit response data and to select a simpler and more adequate model for analysis. The analysis of variance (ANOVA) was performed for each model containing main effects (individual factors) along with interaction terms and the examination of the F-test for the lack of fit was also for the model selected (Eqs. 3.4-3.7, Table. 3.4). In ANOVA analyses, the F-value is the ratio of the model sum of squares and residual sum of squares that shows the relative contribution of the model variance to the residual variance. A large value for this ratio would indicate that more of the variance can be
explained by the model selected whereas a smaller value would suggest that the variance is more a result of noise.

The selected model for deviation of slope for DM included ELSD factors of drift tube temperature (A), and amplifier gain (C) is shown as Eq. 3.4. As shown in Table 3.4, the F-value of 11.02 indicates that the model is significant. In the ANOVA analyses, p-values less than 0.05 indicate that drift tube temperature (A) and amplifier gain (C) have significant effects on the deviation of slope for DM. In the case of HPMC, the model for deviation of slope included the factor of gain (C) (Eq. 3.5). The F-value of 9.17 implies the model is significant and only the amplifier gain (C) has a significant effect on the deviation of slope (p < 0.05) for HPMC.

\[
\ln(\text{Deviation of Slope for DM}) = 11.04 - 0.13[A] - 0.83[C] \quad (3.4)
\]

\[
\ln(\text{Deviation of Slope for HPMC}) = 2.42 - 0.39[C] \quad (3.5)
\]

For the ANOVA analyses for accuracy response (%) for DM, the selected model included ELSD factors of drift tube temperature (A), amplifier gain (C) and, their interaction (AC) (Eq. 3.6). As shown in Table 3.6, the F-value of 56.27 indicates that the model is significant. P-values less than 0.05 indicate that the factors of drift tube temperature (A), amplifier gain (C) and, their interaction have significant effects on the deviation of slope for DM. Accuracy response analyses showed that drift tube temperature and amplifier gain were significant (p < 0.05) while nebulizer pressure (B) had a slight impact; it was not found to be statistically significant. Both factors (A and C) had a positive impact on the deviation of the slope.

\[
\ln(\text{Accuracy of DM}) = 456.84 - 9.01[A] - 37.88[C] + 0.75[AC] \quad (3.6)
\]
For the precision response for HPMC, the selected model included ELSD factors of drift tube temperature (A) and nebulizer pressure (B) (Eq. 3.7). As shown in Table 3.4, the F-value of 9.85 indicates that the model is significant. P-value of less than 0.05 indicates that drift tube temperature (A) and nebulizer pressure (B) have a significant effect on the precision response for HPMC.

\[
\text{Precision for HPMC} = 52.99 - 0.22[A] - 12.88[B]
\]  

(3.7)

ELSD drift tube temperature in an ideal scenario should completely volatilize the mobile phase without any loss of analyte by thermal degradation hence the temperature would need to be optimized such that there is a minimization of the baseline noise occurring at low temperatures while also balancing the lack of sensitivity, precision, and accuracy occurring at higher temperatures [35]. The variable of nebulizer pressure was found to have a significant effect \((p < 0.05)\) on the precision of the assay. Nebulizer pressure is reported to be critical in the effluent atomization from the chromatographic column by allowing the formation of uniform sized droplets with a narrow size distribution that can directly influence assay sensitivity and precision. It was also reported that an increase in droplet size, contributed to an enhancement of the ELSD response. The current results are in agreement with a previous observation [35]. From the response plots and ANOVA results, it was determined that drift tube temperature and amplifier gain of the ELSD instrument were the most important interacting parameters that positively influenced the accuracy of the assay (Figure 3.4). This result indicates that the accuracy of the assay would increase with an increase in the drift tube temperature and amplifier gain. On the other hand, it was observed that the nebulizer pressure impacted the precision of the assay negatively. Thus, a decrease in the nebulizer pressure would be favored to increase the
precision of the assay, and an increase in the drift tube temperature and instrument gain was favored to decrease the deviation of the slope.

3.4. Influence of interactions between DoE factors on assay responses

In the ANOVA analyses described in the earlier section (3.3), interactions between factors were observed. The interaction between factors may be defined as the failure of one variable to produce an identical response at different levels of another variable. Hence, to understand and predict the desired responses, and to select the optimized design space, the putative interactions must be considered.

The results demonstrated that the interaction between the drift tube temperature and instrument gain (termed AC) had significant ($P < 0.05$) impact on the accuracy of the assay (Table 3.3). While a decrease in the accuracy data was seen with an increase in drift tube temperature at amplifier gain value of 10, a slight increase in the accuracy data was seen at the gain value of 12. Therefore, we can conclude that the highest level of drift tube temperature and an intermediate level of the amplifier gain would provide the optimal response with respect to the accuracy of the assay. Overall, the selected models sufficiently described the impact of the drift tube temperature, nebulizer gas pressure and, instrument gain and interactions of these factors on the accuracy, precision, and deviation of the slope of the assay.

3.5. Optimization of design space

Desirability was defined as the optimal conditions of the ELSD instrument when the deviation of slope, precision, and accuracy were not greater than 0.05, 5% RSD and 10%, respectively. Desirability values range from 0 to 1, with 0 being unacceptable and 1 as the most desirable in terms of accuracy, precision, and deviation of slope responses.
From the 3D graphical plots of response surface for desirability obtained from the Stat-Ease® software (Figures 3.4 and 3.5), it can be determined that drift tube temperature and amplifier gain of the ELSD instrument were the most important parameters that significantly and positively (positive coefficient) influenced the accuracy of the assay. Figure 3.5 shows that a desirability index of 0.93 was obtained at a high level of the factors of temperature and pressure and an intermediate level of the instrument gain factor. At a lower gain value of 10 a desirability of only 0.6 could be achieved (Figure 3.4).

3.6. Method validation of an assay for mixed samples of DM and HPMC

The optimized variables obtained from the multifactorial analysis described earlier were employed in the validation of the assay for mixed standards of DM and HPMC. In the method validation process, linearity, precision, accuracy, selectivity, sensitivity, LOD and LOQ of the assay were tested [142].

3.6.1. Linearity

As mentioned earlier, the peak area responses of DM and HPMC are not linear due to the use of ELSD and a wide concentration range. Hence a log-log model as described by Eq. 3.3 was employed [140]. Within the optimized design space, peak areas and analyte concentrations were accurately fit to the log-log model with correlation coefficients of 0.991 and 0.996 for DM and HPMC in the mixed standards, respectively (Table 3.3). In contrast, when a linear model was applied, lower correlation coefficients of 0.955 and 0.966 for DM and HPMC, respectively were obtained (results not shown).

Standard curves for DM and HPMC demonstrated good linearity over a narrow and lower range (1- 32.5 µg) of single and mixed standard solutions (Figure 3.2; Table 3.3). A standard error of 0.007 and 0.004 for the intercept and 0.014 and 0.015 for the slope
between the single and mixed standard solutions was determined for DM and HPMC, respectively.

3.6.2. Sensitivity, limits of detection, and quantification

The sensitivity of the instrument was determined as the slope of linear standard curves obtained at three lowest concentrations within the quantification limit (<50 µg/ml) of DM and HPMC. The limit of detection (LOD) is defined as the lowest concentration of the analyte that can be reliably detected. The FDA guidance (Guidance for Industry, ICH-Q2A) specifies that LOD = 3.3 σ/S; where σ is the standard deviation of responses and S is the sensitivity defined as the slope of standard curves [25-28]. The limit of quantification (LOQ) is similarly defined as the lowest concentration of an analyte that can be quantified with acceptable accuracy and precision. Based on the FDA guidance document, LOQ was calculated as 10 σ/S. The sensitivity, LOD and LOQ values obtained in the optimized design are listed in Table 3.5.

3.6.3. Precision and accuracy

Precision and accuracy were determined for samples of DM and HPMC within the optimized design space as described in section 3.2.2. For DM and HPMC at a low concentration (50 µg/ml), the precision was determined to be 1.4 % RSD and 3.8 % RSD, respectively and at a high concentration (300 µg/ml) was determined to be 1.2% RSD and 4.7% RSD, respectively. The precision in the DM and HPMC samples in mixed standards was 1.8 % RSD and 4.9 % RSD for DM and HPMC, respectively.

Accuracy for DM and HPMC samples were determined in the optimized design space. The recoveries in the standard curve (1- 32.5 µg; amount injected) ranged from 95% to 104% for DM and between 98% and 103% for HPMC analyzed within the
optimized design space. Additionally, the %RSD (n=4) of the accuracy obtained in the optimized design space were 3% and 2% for DM and HPMC, respectively. Accuracy was significantly decreased (9-21 % RSD) when samples were run in ELSD conditions outside the design space. The data show that within the optimized design space the developed assay for the mixed standards of DM and HPMC is robust and reproducible.

4. Conclusions

A fast, robust and accurate assay was developed for the simultaneous quantification of polymer (HPMC) and surfactant (DM) in the pure standards and mixed standards with silica-based nanosuspension formulations. The design of experiments was used successfully to understand the influence of critical parameters of ELSD (drift tube temperature, nebulizer pressure, and instrument gain) on the responses of the assay. An optimized design space was also identified by using a full factorial design of experiments (DoE).

An increase in drift tube temperature and instrument gain increased the accuracy of the assay while a decrease in nebulizer pressure improved the sensitivity of the assay. An increase in drift tube temperature and instrument gain decreased the % deviation of slopes for both DM and HPMC responses. The assay was proven to be robust with respect to all three critical ELSD parameters within the optimized design space. The optimization of the assay using the factorial design of experiments led to the prediction of 93% desirability at the extreme levels of the two factors (drift tube temperature and instrument gain) and an intermediate level of the third factor (nebulizer pressure).

Overall the graphical mapping of the critical factors within the optimized design space helped in identifying the best conditions to develop an assay that is both repeatable
and robust. This method was used to quantify these pharmaceutical excipients (HPMC and DM) in nanosuspension formulations. The sensitivity and accuracy of this method are critical towards developing a mechanistic understanding of the physical stabilization process of nanosuspensions.
### Tables

**Table 3.1: ELSD variables as factors and their levels in full factorial DoE**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Design Levels</th>
<th>Actual Levels Tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature¹</td>
<td>+1</td>
<td>50° C</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>40° C</td>
</tr>
<tr>
<td>Pressure²</td>
<td>+1</td>
<td>3.2 bars</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>3.0 bars</td>
</tr>
<tr>
<td>Gain³</td>
<td>+1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>-1</td>
<td>12</td>
</tr>
</tbody>
</table>

¹ Drift Tube Temperature  
² Nebulizer Pressure  
³ Amplifier Gain
Table 3.2: Full factorial DoE with ELSD variables and responses for standard solutions containing mixtures of DM and HPMC K-4M.

<table>
<thead>
<tr>
<th>Run</th>
<th>A: Temperature °C</th>
<th>B: Pressure bars</th>
<th>C: Gain</th>
<th>Deviation of slope</th>
<th>Accuracy %</th>
<th>Precision % RSD</th>
<th>Deviation of slope</th>
<th>Accuracy %</th>
<th>Precision % RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>3</td>
<td>10</td>
<td>0.18</td>
<td>18.17</td>
<td>1.17</td>
<td>0.22</td>
<td>6.62</td>
<td>6.6</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>3.2</td>
<td>12</td>
<td>0.01</td>
<td>3.44</td>
<td>1.27</td>
<td>0.01</td>
<td>2.20</td>
<td>1.1</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>3</td>
<td>12</td>
<td>0.03</td>
<td>3.95</td>
<td>2.99</td>
<td>0.07</td>
<td>5.03</td>
<td>5.2</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
<td>3.2</td>
<td>10</td>
<td>0.12</td>
<td>4.43</td>
<td>2.99</td>
<td>0.16</td>
<td>2.22</td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>3</td>
<td>12</td>
<td>0.01</td>
<td>5.93</td>
<td>2.48</td>
<td>0.01</td>
<td>6.40</td>
<td>1.8</td>
</tr>
<tr>
<td>6</td>
<td>50</td>
<td>3</td>
<td>10</td>
<td>0.04</td>
<td>5.56</td>
<td>2.99</td>
<td>0.14</td>
<td>8.84</td>
<td>4.7</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>3.2</td>
<td>10</td>
<td>0.11</td>
<td>21.04</td>
<td>21.1</td>
<td>0.14</td>
<td>2.27</td>
<td>3.1</td>
</tr>
<tr>
<td>8</td>
<td>40</td>
<td>3.2</td>
<td>12</td>
<td>0.08</td>
<td>2.60</td>
<td>4.46</td>
<td>0.06</td>
<td>9.63</td>
<td>2.6</td>
</tr>
</tbody>
</table>
Table 3.3: Results of fitting response logarithmic model to peak area response data for single standard solutions of DM, HPMC and DM/HPMC in the mixed standards

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Sample Injected Range (µg)</th>
<th>Logarithmic model&lt;sup&gt;1&lt;/sup&gt;</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DM&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.0 - 32.5</td>
<td>3.5</td>
<td>1.6</td>
</tr>
<tr>
<td>HPMC&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.0 - 32.5</td>
<td>3.6</td>
<td>1.5</td>
</tr>
<tr>
<td>DM&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.0 - 32.5</td>
<td>3.4</td>
<td>1.6</td>
</tr>
<tr>
<td>HPMC&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.0 - 32.5</td>
<td>3.7</td>
<td>1.5</td>
</tr>
</tbody>
</table>

<sup>1</sup> Logarithmic model parameters as described in Eq. 3.2.
<sup>2</sup> Excipient prepared as single standard solution
<sup>3</sup> Excipient prepared as mixed standard solution (DM/HPMC)
Table 3.4: ANOVA results of DoE model and significant terms for responses of DM and HPMC 4M standard solutions

<table>
<thead>
<tr>
<th>DoE Responses</th>
<th>ANOVA test results</th>
<th>DM</th>
<th>HPMC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Significant Model Terms</td>
<td>F-value</td>
<td>p-value&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Deviation of Slope</td>
<td>A, C&lt;sup&gt;2&lt;/sup&gt;</td>
<td>M: 11.02</td>
<td>M: 0.01</td>
</tr>
<tr>
<td></td>
<td>A: 8.18</td>
<td>A: 0.03</td>
<td>C: 13.85</td>
</tr>
<tr>
<td></td>
<td>M: 56.27</td>
<td>M: 0.001</td>
<td></td>
</tr>
<tr>
<td>Accuracy</td>
<td>A, C&lt;sup&gt;4&lt;/sup&gt;</td>
<td>A: 50.00</td>
<td>A: 0.002</td>
</tr>
<tr>
<td></td>
<td>C: 61.90</td>
<td>C: 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC: 57.02</td>
<td>AC: 0.001</td>
<td></td>
</tr>
<tr>
<td>Precision</td>
<td>A, B&lt;sup&gt;5&lt;/sup&gt;</td>
<td>A: 8.20</td>
<td>A: 0.04</td>
</tr>
<tr>
<td></td>
<td>B: 11.50</td>
<td>B: 0.02</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup>p < 0.05 indicate model terms are significant

<sup>2</sup>Model Eq: \( \ln(\text{Deviation of Slope for DM}) = 11.04 - 0.13[A] - 0.83[C] \)

<sup>3</sup>Model Eq: \( \ln(\text{Deviation of Slope for HPMC}) = 2.42 - 0.39[C] \)

<sup>4</sup>Model Eq: \( \ln(\text{Accuracy of DM}) = 456.84 - 9.01[A] - 37.88[C] + 0.75[AC] \)

<sup>5</sup>Model Eq: \( \ln(\text{Precision for HPMC}) = 52.99 - 0.22[A] - 12.88[B] \)
Table 3.5: Summary of retention time, sensitivity, LOD, and LOQ for DM and HPMC standard solutions in optimized design space

<table>
<thead>
<tr>
<th>Excipient</th>
<th>Retention time (^1) (min)</th>
<th>Sensitivity (mV/µg)</th>
<th>LOD (µg)</th>
<th>LOQ (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (^2)</td>
<td>15.65</td>
<td>24,691</td>
<td>0.30</td>
<td>0.92</td>
</tr>
<tr>
<td>HPMC (^2)</td>
<td>4.99</td>
<td>34,275</td>
<td>0.11</td>
<td>0.35</td>
</tr>
<tr>
<td>DM (^3)</td>
<td>15.90</td>
<td>24,749</td>
<td>0.33</td>
<td>1.00</td>
</tr>
<tr>
<td>HPMC (^3)</td>
<td>4.91</td>
<td>33,733</td>
<td>0.12</td>
<td>0.36</td>
</tr>
</tbody>
</table>

\(^1\)Retention time of standards is the average of n=10 over a concentration range of 1.0–32.5µg with %RSD <2% for DM and HPMC
\(^2\)Excipient prepared as a single standard solution
\(^3\)Excipient prepared as mixed standards solution
Figures

(a) \( \text{HO} \quad \text{HO} \quad \text{HO} \quad \text{HO} \quad \text{OCH}_2(\text{CH}_2)_10\text{CH}_3 \)

(b) \( \text{(RO}_n\text{OR})_n \)

\[ R = \text{H} \quad \text{or} \quad \text{CH}_3 \quad \text{or} \quad \text{CH}_2\text{CH(OH)}\text{CH}_3 \]

Figure 3.1. Structures of a model surfactant and a polymer: (a) dodecyl \( \beta \)-D-maltoside (DM) and (b) hydroxypropyl methylcellulose (HPMC).
Figure 3.2. Representative SEC-ELSD chromatograms for (a) dodecyl β-D-maltoside (DM), (b) hydroxypropyl methylcellulose (HPMC K-4M), and (c) DM and HPMC K-4M.
Figure 3.3. Standard curves for (a) hydroxypropyl methylcellulose (HPMC) and (b) dodecyl β-D-maltoside (DM) in standard solutions containing mixtures of DM and HPMC. Inset in (a) and (b) show sensitivity of the assay for HPMC and DM, respectively.
Figure 3.4. The 3-Dimensional plot of the desirability index for responses (% deviation of slope, accuracy, precision, and sensitivity) with respect to two significant ELSD variables (pressure and temperature) at an instrument gain value of 10.
Figure 3.5. The 3-Dimensional plot of the desirability index for responses (% deviation of slope, accuracy, precision, and sensitivity) with respect to two significant ELSD variables (pressure and temperature) at an instrument gain value of 12.
CHAPTER 4

Thermodynamics of Aggregate Formation between a Non-Ionic Polymer and Ionic Surfactants: an Isothermal Titration Calorimetric Study

1. Introduction

Aggregate formation between polymers and ionic surfactants is linked with stabilization of nanoparticulate drug delivery systems (NDDS) [8, 16]. NDDS often encounter varying degrees of thermodynamic instability due to the extensive surface area which can lead to nanoparticle aggregation [1, 6, 57]. The higher surface area is accompanied by a large positive free energy, and without any effort to dampen the surface energy, the system tends to move to an equilibrium state of the lowest free energy via aggregation of the smaller particles into larger particles [11, 16, 60]. The adsorption of excipients such as surfactants and polymers onto the surface of NDDS could potentially decrease the surface energy leading to stabilization of the nanoparticles [6, 56].

Several literature studies have explored the effect of surfactant concentration on polymer-surfactant aggregate formation [72, 82, 83]. In general, there are three critical concentrations: (1) critical aggregation concentration (CAC) representing the formation of polymer-surfactant aggregates, (2) polymer saturation concentration (C_{sat}) representing the saturation of the available polymer sites where additional binding of surfactant is not favorable, and (3) critical micelle concentration (CMC) where the formation of surfactant micelles is favorable [84]. While the concentration effect has been well understood, the thermodynamics of polymer-surfactant interactions and related aggregate formation need to be further explored in order to predict NDDS stability.
Most of the literature studies on polymer-surfactant aggregation have focused on non-pharmaceutically relevant systems containing polymers such as poly(2-(dimethylamino)ethyl methacrylate [85, 86], ethylene oxide (EO) copolymer [87, 88], polyethylene oxide (PEO) [89-93], and polyacryl amide [94, 95] as well as surfactants such as lithium dodecylsulfate (LiDS) [92, 96], gemini cationic surfactants [97-99], sodium dodecylbenzenesulfonate [19, 100], and sodium dodecylsulfonate dodecylamine hydrochloride [95]. In the case of pharmaceutically relevant systems, the main body of work has focused on excipients such as sodium dodecyl sulfate (SDS) [101], polyvinylpyrrolidone (PVP) [91, 102, 103], polyethylene oxide (PEO) [92, 103-107], polyethylene glycol (PEG) [102, 108], and hydroxypropyl methylcellulose (HPMC) [27, 109].

The SDS-HPMC system was selected not only because it is pharmaceutically relevant but also due to several gaps that were identified from the review of literature studies containing HPMC such as: (1) the higher concentrations of HPMC typically utilized in NDDS (0.25-1 %w/w) [8, 11, 58] have not been evaluated, (2) the influence of HPMC molecular weight on the number of available binding sites constraints has not been evaluated, (3) the effects of ionic strength and surfactant properties (i.e., head group, chain length) on the interaction between surfactants with HPMC have not been investigated, and (4) relatively less sensitive and selective techniques such as tensiometry, fluorescence and viscometry have been used [102, 123, 124]. In studies where more sensitive techniques such as microcalorimetry were used, the calorimetric data treatment could be further optimized. The use of modern isothermal titration calorimetry (ITC) has gained momentum particularly due to its increased sensitivity and selectivity. Using ITC,
thermodynamic parameters (i.e., enthalpy, free energy, and entropy) for polymer-surfactant aggregate formation and structural rearrangement information can be obtained from a single experiment.

Nilsson [27] evaluated binding of SDS to HPMC in water using viscometry, equilibrium dialysis, dye solubilization and fluorescence techniques for a dilute range of HPMC (0.05-0.2% w/w). The author reported the critical concentration parameters for binding of SDS to HPMC. Although Nilsson’s work applied only to dilute concentrations of HPMC and did not provide significant thermodynamic information, it has a major advantage over other work in that SDS monomer concentrations were measured, giving a basic platform for interpretation of data from other studies including microcalorimetry used in this work. Singh et al. [29] studied the HPMC/SDS system by microcalorimetry and reported thermodynamic parameters for the system. However, the data treatment used in these studies did not take into consideration the formation of various species during the calorimetric titration experiment. Another study by Ridell et al. [109] also used microcalorimetry to determine the effect of counterions on the SDS-HPMC aggregation. They reported that the type of counterion significantly changed the critical concentration parameters and the nature of the aggregates. However, the data treatment lacked the appropriate adjustments needed to account for speciation occurring during titration. In the above-mentioned studies, accurately accounting for the enthalpies of dilution of all species could have allowed for more accurate measurements of the energetics of interactions and interpretation of the enthalpy plots than those reported.

The goal of this work was to determine the energetics of aggregate formation between the model non-ionic polymer, HPMC, and model ionic surfactants, including
SDS, hexadecyltrimethylammonium bromide (CTAB) and dodecyltrimethylammonium bromide (DTAB) using ITC. A novel data treatment was used in conjunction with ITC that increased the accuracy of the measured thermodynamic parameters of aggregation and subsequent interpretation. This treatment involved the identification and appropriate accounting for the concentration-dependent species (i.e., surfactant monomers and micelles, and surfactant-HPMC aggregates). More detailed descriptions of the species formed and the process of accounting for the enthalpies of dilution are provided in the ITC method validation section. Additionally, the influence of ionic strength, the HPMC molecular weight, and the type of ionic surfactant head group on the aggregate formation process was explored. The understanding from this study was used in subsequent studies to characterize the nature of HPMC-ionic surfactant aggregates (Chapter 5) and their adsorption onto the surface of nanoparticles (Chapter 6).

2. Materials and Methods

2.1. Materials

Sodium dodecyl sulfate (>98%) was obtained from Sigma-Aldrich (St. Louis, MO) and was further purified by solid phase extraction by passing a 1% w/w aqueous solution of SDS through a Waters SEP-PAK® plus C18 environmental cartridge. The purified SDS solution was then lyophilized, and surface tension measurements were conducted for reconstituted SDS to assess the presence of any local minimum near the critical micelle concentration (CMC) of the surfactant by the du Noüy ring tensiometer. An absence of such a local minimum showed that the purified SDS was not found to be affected by the presence of trace amounts of dodecanol that are commonly found in the commercially available SDS, thus allowing the usage of purified SDS. The cationic surfactants including
DTAB and CTAB were purified in the same way as SDS with the Waters SEP-PAK® plus C18 environmental cartridges, and the extracted solution was then lyophilized and examined for the presence of any local minimum near the CMC. The absence of local minima near the respective CMCs of DTAB and CTAB during the surface tension measurements for both purified and unpurified DTAB and CTAB resulted in the use of these chemicals as received from Sigma-Aldrich (St. Louis, MO). HPMC polymers varying in molecular weight (Benecel® K-4M, K-15M and K-100M) were obtained from Ashland Aqualon Functional Ingredients, Ashland Inc. (Wilmington, DE) and used as received. Sodium chloride (NaCl) was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. All solutions were prepared using purified water (18.2 megohm-cm) obtained from a Milli-Q water purification system (Millipore, Billerica, MA).

2.2. Methods

The calorimetry experiments were conducted using a TAM III isothermal titration calorimeter (ITC) manufactured by TA Instruments (New Castle, DE). The TAM III calorimeter operates in power compensation mode (Figure 4.1) wherein the temperature of the sample cell is maintained constant using a temperature sensor with a feedback system utilizing a reference cell. When an endothermic or exothermic event (i.e., chemical reaction, molecular reorganization, binding, solubilization) occurs during titration, the power supplied to a heater or cooler to maintain isothermal conditions is directly measured [118]. Surfactant solutions at 10x CMC concentrations (i.e., 87.3 mM for SDS, 129.7 mM for DTAB and 9.1 mM for CTAB, respectively) were prepared and loaded into either a 1 ml or 5 ml syringe mounted on a precision pump. The surfactant solutions were then titrated into sample cells containing known quantities of either deionized purified water.
(2.1 ml or 2.4 ml) or HPMC solution. At the start of an experiment, the reference cell contained the same solution as the sample cell. Titrations at each predetermined time interval were performed by a syringe under computer control that injected either 20 or 25 µl of surfactant solution into the sample cell. Usually, 5 to 7 minutes were provided between injections to allow time for thermal equilibration while the sample cell was continuously stirred (60 rpm) with a turbine stirrer. Thus, in a given ITC measurement, the energetics associated with a process was directly measured at a constant temperature. For example, if an exothermic event occurred during the titration, the compensation mode (feedback loop) would cool the sample until the temperature of the sample cell was brought to the temperature of the reference cell. The signal (peak) thus obtained from the feedback loop was integrated directly to yield the heat associated (Q) with that event. As shown in Figure 4.2a, the heat signal (Q) was directly related to the concentration and volume of the titrant in each injection, which was normalized with respect to the moles (δn) of surfactant added to the sample cell. The signal was further analyzed to obtain the apparent enthalpy change (ΔH_{app} = Q/δn) using TAM III Lab Assistant Software provided by TA Instruments. The apparent enthalpy change (ΔH_{app}) was plotted against the surfactant concentration as illustrated by the enthalpograms in Figure 4.2b. All experiments were repeated at least twice to confirm reproducibility of the measurements. Representative SDS-HPMC interaction data are shown in Appendix.1.

3. Results and Discussion

The goal of this work was to determine the effect of various parameters including temperature, ionic strength, and excipient properties (i.e., the molecular weight of polymer,
head-group and chain length of surfactant) on the energetics of surfactant-HPMC aggregation using ITC with a novel data treatment method.

3.1. ITC Method Validation using Ionic Surfactant Micellization Parameters

The properties of the micelles of three model ionic surfactants, SDS, CTAB, and DTAB, have been studied previously using techniques such as ITC, NMR and surface tensiometry [31, 144, 145]. Hence the ITC methodology employed herein was first validated by comparing the values of micellization parameters (i.e., CMC, enthalpy, entropy, and free energy of micellization) determined in the present study with those reported in the literature [146-148].

In all experiments, the micellar solution of a model surfactant (~10x CMC) was added into a thermodynamically isolated cell containing deionized water at a selected temperature. The apparent enthalpy ($\Delta H_{app}$) determined from heat signal (see “Methods” for more details) was plotted against surfactant concentration. The enthalpograms ($\Delta H_{app}$ vs. surfactant concentration) for CTAB, SDS, and DTAB are presented in Figures 4.2b, 4.3a, and 4.3b, respectively. As a micellar surfactant solution is mixed with deionized water in the sample cell, an extremely dilute solution of surfactant monomers is formed, a process referred to as demicellization. In Figures 4.2b and 4.3, the first plateau seen in the enthalpogram is denoted as demicellization plateau which is attributed to cumulative heat changes associated with demicellization, dilution of surfactant monomers and interactions with their counterions [149, 150]. As surfactant concentration in the sample cell reaches the critical micelle concentration (CMC), a sharp decrease in the apparent enthalpy is observed at a concentration of ~8 mM for SDS, ~14 mM for DTAB, and ~1 mM for CTAB, respectively (Figures 4.2b and 4.3). After this point, the process mainly involves titrating
the micellar surfactant solution in the syringe into the micellar solution already present in
the sample cell as indicated by a second plateau in the enthalpograms (Figures 4.2b and
4.3). This plateau is associated with the apparent enthalpies of dilution of micelles. Similar
ITC profiles have been reported for anionic surfactants such as SDS, SDES (sodium
decylsulfate), sodium dodecyl benzene sulfonate (SDBS) and cationic surfactants such as
CTAB, DTAB and TTAB [148, 151]. Blandamer et al. [124] described the shape of the
ITC plot for SDS and SDES in terms of demicellization titration. Some deviations from
the ideal enthalpy were observed that were correlated to the extent to which the properties
of the solution present in the sample cell deviated from the ideal state. Beyer et al. [148]
studied the demicellization of alkyltrimethylammonium bromides in 0.1 M sodium
chloride solution by ITC. The endothermic part of the enthalpogram was considered to be
the result of the demicellization of ionic micelles and the subsequent dilution of the
resultant surfactant monomers followed by dilution of the micelles formed at
concentrations higher than the CMC.

The consistent view in the literature is that the change in apparent enthalpy for
surfactant micellization may include various components such as shown below in Eq. 4.1.
The enthalpy of demicellization ($\Delta H_{demi}^{\circ}$) is equal in magnitude to the enthalpy of
micellization although it bears an opposite sign [118, 148]. The components described in
Eq. 4.1 would vary depending on the species expected at equilibrium in the sample cell in
different regions of the surfactant micellization enthalpogram. For example, for the
titrations where surfactant monomers are expected to predominate in the sample cell,
apparent enthalpy ($\Delta H_{app}$) is described by Eq. 4.1. The region where a fraction of
surfactant micelles are expected to dissociate is described by Eq. 4.2 and finally in the
region near the CMC (i.e., 7-10 mM SDS at 25°C) where titrations consist of mere dilution of micelles (e.g. >10 mM SDS concentration at 25°C; second plateau) in the sample cell, $\Delta H_{\text{app}}$ can be described by Eq. 4.3.

$$\Delta H_{\text{app}} = \Delta H_{\text{dil}}(\text{mon},s) - \Delta H_{\text{mic}}^°$$  \hspace{1cm} (4.1)  

$$\Delta H_{\text{app}} = \Delta H_{\text{dil}}(\text{mon},s) - (1 - f)\Delta H_{\text{mic}}^° + \Delta H_{\text{dil}}(\text{mic},s)$$  \hspace{1cm} (4.2)  

$$\Delta H_{\text{app}} = \Delta H_{\text{dil}}(\text{mic},s)$$  \hspace{1cm} (4.3)  

where $\Delta H_{\text{dil}}(\text{mon},s)$ and $\Delta H_{\text{dil}}(\text{mic},s)$ are the enthalpies of dilution of the surfactant monomers and surfactant micelles above the CMC, respectively. $(1 - f)$ is the fraction of micelles that dissociate in the sample cell and the value of $f$ increases from 0 to 1 when the SDS concentration increases from 6-11 mM with $f = 0.5$ at CMC. The $\Delta H_{\text{dil}}(\text{mon},s)$ could also be a cumulative effect of dilution of monomers and the formation of smaller self-associated aggregates such as dimers, trimers etc. $\Delta H_{\text{dil}}(\text{mon},s)$ and $\Delta H_{\text{dil}}(\text{mic},s)$ were measured independently by direct experimentation utilizing ITC. The values of $(1 - f)\Delta H_{\text{mic}}^°$ are directly obtained from the top and bottom of plateau regions of the SDS enthalpogram. $\Delta H_{\text{mic}}^°$ represents the standard enthalpy of micellization per mole of surfactant monomer unit. To determine $\Delta H_{\text{mic}}^°$ directly from the enthalpograms, lines are drawn to fit the two plateaus above and below the observed inflection point at the CMC. A line is drawn perpendicular to the x axis and $\Delta H_{\text{mic}}^°$ is measured from the length of the segment connecting the two extrapolated lines (Figure 4.2b). The $\Delta H_{\text{mic}}^°$ for SDS at 25°C was determined to be -0.68 kJ/mol (Table 4.1), which is close to the values of -0.75 kJ/mol reported by Woolley et al. [146] and -0.5 kJ/mol reported by Singh et al. [29]. The
\( \Delta H_{mic} \) for DTAB, at 25 °C was determined to be -1.7 kJ/mol (Table 4.2), which is similar to the value of -1.9 kJ/mol reported by Beyer et al [148].

The CMC value is determined from the extremum (highest peak) of the first derivative of the \( \Delta H_{app} \) vs. surfactant concentration (Figure 4.2c). For SDS, the CMC was determined to be 8.3 mM (Table 4.1), in agreement with the values reported by Philips et al. (8.1 mM) and Horin et al. (8.2 mM) [46, 152, 153]. The CMC’s for CTAB and DTAB at 25°C were determined to be 1.25 mM and 14.49 mM, respectively (Figure 4.2b and Table 4.2). Blandamer et al. [154] reported the CMC for CTAB at 25°C to be 0.97 mM utilizing ITC while Beyer et al. [148] utilizing the same technique reported the CMC for DTAB at 25°C as 13.5 mM, both of which are similar to the values determined here. Overall, the micellization parameters such as CMC and \( \Delta H_{mic} \) for three model ionic surfactants determined in this study were in good agreement with the literature values, which validated the ITC methodology.

3.2. Thermodynamics of Model Ionic Surfactant Micellization: Phase Separation Model

Additional thermodynamic parameters such as standard free energy (\( \Delta G_{mic}^o \)), standard enthalpy (\( H_{mic}^o \)), standard entropy (\( \Delta S_{mic}^o \)), and heat capacity (\( \Delta C_p \)) of micellization for the model ionic surfactants SDS and DTAB were determined using the phase separation model [85]. According to the phase separation model, micellization is assumed to be a highly cooperative (rather than a progressive stepwise association of surfactant monomers), the one-step process leading to the formation of a separate phase. In other words, at or above CMC, a surfactant system contains two separate phases (i.e., surfactant monomers and surfactant micelles) that are in equilibrium. The phase separation
model has been shown to be a special case of the mass action model when the aggregation number is large [155]. The micellization process can be represented by Eq. 4.4.

\[ nS^- + mX^+ \overset{K}{\leftrightarrow} [S_nX_m]^{(n-m)-} \tag{4.4} \]

where each micelle ([S_nX_m]^{(n-m)-}) formed is assumed to contain \( n \) surfactant ions (\( S^- \)) and \( m \) dissociated counterions (\( X^+ \)) with the fraction of charge for surfactant ions of \( \frac{m}{n} = \alpha \) (i.e., degree of ionization) in each micelle to give a net electroneutrality. The equilibrium constant \( (K) \) can be expressed as Eq. 4.5

\[ K = \frac{[S_nX_m]^{(n-m)-}}{[S^-]^n[X^+]^m} \tag{4.5} \]

The standard free energy of micellization (\( \Delta G^\circ_{mic} \)) is the standard free energy per mole of surfactant monomer or the free energy of micellization for 1 M SDS at 25°C, \( \Delta G^\circ/n \) as determined by Eq. 4.6

\[ \Delta G^\circ_{mic} = \frac{\Delta G^\circ}{n} = \frac{-RT}{n \ln K} \tag{4.6} \]

where \( n \) is the micelle aggregation number. As per the phase separation model, at CMC, \([S^-]= [X^+]= \text{CMC} \) [146, 156]. Thus, the CMC Eq. 4.6 can be computed as

\[ \Delta G^\circ_{mic} = -RT \left[ \frac{1}{n} \ln[S_nX_m]^{(n-m)-} - \ln(CMC) - \frac{m}{n} \ln(CMC) \right] \tag{4.7} \]

In Eq. 4.7, the first term can be neglected due to its negligible influence [149] on the thermodynamic parameters and Eq. 4.7 is computed as

\[ \Delta G^\circ_{mic} = RT \left( 1 + \alpha \right) \ln(CMC) \tag{4.8} \]
where $\frac{m}{n} = \alpha$ is the counterion association for the micelle, $\alpha$, and is assumed to be one by accounting for 100% counterion binding on the micelle for ionic surfactants [72]. Thus, Eq. 4.8 becomes

$$\Delta G_{mic}^\circ = 2RT \ln(CMC) \quad (4.9)$$

In Eq. 4.7 it is assumed that the ionic micelle has net electroneutrality. This is attributed to the self-aggregation of ionic monomers and their binding with an equal number of counterions to form neutral micelles [149]. The effect of temperature on the CMC and $\Delta H_{mic}^\circ$ for SDS and DTAB were determined at 25°C, 32°C, 40°C and 50°C (Figures 4.3 and Table 4.1-4.2). The thermodynamic parameters such as $\Delta G_{mic}^\circ$ and $\Delta S_{mic}^\circ$ for SDS and DTAB were obtained from Eq. 4.9 and 4.10 and Tables 4.1 and 4.2. The $\Delta G_{mic}^\circ$ is twice that of a nonionic micelle having the same CMC since two species bind together to form a micelle for ionic surfactants.

$$\Delta G_{mic}^\circ = \Delta H_{mic}^\circ - T\Delta S_{mic}^\circ \quad (4.10)$$

The values of $\Delta G_{mic}^\circ$ of SDS and DTAB micellization at 25°C were determined to be strongly negative and remained favorable at higher temperatures (Tables 4.1 and 4.2). The values of $\Delta S_{mic}^\circ$ and $\Delta H_{mic}^\circ$ of SDS and DTAB micellization were observed to decrease and increase with temperature, respectively. Due to the enthalpic-entropic compensation, the overall free energy of micellization of SDS and DTAB remains fairly constant with temperature [100, 148]. In comparison to CTAB, SDS micellization is more enthalpically favored with increasing temperatures as indicated by the magnitude of change in $\Delta H_{mic}$. The CMCs of both SDS and DTAB shifted slightly with increasing temperature indicating that the micellization of SDS and DTAB is mainly driven by hydrophobic interactions.
Another thermodynamic parameter evaluated in this study was the heat capacity of micellization ($\Delta C_p^\circ$), which was obtained from the slope of $\Delta H_{mic}^\circ$ vs. $T$. As shown in Tables 4.1 and 4.2, $\Delta C_p^\circ$ is -0.5 kJ/mol and -0.4 kJ/mol for SDS and DTAB micellization, respectively. The negative $\Delta C_p^\circ$ for SDS and DTAB micelles is consistent with the transfer of hydrophobic SDS/DTAB monomers from an aqueous phase into a more hydrophobic micellar phase [160, 161]. These results are in agreement with the literature wherein the hydrophobic effect is considered as a predominant driving force for the micellization of ionic surfactants [146, 161].

3.3. Influence of HPMC on the Energetics of Ionic Surfactant-HPMC Aggregation Process

To investigate the energetics of interactions between HPMC and model ionic surfactants (SDS and DTAB), ITC and the phase separation model were utilized. Figure 4a shows enthalpograms for the titration of an SDS micellar solution (10x CMC; ~87.3mM) into a sample cell containing 0.25% w/w or 0.5% w/w HPMC K-4M solution at 25°C. A distinct endothermic peak is observed upon titration of SDS into HPMC K-4M, indicating that the enthalpograms obtained in the presence of HPMC differ from those obtained without HPMC as described earlier for SDS/water (Figures 4.3 and 4.4a). This difference could be attributed to interactions between SDS and HPMC [104]. As shown in Fig 4.4a, the change in apparent enthalpy for SDS-HPMC interactions ($\Delta H_{app}$) over the entire curve (cumulative of all regions of the enthalpogram) may reflect various contributions as shown in the model below, Eq. 4.11 [100, 110, 118].

$$\Delta H_{app} = \Delta H_{dil(mic,s)} + \Delta H_{(p-s)} + \Delta H_{dil(p-s)} - \Delta H_{mic}^\circ + \Delta H_{dil(mic,s)} + \Delta H_{dil(p)}(4.11)$$
where $\Delta H_{dil(p)}$ is the enthalpy of dilution for HPMC, $\Delta H_{(p-s)}$ is the enthalpy for SDS-HPMC interactions and $\Delta H_{dil(p-s)}$ is the enthalpy of dilution of the SDS-HPMC aggregates and the remaining terms are applicable to the micellization of pure SDS as described in the previous sections.

In previous studies with SDS-polymer systems, the treatment of raw ITC data either including no correction [91, 103] or with the corrected $\Delta H_{app}$ ($\Delta H_{corr}$) by subtracting the SDS enthalpy of dilution curve (demicellization enthalpogram; Figure 4.4a (squares)) in the absence of polymer [29]. $\Delta H_{corr}$ in all corrected equations below correspond to the terms $\Delta H_{(p-s)} + \Delta H_{dil(p-s)}$. Although a correction of the raw ITC data is required for an accurate interpretation, a single subtraction of the SDS demicellization enthalpogram may not be appropriate in all regions of the SDS-HPMC enthalpogram because the presence of HPMC significantly alters the SDS monomer and micellar fractions [27]. For example, as shown in Figure 4.4a, the CMC for SDS is lower (e.g. >8 mM at 25°C) than SDS-HPMC system (e.g. >17 mM at 25°C and 0.25% HPMC). Thus, a simple subtraction of the SDS demicellization enthalpogram without careful consideration of the species present could result in inaccurate values of the thermodynamic parameters associated with the aggregation and micellization.

For a more accurate interpretation of the ITC data, a novel correction approach was used in this study by accounting for the various species expected at equilibrium in the sample cell. At the start of titration where SDS monomers, smaller self-associated aggregates such as dimers, trimers etc. or both are expected to predominate (Figure 4.4b, A→C region), the corrected enthalpy ($\Delta H_{corr}$) value is obtained using Eq. 4.12.
\[ \Delta H_{\text{corr}(A\rightarrow C \text{ region})} = \Delta H_{\text{app}} - \left( \Delta H_{\text{mic}}^o + \Delta H_{\text{dil}(\text{mon,s})} + \Delta H_{\text{dil}(p)} \right) \]  

Similarly, in the region where the formation of SDS micelles is expected (Figure 4.4b, C→D region), \( \Delta H_{\text{corr}} \) value obtained is processed as per Eq. 4.13.

\[ \Delta H_{\text{corr}(C\rightarrow D \text{ region})} = \Delta H_{\text{app}} - (\Delta H_{\text{dil}(\text{mon,s})} + (1 - f)\Delta H_{\text{mic}}^o + \Delta H_{\text{dil}(p)}) \]  

In C→D region, the initial part of the curve until the inflection point (i.e., CMC) contains the \( \Delta H_{\text{dil}(\text{mon,s})} \) and \( \Delta H_{\text{dil}(p)} \) terms followed by the \( \Delta H_{\text{mic}}^o \) and \( \Delta H_{\text{dil}(p)} \) terms along with a third term \( (1 - f)\Delta H_{\text{mic}}^o \), where \( (1 - f) \) is the fraction of micelles that dissociate in the sample cell and the value of \( f \) increases from 0 to 1 when the SDS concentration increases from 7-21 mM with \( f = 0.5 \) at CMC. The \( \Delta H_{\text{dil}(\text{mon,s})} \) could also be a cumulative effect of dilution of monomers and the formation of smaller self-associated aggregates such as dimers, trimers etc. Thus, the overall \( \Delta H_{\text{corr}} \) in the C→D region is processed by Eq. 4.13.

Finally, in the region where predominantly all the SDS being titrated into the sample cell is expected to remain as SDS micelles (Figure 4.4b; D region), \( \Delta H_{\text{corr}} \) value obtained is processed as per Eq. 4.14.

\[ \Delta H_{\text{corr}(\geq D \text{ region})} = \Delta H_{\text{app}} - (\Delta H_{\text{dil}(\text{mic,s})} + \Delta H_{\text{dil}(p)}) \]  

Overall, this approach should address the over and under correction of the raw data mentioned above where the corrected enthalpy (\( \Delta H_{\text{corr}} \)) value corresponds to \( \Delta H_{(p-s)} + \Delta H_{\text{dil}(p-s)} \) accurately accounting for the various species present in sample cell.

For the \( \Delta H_{\text{corr}} \) determination in this study, the enthalpy of dilution was measured independently by ITC and subtracted as per Eq. 4.12, 4.13 and 4.14. Figure 4.4a shows the enthalpograms at 25°C for pure SDS in water (0% HPMC) and SDS-HPMC systems.
(0.25% and 0.5% HPMC). Figure 4.4b shows the corrected enthalpy ($\Delta H_{corr}$) plotted as a function of SDS concentration at 25°C, the corrected enthalpograms preserve the shape of uncorrected enthalpograms lending further to the selection of 25°C as the ideal temperature for investigating the SDS-HPMC interactions. For the SDS-HPMC interactions investigated at higher temperatures of 32°C and 40°C, the corrections were carried out as per Eq. 4.12, 4.13 and 4.14 however, slight adjustments were made to account for the $f$ values.

In the A→B region (Figure 4.4b), a sharp increase in $\Delta H_{corr}$ at ~4 mM SDS concentration shows the first critical concentration denoted as the critical aggregation concentration (CAC) [90]. As SDS concentration increases above the CAC, an endothermic maximum is observed indicating increasing interactions between HPMC and SDS. The sudden and sharp increase in $\Delta H_{corr}$ in this region could signal cooperative SDS-HPMC interactions at 25°C due to the availability of multiple sites for interactions between SDS and HPMC [162]. The concentration of HPMC influences the CAC, with the slightly lower CAC and a sharper slope of the endothermic curve at 0.5% w/w as compared to 0.25% w/w of HPMC reflecting a slight increase in cooperativity (Figure 4.4b & Table 4.3) [27].

SDS and HPMC could exhibit hydrophobic and hydrogen bonding interactions since it is known that along with being moderately hydrophobic in nature, HPMC also has both hydrogen bond acceptor and donor groups while the SDS head group can accept hydrogen bonds. Therefore, the question in this case would be which of the two interactions is dominant in SDS-HPMC aggregate formation. Considering the moderately hydrophobic nature of HPMC, it was postulated that these interactions might be driven by
the hydrophobic effect. In order to support this hypothesis, the influence of temperature on the CAC and the endothermic peak of the SDS-HPMC interactions was investigated. Previous studies have also shown that if polymer-surfactant interactions are driven by the hydrophobic effect, CAC and endothermic peak should diminish with increasing temperature due to the breakdown of hydration shell (water structure) surrounding the hydrophobic regions of polymers and surfactants at higher temperatures [147, 160, 163].

In Figure 4.5, while the general shapes of titration curves remain similar, as the temperature increases from 25°C to 40°C, the CAC is no longer as pronounced, and the endothermic peak is almost undistinguishable at 40°C. The temperature dependence of the CAC and the endothermic peak for SDS-HPMC systems support the hypothesis that the hydrophobic effect is a driving force for the HPMC and SDS interactions.

In B→C region (Figure 4.4b), as SDS concentration increases, $\Delta H_{corr}$ decreases. This decrease in $\Delta H_{corr}$ could be indicative of decreased hydrophobic interactions. Figure 4.5 shows that the B→C region in SDS-HPMC enthalpograms vary with temperature suggesting hydrophobic interactions could be involved [147, 160, 163]. However, the enthalpy change becomes more endothermic with an increase in temperature, which is the opposite of what is generally expected with hydrophobic interactions [82]. This inverse temperature dependence may also suggest a decrease in hydrophobic interactions.

As shown in Figure 4.4b, a sharp almost linear decrease in the endothermic peak is seen leading to an exothermic minimum at ~17 mM SDS concentration (0.25% HPMC) at 25°C. The decrease in endothermic peak and the exothermic nature of the curve may be attributed to the restructuring of SDS-HPMC aggregates upon higher adsorption of SDS on the HPMC chains [82, 157, 164]. Moreover, the rehydration of hydrophobic groups of
HPMC such as hydroxypropyl and methyl groups is expected to be exothermic based on the reported enthalpy of hydration of -10 kJ/mol for propan-1-ol [165] and 0 kJ/mol for methyl [166] groups at 25°C. The restructuring of the SDS-HPMC aggregate network at higher SDS concentrations could be attributed to the expansion and rehydration of the HPMC chains caused by the electrostatic repulsion exerted by the anionic head groups of the densely adsorbed SDS (43, 44). Using a different methodology based on equilibrium dialysis and intrinsic viscosity measurements, Nilsson [27] showed that the aggregation number of SDS-HPMC aggregates increased linearly from <10 to 50 while the SDS monomer concentration remained constant when SDS concentration increased from 5 mM-16 mM (0.2% w/w HPMC), which was attributed to the restructuring of SDS-HPMC aggregate network and intermolecular networking capability of HPMC [27]. For other surfactant-polymer systems such as PVP-SDBS and PEG-SDS, authors have reported an expansion of polymer chains due to the electrostatic repulsion of the adsorbed surfactant molecules and rehydration of polymer chains at higher surfactant concentrations [82, 167].

In Figure 4.4b, the exothermic minima (i.e., 18 mM for 0.25% w/w HPMC at 25°C) may indicate saturation of HPMC chains with the adsorbed SDS, which is also known as the polymer saturation concentration (C_{sat}) [27, 72]. The C_{sat} shifts from 18 mM at 0.25% w/w HPMC to 21 mM at 0.5% w/w HPMC concentration, which lends support that more SDS is needed to saturate a greater number of HPMC binding sites. The amount of SDS needed to saturate twice the amount of HPMC (from 0.25% w/w to 0.5% w/w) is only ~3 mM and thus it is assumed that the number of sites binding sites on the HPMC may not be proportional to the total amount of HPMC in solution and could be related to conformational changes of HPMC with increasing concentration.
Finally, in the last C→D region of the titration curve, the $\Delta H_{corr}$ increases somewhat with increasing SDS concentrations and reaches a plateau at approximately $\Delta H_{corr}$ values close to zero (Figure 4.4b). This may be signaling that after the available binding sites on HPMC are saturated with SDS, the newly added SDS monomers mutually begin to interact until a CMC ($C_m$) for SDS is achieved and pure SDS micelles begin to form. As shown in Figure 4.4b, the $C_m$ values for SDS were 21mM and 25 mM for 0.25% w/w HPMC and 0.5% w/w HPMC, respectively. The $\Delta H_{corr}$ in this region for SDS-HPMC system is approximately zero and superimposes on the $\Delta H_{corr}$ of pure SDS micelle dilution region in the enthalpograms. It was the least influenced by temperature, which supports the formation of pure SDS micelles above $C_m$.

3.4. Thermodynamic Parameters of SDS-HPMC Aggregation

For a more in-depth understanding of the driving forces for the SDS-HPMC interactions, the standard free energy of aggregation ($\Delta G_{agg}^{\circ}$), standard enthalpy of aggregation($H_{agg}^{\circ}$) and standard entropy of aggregation ($\Delta S_{agg}^{\circ}$) are determined (Table 4.3). The $\Delta G_{agg}^{\circ}$ was computed using the phase separation [85] model and Eq. 4.9 as described in the previous section. The standard free energy of aggregation per mole of surfactant monomer unit, $\Delta G^{\circ}/n$, is

$$\Delta G_{agg}^{\circ}/n = RT/n \ln K \tag{4.15}$$

where $n$ is the number of moles of surfactant and $[S^-]= [X^+]=CAC$; thus, at CAC it can be computed as Eq. 4.15 that can be further rearranged to Eq. 4.17.

$$\Delta G_{agg}^{\circ} = 2RT \ln(CAC) \tag{4.16}$$

$$\Delta G_{agg}^{\circ} = \Delta H_{agg}^{\circ} - T\Delta S_{agg}^{\circ} \tag{4.17}$$
As shown in Table 4.3, the value of $\Delta G_{agg}^\circ$ for SDS-HPMC aggregation process is found to be strongly negative indicating an energetically favorable process. The values of $\Delta G_{agg}^\circ$ for SDS-HPMC aggregation is not observed to be sensitive to temperature (Table 4.3), which may indicate the mechanism of enthalpy-entropy compensation for the SDS-HPMC aggregation process [82]).

Furthermore, as the $\Delta G_{agg}^\circ$ does not provide a complete picture of the free energy for the SDS-HPMC aggregation process at high SDS concentrations, the phase separation model with the ratio of $\frac{C_{AC}}{C_{MC}}$ is utilized for determining the free energy ($\Delta G_{Tr}^\circ$) associated with transfer of surfactant molecule from micelles to a binding site on the polymer. This equation provides information on the strength of interactions between HPMC and surfactant at a specified temperature [68] and is given below

$$\Delta G_{Tr}^\circ = \Delta G_{agg}^\circ - \Delta G_{mic}^\circ = RT \ln \frac{C_{AC}}{C_{MC}}$$

where $\Delta G_{Tr}^\circ$ is the standard free energy of transfer of one mole of SDS from pure to SDS-HPMC mixed micelles. The $C_{MC}$ is the inflection point of the curve and the $C_{AC}$ is the breakpoint observed, indicating increased interactions between HPMC and SDS (Figure 4.4b). For SDS-HPMC aggregates, the ratio was found to be dependent on the polymer concentration and became slightly more negative with increasing polymer concentration. The $\Delta G_{Tr}^\circ$ increased slightly from -42.2 kJ/mol to -43.1 kJ/mol for 0.25% and 0.5% w/w HPMC, respectively as the CAC decreased to a slightly lower SDS concentration while the endothermic maxima increased with an increase in the HPMC concentration (Figure 4.4).
The interaction behavior between SDS and HPMC consists of a few other thermodynamic components (i.e., \( \Delta H^\circ_{agg} \) and \( \Delta S^\circ_{agg} \)) that are of considerable importance towards understanding the mechanism of the interaction process. Additionally, the standard enthalpy of aggregation (\( \Delta H^\circ_{agg} \)) is directly obtained from the enthalpogram (\( \Delta H_{corr} \) vs. surfactant concentration) and is defined as the standard enthalpy of aggregation per mole of surfactant monomer unit (Figure 4.4b). For the determination of \( \Delta H^\circ_{agg} \), lines are drawn to fit the start of interaction or CAC (shown as dotted line A) and the peak point (shown as dotted line B) of the endothermic curve, a line is drawn perpendicular to the x axis and \( \Delta H^\circ_{agg} \) is measured from the length of the segment connecting the lines. A similar approach to determine \( \Delta H^\circ_{agg} \) was reported by Torn et al. [82] while investigating the aggregation behavior between PVP and SDBS by ITC. \( \Delta H^\circ_{agg} \) for SDS-HPMC aggregates in this study is determined to be 2.8 kJ/mol reflecting cooperative interactions between SDS and HPMC that decreased with increasing temperature (Table 4.3).

As discussed in the previous section since the SDS-HPMC aggregation process is not favored enthalpically, an increase of overall entropy is required to compensate unfavorable enthalpy of the aggregate formation [168]. The standard entropy (\( \Delta S^\circ_{agg} \)) can be obtained from Eq. 4.17. The breaking of the H-bonding network of the water structure at it reorganizes during the interaction process may reflect the increase in the overall \( \Delta S^\circ_{agg} \) (100.1 J/K mol at 25°C) for the SDS-HPMC system. The relative gain in entropy of the system as a result of the hydrophobic interactions at higher temperatures is expected to be less as the water molecules already possess a higher state of disorder [163, 168].

3.5. Effect of Molecular Weight of HPMC on the Energetics of Surfactant-HPMC Aggregation Process
The molecular weight (M_w) of a polymer may influence the energetics of polymer-surfactant interactions that are associated with the conformational changes of polymer to attain the most stable aggregate structures [169]. In the previous section, it was determined that C_{sat} increased modestly with the concentration of HPMC, which could be attributed to the number of available sites on the HPMC surface for SDS-HPMC interactions to occur. Hence it is reasonable to expect an influence of M_w of HPMC on SDS-HPMC interactions. The effect of HPMC M_w on the energetics of SDS-HPMC aggregation is presented in Figure 4.6 (0.25% w/w HPMC). From the enthalpogram, it is evident that the titration curves for all three M_w s of HPMC K-4M, K-15M, and K-100M followed the same profile and showed similarly shaped curves. However, the enthalpogram of the highest M_w HPMC (i.e., K-100M) was different from the lower M_w HPMC’s (i.e., K-4M and K-15M). Similar to HPMC K-4M (previous section), the SDS-HPMC enthalpograms for the two higher M_w grades of HPMC (K-15M and K-100M) show an endothermic maximum (A→B) followed by an exothermic minimum (B→C) before increasing again and merging with the pure SDS micelles (C_m) dilution curve at approximately zero enthalpy change (C→D) (Figure 4.6).

The number average molecular weights (M_n) for HPMC K-4M, K-15M, and K-100M are 86 kDa, 120 kDa and >240 kDa, respectively. Moreover, the substitution patterns for HPMC K-grades used in this study are similar, consisting of the same methyl (19-24%) and hydroxypropyl (7-12%) groups. Hence, the hydrophobicity of these three HPMC grades is expected to be similar. Since the CAC is known to be sensitive to the hydrophobicity of polymer, it should not alter the change in HPMC M_w [167]. As shown in Figure 4.6, the CACs for the two lower M_w HPMC (K-4M and K-15M) are similar,
while the CAC for the higher M\textsubscript{w} HPMC (K-100M) is slightly lower. The slight decrease in the CAC at higher M\textsubscript{w} may be attributed to conformational differences between the low and high M\textsubscript{w} grades of HPMC with the HPMC K-100M having less accessible non-polar surfaces [105]. The \( C_{\text{sat}} \) and \( C_{\text{m}} \) of the higher M\textsubscript{w} HPMC-100M are lower than those of the lower M\textsubscript{w} HPMCs (K-4M and K-15M) (Figure 4.7). The \( C_{\text{sat}} \) and \( C_{\text{m}} \) for HPMC K-100M are \( \sim13 \) mM and 15 mM, respectively, as compared to \( \sim17 \) mM and \( \sim20 \) mM for HPMC K-4M and HPMC K-15M, respectively. Overall, the molecular weight of HPMC is determined to influence \( C_{\text{sat}} \) and \( C_{\text{m}} \). To the best of our knowledge, these are the first reported values to show the influence of HPMC M\textsubscript{w} on the SDS-HPMC aggregate formation, which attempts to fill the gap in developing an in-depth understanding for this system [29].

When normalized to HPMC M\textsubscript{w}, the values of \( C_{\text{sat}} \) and \( C_{\text{m}} \) for SDS-HPMC systems remain distinctly lower for the highest M\textsubscript{w} HPMC K100M, which indicates that the number of bound SDS is not proportional to the chain length of HPMC. The lower values of \( C_{\text{sat}} \) and \( C_{\text{m}} \) for the highest M\textsubscript{w} HPMC K-100M suggests that the number of binding sites available for SDS adsorption on HPMC K-100M could be lower than those for the other two lower M\textsubscript{w} HPMCs. This may be attributed to the conformation difference for HPMC K-100M consisting of more buried chains thus providing considerably fewer binding sites for SDS [103]. Dai et al. [167] also showed for PEG-SDS aggregate an inverse dependence of molecular weight of PEG on the SDS and PEG interaction that was attributed to the lower number of available sites for higher M\textsubscript{w} PEG as compared to lower M\textsubscript{w} PEG.

3.6. Influence of Ionic Strength on the Energetics of Surfactant-HPMC Aggregation Process
Figure 4.7 shows the SDS-HPMC enthalpograms at three ionic strengths using 0.1, 0.3 and 0.6% w/w NaCl at 25°C. Although the amount of NaCl added did not change the shape of the curves significantly, the CAC values decreased significantly to lower SDS concentrations as NaCl concentration increased, which may reflect a decrease in the repulsive forces (charge shielding effect) between SDS molecules promoting the SDS-HPMC aggregation at lower concentrations [46]. As a result, the cooperative binding of SDS is enhanced as more SDS molecules are adsorbed onto HPMC.

The concentration of NaCl also influences $\Delta H_{agg}^\circ$, as the NaCl concentration increases from 0.1% to 0.6% w/w, the values of $\Delta H_{agg}^\circ$ were determined at these NaCl concentrations as described in the thermodynamic parameters of SDS-HPMC aggregation section and were determined to increase from 2.1 kJ/mol to 2.9 kJ/mol at 25°C, respectively, which may suggest that the interactions between HPMC and SDS are stronger at higher NaCl concentrations. This may be attributed to the charge shielding effect. The influence of NaCl on the exothermic region (B→C) was also evaluated (Figure 4.7). The value of $C_{sat}$ was lower at the highest concentration of NaCl (0.6% w/w) may be suggesting a change in conformation of HPMC resulting in a lower number of available binding sites at the highest NaCl concentration.

3.7. Influence of Surfactant Headgroup on the Energetics of Surfactant-HPMC Aggregation Process

The influence of surfactant headgroup on the surfactant-HPMC interactions was studied by determining the thermodynamic parameters for DTAB-HPMC interactions since the chain length of DTAB is the same as that of SDS. Similar to SDS, the studies were conducted by titrating a micellar solution of DTAB (10x CMC; 129.7mM) into
HPMC K-4M solution. However, unlike the SDS/HPMC system where the novel data treatment method was applied, a lack of available data associated with DTAB and HPMC binding prevented the same data treatment to be applied here and the parameters were extracted from the uncorrected enthalpograms. The DTAB-HPMC enthalpogram does not show a distinct endothermic peak as seen with the SDS-HPMC (Figure 4.8), which is attributed to a lack of or weak interactions between DTAB and HPMC. Weak or a lack of interactions between cationic surfactants and nonionic polymers (i.e., PEO, PVP) have been reported. The larger size of the cation may deter the interaction with nonionic polymers [170].

4. Conclusions

Isothermal titration calorimetry (ITC) was utilized to study the solution-state interaction between ionic surfactants and HPMC. The interaction of SDS with HPMC was determined to be stronger than DTAB and HPMC. The interaction between SDS and HPMC was endothermic and cooperative in nature and dependent on temperature and ionic strength of the solution. The effect of temperature, HPMC molecular weight and ionic strength was utilized to postulate the mechanism of SDS-HPMC aggregate formation at a critical aggregate concentration (CAC). The driving force for the SDS-HPMC interactions is suggested to be the hydrophobic effect. At the highest molecular weight and concentration of HPMC, the critical concentration parameters $C_{\text{sat}}$ and CMC are significantly altered and shift to a higher concentration of SDS. Ionic strength significantly influenced SDS-HPMC aggregation. Specifically, the critical concentration parameters (CAC and CMC) decreased with increasing ionic strength for both anionic and cationic surfactant-HPMC systems suggesting stronger interactions.
Overall, the interpretation of the microcalorimetric studies at different temperatures and ionic strengths while varying the properties of polymer and surfactant was very effective in developing insights into the nature and energetics of HPMC and ionic surfactant interactions. This study focused on understanding the thermodynamics of surfactant-HPMC aggregate formation; the next chapter focused on exploring the structural aspects of the surfactant-HPMC aggregates. The knowledge gained from these two studies was utilized in subsequent studies to understand the adsorption behavior of HPMC and ionic surfactants onto model solid surfaces.
### Tables

#### Table 4.1: Thermodynamic parameters for SDS micellization

<table>
<thead>
<tr>
<th>Temp.</th>
<th>CMC&lt;sup&gt;a&lt;/sup&gt; (°C)</th>
<th>CMC (mM)</th>
<th>$\Delta H_{mic}$&lt;sup&gt;a&lt;/sup&gt; (kJ/mol)</th>
<th>$\Delta H_{mic}$ (kJ/mol)</th>
<th>$\Delta G_{mic}$° (kJ/mol)</th>
<th>$\Delta S_{mic}$° (J/°K)</th>
<th>$\Delta C_p$ (kJ/°mol K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>8.3</td>
<td>7.2-8.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.6</td>
<td>-0.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-23.7</td>
<td>77.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.1&lt;sup&gt;b&lt;/sup&gt;(8.1&lt;sup&gt;d&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>32°C</td>
<td>8.2(±0.2)</td>
<td>--</td>
<td>-3.5(±0.1)</td>
<td>--</td>
<td>-24.3(±0.2)</td>
<td>67.7</td>
<td>-0.5</td>
</tr>
<tr>
<td>40°C</td>
<td>7.7(±0.2)</td>
<td>7.9-8.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-7.5(±0.0)</td>
<td>-7.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-25.3(±0.2)</td>
<td>56.2</td>
<td></td>
</tr>
<tr>
<td>50°C</td>
<td>8.1(±0.0)</td>
<td>8.1-9.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-10.7(±0.3)</td>
<td>-11.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-25.8(±0.0)</td>
<td>45.9</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Experimental data from this study

<sup>b</sup> ITC generated values; Ref [29]

<sup>c</sup> values of CMC from surface tension method; Ref [171]

<sup>d</sup> values of CMC from the conductance method are shown in the parentheses; Ref [171]
Table 4.2: Thermodynamic parameters for DTAB micellization

<table>
<thead>
<tr>
<th>Temp.</th>
<th>CMC (mM)</th>
<th>CMC$^a$ (mM)</th>
<th>$\Delta H_{mic}^\circ$ a (kJ/mol)</th>
<th>$\Delta H_{mic}^\circ$ (kJ/mol)</th>
<th>$\Delta G_{mic}^\circ$ (kJ/mol)</th>
<th>$\Delta S_{mic}^\circ$ (J/°K)</th>
<th>$\Delta C_p$ (kJ/°mol K)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>14.5(±0.4)</td>
<td>13.5</td>
<td>-1.9</td>
<td>-1.7(±0.1)</td>
<td>-20.9(±0.1)</td>
<td>64.3(±0)</td>
<td></td>
</tr>
<tr>
<td>32°C</td>
<td>14.9(±0.3)</td>
<td>--</td>
<td>--</td>
<td>-4.4(±0.0)</td>
<td>-21.3(±0.1)</td>
<td>53.2(±0)</td>
<td>-0.4</td>
</tr>
<tr>
<td>40°C</td>
<td>15.9(±0.3)</td>
<td>--</td>
<td>--</td>
<td>-7.4(±0.1)</td>
<td>-21.5(±0.3)</td>
<td>41(±0)</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Ref [148]
Table 4.3: Thermodynamic parameters for SDS-HPMC aggregation

<table>
<thead>
<tr>
<th>HPMC Conc.</th>
<th>Temp.</th>
<th>CAC&lt;sup&gt;a&lt;/sup&gt;</th>
<th>$\Delta H_{agg}^o$</th>
<th>$\Delta G_{agg}^o$</th>
<th>$\Delta S_{agg}^o$</th>
</tr>
</thead>
<tbody>
<tr>
<td>% w/w</td>
<td>°C</td>
<td>mM</td>
<td>kJ/mol</td>
<td>kJ/mol</td>
<td>J/K</td>
</tr>
<tr>
<td>0.25</td>
<td>25°C</td>
<td>4.1(±0.2)</td>
<td>1.9(±0.1)</td>
<td>-27.1(±0.3)</td>
<td>97.4</td>
</tr>
<tr>
<td>0.5</td>
<td>25°C</td>
<td>4.2(±0.2)</td>
<td>2.2(±0.1)</td>
<td>-27.1(±0.2)</td>
<td>98.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>CAC: critical aggregation concentration
Figure 4.1. Schematic of isothermal titration calorimetry for exploring the energetics of surfactant micelles and surfactant-HPMC aggregates. Inset: Raw heat signals measurement over time [118].
Figure 4.2. Representative calorimetric data transformation to determine critical concentrations: (a) raw data directly obtained from isothermal titration calorimetry (heat flow (µW) vs. time), (b) enthalpograms depicting apparent enthalpy change (ΔH_{app}) as a function of model surfactant (CTAB) concentration, and (c) the first derivative of the curve (b).
Figure 4.3. Apparent enthalpy change (ΔH_{app}) for the titration of the micellar solution of SDS (a) and DTAB (b) in water at 25°C (circles), 32°C (triangles), 40°C (diamonds) and 50°C (squares).
Figure 4.4. Plot of (a) apparent enthalpy change ($\Delta H_{\text{app}}$) and (b) corrected enthalpy change ($\Delta H_{\text{corr}}$) as a function of total SDS concentration in the presence of 0% HPMC K-4M (squares), 0.25% HPMC K-4M (diamonds) and 0.5% HPMC K-4M (triangles) at 25°C.
Figure 4.5. Effect of temperature on (a) apparent enthalpy change (ΔHapp) and (b) corrected enthalpy change (ΔHcorr) as a function of total SDS concentration in the presence of .25% w/w HPMC K-4M at 25°C (squares), 32°C (triangles) and 40°C (crosses for ΔHapp and diamonds for ΔHcorr).
Figure 4.6. Plot of (a) apparent enthalpy change ($\Delta H_{\text{app}}$) and (b) corrected enthalpy change ($\Delta H_{\text{corr}}$) as a function various molecular weight of 0.25% w/w HPMC at 25°C, HPMC K-4M (squares), HPMC K-15 (triangles for $\Delta H_{\text{app}}$ and diamonds for $\Delta H_{\text{corr}}$) and, HPMC K-100 (circles).
Figure 4.7. Enthalpogram for the effect of NaCl on SDS-HPMC aggregation behavior for 0.25% w/w HPMC at 25°C (a) apparent enthalpy change ($\Delta H_{\text{app}}$) and (b) corrected enthalpy change ($\Delta H_{\text{corr}}$) at 0 % NaCl, (cross), 0.1% NaCl (squares), 0.3% NaCl (triangles) and 0.6% NaCl (diamonds).
Figure 4.8. Plot of (a) apparent enthalpy change ($\Delta H_{\text{app}}$) and (b) corrected enthalpy change ($\Delta H_{\text{corr}}$) as a function of total DTAB concentration in the presence of 0% HPMC K-4M (squares) and 0.25% HPMC K-4M (diamonds) at 25°C.
CHAPTER 5

Exploring Factors Influencing Structural Characteristics of Surfactant-Polymer Aggregates using a Fluorescence Probe Technique

1. Introduction

Nanoparticle drug delivery systems (NDDS) that utilize pharmaceutical excipients such as surfactants and polymers are generally effective in increasing their stability by preventing particle aggregation [50]. A combination of polymer and surfactant could provide a synergistic effect towards the stabilization of nanoparticles through the adsorption of polymer-surfactant aggregates onto the surface of nanoparticles [51-55]. For a rationale, a priori selection of excipients during NDDS development, a comprehensive understanding of the role of surfactant-polymers aggregates in stabilizing nanoparticles as well as the factors that influence their structural characteristics is essential.

The type of interactions between surfactants and polymers as well as their properties play a significant role in the formation of aggregates [64, 72-74]. Two type of interactions are generally observed: (1) cooperative interactions where the binding of a ligand such as a surfactant molecule at an adsorbate site affects the binding of ligands at other binding sites of the same adsorbate [48] [110], and (2) competitive interactions where a ligand could preferentially displace another molecule from a binding site of the macromolecule [111]. Cooperative adsorption of sodium dodecyl sulfate (SDS) on polyethylene oxide (PEO) was reported in the formation of PEO-SDS [107]. While Nilsson [27], using an equilibrium dialysis technique, reported that the cooperative interactions between hydroxypropyl methylcellulose (HPMC) and SDS formed SDS-HPMC aggregates, Hammarstrom et al. [112] utilizing a nuclear magnetic resonance
(NMR) technique found that the size and shape of SDS-HPMC aggregates did not change in the presence of HPMC.

Although the reported interactions between anionic surfactants and some polymers such as PEO, HPMC, polyvinyl pyrrolidone (PVP), and ethyl hydroxyethyl cellulose (EHEC) may be cooperative, not much information on the role of the properties of these excipients, their interactions with other cationic surfactants and mechanisms are available [99]. Moreover, while the combinations of surfactant and polymer have been reported to provide improved stability to NDDS as compared to the utilization of either a surfactant or a polymer alone [6, 113], the mechanism of these observations and the role of the solution-state environment remains unclear. Therefore, despite the above-mentioned studies, formulation scientists generally use an empirical, screening-based approach to select polymers and surfactants for NDDS development. The impact of various formulation parameters such as the nature of surfactant head-group (i.e., charge and size), hydrophobicity or chain length of surfactant, and ionic strength of the solution-state on the formation and structural properties of HPMC-ionic surfactant aggregates and, in turn, the adsorption of these aggregates to the surface of nanoparticles is still not well understood.

A variety of techniques such as a fluorescence probe method, surface tensiometry, isothermal calorimetry, dynamic light scattering, nuclear magnetic resonance spectroscopy, neutron scattering, and equilibrium dialysis have been used to study interactions between polymers and surfactants [[46, 103, 104, 107, 172, 173]. While these techniques are useful in studying interactions, the fluorescence probe method has been proven to be a very effective tool in determining the microstructural information, critical aggregation parameters of polymer-surfactant aggregates [174, 175], and to quantify the
influence of various formulation parameters on the structural properties of these aggregates [152, 176-178].

In general, some gaps were identified from the review of previous studies containing HPMC: (1) pharmaceutically relevant higher concentrations of HPMC typically utilized in formulation of NDDS (0.25-1 %w/w) [27-29] have not been evaluated, (2) detailed investigation of the factors influencing the structural characteristics of aggregates such as ionic strength of solution and surfactant properties (i.e., head group, chain length) on the interaction between ionic surfactants with HPMC has also not been investigated to the best of our knowledge, (3) detailed investigation of the influence of the ionic strength in potentially manipulating the solution-state environment to enhance the interactions between surfactants and HPMC and, (4) systematic investigation of aggregation number of surfactant-HPMC aggregates using excimerization and modelling of fluorescence data without the use of a fluorescence quenchers and the various assumptions that need to be considered with their use (i.e., solubilization and distribution of quenchers relative to the probe molecule) [46, 118].

The objectives of this chapter were to study: (1) the effect of a model non-ionic polymer, HPMC on the state of aggregation of model anionic and cationic surfactants, and (2) the impact of surfactant properties (i.e., type of head-group and chain length) and solution properties (i.e., ionic strength) on the structural characteristics (i.e., aggregation number/size and microenvironment) of surfactant-HPMC aggregates. The fluorescence probe method using pyrene as a hydrophobic probe molecule was used to determine the structural characteristics of the ionic surfactant-HPMC aggregates as pyrene is preferentially solubilized within hydrophobic microdomains (such as micelles and
aggregates), which can result in a change in the intensity of emission (monomer) or excimer (dimer or higher) peaks of pyrene; this change in intensity was used to determine the characteristics of ionic surfactant-HPMC aggregates. The understanding gained from this work was applied in subsequent studies towards exploring the impact of solution-state interactions on the mechanism of adsorption of surfactant- HPMC aggregates onto the surface of nanoparticles.

2. Materials and Methods

2.1. Materials

Sodium dodecyl sulfate (SDS), dodecyltrimethylammonium bromide (DTAB), tetradecyltrimethylammonium bromide (TTAB), and hexadecyltrimethylammonium bromide (CTAB) were obtained from Sigma-Aldrich (St. Louis, MO). The purified (Chapter 4) and unpurified surfactant solutions were also analyzed for pyrene solubilization and were found to be similar. Hence the surfactants purchased from Sigma-Aldrich (St. Louis, MO) were used as received. Hydroxypropyl methylcellulose (Benecel® K-4M) was obtained from Ashland Aqualon Functional Ingredients, Ashland Inc (Wilmington, DE) and was used as received. Pyrene (>99% pure) was purchased from Sigma-Aldrich (St. Louis, MO) and differential calorimetric analysis was conducted to assess the high purity of pyrene and was then used as received. Methanol and NaCl were purchased from Sigma-Aldrich (St. Louis, MO) and used as received. All solutions were prepared using purified water (18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore, Billerica, MA).
2.2. Methods

2.2.1. Fluorescence Spectroscopic Measurements

Steady-state fluorescence measurements were performed using Varian Cary Eclipse fluorescence spectrophotometer (Varian, Santa Clara, CA). All measurements were carried out with the excitation wavelength of 340 nm, and the emission spectrum was recorded between 340 and 550 nm in a 10 mm path length quartz cuvette. The excitation and emission slit widths of 5 nm and 1.5 nm, respectively were used. Data was acquired using Cary Eclipse software from Varian (Varian, Santa Clara, CA). The molar concentrations of pyrene used in this study were evaluated carefully to select concentrations that would give the most optimum signal with minimum perturbation to the aggregate or micellar states formed. Stock solutions of pyrene of 5, 10 and 20µM were prepared in methanol. An appropriate aliquot of the stock solution was mixed with a surfactant-HPMC solution in 20 ml scintillation vial. This solution was allowed to equilibrate in the dark for 10 hours before centrifuging the sample to remove any excess pyrene microcrystals. A control solution was prepared the same way without the addition of pyrene and was found to have negligible background intensity or scatter. The trace amounts of methanol used to prepare pyrene solutions do not influence the solubilization of pyrene or its fluorescence characteristics [47, 119, 179, 180]. All experiments and sample preparations were carried out at ambient temperature (25 ±2°C).

2.2.2. UV-Vis Spectroscopic Measurements

UV-Vis spectroscopy measurements were conducted to determine the total amount of solubilized pyrene using Cary-50 Bio UV-Visible spectrophotometer (Varian, Santa
All samples were prepared by the addition of an excess amount of pyrene microcrystals to surfactant and HPMC solutions. The ionic strength was adjusted using NaCl in 20 ml scintillation vials. These samples were then sonicated and allowed to equilibrate for 12 hours before being centrifuged at 5000 rpm for 1 hour to sediment any excess pyrene microcrystals. The supernatant from the centrifuged vials was then diluted in surfactant and surfactant-HPMC solutions in order to assay the total amount of pyrene solubilized in surfactant micelles or surfactant-HPMC aggregates using UV-Vis spectroscopy, respectively. The dilutions were carried out using the solutions with significantly higher surfactant concentrations than CMC to ensure that the solubilized pyrene did not precipitate out during UV absorbance measurement. The absorbance intensity was recorded at 336 nm with the molar absorptivity of $2.06 \times 10^{-5}$ M/cm. The molar absorptivity for pyrene solubilized in micelles was calculated and has also been reported [119].

### 3. Results and Discussion

The effect of surfactant properties (type of head-group and chain length) and solution properties (i.e., ionic strength) on the structural characteristics of ionic surfactant-HPMC aggregates have been explored in this study. The pyrene fluorescence method with the Poisson distribution data analysis was selected to study the properties of micelles and surfactant-HPMC aggregates due to the following advantages: (1) it provides quantitative information on critical concentration parameters and aggregation number of micelles and aggregates without the use of fluorescence quenchers, and (2) it provides qualitative information on the microenvironment of micelles and aggregates. Since the properties of SDS micelles have been studied previously by this laboratory using isothermal titration
calorimetry and by others using techniques such as NMR and surface tensiometry, the pyrene fluorescence method was first validated by comparing the CMC of SDS determined by the current method as well as the same reported in the literature [46, 47]. Upon validation, the pyrene method was used to determine the critical structural characteristics (CAC and aggregation number), and the microenvironment of ionic surfactant-HPMC aggregates.

3.1. Pyrene Fluorescence Method Validation using SDS Micellization Parameters

Steady-state fluorescence spectroscopy with probe molecules such as pyrene has been proven to be a powerful tool to quantitatively study the aggregation process between polymers and surfactants [174, 175]. Pyrene is an extremely hydrophobic molecule, which gets solubilized in the core and interfacial regions of micelles and polymer-surfactant aggregates [181, 182]. Pyrene has photophysical characteristics that are sensitive to microenvironmental changes and hence the alteration of its spectra can be used to quantitatively study the micellization or aggregation process [183]. The emission spectrum of pyrene (Figure 5.1) has several peaks and the ratio of the first (~373 nm, I₁ or I_mon) and the third (~384 nm, I₃) vibronic peak intensities (i.e., I₁/I₃ ratio) has been reported to be extremely sensitive to the polarity of solvent or local environment where pyrene molecules reside [177, 184]. The I₁/I₃ ratio, also known as the micropolarity index, decreases with a decrease in the polarity of the environment experienced by pyrene molecules. The micropolarity index (I₁/I₃ ratio) has been applied to understand the interaction between surfactants and polymers by characterizing the polarity of an unknown microenvironment [72, 152].
Representative plots of the pyrene micropolarity index \((I_1/I_3\) ratio) as a function of total SDS concentration at various ionic strengths are shown in Figure 5.2. The value of \(I_1/I_3\) ratio at low SDS concentration is \(\sim 1.9\), which is in agreement with the values of \(I_1/I_3\) ratio \((\sim 1.9)\) reported in the literature for pyrene dissolved in water [183]. As SDS concentration increases, the \(I_1/I_3\) ratio sharply decreases above a critical SDS concentration, which is attributed to the preferential solubilization of pyrene in SDS micelles where the solubilized pyrene experiences a less polar microenvironment resulting in a lower micropolarity index [90, 185]. From the inflection point of the sharply declining \(I_1/I_3\) curve, the CMC of SDS is determined to be 8.1 mM at 0% NaCl (Figure 5.2; squares and Table 5.1). This CMC value is in agreement with our previous determination utilizing an ITC methodology (8.3 mM) and the values reported by Philips et al. (8.1 mM), Horin et al. (8.2 mM) and Hu et al. (8.0 mM) [46, 152, 153].

Within the micellar and aggregate microstructures, an excited pyrene molecule could bind to another pyrene molecule in its ground state, leading to the formation of an excited pyrene dimer (excimer) [44]. This results in a broad excimer fluorescence peak \((I_e; 470 \text{ nm})\) as shown in Figure 5.1. The ratio of excimer to monomer peak (i.e., \(I_e/I_{mon}\) ratio) has been reported to be sensitive to the distribution of pyrene within hydrophobic microdomains of micelles and aggregates [186-188]. The \(I_e/I_{mon}\) ratio with Poisson and Binominal statistical distribution models have been used to determine the structural characteristics such as the aggregation number and the microenvironment of surfactant-polymer aggregates and pure surfactant micelles [39-42]. The binomial distribution model has been utilized to describe the distribution statistics of solubilizates (e.g., pyrene) when higher concentrations of solubilizate are used [186]. Whereas, the Poisson distribution
model is favored at low solubilizate concentrations where a random and low occupancy of solubilizate (~less than 5) in micelles or surfactant-polymer aggregates is expected [178, 188-191].

The Poisson distribution model is better suited for the present study since low concentrations of pyrene are used. According to the Poisson distribution model [47, 189], the fraction of micelles or aggregates that contain \( i \) pyrene molecules is

\[
\frac{[M_i]}{[M]} = \frac{\bar{n}^i e^{-\bar{n}}}{i!}
\]  

(5.1)

where \([M]\) is the total concentration of micelles, \([M_i]\) is the concentration of micelles that contain \( i \) pyrene molecules, and \( \bar{n} \) is the average number of pyrene molecules solubilized by micelles. \( \bar{n} \) can be further described as

\[
\bar{n} = \frac{[Py]_m \ N_{agg}}{[S]_t - C_{crit}}
\]

(5.2)

where \([Py]_m \) is the total concentration of pyrene assumed to be solubilized by micelles, \( N_{agg} \) is the aggregation number of micelles, \([S]_t \) is the total surfactant concentration, and \( C_{crit} \) is the critical surfactant concentration (i.e., CAC for aggregates and CMC for micelles).

The fluorescence intensities transcribed by pyrene in its monomeric and excimeric states when solubilized in micelles (or aggregates) can be described by Eq. 5.3 and 5.4, respectively[188].

\[
I_{mon} = K_m \bar{\phi}_m^n R \sum_{i=1}^{\infty} \left( \frac{i}{R + i - 1} \right) \frac{[M_i]}{[M]}
\]

(5.3)
\[
I_e = K_m \phi_m^m \sum_{i=2}^{\infty} \left( \frac{i(i-1)}{R+i-1} \right) \frac{[M_i]}{[M]}
\]

(5.4)

where \(K_m\) is the proportionality factor that depends on instrument parameters such as wavelength and absorptivity, \(\phi_m^m\) and \(\phi_e^m\) are the monomer and excimer quantum yields within micellar structures, respectively, and \(R\) is the fluorescence kinetic factor. The “\(m\)” superscript denotes the pyrene fraction solubilized in micelles. At low surfactant concentrations, the fluorescence intensity is assumed to be mainly constituted by the pyrene residing in the aqueous phase. As surfactant concentration increases beyond the CMC, the fluorescence intensity is predominantly expected to arise from the pyrene solubilized in micelles. In order to study the microenvironment and aggregation number of the micelles, we have focused on the fluorescence intensity data at the surfactant concentrations above CMC while assuming that the fluorescence contribution from pyrene in the aqueous phase is insignificant [188]. Hence, the ratio of experimentally measured excimer and monomer fluorescence intensities of pyrene can be expressed as

\[
\frac{I_e}{I_{mon}} = \frac{\phi_e^m}{\phi_m^{mon}} \sum_{i=2}^{\infty} \left( \frac{i(i-1)}{R+i-1} \right) \frac{[M_i]}{[M]}
\]

(5.5)

By substituting Eq. 5.1 and 5.2 in Eq. 5.5, the aggregation number of micelles \(N_{agg}\) can be determined from the non-linear regression analysis of \(I_e/I_{mon}\) ratio measured at different surfactant concentrations \([S]_t\) above CMC while treating \(R, \phi_m^{mon}, and \phi_e^m\) as unknown parameters [188, 189] and \([Py]_m\) and \(C_{crit}\) as known parameters.

Figure 5.3 shows representative curves of the \(I_e/I_{mon}\) ratio of pyrene vs. SDS concentration at various ionic strengths. As SDS concentration increases to CMC, the
I_e/I_{mon} ratio increases sharply reaching a maximum followed by a monotonic decrease at higher SDS concentrations. This maximum is indicative of the highest solubilization of pyrene within hydrophobic micelles followed by subsequent dilution of the solubilized pyrene as the number of micelles increases at higher SDS concentrations. Using non-linear regression analysis (Scientist® software, Micromath Inc., St. Louis, MO, USA) and Eqs. 5.1, 5.2 and 5.5, the aggregation number (N_{agg}) of SDS micelles at 0% NaCl concentration was determined to be 68 ±4 (95% CI), which is slightly lower than the previously reported value of 80 measured by dynamic light scattering [46] and 76 as measured by neutron scattering [192]. The slightly lower value of N_{agg} may be attributed to the difference in experimental techniques. The non-linear regression analysis method will be used in subsequent sections to determine the influence of HPMC and ionic strength on the aggregation number of ionic surfactant-HPMC aggregates.

3.2. Formation and Structural Characterization of Surfactant-HPMC Aggregates: Aggregation Number and Microenvironment

The micropolarity index (I_1/I_3 ratio), the excimerization (I_e/I_{mon} ratio), and the solubilization of pyrene by UV-Vis were used to understand the formation and structural characteristics (i.e., aggregation number and microenvironment) of SDS-HPMC aggregates. As shown in Figure 5.4, the micropolarity index (I_1/I_3 ratio) of SDS-HPMC systems is lower than that of pure SDS systems. This could be attributed to the lower apparent polarity experienced by pyrene in the presence of HPMC [47]. The I_1/I_3 ratio of 1.6 for SDS-HPMC systems remains steady upon every successive addition of SDS until a critical SDS concentration of 4.3 mM, where a sudden decrease (breakpoint) in the I_1/I_3 ratio is observed that is defined as a critical aggregation concentration (CAC) (Figure 5.4
and Table 5.2). The steady decline in the micropolarity index (I$_1$/I$_3$ ratio) indicates that the microenvironment being experienced by pyrene is becoming increasingly more hydrophobic.

In the presence of HPMC, the aggregation behavior is altered with two break points with SDS-HPMC aggregates forming at CAC and free SDS micelles forming at CMC as compared to the SDS-water system where only one break point, i.e., the CMC of SDS micelles was observed. The CMC of SDS in the presence of HPMC is higher (~9.5 mM) as compared to the same with no HPMC (~8.1 mM). This suggests that the higher concentration of SDS is needed for the interaction of SDS with HPMC before the formation of free SDS micelles [84]. The micropolarity index (I$_1$/I$_3$ ratio) for free SDS micelles in the absence and presence of HPMC were similar wherein the plateau values are in the ~1.1-1.2 range, indicating that the partitioning behavior of pyrene is similar for both systems.

When CAC is below CMC, synergism or cooperativity (i.e., binding of SDS at one site influences the binding of molecules at other sites) could exist between polymer and surfactant, whereas when CAC is above CMC, antagonism or competition could exist [193, 194]. This study shows that cooperativity between HPMC and SDS could be assumed as the CAC for SDS-HPMC aggregates is below the CMC for SDS micelles. This is in good agreement with previous results obtained by this lab using an ITC method. A similar cooperative aggregation behavior was observed for SDS-EHEC system using micropolarity index [89] and NMR spectroscopy [195].

The microenvironment of SDS-HPMC aggregates was further investigated by pyrene solubilization. Figure 5.7 shows pyrene solubilization measured at various SDS concentrations using UV-Vis measurements for SDS micelles (Figure 5.7a) and SDS-
HPMC aggregates (Figure 5.7b). The onset of pyrene solubilization occurs at CMC (~8.4 mM) and CAC (~4.3 mM) as SDS micelles, and SDS-HPMC aggregates are being formed at 0% NaCl, respectively. The values of CMC & CAC measured by UV-Vis are consistent with those determined using pyrene fluorescence spectroscopy (I/I₃ curves, Figure 5.4). The pyrene solubilizing power of SDS micelles & SDS-HPMC aggregates were obtained from the slope of the curve beyond CMC (Figure 5.7a, Table 5.1) and CAC (Figure 5.7b, Table 5.2), respectively. As shown in Table 5.2, the solubilizing power of SDS-HPMC aggregates at 0% NaCl is approximately ~35% more than that of SDS micelles alone. This provides further insight towards a more hydrophobic microenvironment within SDS-HPMC aggregates.

The aggregation number of SDS-HPMC aggregates was determined from pyrene excimerization. A representative curve of the pyrene excimerization or Iₑ/Iₘ₀n ratio vs. SDS concentration for SDS-HPMC systems (0% NaCl) is shown in Figure 5.6. As SDS concentration increases beyond the CAC, a sharp increase in the Iₑ/Iₘ₀n ratio is observed and with further increase in SDS concentration beyond a maximum, the Iₑ/Iₘ₀n ratio decreases asymptotically. By regressing the data (Eq. 5.1, 5.2 and 5.5) after the onset of HPMC and SDS interactions (i.e., from the maximum to the asymptotic decrease in the Iₑ/Iₘ₀n ratio) where we can assume the solubilized pyrene resides in SDS-HPMC aggregates, the aggregation number for SDS-HPMC aggregates was determined (Table 5.2). The values of parameters R=0.4 and ϕₑ/ϕₑₘ₀n=0.68 used as constants in the regression analysis were similar to the values reported earlier for the steady state fluorescence of pyrene and pyrene excimer [189]. The aggregation number of SDS-HPMC aggregates at 0% NaCl determined by the regression of the Iₑ/Iₘ₀n curve (Figure 5.6) was
determined to be 34±7 (± 95 CI), which is approximately half the aggregation number of pure SDS micelles (Table 5.1), whereas the pyrene solubilizing power of SDS-HPMC aggregates was 35% higher than that of SDS micelles. Despite the smaller size, the higher solubilizing power could be attributed to a more hydrophobic environment of the SDS-HPMC aggregates. As SDS concentration increases, the I_e/I_mon ratio decreases suggesting that the occupancy of pyrene in the aggregates decreases as more and more SDS-HPMC aggregates and free SDS micelles are formed and pyrene is subsequently diluted, making it unlikely for the aggregates/micelles to contain enough pyrene to form excimers.

### 3.3. Effect of Ionic Strength on the Structural Characteristics of SDS Micelles & SDS-HPMC Aggregates

To determine the influence of ionic strength on the structural characteristics of SDS-HPMC aggregates, we first determined the effect of ionic strength on SDS micellization parameters (i.e., CMC, aggregation number, and solubilization power). The CMC of SDS at different ionic strengths (0.1-0.6% w/w NaCl) was determined from the inflection point of sharply decreasing regions of I_1/I_3 curves (Figure 5.2 and Table 5.1). The CMC of SDS was observed to decrease with ionic strength (Table 5.1), which agrees with our previous ITC study (Chapter 4). At higher ionic strengths, NaCl is expected to decrease electrostatic repulsion between negatively charged SDS head groups due to charge shielding thus facilitating micelle formation at lower SDS concentrations [152].

As shown in Figure 5.3, the peak positions (maxima) for I_e/I_mon curves shift to the left at lower concentrations of SDS as ionic strength increases. There is a significant increase in the peak height of I_e/I_mon ratio with increasing ionic strength that reflects the increasing solubilization capacity of SDS micelles. Similarly, as ionic strength increases,
the aggregation number of SDS micelles increases from 68±4 (± 95 CI) at 0% NaCl to 134 ±16 (± 95 CI) at 0.6% NaCl (Table 5.1). Due to charge shielding, the interface of micelles is expected to be more tightly packed allowing less water to penetrate these micro-structures resulting in a more hydrophobic environment for greater solubilization of the pyrene [21].

The influence of ionic strength on the structural characteristics of SDS-HPMC aggregates was examined. The change in micropolarity index (I/I3 ratio) at different ionic strengths (0, 0.1%, 0.3% or 0.6% NaCl) is shown in Figure 5.5. The CAC values move progressively to lower SDS concentrations with increasing ionic strength (Table 5.2). Similarly, Ic/I_mon maxima shifted to progressively lower SDS concentrations with increasing ionic strength (Figure 5.6). A significant increase in the Ic/I_mon maxima at higher ionic strengths was also observed, which may indicate an increase in pyrene solubilization by SDS-HPMC aggregates. The solubilizing capacity for SDS-HPMC aggregates determined using UV-Vis spectroscopy increased by nearly 3-fold from 0.27 to 0.83 as NaCl concentration increased from 0% to 0.6% (Table 5.2, Figure 5.6b). The pyrene solubilization power of SDS-HPMC aggregates also increased with ionic strength (Table 5.2). The significant increase in the solubilization capacity suggests that the hydrophobic volume provided SDS-HPMC aggregates for pyrene solubilization increases with ionic strength [196]. Thus, with the addition of NaCl, the structure and properties of SDS-HPMC aggregate change possibly due to the greater charge shielding of negatively charged SDS headgroups provided by NaCl and thus allowing for tighter packing [152].

3.4. Influence of Surfactant Properties on the Structural Characteristics of Surfactant-HPMC Aggregates
The influence of surfactant properties such as head group and chain length on the structural characteristics of surfactant-HPMC aggregates was examined using several ionic surfactants: SDS, DTAB, TTAB, and CTAB at 0.25% w/w HPMC. Between SDS and DTAB, the chain length (C₁₂) of both surfactants remains constant while the head group varies in size and charge. The chain length varies from C₁₂ to C₁₆ among the three cationic surfactants: DTAB, TTAB, and CTAB.

3.4.1. Effect of head-group

As shown in Figure 5.8, the micropolarity index (I₁/I₃ ratio) at low surfactant concentrations for DTAB-HPMC and SDS-HPMC systems were determined to be 1.9 and 1.6, respectively. The higher I₁/I₃ ratio for DTAB-HPMC system as compared to SDS-HPMC system suggests a more polar environment with DTAB-HPMC aggregates. As the surfactant concentration gradually increases, the I₁/I₃ ratio begins to decrease and signals the onset of CAC. The less steep decline in the I₁/I₃ ratio for DTAB as compared to SDS indicates weaker interactions between DTAB and HPMC, which is further observed in higher CAC for DTAB (~8.4 mM) as compared to the same for SDS-HPMC (~4.3 mM). This may be attributed to the looser packing and greater penetration of water into DTAB-HPMC aggregates due to steric constraints provided by the bulkier trimethylammonium bromide head groups.

This hypothesis was further confirmed using the pyrene solubilizing power of aggregates. The solubilizing power of DTAB-HPMC aggregates (2.42 µM pyrene/mM) was approximately 4x lower than that of the SDS-HPMC aggregates (8.18 µM pyrene/mM) suggesting the presence of more polar microdomains in DTAB-HPMC aggregates. This in agreement with our previous study utilizing an isothermal titration calorimetric approach.
where the weaker interactions between DTAB and HPMC were also observed (Chapter 4).

Overall, the results from this study indicated that the type of surfactant head-group significantly influenced the structural characteristics including aggregation behavior, microenvironment, and solubilization power of surfactant-HPMC aggregates.

3.4.2. Effect of chain length

Figure 5.9 shows the $I_1/I_3$ ratio curves for $(C_n)$TAB-HPMC systems with varying surfactant chain lengths. The cationic surfactants ($(C_n)$TAB) in this study included DTAB ($C_{12}$), TTAB ($C_{14}$) and CTAB ($C_{16}$). Amongst the three systems investigated, at lower surfactant concentrations, the CTAB-HPMC system showed the lowest $I_1/I_3$ ratio, which indicated a more hydrophobic microenvironment. The hydrophobicity of $(C_n)$TAB-HPMC aggregates was further examined using pyrene solubilization and $I_e/I_{mon}$ peak height. Both, the pyrene solubilization and the $I_e/I_{mon}$ peak height were the highest for CTAB-HPMC aggregates as compared to DTAB-HPMC and TTAB-HPMC aggregates indicating that the longer surfactant chain length of CTAB resulted in a more hydrophobic microenvironment for CTAB-HPMC aggregates.

The CAC decreases with an increase in chain length with the following rank order: DTAB (8.4 mM) < TTAB (2.5 mM) < CTAB (0.6 mM). The CAC-values of CTAB-HPMC and TTAB-HPMC are much closer to each other and well separated from DTAB-HPMC system; this may suggest that the strength of the interaction between HPMC and surfactant are in the order of CTAB > TTAB > DTAB with the weakest interaction between DTAB and HPMC. The CAC/CMC ratio has been reported to successfully estimate the change in the free energy of aggregation for polyelectrolytes-surfactant aggregates [197, 198]. In this study, the CAC/CMC ratio was determined for all three systems explored.
The strength of solution-state interaction between HPMC and surfactant (R=CAC/CMC) The CAC/CMC ratio denoted as R was observed to decrease with an increase in the chain length since the CAC shifted significantly to lower concentrations hence the strength of interactions follows the rank order: DTAB< TTAB< CTAB.

4. Conclusions

The influence of surfactant properties and ionic strength on the formation and structural characteristics of surfactant-HPMC aggregates were examined using a pyrene steady-state fluorescence probe method. The pyrene fluorescence method was effective in quantitatively determining surfactant-HPMC interaction parameters (CAC and CMC) and structural characteristics (e.g., aggregation number and microenvironment) of surfactant-HPMC aggregates and free surfactant micelles at pharmaceutically relevant concentrations.

The presence of HPMC significantly altered the structural characteristics of surfactant-HPMC aggregates. The microenvironment of the SDS- HPMC aggregates was determined using the micropolarity index and the pyrene solubilization power and was found to be more hydrophobic as compared to SDS micelle. The pyrene solubilization power and solubilization capacity of SDS-HPMC aggregates as compared to SDS micelles were ~35% higher. The aggregation number (N_{agg}) of SDS-HPMC aggregates was approximately half of that of SDS micelles. Hence, the overall increase in pyrene solubilization power of SDS-HPMC aggregates may be due to a higher number of aggregates with a more hydrophobic environment.

At higher ionic strengths, critical aggregation concentrations (CAC and CMC) of SDS-HPMC aggregates were observed to shift to lower surfactant concentrations. The increase in pyrene solubilization power at increasing ionic strengths reflected a more
hydrophobic microenvironment provided by the SDS-HPMC aggregates and provides insights into manipulating the formation and structural characteristics of these aggregates with the addition of NaCl.

In addition to SDS, the effects of surfactant properties such as head group and chain length on the state of aggregation were also determined. The HPMC-cationic surfactant aggregates were observed to be less polar than those formed with an anionic surfactant, SDS. Moreover, as the surfactant hydrophobicity (i.e., increase in chain length) increased the strength of the HPMC-cationic surfactant interactions increased, with the CAC shifting significantly to lower surfactant concentrations. Finally, the current understanding of the influence of ionic strength and the properties of ionic surfactants on the formation and structural characteristics of polymer-surfactant aggregates was utilized in subsequent studies to explore the adsorption of model polymer-surfactant aggregates to a model nanoparticle surface (silica and carbon black).
Table 5.1: Aggregation and Solubilization Characteristics of SDS Micelles at Various Concentrations of NaCl at 25°C

<table>
<thead>
<tr>
<th>NaCl (% w/w)</th>
<th>CMC (mM)</th>
<th>$N_{agg}$</th>
<th>Solubilizing Power Pyrene/surfactant</th>
<th>Solubilizing Capacitya Pyrene/micelle</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.1</td>
<td>68 ±4(95%CI)</td>
<td>6.09(±0.01) × 10^{-3}</td>
<td>0.41</td>
</tr>
<tr>
<td>0.1</td>
<td>4.3</td>
<td>100 ±9(95%CI)</td>
<td>6.42(±0.02) × 10^{-3}</td>
<td>0.64</td>
</tr>
<tr>
<td>0.3</td>
<td>2.4</td>
<td>120 ±9(95%CI)</td>
<td>7.71(±0.01) × 10^{-3}</td>
<td>0.92</td>
</tr>
<tr>
<td>0.6</td>
<td>1.2</td>
<td>134 ±16(95%CI)</td>
<td>8.22(±0.03) × 10^{-3}</td>
<td>1.09</td>
</tr>
</tbody>
</table>

a: $N_{agg}$ × solubilizing power
### Table 5.2: Effect of Ionic Strength on Aggregation and Solubilization

**Characteristics of SDS-HPMC Aggregates at 25°C.**

<table>
<thead>
<tr>
<th>NaCl (% w/w)</th>
<th>CAC (mM)</th>
<th>Solubilizing Power Pyrene/surfactant</th>
<th>$N_{agg}$</th>
<th>Solubilizing Capacity&lt;sup&gt;a&lt;/sup&gt; Pyrene/micelle</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>4.3</td>
<td>$8.18(±0.03) \times 10^{-3}$</td>
<td>$34 ±7(95%CI)$</td>
<td>0.27</td>
</tr>
<tr>
<td>0.1</td>
<td>2.6</td>
<td>$11.32(±0.04) \times 10^{-3}$</td>
<td>$47 ±3(95%CI)$</td>
<td>0.53</td>
</tr>
<tr>
<td>0.3</td>
<td>1.2</td>
<td>$12.37(±0.04) \times 10^{-3}$</td>
<td>$49 ±9(95%CI)$</td>
<td>0.60</td>
</tr>
<tr>
<td>0.6</td>
<td>0.9</td>
<td>$14.95(±0.05) \times 10^{-3}$</td>
<td>$56 ±13(95%CI)$</td>
<td>0.83</td>
</tr>
</tbody>
</table>

<sup>a</sup>: $N_{agg} \times$ solubilizing power
Figure 5.1. Representative pyrene fluorescence emission spectra in SDS solution.
Figure 5.2. Micropolarity index \((I_1/I_3)\) for pyrene fluorescence as a function of SDS concentration in SDS-water systems with 0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles) NaCl.
Figure 5.3. Ratio of excimer to monomer peak intensities ($I_e/I_{mon}$) of pyrene fluorescence as a function of SDS concentration in SDS-water systems with 0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles) NaCl. (Inset: $I_e/I_{mon}$ ratio after maximum peak as a function of SDS conc. at various concentrations of NaCl).
Figure 5.4. Micropolarity index ($I_1/I_3$) for pyrene fluorescence as a function of SDS concentration in SDS-water (squares) and HPMC (0.25%)-SDS-water (diamonds) systems.
Figure 5.5. Micropolarity index ($I_1/I_3$) for pyrene fluorescence as a function of SDS concentration in HPMC (0.25%)-SDS-water systems with 0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles) NaCl.
Figure 5.6. Ratio of excimer to monomer peak intensities ($I_e/I_{mon}$) of pyrene fluorescence as a function of SDS concentration in SDS-HPMC-water systems with 0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles) NaCl.
Figure 5.7. Pyrene solubilization power of (A) SDS micelles and (B) SDS-HPMC aggregates plotted as a function of SDS concentration at various concentrations of NaCl [0% (squares), 0.1% (triangles), 0.3% (diamonds) and 0.6% (circles)].
Figure 5.8. Micropolarity index ($I_1/I_3$) for pyrene fluorescence as a function of surfactant concentration in HPMC-DTAB (squares) and SDS-HPMC (diamonds) systems.
Figure 5.9. Micropolarity index ($I_1/I_3$) for pyrene fluorescence as a function of hydrophobicity (chain length) of cationic surfactants in surfactant-HPMC systems. DTAB-HPMC (squares), TTAB-HPMC (triangles) and CTAB-HPMC (diamonds) systems.
Mixed Adsorption of Model Ionic Surfactants with Hydroxypropyl Methylcellulose on a Model Nanoparticle Surface, Colloidal Silica

1. Introduction

Mixed adsorption process involves an interplay of interactions between polymers, surfactants, solvents, and the surface [17]. An in-depth understanding of the mixed adsorption process is essential to select the type and levels of surfactants and polymers to maximize the extent of adsorption on nanoparticle type surfaces [114]. In order to understand the mixed adsorption isotherms of polymers and surfactants, the bulk solution-state interactions between polymers and surfactants need to be explored. Moreover, the adsorption of a single component such as polymers or surfactants could also be utilized.

Polymer and surfactant adsorption has been extensively studied due to its various industrial application such as cosmetics, petroleum products, pharmaceuticals, and food items [75, 76]. The mixed adsorption of polymers and surfactants is dependent on attractive or repulsive interactions between polymers, surfactants, and surfaces [77]. Mixed systems consisting of ionic surfactants and non-ionic polymers such as polyvinyl pyrrolidone (PVP), polyethylene oxide (PEO), and polystyrene sulfonate as well as their adsorption on oxide surfaces have been studied [78-81, 111]. While the mixed adsorption of several polymers and surfactants have been studied, the complexity of polymer-surfactant interactions hinders rationale selection of the type and levels of polymer and surfactants during the development of pharmaceutical products containing nanoparticles. In general, empirical approaches have been used to develop nanoparticle-based formulations, which highlights the need to mechanistically understand the parameters that...
govern the mixed adsorption of pharmaceutically relevant polymers and surfactants on model surfaces. This report examines the extent to which solution-state interactions between a nonionic polymer hydroxypropyl methylcellulose (HPMC) and sodium dodecyl sulfate (SDS) modulate the adsorption on a model nanoparticulate solid surface (silica). Isothermal titration microcalorimetry (ITC), solution depletion techniques and ELSD-SEC were applied for this purpose. ITC was used to directly measure the change in heat flow as various interactions occurred between the HPMC, SDS, and silica, that were used to quantitatively determine critical thermodynamic parameters of processes such as aggregation of SDS with HPMC and adsorption of various species on the solid-liquid interface in the presence of silica. Furthermore, the influence of ionic strength on this aggregation behavior, adsorption enthalpies were also quantitatively determined, and probable mechanisms have been discussed.

2. Materials and Methods

2.1. Materials

Sodium dodecyl sulfate (>98%) were obtained from Sigma-Aldrich (St. Louis, MO). The SDS thus obtained were further purified by the solid phase extraction process by passing a 1%w/w aqueous solution of SDS through a Waters SEP-PAK® plus C18 environmental cartridge, the extracted solution was then lyophilized. Surface tension measurements were conducted for lyophilized sodium dodecyl sulfate to assess the presence of any local minimum near the critical micelle concentration (CMC) of the surfactant by the du Noüy ring tensiometer. Hence the SDS purchased from Sigma-Aldrich (St. Louis, MO) was purified before use. The cationic surfactants including DTAB were examined for the presence of any local minimum near the CMC, and the absence of the
same resulted in using these chemicals as received from Sigma-Aldrich (St. Louis, MO). Hydroxypropyl methylcellulose (Benecel® K-4M, molecular weight: ~86000 g/mol) was obtained from Ashland Aqualon Functional Ingredients, Ashland Inc (Wilmington, DE) and was used as obtained. Colloidal silicon dioxide (specific surface area ~202 m²/gm) is non-porous fumed particles (Cab-O-Sil® EH-5), and carbon black were purchased from Cabot Corp, MA. NaCl was purchased from Sigma-Aldrich (St. Louis, MO) and used as received. Deionized water (18.2 megohm-cm) obtained from a Milli-Q water purification system (Millipore, Billerica, MA).

2.2. Methods

2.2.1. Size Exclusion Chromatography Method with ELSD Detector

A normal phase size exclusion chromatography (SEC) with evaporative light scattering detector (ELSD) technique was utilized to develop an accurate and robust assay for simultaneous quantification of HPMC and SDS in a nanosuspension formulation. All studies in this work were carried out on the HPLC system that consisted of a Waters 2695 Separations Module (Waters, Milford, MA) coupled with a Sedex 85 low-temperature evaporative light scattering detector (SEDERE, France). The signal was acquired and processed with Millennium software (Waters, Milford, MA). A Waters Ultrahydrogel® 120 size exclusion column (5 µm, 300 mm x 7.8 mm) with a pore size of 120Å (Waters Corporation, Milford, MA) was used to separate ionic surfactants including SDS, DTAB, and HPMC. This method was used to quantitatively analyze the sample obtained from the solution depletion experiments.

2.2.2. Solution Depletion Method for Adsorption Isotherms
The solution depletion method was used to determine adsorption isotherms for various adsorbates including SDS, HPMC, and DTAB on silica at 25°C. In the solution depletion method, appropriate amounts of adsorbates were equilibrated with aqueous dispersions of silica in centrifuge tubes using a mechanical shaker. For example, 200 mg of silica was dispersed in 10 mL of HPMC or surfactant-HPMC solution at different concentrations. The equilibration time for the adsorption samples was determined to be 36 hours for all three adsorbates. Upon equilibration, the samples were centrifuged at 5000 RPM for 45 minutes to separate the silica particles, and the adsorbate concentration in the supernatant was measured after equilibration using the SEC-ELSD method.

**2.2.3. Isothermal Titration Calorimetry (ITC) Method**

The calorimetry experiments were performed using a TAM III isothermal titration calorimeter (ITC) manufactured by TA Instruments (New Castle, DE). The TAM III calorimeter operates in the power compensation mode wherein the temperature of the sample cell is maintained constant using a temperature sensor with a feedback system utilizing a reference cell. When an endothermic or exothermic event (i.e., chemical reaction, molecular reorganization, binding, solubilization) occurs during the titration, the power supplied to a heater or cooler to maintain isothermal conditions is directly measured [118]. Surfactant solutions at concentrations of 10x CMC (i.e., 87.3 mM for SDS and 129.7 mM for DTAB, respectively) were prepared and loaded into either a 1 ml or 5 ml syringe mounted on a precision pump. This surfactant solution was then titrated into a sample cell containing a known quantity of either deionized purified water (2.1 ml or 2.4 ml) or HPMC solution (0.25% w/w and 0.5% w/w) at various ionic strengths. At the start of each experiment, the reference cell contained the same solution as the sample cell.
Titrations at each predetermined time interval were performed by the syringe under computer control that injected either 20 or 25 µl of surfactant solution into the sample cell. Usually, 5 to 7 minutes were provided between injections to allow time for thermal equilibration while the sample cell was continuously stirred (60 rpm) with a turbine stirrer. Thus, in a given ITC measurement, the energetics associated with a process was directly measured at a constant temperature. For example, if an exothermic event occurred during the titration, the compensation mode (feedback loop) would cool the sample until the temperature of the sample cell was brought to the temperature of the reference cell. The signal (peak) thus obtained from the feedback loop was integrated directly to yield the heat associated (Q) with that event. The heat signal (Q) was directly related to the concentration and volume of the titrant in each injection, which was normalized with respect to the moles (δₙ) of surfactant added to the sample cell. The signal was further analyzed to obtain the apparent enthalpy change (ΔHₚₛ = Q/δₙ) using TAM III Lab Assistant Software provided by TA Instruments. The apparent enthalpy change (ΔHₚₛ) was plotted against the surfactant concentration, i.e., enthalpograms. To determine the enthalpy of micellization (ΔHₘᵢₖ), lines are drawn to fit two plateaus that were observed above and below the inflection that are extended beyond the inflection. A line is drawn a perpendicular to the x-axis at the CMC, and the enthalpy of demicellization is measured from the length of the segment connecting the lines. The CMC value is defined as the extreme value of the first derivative of the ΔHₚₛ as a function of total surfactant concentration plot. All experiments were repeated at least twice to check for reproducibility of the measurements.

2.2.4. BET Surface Area Measurement
The surface area of silica was measured utilizing the Brunauer–Emmett–Teller (BET) method where nitrogen was used as a model adsorbate and the adsorption of nitrogen was measured using Tristar 3000 (Micromeritics, USA). The silica samples (triplicate) were purged with nitrogen (~4 hours) and degassed (120°C) prior to analysis.

2.2.5. Data Analyses

Microsoft Excel program was used for the Student t-test and ANOVA. The non-linear regression analyses were carried out using Scientist program (Micromath Inc., St. Louis, MO, USA). The heat signal data were analyzed using TAM III Lab Assistant Software provided by TA Instruments.

3. Results and Discussion

3.1. SEC-ELSD Method for Simultaneous Quantification of SDS, DTAB, and HPMC in Nanoparticulate Formulations

An SEC-ELSD method was developed in our previous study (Chapter 3) where simultaneous detection and quantification of excipients such as surfactants like dodecyl maltoside (DM) and HPMC were achieved in a single chromatogram. The method was further optimized using a design of experiments while varying several parameters including instrument gain, nebulizing pressure, and drift tube temperature. The optimized method was used in the present study to simultaneously quantify SDS, DTAB, and HPMC in the nanoparticulate formulation samples obtained from the solution depletion experiments which in turn, were utilized to determine the amount of SDS, HPMC, and DTAB adsorbed onto a model nanoparticulate surface, silica.

A representative SEC-ELSD chromatogram of SDS and HPMC is shown in Figure 6.1. The method successfully resolved the peaks of SDS and HPMC in a single
chromatogram. DM was not selected as a model surfactant in the present study as preliminary experiments showed no significant interaction between DM and HPMC in solutions at various ionic strengths. Additionally, DM did not show significant adsorption onto the model surface, silica. SDS and DTAB were selected as model ionic surfactants to determine any correlation between their solution-state interactions and the adsorption onto silica.

3.2. Adsorption of Ionic Surfactants and HPMC on Model Surfaces: Individual Adsorption Isotherms

The study of adsorption isotherms is essential in understanding the interactions of model adsorbates SDS, DTAB, and HPMC with model adsorbent surfaces such as silica and carbon black. The HPMC adsorption isotherm for silica is shown in Figure 6.2. The HPMC adsorption isotherm is typical a high-affinity isotherm type. The adsorption isotherm was modeled using the Langmuir adsorption isotherm model (Eq. 1) [65, 199]. The model assumes a monolayer formation when adsorbates interact with free, identical, and limited adsorbent sites.

$$\frac{\theta}{\theta_{\max}} = \frac{Kc}{1 + Kc}$$

(6.1)

where $\theta$ is the amount of HPMC adsorbed per unit surface area of silica (mg/m$^2$) at a specific concentration of HPMC, $c$ (mg/L). $K$ is the Langmuir affinity constant (L/mg). $\theta_{\max}$ is the maximum amount of HPMC adsorbed per unit surface area of silica, which is commonly termed as the extent of HPMC adsorption. As shown in Figure 6.2 (solid line), the Langmuir adsorption isotherm model fit the HPMC isotherm. The extent of HPMC adsorption for silica was 0.9 ± 0.1 mg/m$^2$. The Langmuir constant for HPMC was obtained from the model fit as 19 ± 9 mL/mg. The smoothness of the isotherm between lower
adsorption values and the plateau is in accordance with the effect of HPMC molecular weight distribution [61]. This may indicate that the long chains of HPMC preferentially adsorbed to silica as compared to short (low molecular weight) chains by displacing them from the surface [61]. The adsorption of HPMC onto silica can be attributed to hydrophobic interactions and hydrogen bonding.

The adsorption isotherm of SDS, a model ionic surfactant, onto silica is shown in Figure 6.3a (no NaCl). The extent of SDS adsorption onto silica was 0.05 mg/m$^2$. SDS did not show as high adsorption onto silica as seen with HPMC. This could be due to the negative charge of silica and SDS in the sample. The pH of the sample was around 8, which exceeded the isoelectric point of silica of 2.5 [200]. The negatively charged silanol groups on the silica surface would create charge repulsion from the negatively charged SDS and hence would result in the lower extent of adsorption of SDS onto silica. This hypothesis was confirmed by (1) exploring the extent of adsorption of a model cationic surfactant, DTAB, on the negatively charged silica surface, (2) conducting SDS adsorption experiments with silica at higher ionic strengths, and (3) determining the extent of adsorption of SDS onto a model non-ionic surface, carbon black.

If the negatively charged surface of silica is replaced with a neutral adsorbent surface such as carbon black, the extent of SDS adsorption should increase. As shown in Figure 6.3b, the extent of SDS adsorption onto carbon black was much higher (~0.6 mg/m$^2$) than that with silica, which confirmed the hypothesis that the anionic nature of SDS could lower its adsorption onto silica. The extent of adsorption of positively charged DTAB onto the negatively charged silica was determined. As shown in Figure 6.2b, the extent of DTAB adsorption onto silica was significantly higher than the of the negatively charged
SDS (2.2 vs. 0.05 mg/m²). This could be attributed to stronger interactions between the negatively charged silica and positively charged DTAB [201]. Additionally, the shielding of the negative charge of SDS would increase the extent of adsorption onto silica. As shown in Figure 6.3a, the presence of NaCl significantly increased SDS adsorption onto silica. The amount of SDS adsorbed increased from 0.05 mg/m² (no NaCl) to 0.12 mg/m² (0.01M NaCl), 0.42 mg/m² (0.05M NaCl), and 0.44 mg/m² (0.16 M NaCl). Contrastingly, the effect of ionic strength on the adsorption of SDS on carbon black was negligible (Figure 6.3b), which was attributed to the neutral charge of carbon black. The extent of SDS adsorption did not change significantly when the ionic strength was increased by ~3-fold. This could indicate that the surface coverage of silica by SDS reaches its maximum at ~0.4 mg/m².

To further explore the surface coverage of silica by SDS, the extent of SDS adsorption was estimated theoretically. The theoretical estimation assumed that (1) SDS was adsorbed in a compact monolayer, (2) the adsorbed SDS molecules were in tail conformation and attached to the surface at one end and the other end, i.e., dodecyl chain is extended out in bulk solution, and (3) the surface area occupied by one SDS molecule would be the same as the topological polar surface area of SDS (74.8 Å²) [202]. The tail conformation of SDS would be a reasonable assumption considering the hydrophilicity of negatively charged head groups and their selective hydration by water. The rationale behind the selection of the topological polar surface area of SDS is that SDS molecules that are adsorbed onto silica would be separated by the negatively charged head groups and hence the minimum surface area occupied would be similar to the polar surface area. The theoretical extent of SDS adsorption is ~0.6 mg/m², which was similar to the extent of SDS
adsorption onto carbon black. The extent of SDS adsorption onto silica was slightly lower than the maximum surface coverage possible while assuming compact monolayer (0.4 vs. 0.6 mg/m²). This indicates that even at the highest ionic strength the SDS molecules adsorbed on silica does not form as compact monolayer as the SDS molecules adsorbed on carbon black.

3.3. Mixed Adsorption of SDS and HPMC on Silica

Another objective of this study was to determine if the adsorption behavior of SDS for silica was modified in the presence of HPMC. Figure 6.4 illustrates the adsorption of SDS in the presence of HPMC (5 mg/mL). The amounts of SDS adsorbed on silica at 4 mM SDS concentration were 0.02 and 0.2 mg/m² without and with HPMC, respectively. Thus, the extent of SDS adsorption increased by 10-fold at 4 mM SDS concentration in the presence of HPMC.

The dramatic increase in SDS adsorption in the presence of HPMC at low SDS concentrations correlated well with the critical aggregation concentration (CAC) of SDS-HPMC aggregates ~4 mM as reported in Chapters 4 and 5. This indicates that the formation of SDS-HPMC aggregates facilitates the adsorption of SDS on silica. This behavior may be attributed to the aggregation process between HPMC and SDS in solution. As a result of the correlation and our findings from the previous studies, it is suggested that the increase in SDS adsorption onto silica in the presence of HPMC is related to the cooperative binding between SDS and HPMC, which is predominantly driven by hydrophobic interactions (Chapter 4; Figure 6.3). This combined with the high affinity of HPMC for the silica surface will allow the SDS-HPMC aggregates to cooperatively adsorb
onto the silica surface. Here too, the adsorption process is proposed to be driven mainly by hydrophobic interactions between SDS-HPMC aggregates and silica.

However, at higher concentrations of SDS (>>CAC), a significant decrease in the adsorbed amount of SDS on silica was determined. The adsorption isotherm shows an almost linear decrease in the adsorption of SDS as the concentration of SDS is approaching the previously determined critical saturation concentration for the binding of SDS to HPMC (C_{sat}) of ~15 mM and critical SDS concentration for free micelle formation (CMC) of ~20 mM. Previous studies have shown that in this region, the breakdown of the SDS-HPMC aggregate structures may occur due to electrostatic repulsion of the concentrated negatively charged SDS head groups at higher SDS concentrations. This may also cause unfolding and rehydration of HPMC chains containing adsorbed SDS molecules [82, 102, 203]. This may lead to decreased adsorption of SDS at concentrations above CAC (Figure 6.4).

Overall, these results indicate that the adsorption of SDS onto silica is significantly enhanced in the presence of HPMC. This increase in adsorption may be due to the formation of SDS-HPMC aggregates at SDS concentrations at or below CAC (cooperative adsorption) and the adsorption of SDS decreases significantly at higher SDS concentrations above CAC may be due to the charge repulsion of SDS head group and the unfolding of polymer (competitive adsorption).

3.4. Energetics of Mixed Adsorption of SDS and HPMC on Silica

To further confirm the above-mentioned hypothesis for the SDS adsorption on silica in the presence of HPMC, the enthalpy changes during titration that includes the formation of SDS-HPMC aggregates and their adsorption to the silica surface as a function
of SDS concentration were determined using ITC. Similar to the SDS-HPMC system described in Chapter 4, the change in apparent enthalpy for polymer-surfactant-silica interactions ($\Delta H_{app}$) may consist of various components shown in the model below in Eq. 6.2 [118, 148, 154].

$$\Delta H_{app} = \Delta H_{dil(mon,s)} - \Delta H_{mic} + \Delta H_{dil}(p) + \Delta H_{(p-s)} + \Delta H_{dil(p-s)} + \Delta H_{dil(mic,s)} + \Delta H_{s-sli} + \Delta H_{dil(s-sli)} + \Delta H_{p-sli} + \Delta H_{dil(p-sli)} + \Delta H_{p-s-sli} \tag{6.2}$$

where $\Delta H_{dil(mon,s)}$ and $\Delta H_{dil(mic,s)}$ are the enthalpy of dilution of surfactant monomers or smaller self-associated aggregates and surfactant micelles, respectively. $\Delta H_{dil(p)}$ and $\Delta H_{dil(p-s)}$ are the enthalpy of dilution of polymer and polymer-surfactant aggregates, respectively. $\Delta H_{mic}$ is the enthalpy of micellization, $\Delta H_{(p-s)}$ is the enthalpy of polymer-surfactant aggregation, and $\Delta H_{dil(p-s)}$ is the enthalpy of dilution of SDS-HMC aggregates. The $\Delta H_{s-sli}$ and $\Delta H_{dil(s-sli)}$ represent the enthalpy change of surfactant-silica interaction and the enthalpy of dilution of the surfactant adsorbed onto silica.

The term $\Delta H_{p-sli}$ is the enthalpy of polymer and silica interaction or adsorption, however, since the polymer and silica are already present in the sample cell at equilibrium, this enthalpy change can be considered negligible and thus be ignored in this model. $\Delta H_{dil(p-si)}$ is the enthalpy of dilution of the polymer adsorbed onto silica was found to be negligible in the concentration range (0.5 mg/ml) of HPMC selected for this ITC experiment and can thus also be ignored. The terms $\Delta H_{s-sli}$ and $\Delta H_{dil(s-sli)}$ are considered negligible because of the lack of interaction observed between SDS and silica (Figure 6.3a; diamonds). $\Delta H_{p-s-sli}$ and $\Delta H_{dil(p-s-sli)}$ represent the enthalpy change of polymer-
surfactant-silica interaction and the enthalpy of dilution of the polymer-surfactant aggregates adsorbed onto silica. Thus, the above Eq. 6.2 can be written as Eq. 6.3.

\[
\Delta H_{\text{app}} = \Delta H_{\text{dil(mon},s)} - \Delta H_{\text{mic}} + \Delta H_{\text{dil}(p)} + \Delta H_{(p-s)} + \Delta H_{\text{dil}(p-s)} + \Delta H_{\text{dil}(p-s)} + \Delta H_{p-s-sl} + \Delta H_{\text{dil}(p-s-sl)}
\]  

(6.3)

For the more accurate determination of SDS-HPMC-silica interactions (i.e., \(\Delta H_{p-s-sl} + \Delta H_{\text{dil}(p-s-sl)}\)), a novel correction approach, similar to the one used in Chapter 4, was utilized that accounted for various species expected in the sample cell during titration. The various enthalpy of dilutions shown in Eq. 6.3 were measured independently by direct experimentation utilizing ITC and subtracted based on the different regions of the interaction enthalpogram. The interaction curve can be divided into three regions: (i) A→C region, (ii) C→D region, and (iii) ≥ D region as described in Chapter 4. In the A→C region of the SDS-HPMC-silica interaction curve, SDS monomers and or smaller self-associated aggregates such as dimers, trimers etc. are expected to predominate (e.g., <17 mM SDS at 0.25% w/w HPMC and 25°C). Cooperative adsorption of SDS-HPMC aggregates onto to the silica surface is also expected to predominate in this region and will be described in detail the later section. The corrected enthalpy (\(\Delta H_{\text{corr}}\)) for this region is shown as Eq. 6.4.

\[
\Delta H_{\text{corr(A→C region)}} = \Delta H_{\text{app}} - (\Delta H_{\text{dil(mon},s)} + \Delta H_{\text{mic}} + \Delta H_{\text{dil}(p)} + \Delta H_{(p-s)} + \Delta H_{\text{dil}(p-s)})
\]  

(6.4)

In the next C→D region, SDS micellization is expected to occur along with the competitive adsorption of the SDS-HPMC aggregates on the silica surface (described in detail in the next section). The corrected enthalpy (\(\Delta H_{\text{corr}}\)) for this region is shown as Eq. 6.5.
\[ \Delta H_{corr}(C \rightarrow D\ region) = \Delta H_{app} - (\Delta H_{dil(mon,s)} + (1 - f)\Delta H_{mic}^\circ + \Delta H_{dil(p)} + \Delta H_{(p-s)} + \Delta H_{dil(p-s)}) \]  

(6.5)

where \((1 - f)\) is the fraction of micelles that dissociate in the sample cell and the value of \(f\) increases from 0 to 1 when the SDS concentration increases from 7-21 mM with \(f = 0.5\) at the observed CMC. The \(\Delta H_{dil(mon,s)}\) could also be a cumulative effect of dilution of monomers and the formation of smaller self-associated aggregates such as dimers, trimers etc. The final two terms of the equation are associated with the SDS-HPMC interaction i.e., \(\Delta H_{(p-s)}\) and \(\Delta H_{dil(p-s)}\), enthalpy of polymer-surfactant aggregation, and enthalpy of dilution of SDS-HMC aggregates.

The next region corresponds to \(\geq D\) region, where the micellar solution of SDS being titrated into the sample cell is expected to remain as SDS micelles and \(\Delta H_{corr}\) can be shown as Eq. 6.6.

\[ \Delta H_{corr}(\geq D\ region) = \Delta H_{app} - (\Delta H_{dil(mic,s)} + \Delta H_{dil(p)} + \Delta H_{(p-s)} + \Delta H_{dil(p-s)}) \]  

(6.6)

where \(\Delta H_{dil(mic,s)}\) is the enthalpy of dilution of the surfactant micelles above the CMC.

Figure 6.5 shows the corrected enthalpy \((\Delta H_{corr})\) plotted as a function of SDS concentration at 25°C for the titration of a micellar solution of SDS into the sample cell containing the aqueous dispersion of HPMC K4M (0.5% w/w) and silica (2% w/w). From previous studies, it was determined that 25°C was a suitable temperature to study SDS/HPMC interactions since the dilution enthalpy of both SDS and HPMC \((\Delta H_{dil(mon,s)} + \Delta H_{dil(mic,s)} + \Delta H_{dil(p)})\) are negligible and hence all adsorption studies were conducted at this temperature. The overall shape of the enthalpogram of SDS-HPMC-Silica system \(\Delta H_{app}\) is similar to that of SDS-HPMC (Chapter 4. Figure 6.4.).
A distinct region above ~4 mM SDS concentration where apparent enthalpy change increases sharply reaching a maximum. This could be attributed to the formation of SDS-HPMC aggregates and their adsorption on silica (cooperative adsorption) [154, 162, 203].

The enthalpy of cooperative adsorption of SDS-HPMC aggregates on silica is determined from the first endothermic peak of 1.25 kJ/mole (Figure 6.5), whereas the adsorption enthalpy for SDS on silica (no HPMC) was negligible (~0.1 kJ/mole). The ~12-fold increase in the adsorption enthalpy of SDS-HPMC aggregates correlated well with ~10-fold increase in the SDS adsorption as determined by the solution-depletion method.

The competitive adsorption region above ~6 mM SDS concentration where SDS adsorption on silica decreases as the bulk SDS concentration increases is reflected as an exothermic linear decline in the enthalpograms reaching a minima (Figure 6.5.a and 6.5.b). From Figure 6.5., the enthalpy of the competitive adsorption process is determined to be -1.95 kJ/mole. As more SDS molecules are being incorporated into SDS-HPMC aggregates, a greater density of negatively charged head groups of SDS may cause electrostatic repulsion resulting in the unfolding of HPMC chains and expulsion of SDS monomers from the aggregates resulting in hydration of HPMC chains and SDS monomers and related exothermic enthalpy change [154, 162, 203]. This supports the hypothesis that the rehydration of HPMC and SDS (as reflected as an exothermic process) due to the head group charge repulsion and subsequent unfolding of HPMC chains could be attributed to the decrease or displacement in SDS adsorption above bulk SDS concentration of ~6 mM.

Next, the adsorption enthalpy changes for DTAB adsorption on silica in the presence and absence of HPMC were determined. The purpose here was to determine if the bulk solution-state interactions between HPMC and the model cationic model
surfactant, DTAB, influenced its adsorption on negatively charged silica surface as observed earlier in the case of the model anionic surfactant, SDS, where the bulk solution-state interactions significantly increased its adsorption on silica. The enthalpy of adsorption of DTAB on silica is ~20-fold higher than that for SDS adsorption on silica (2 kJ/mole vs. 0.1 kJ/mole). This explains higher adsorption values of DTAB for silica as compared SDS adsorption values (Figure 6.2.b and 6.3.a). However, unlike the influence of HPMC on the SDS adsorption enthalpy for silica, the presence of HPMC did not change the adsorption enthalpy of DTAB for silica (Figure 6.6.a and 6.6.b). This observation was further investigation by understanding the bulk solution-state interactions between DTAB and HPMC. As shown in Figure 6.7.a, the enthalpogram for the DTAB-HPMC-water system was not different from the enthalpogram for DTAB-water system.

Moreover, the change in HPMC concentration did not influence the enthalpograms for DTAB-HPMC-water system. This indicates that there were no significant bulk solution-state interactions between DTAB and HPMC in water. This observation is in accordance with previous studies in the literature where no significant interactions between cationic surfactants and non-ionic polymers in water have been reported [204-206]. The lack of interactions between DTAB and HPMC in water may be attributed to the head group charge and its impact on the size of the head group. Higher repulsion between head groups could decrease the packing density of surfactant molecules resulting in larger and less densely packed aggregates (Chapter 5). To further support this hypothesis, we determined the effect of charge shielding on the interaction between DTAB and HPMC. As shown in Figure 6.7b, the enthalpograms with 0.1% NaCl showed an endothermic peak at lower DTAB concentrations, which is indicative of weak interactions between DTAB
and HPMC. This may suggest that the lack of solution-state interactions between DTAB and HPMC could be due to the properties of DTAB head group. Finally, the bulk-solution state interactions between ionic surfactants and HPMC could vary depending on the properties of the surfactant head group, and that could be modulated by changing solution properties such as ionic strength. The bulk-solution interactions could vary with surfactant concentrations and thereby influence the formation of surfactant-polymer aggregates and their adsorption on model nanoparticle surfaces of the NDDS system.

4. Conclusions

The extent and mechanism of the mixed adsorption of HPMC and ionic surfactants (SDS and DTAB) on model nanoparticle surfaces (silica and carbon black) were determined utilizing ITC, solution depletion, and ELSD-SEC techniques. The adsorption of SDS, a model anionic surfactant, on the negatively charged silica surface was found to be negligible. However, the addition of electrolyte (NaCl) had a significant effect on enhancing the adsorption of SDS on silica. HPMC was determined to have a strong affinity for the silica surface and followed the Langmuir adsorption behavior. The presence of HPM increased interactions between SDS-HPMC aggregates and silica. SDS adsorbed onto silica in the presence of HPMC in a cooperative manner at low SDS concentrations, and in a competitive manner at high SDS concentrations. This behavior was correlated to the bulk solution-state interaction between SDS and HPMC.

For DTAB, a model cationic surfactant, the adsorption on silica was higher as compared to SDS and could be driven by electrostatic interactions. The lack of adsorption of DTAB-HPMC aggregates onto silica correlated well with the weak interactions between DTAB and HPMC in solution. Overall, this study shows that the mixed adsorption of
polymers and surfactants could be studied using techniques such as the solution-depletion method (SEC-ELSD) and ITC. The understanding of solution-state interactions and formation of surfactant-polymer aggregates could be utilized to enhance their adsorption and stabilization of nanoparticulate drug delivery systems.
Figure 6.1. Representative SEC-ELSD chromatograms for HPMC and SDS.
Figure 6.2. Adsorption isotherms of (a) HPMC and (b) DTAB on silica.
Figure 6.3. Effect of ionic strength on the adsorption of SDS on (a) silica and (b) carbon black.
Figure 6.4. Mixed adsorption isotherm of SDS on silica surface in the presence of HPMC.
Figure 6.5. Plot of corrected enthalpy ($\Delta H_{corr}$) for SDS adsorption on silica as a function of SDS concentration in the presence of 0.5% HPMC K-4M at 25º C.
Figure 6.6. Plot of corrected adsorption enthalpy for DTAB adsorption on silica as a function of DTAB concentration in the presence of (a) no HPMC and (b) 0.5% HPMC K-4M at 32°C.
Figure 6.7. Apparent enthalpy ($\Delta H_{\text{app}}$) for DTAB-HPMC interactions as a function of DTAB concentration in the presence of (a) no NaCl at 32º C and (b) 0.1% NaCl at 25º C.
CHAPTER 7

Conclusions

A unique approach utilizing fluorescence, solution calorimetry, HPLC-ELSD and adsorption isotherms were applied to tease apart the effect of solution state interactions of polymer and surfactant on the extent of simultaneous adsorption of the two excipients on a model surface. A fast, robust and accurate assay was developed for the simultaneous quantification of polymer (HPMC) and surfactant (DM) in the pure standards and mixed standards with silica-based nanosuspension formulations. The design of experiments was used successfully to understand the influence of critical parameters of ELSD (drift tube temperature, nebulizer pressure, and instrument gain) on the responses of the assay. An optimized design space was also identified by using a full factorial design of experiments (DoE). An increase in drift tube temperature and instrument gain increased the accuracy of the assay while a decrease in nebulizer pressure improved the sensitivity of the assay. An increase in drift tube temperature and instrument gain decreased the % deviation of slopes for both DM and HPMC responses. The assay was proven to be robust with respect to all three critical ELSD parameters within the optimized design space. The optimization of the assay using the factorial design of experiments led to the prediction of 93% desirability at the extreme levels of the two factors (drift tube temperature and instrument gain) and an intermediate level of the third factor (nebulizer pressure). This method was used to quantify these pharmaceutical excipients (HPMC and DM) in nanosuspension formulations.

Isothermal titration calorimetry (ITC) with a novel data treatment was utilized to study the solution-state interaction between ionic surfactants and HPMC. The novel data
treatment increased the accuracy of the measured thermodynamic parameters of surfactant-HPMC aggregation and subsequent interpretation. This treatment involved the identification and appropriate accounting for the concentration-dependent species (i.e., surfactant monomers and micelles, and surfactant-HPMC aggregates) and related enthalpies. The interaction of SDS with HPMC was determined to be stronger than DTAB and HPMC. The interaction between SDS and HPMC was endothermic and cooperative in nature and dependent on temperature and ionic strength of the solution. The effect of temperature, HPMC molecular weight, and ionic strength was utilized to postulate the mechanism of SDS-HPMC aggregate formation at a critical aggregate concentration (CAC). The driving force for the SDS-HPMC interactions is suggested to be the hydrophobic effect. At the highest molecular weight and concentration of HPMC, the critical concentration parameters $C_{\text{sat}}$ and CMC are significantly altered and shift to a higher concentration of SDS. Ionic strength significantly influenced SDS-HPMC aggregation. Specifically, the critical concentration parameters (CAC and CMC) decreased with increasing ionic strength for both anionic and cationic surfactant-HPMC systems suggesting stronger interactions.

The influence of surfactant properties and ionic strength on the formation and structural characteristics of surfactant-HPMC aggregates were examined using a pyrene steady-state fluorescence probe method. The pyrene fluorescence method was effective in quantitatively determining surfactant-HPMC interaction parameters (CAC and CMC) and structural characteristics (e.g., aggregation number and microenvironment) of surfactant-HPMC aggregates and free surfactant micelles at pharmaceutically relevant concentrations. The presence of HPMC significantly altered the structural characteristics of surfactant-
HPMC aggregates. In the presence of HPMC, the structures of the aggregates formed were much smaller with an aggregation number \(N_{agg}\) of 34 as compared to micelles \(N_{agg} \sim 68\) formed in the absence of HPMC. The microenvironment of SDS-HPMC aggregates was determined using the micropolarity index and the pyrene solubilization power and was found to be more hydrophobic as compared to SDS micelle. The pyrene solubilization power of SDS-HPMC aggregates as compared to SDS micelle was 35% higher. The aggregation number \(N_{agg}\) of SDS-HPMC aggregates was approximately half of that of SDS micelles. Hence, the overall increase in pyrene solubilization power of SDS-HPMC aggregates may be due to a higher number of aggregates with a more hydrophobic environment. At higher ionic strengths, critical aggregation concentrations (CAC and CMC) of SDS-HPMC aggregates were observed to shift to lower surfactant concentrations. The increase in pyrene solubilization power at increasing ionic strengths reflected a more hydrophobic microenvironment provided by the SDS-HPMC aggregates and provides insights into manipulating the formation and structural characteristics of these aggregates with the addition of NaCl. In addition to SDS, the effects of surfactant properties such as head group and chain length on the state of aggregation were also determined. The HPMC-cationic surfactant aggregates were observed to be less polar than those formed with an anionic surfactant, SDS. Moreover, as the surfactant hydrophobicity (i.e., increase in chain length) increased the strength of the HPMC-cationic surfactant interactions increased, with the CAC shifting significantly to lower surfactant concentrations. Finally, the current understanding of the influence of ionic strength and the properties of ionic surfactants on the formation and structural characteristics of polymer-surfactant aggregates was utilized
in subsequent studies to explore the adsorption of model polymer-surfactant aggregates to a model nanoparticle surface (silica and carbon black).

The extent and mechanism of the mixed adsorption of HPMC and ionic surfactants (SDS and DTAB) on model nanoparticle surfaces (silica and carbon black) were determined utilizing ITC, solution depletion, and ELSD-SEC techniques. The adsorption of SDS, a model anionic surfactant, on the negatively charged silica surface was found to be negligible. However, the addition of electrolyte (NaCl) had a significant effect on enhancing the adsorption of SDS on silica. HPMC was determined to have a strong affinity for the silica surface and followed the Langmuir adsorption behavior. The solution depletion and HPMC/ELSD methods showed a marked increase in the adsorption of SDS onto silica in the presence of HPMC. However, at high SDS concentrations, a significant decrease in the adsorbed amount of HPMC onto silica was determined. This suggested that the decrease in adsorption of HPMC onto silica at high SDS concentrations was due to SDS-HPMC competitive adsorption. At low SDS concentrations, an increase in adsorption of SDS was due to cooperative adsorption. A strong adsorption enthalpy of 1.25 kJ/mol was determined for SDS adsorption onto silica surface in the presence of HPMC as compared to the negligible adsorption enthalpy of 0.1 kJ/mol for SDS alone on the silica surface. For DTAB, a model cationic surfactant, the adsorption on silica was higher as compared to SDS and could be driven by electrostatic interactions. The lack of adsorption of DTAB-HPMC aggregates onto silica correlated well with the weak interactions between DTAB and HPMC in solution. This adsorption behavior confirmed our hypothesis that the solution-state interactions between pharmaceutical excipients such
as polymers and surfactants would significantly impact the affinity and capacity of adsorption of these excipients on NDDS surfaces.

Overall, the graphical mapping of the critical factors within the optimized design space helped in identifying the best conditions to develop an assay that is both repeatable and robust. The sensitivity and accuracy of this method are critical towards developing a mechanistic understanding of the physical stabilization process of nanosuspensions. The interpretation of the microcalorimetric studies at different temperatures and ionic strengths while varying the properties of polymer and surfactant was very effective in developing insights into the nature and energetics of HPMC and ionic surfactant interactions. The pyrene solubilization method helped in exploring the structural aspects of the surfactant-HPMC aggregates. The presence of HPMC increased interactions between SDS-HPMC aggregates and silica. SDS adsorbed onto silica in the presence of HPMC in a cooperative manner at low SDS concentrations, and in a competitive manner at high SDS concentrations. The understanding of solution-state interactions and formation of surfactant-polymer aggregates could be utilized to enhance their adsorption and stabilization of nanoparticulate drug delivery systems.
APPENDICES

Appendix A. Representative data transformation and statistics (std. dev and CI) to determine critical thermodynamic parameters obtained by processing raw data directly obtained from isothermal titration calorimetry (peak area) for SDS-HPMC enthalpograms.
### Appendix B. Abbreviations

- **NDDS**- Nanoparticle drug delivery systems
- **CAC**- Critical aggregation concentration
- **CMC**- Critical micelle concentration
- **HPMC**- Hydroxypropyl methylcellulose
- **SDS**- Sodium dodecyl sulfate
- **DM**-dodecyl β-D-maltoside
- **DTAB**- Dodecytrimethylammonium bromide
- **TTAB**- Tetradecyltrimethylammonium bromide
- **CTAB**- Hexadecyltrimethylammonium bromide
- **PEO**- Polyethylene oxide
- **PVP**- Polyvinyl pyrrolidone
- **EHEC**- Ethyl hydroxyethyl cellulose
- **NaCl**- Sodium chloride
- **LiDS**- Lithium dodecylsulfate
- **NMR**- Nuclear magnetic resonance
- **BET**-Brunauer–Emmett–Teller
- **FPT**- Fluorescence probe technique
- **UV-Vis**- Ultraviolet–visible
- **ITC**- Isothermal titration calorimetry
- **HPLC**-high pressure liquid chromatography
- **ELSD**- evaporative light scattering detector
- **SEC**-Size exclusion chromatography
- **DoE**-Design of experiments
- **LCMS**-Liquid chromatographic mass spectroscopy
- **UV-Vis**-Ultraviolet Visible

\[ \Delta G_{mic}^\circ \text{- Standard enthalpy} \]
\[ \Delta H_{app}^\circ \text{- Apparent enthalpy} \]
\[ \Delta H_{corr}^\circ \text{- Corrected enthalpy} \]
\[ \Delta H_{mic}^\circ \text{- Standard enthalpy of micellization} \]
\[ \Delta H_{demic}^\circ \text{- Enthalpy of demicellization} \]
\[ \Delta S_{mic}^\circ \text{- Standard entropy} \]
\( \Delta C_p \)-Heat capacity
\( \Delta H_{dil(\text{mon},s)} \)-Enthalpy of dilution of surfactant monomers
\( \Delta H_{dil(\text{mic},s)} \)-Enthalpy of dilution of surfactant micelles
\( \Delta H_{(p-s)} \)-Enthalpy for SDS-HPMC interactions
\( \Delta H_{dil(p-s)} \)-Enthalpy of dilution of SDS-HPMC aggregates

\( C_{\text{sat}} \)- Polymer saturation concentration
\( C_m \)- Critical micelle concentration in the presence of HPMC

\( \Delta G_{agg}^\circ \)-Standard free energy of aggregation
\( H_{agg}^\circ \)-Standard enthalpy of aggregation
\( \Delta S_{agg}^\circ \)-Standard entropy of aggregation

\( \Delta H_{s-si} \)-Enthalpy change of surfactant-silica interaction
\( \Delta H_{dil(s-si)} \)-Enthalpy of dilution of surfactant adsorbed onto silica

\( M_w \)-Molecular weight

Cab-O-Sil®EH-5-Colloidal silicon dioxide or silica
REFERENCES


24. Whelan, M.R., J.L. Ford, and M.W. Powell, *Simultaneous determination of ibuprofen and hydroxypropylmethylcellulose (HPMC) using HPLC and


60. Elsayed, I., A.A. Abdelbary, and A.H. Elshafeey, *Nanosizing of a poorly soluble drug: technique optimization, factorial analysis, and pharmacokinetic study in*


89. Vanstam, J.A., M; Lindblad,C, *Sodium dodecyl sulfate-poly(ethyleneoxide) interactions studied by time resolved fluorescence quenching* Trends in Colloid and Interface Science V ed. M.M. Corti, F. Vol. 84. 1991, Conf of the European Colloid and Interface Soc Location: ITALY Date: Sep, 1990 European Colloid and Interface Soc ; CNR; Messina Univ, Dept Phys; Assessorato Agr Reg Clabria; IBM Italy; Spectra Phys; Chemifarm


130. Zhang, R., L. Zhang, and P. Somasundaran, *Study of mixtures of n-dodecyl-β-D-maltoside with anionic, cationic, and nonionic surfactant in aqueous solutions*


181. Binanalimbele, W. and R. Zana, *Fluorescence probing of microdomains in aqueous-solutions of polysoaps.1. Use of pyrene to study the conformational state*


VITA

Salin Gupta Patel

Educational Institutions and Degrees:

Master of Science (Pharmaceutical Sciences)
St. John’s University, NY.

Bachelors in Pharmacy
Prin. K. M. Kundanani College of Pharmacy, University of Bombay, Mumbai, India

Professional Positions:

Manager-Senior Manager, 2016-Present
Otsuka Pharmaceutical, Princeton, NJ

Senior Scientist-Associate Principal Scientist, 2011-2015
Merck & Co. Inc., Kenilworth, NJ

Scientist-Associate Scientist, 2002-2006
GlaxoSmithKline Inc., Research Triangle Park, NC

Scholastic & Professional Honors:

1998 Intercollegiate award for a project on small volume parenterals
2000-present Member, American Association of Pharmaceutical Scientist (AAPS)
2003 GSK Silver Award for Excellence
2007 Pfizer research grant
2007-2009 Executive Member, Graduate Student Congress, University of Kentucky
2007-2008 Secretary and Executive Council Member, AAPS Student Chapter, University of Kentucky
2008-2009 Vice Chair, AAPS Student Chapter, University of Kentucky
2009-2010 Chair, AAPS Student Chapter, University of Kentucky
2009 Outstanding student chapter award from AAPS
2012 Merck Award of Excellence for Solubilization course
2013 Merck Award of Excellence for Enhancing Collaboration
2013 George W Merck PSCS leadership Award
2016 Otsuka SPARK Award
2017 Otsuka SPARK Award
2018 Otsuka SPOT Award
Professional Publications:


Patel, S.G. and P.M. Bummer, Development of a Robust Method for Simultaneous Quantification of Polymer (HPMC) and Surfactant (Dodecyl beta-D-Maltoside) in Nanosuspensions. AAPS PharmSciTech, 2015, Issue 7.


Patel, S.G. and P.M. Bummer, Exploring Factors Influencing Structural Characteristics of Surfactant-Polymer Aggregates using a Fluorescence Probe Technique. (Pharmaceutical Research, under review-minor comments received)

Patel, S.G. and P.M. Bummer, Mixed Adsorption of Model Ionic Surfactants with Hydroxypropyl Methylcellulose on a Model Nanoparticle Surface, Colloidal Silica. (submitted to International Journal of Pharmaceutics)