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Quartz Crystal Microbalance as a Predictive Tool for Drug-Material of Construction Interactions in Intravenous Protein Drug Administration

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ABSTRACT

As a growing number of protein drug products are developed, formulation characterization is becoming important. An IgG drug product is tested at concentrations from 0.0001−0.1 mg/mL for adsorption behavior to polymer surfaces polyvinyl chloride (PVC) and polypropylene (PP) upon dilution in normal saline (NS) using quartz crystal microbalance with dissipation (QCM-D). The studies mimicked IgG antibody interaction during IV administration with polymeric surfaces within syringes, lines, and bags. Drug product was characterized with excipients, with focus on surfactant. Drug solutions were run over polymer-coated sensors to measure the adsorption behavior of the formulation with emphasis on the behavior of each of the formulation's components. Over 60 sensorgram data sets were correlated with assayed protein solution concentrations in mock NS-diluted infusions of drug product in the equivalent concentrations to QCM experiments to build a preliminary predictive model for determining fraction of drug and surfactant adsorbed and lost at the hydrophobic surface during administration. These results create a method for reliably and predictively estimating drug product adsorption behavior and protein drug dose loss on polymers at different protein drug concentrations.

Introduction

Monoclonal antibodies (mAbs) and biologics are used in various formulations and concentrations to treat an ever-growing number of diseases. Projected sales in 2024 have been estimated to reach greater than $380 billion.[1,2] As formulation development progresses for each of these protein drugs, a focus has developed on stability from shelf-life and in-use time.[3] Interest has grown in formulation testing around protein aggregation and adsorption to polymer materials of construction.[4-6] Aggregation and adsorption can affect the dose due to the loss of drug substance on materials in aggregates and proteinaceous immunogenic complexes which could lead to adverse events.[4,6-9] A transition from commonplace intravenous administration, which is still often a focus, to more and more subcutaneous administration is also occurring.[10,11] Significant challenges remain to optimize formulation with each protein and regulatory-mandated testing of compatibility.[3,12]

Antibodies must retain an active conformation of their tertiary structure in the face of interfacial stressors to have their pharmacological effect. This structure may be lost before, during, or after adsorption to solid surfaces, leading to possible drug loss and aggregation if not reversible or mitigated.[13-18] Techniques are available to characterize the air-liquid and solid-liquid interfaces at which stress occurs for the protein drugs and quartz crystal microbalance with or without dissipation (QCM or QCM-D if dissipation is discussed as well, which is not the focus of this study) technology; is a field yet to be primarily explored and has many advantages.[19] This technology is a resonating piezoelectric A-T cut quartz crystal (Fig. 1) and has many application opportunities to characterize adsorption to benefit formulation development and drug in-use testing.[19,20]

At any dose, some of the protein is left behind, and this occurs as either aggregate which may not pass through the filter if filtered, or it is lost on internal surfaces of the administration materials due to adsorption.[21-23] Some studies have concluded adsorption could play a role in aggregate formation in all administration routes.[23-26] Data from pre-filled syringes shows molar ratio and time were two contributors to particle formation in un-siliconized syringes of similar formulations as explored here, which could, though these experiments were over many days instead of hours, still inform shorter but nonetheless present compounded IV bag and line in-use times.[26] Techniques for minimizing adsorption should be employed because

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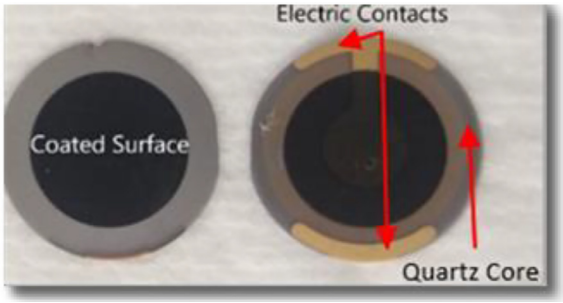

Figure 1. QCMD Sensor Top (Left) and Bottom (Right).

inattention to protein adsorption effects could lead to dosing errors due to loss of active drug confirmation or protein on exposed surfaces.[15,16,27-29] Studies on adsorption to IV set materials have been done with substances such as antibodies themselves, insulin, or polysorbate.[19,30-32,19, 29-31] During these studies, it is unclear where and how the protein in solution adsorbs in the administration fluid path and which component could be changed for less loss, with newer polymer coating experiments exploring modification of the fluid path.[33] Aside from the referenced studies, few models and experiments estimate protein loss from predictive experiments, especially at low doses or in the presence or absence of surfactant, to filters, catheters, lines, and IV bags when a diluent is used.

There are a few ways to approach adsorption problem. Two strategies, either changing the structure of protein drugs or changing the environment around the molecule by changing the concentration of excipients in the solution, have been explored to mitigate adsorption.[21,22,33-35] Changing the fluid path has even been employed, using hydrophobic or surfactant-like pre-coatings like polydimethylsiloxane with polyethylene glycol modification, but this complicates administration and may lead to the polymer itself being administered as well as tubing changes.[33] Changing the drug solution environment to account for exclusion effects and other forces a protein can experience is crucial to maintaining protein structure and optimizing solution conditions.[36-38] Here again, pre-filled syringe data may be translational, as it was found pH, surfactant concentration, material of construction in the barrel, and salt concentration all play roles in protein adsorption in an adlayer.[38] One of the most common methods, employing surfactants like polysorbate 20 or 80 (here, polysorbate 20 or simply 'PS' or 'PS20') in the more common solution environment modulation strategy, has effects not largely due to protein-surfactant complexes.[39] Instead, reduced interfacial affinity of the protein towards either the air-liquid, or liquid-solid interface due to blocking properties render surfactants or other blocking molecules effective for adsorption prevention .[22,33,38,40] Surfactants at low concentrations are subject to minimal preferential exclusion effects and have a higher affinity for the interface due to their amphoteric nature and molecular properties.[39]

Assuming the protein solution is optimized for minimizing interfacial stress, aggregation and adsorption still may occur, which could affect patient dose and immunogenicity.[5,9] Concerns manifest when protein drug solutions have a large surface area of many polymers in their fluid path to interact with, the dose of the therapeutic is small, or both.[41] Studying interactions is essential to patient safety and dose accuracy.[4] These studies on therapeutics which elicit immune responses both wanted (targeting the immune system towards an unwanted entity) and unwanted (the immune system targeting and reacting to the therapeutic) that are dosed incorrectly or aggregate grossly due to adsorption or aggregation could lead to patient safety issues.[4,7,8,42] Aside from altering the formulation or therapeutic, a few studies have characterized mAb interactions and orientations when exposed in solution with and without surfactant to hydrophobic or other surfaces using different techniques including, QCM.[24,26,38,43-45]

QCM involves a resonating piezoelectric A-T cut quartz crystal where resonance is measured at different harmonics of the base resonance frequency, and changes in mass and thickness of adlayers at the surface can be found.[46] QCM can accurately predict the mass and viscoelasticity of the adsorbed layer and here the mass is the focus of this study since drug adsorption loss is being investigated.[47] The Sauerbrey equation is a relationship that applies when dealing with the mass of protein adlayers in the formulation, as seen here using QCM and as explored in other studies due to the near zero change in dissipation and heavy overlap in harmonics during the sample periods in the current study.[48,49] The Sauerbrey equation (Eq. (1)) relates the change in the resonance frequency proportionally to the change in the total adsorbed sensor surface mass where ρ_q and μ_q are the density (2.648 g·cm^{-3}) and shear modulus of quartz (2.947 × 1011 g·cm^{-1}·s^{2}), respectively, A is the crystal piezoelectrically active geometrical area, defined by the area of the deposited film on the crystal, f_0 is the unloaded crystal frequency, and Δm and Δf are the mass and system frequency changes.[19,48,50] The derived Kanazawa-Gordon equation (Eq. (2)), where f_0 is the unloaded crystal frequency, μ_q is the shear modulus of quartz, ρ_q is the density of quartz, and η and ρL are the liquid viscosity and the density, respectively, deals with when one side of the quartz crystal is immersed in liquid and accounts for the liquid's viscous damping effects while mass is adsorbing and measurement takes place.[19,48] Both equations are useful in the current study to predict the mass adsorption in a low-viscosity liquid in the current study of <10 cP.

$$\Delta f = -\frac{2f_0^2}{A\sqrt{\rho_q \mu_q}}\Delta m \tag{1}$$

$$\Delta f = -f_0^{\left(\frac{3}{2}\right)}\sqrt{\frac{\rho_L \eta}{\pi \rho_q \mu_q}} \tag{2}$$

The assumptions for these relationships to exist and produce meaningful data, which apply in the present study, are that the adsorbed mass must be small relative to the mass of the quartz crystal, the mass adsorbed is a rigid, non-slipping film, and the mass adsorbed is evenly distributed over the area of the crystal.[46,48,49,51]

Published articles exist on peptides and proteins analyzed in many different manners including QCM, and few QCM studies are dedicated to the research and clinical development of protein drug products.[19] With current formulations' unavoidable administration risks, translational knowledge of the solid-liquid interface related to adsorption, aggregation, and protein molecular properties to characterize interaction is useful.[13,19,37,48,52] The current study focuses on novel bridging of this surface interaction QCM knowledge to clinical and formulation development. The goal is to measure an investigational IgG protein drug's behavior when a formulation is tested via QCM on different hydrophobic polymer surfaces and correlate it with infusion ECLIA results during the formulation process to build a model to predict adsorption and loss behavior at many concentrations. By measuring the adsorption using QCM, qualitatively and quantitatively backed decisions on polymers in administration supplies can be made based on adsorption data, and modeling of adsorbed mass loss can occur predicting behavior for a specific drug and its formulations or dilutions containing a wide range of protein drug concentrations.[37]

Materials

General Formulation Materials

Glacial acetic acid (99%), Ethylenediaminetetraacetic acid (EDTA), and sodium acetate trihydrate were all from JT Baker/Avanator VWR

(Allentown, Pennsylvania). Sucrose was from Pfanstiehl (Waukegan, Illinois). Polyoxyethylene (20) sorbitan monolaurate (Polysorbate 20, Non-formulary, Noncompendial), Methionine, and sodium chloride were from Merck (Darmstadt, Germany). The mAb drug used in both purified, preformulated bulk form and a fully formulated form was obtained as an investigational agent from Janssen Pharmaceuticals, a Johnson & Johnson company (Beerse, Belgium).

QCM Materials

Polypropylene and polyvinyl chloride sensors, sensor holders, and QSense Pro QCM instrument were from Biolin Scientific (Gothenburg, Sweden). Pipettors and balances were from Mettler-Toledo Rainin (Oakland, CA). Falcon tubes for solutions from Avanator VQR (Allentown, PA). Deionized, filtered water was used for all solution preparation using the Milli-Q® IQ 7003/05/10/15 Ultrapure and Pure Lab Water Purification System from Merck (Darmstadt, Germany). Cleaning solutions for sensors and QCM instrument which were 100% ethanol, 2% sodium dodecyl sulfate (SDS), and Deconex 11 from Avanator VWR (Allentown, Pennsylvania).

Electrochemiluminescent Immunoassayed Infusion Experiment Materials

PVC IV administration set with PES 0.2-micron filter from BBraun (Melsungen, Germany). 100 mL NS PVC IV bag from Baxter (Deerfield, Illinois). 22 G x 1.5-in. needle and 10 mL PP syringe from BD Biosciences (San Jose, California). The mAb drug substance was from Janssen Biopharmaceuticals (Beerse, Belgium). Polyethylene terephthalate glycol (PETG) bottle to collect infusate and sample and sample solution falcon tubes from Avanator VWR (Allentown, Pennsylvania). Plate reader from Meso Scale Discovery (MSD; Rockville, MD) and plate shaker from IKA (Satufen, Germany). Plate washer from BioTek (Winooski, VT). Pipettors were from Mettler-Toledo Rainin (Oakland, CA), sterile liquid vials from Patheon (New York, NY), and pipette tips from Eppendorf (Hamburg, Germany). ECLIA assay buffer and other solutions prepared day of in-house mock infusion sampling. Experimental solutions and materials prepared in-house included 10% saline and assay buffer, standard antibody for comparison to samples, the quality control mAbs, wash buffer, biotinylated specific antibody receptor ligand, and assay buffer. The ruthenium-R10 reagent was from Meso Scale Discovery (Rockville, MD). Cell culture grade water from Corning (Corning, NY). Read buffer and streptavidin-coated gold plates from Meso Scale Discovery (Rockville, MD).

Methods

QCM Experiments

Quartz sensors coated with either PVC or PP were pre- and post-run cleaned by 30 min soak in 1% Deconex Solution, by a minimum 2 hr soak in DI water (usually overnight), rinsed with DI water and 99% ethanol three times, and blown dry by medical grade nitrogen gas. The sensor was inserted into the QSense Pro with sample solution, NS solution, and water. Runs were configured, and data were collected using QSoft Pro program from Biolin Scientific (Gothenburg, Sweden). Experimental data was then transformed from frequency to mass data using Dfind analysis software from Biolin Scientific (Gothenburg, Sweden). Measurement of frequency and dissipation occurred as follows for all runs during each period with all flow rates for every liquid set at 10 μl/min (also seen in Fig. 2):

1. Baseline in water (priming sequence ~5 min + 10 min)
2. Baseline in NS (15 min)
3. Sample solution run (10 min)
4. NS washing (10 min)
5. System water wash [(10 min) + probe and sample port cleaning]
6. Sensor and instrument cleaning[53]

Elements were influenced from published procedures, but have unique steps and periods as well as specific sensor cleaning procedures.[54] Sensors were used interchangeably and randomly after cleaning overall conditions and tested for reproducibility by multiple runs of the same conditions on different sensors of the same composition.

The sample solutions in period/step 3 were one of 3 categories of solutions and any one of 12 possibilities in any one run detailed in Table 1 simulating four levels of NS dilution that could be seen in-clinic. Normal saline and formulation solutions were made the day of corresponding experimental QCM runs. USP <797> aseptic technique was used when preparing solutions to mimic a hospital preparation environment.[55]Over 60 sensorgrams were analyzed with Dfind software using a composite sauerbrey fit that took into account overtones 3 through 13. The mass adsorbed during the sample period was of primary interest, and this was measured by subtracting the average mass recorded and calculated during the NS period where an ionic liquid had effect on resonance (period 2 above) from the average mass shift recorded and calculated during the sample period in duplicate during each 6-step run sequence (period 3 in Fig. 2 above). Mass was determined in this manner for all three variables in Eqs. (3) and (4) for each run and then the masses were averaged. At each concentration for either PP or PVC, an adsorbed mass in the three separate conditions was determined over all available runs with the three above-defined solutions during the sample period. These adsorbed masses were then compared against each other within the same and between different materials at the same and different concentrations.

Different conditions and adsorbed masses were used to estimate both mass composition at the adsorbed surface of the protein in ng/cm^2 (Eq. (3)) and mass composition at the adsorbed surface of PS20 in ng/cm^2 (Eq. (4)) when protein and polysorbate were both exposed simultaneously to the hydrophobic polymer surfaces. This allows estimation of the protein component and its relationship with the increasing concentrations of surfactant in the layer by simple mathematical comparison of each substance's propensity to contribute mass at the surface individually. In both equations, x is the measured adsorbed mass in ng/cm^2 when IP (investigational product) diluted in NS without PS20 but with all other excipients and protein drug is sampled, y is the measured adsorbed mass in ng/cm^2 when IP diluted in NS with PS20 and all other excipients but no protein drug is sampled, and z is the measured adsorbed mass in ng/cm^2 when IP diluted in NS with PS20 and all other excipients and protein drug is sampled.

Eq. (3)'s masses were taken from the average measurement of the equation-transformed frequency shift during each period. The masses estimated using Eq. (3) were correlated with solution protein concentration and the amounts lost at the same concentrations of drug product in NS IV bags from the ECLIA-assayed infusion experiments, and a natural log-linear function model was developed to predict loss of drug results at a broader range of concentrations for PVC administration materials. The estimates over a wide concentration range were determined for a 100 mL and 250 mL bag. The model also predicted loss based on a sample of actual bag volume fills as the bag volume can vary by a set number of mL around the nominal amount specified on the bag. In a small number of experiments, the QCM-measured adsorbed mass in ng/cm^2 when fully formulated IP diluted in NS without PS20 or protein drug but with all other excipients (which is not a true mass, but rather the liquid effects on the sensor) was compared to NS period 2 above to verify the mass adsorbed at the sensor surface was composed of almost entirely PS20 or protein when adsorption was observed in experiments conducted.

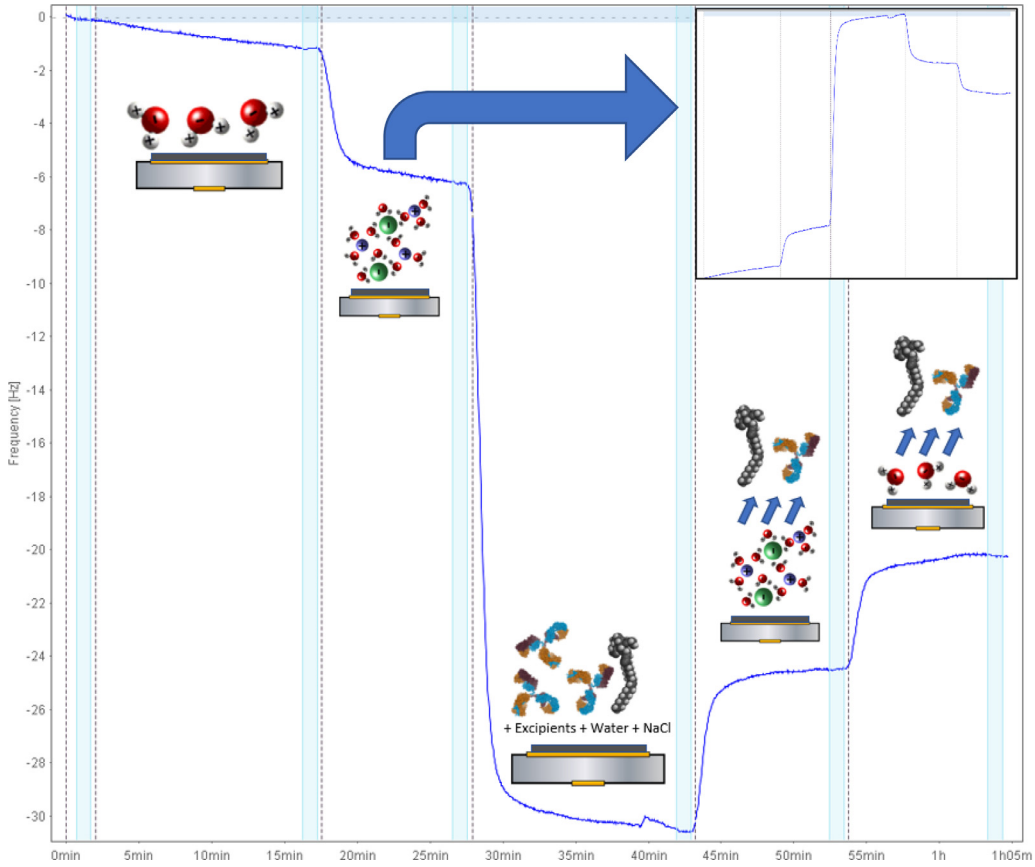

Figure 2. A Typical QCM Experimental Run Composite Sauerbrey Fit to Determine Mass Adsorbed at a Hydrophobic Polymer Surface – From periods left to right, separated by dashed lines: water baseline period, 0.9% sodium chloride (NS) baseline to account for the effect NS has on resonance, sample period in which drug antibody in formulation diluted in NS to mimic parenteral administration, NS wash off period to determine reversible binding and cleaning of sensor surface, and water wash off period to determine reversible binding and cleaning of sensor surface. The sample period contained protein with formulation excipients solution either with or without polysorbate 20 diluted in NS or contained no protein with formulation excipients solution but with polysorbate 20. Cut out section of the data depicts it transformed from frequency to mass data in ng/cm^2 using the composite sauerbrey approach, which uses a weighted average of all data from each overtone.

Mass Contribution Estimate of Protein at Material Surface

$$= z\left(1 - \frac{x}{y}\right) \tag{3}$$

Mass Contribution Estimate of PS20 at Material Surface

$$= z \times \frac{x}{y} \tag{4}$$

Electrocheminuminescent Immunoassayed Infusion Experiment Methods

Experiments were conducted in 100 and 250-mL NS IV bags with administration sets. The formulation containing the drug and PS20, and the other included excipients, were diluted by admixture into the IV bag in an ISO Class 5 vertical laminar flow hood aseptically.[55] The bags were left at ambient temperature and light for 24-hours then infused into PETG bottles. The samples were diluted 1:10 in ECLIA assay buffer.

ECLIA assay, samples, and wash buffers were prepared on the day of the experiment. The ECLIA active protein content method was a sandwich immunoassay based on capture by the receptor-ligand and detection with a generic antibody detection reagent utilizing electrochemiluminescence. A streptavidin-coated plate was loaded with receptor containing modified Biotin, then standard curve calibrators for a 10-point standard curve were added, and the points were established; quality controls were run for concentration comparison, then diluted samples were added. After incubation, the assay plate was washed, and the fluorophore-labeled detection reagent was added to the assay plate. Following incubation, the assay plate was washed and then read on a plate reader after addition of read buffer. The standard curve is used to determine the concentration of the sample. Samples were run in duplicate with the allowance of variation of ±20% between each sample. Sample concentrations were then analyzed against $a \geq 70\%$ recovery acceptance criteria.

Table 1
List of solutions tested on PVC and PP sensor surfaces.

	Solution Protein Component	Solution PS20 Component
Protein + PS20 Solutions	0.1 mg/mL	0.04%
	0.01 mg/mL	0.004%
	0.001 mg/mL	0.0004%
	0.0001 mg/mL	0.00004%
Protein Only Solutions	0.1 mg/mL	0%
	0.01 mg/mL	0%
	0.001 mg/mL	0%
	0.0001 mg/mL	0%
PS20 Only Solutions	0 mg/mL	0.04%
	0 mg/mL	0.004%
	0 mg/mL	0.0004%
	0 mg/mL	0.00004%

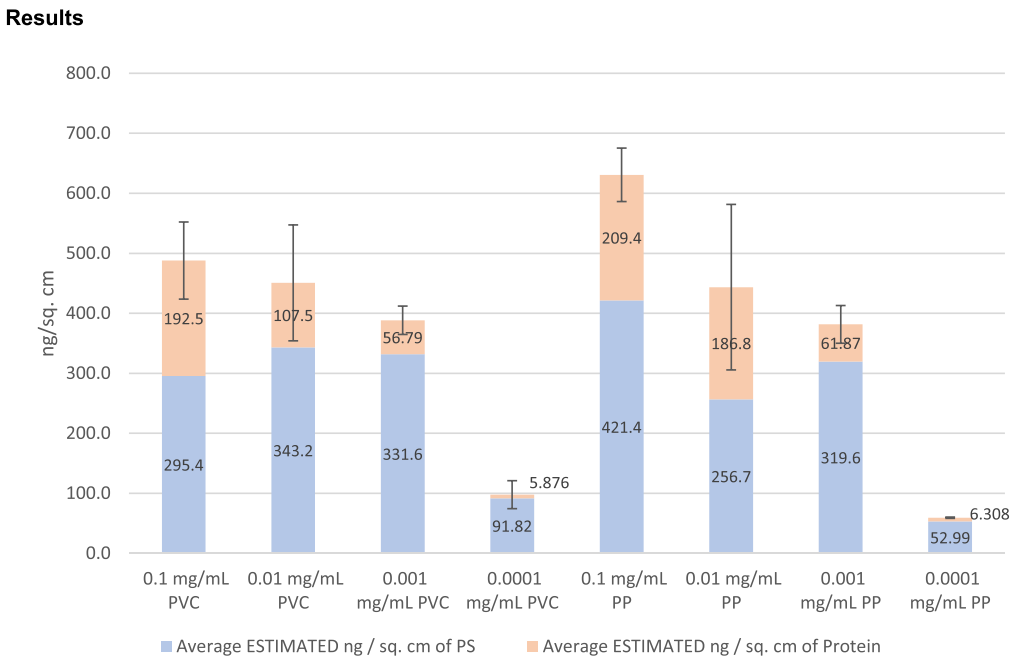

Figure 3. Estimates of Mass Contributions of PS20 and Antibody to Layer at PVC and PP Sensor Surface at Different Concentrations – Each set of four bars from left to right are the total mass adsorbed at 0.1 mg/mL (10 mg dose), 0.01 mg/mL (1 mg dose), 0.001 mg/mL (0.1 mg dose), and 0.0001 mg/mL (0.01 mg dose) on either PVC (right four bars) or PP (left four bars) split out into the estimated mass contributions. Each dose is calculated assuming drug is admixed to the four concentrations tested using a 100 mL NS IV bag for dilution and subsequent infusion. Error bars are included for the total mass adsorbed when PS20 and protein are exposed to the surface during the sample period. All run average masses for protein with formulation excipients solution either with or without polysorbate 20 diluted in NS or formulation excipients solution without protein but with polysorbate 20 can be seen in Fig. 4.

The percent recovery measured was compared against the solution's initial concentrations before mock infusion using an IV line. NS bags were weighed before and after admixture and post-infusion. This allowed controlling fill volumes of individual IV bag and concentration of IP preparation corresponding to 0.1 – 0.0001 mg/mL. The IV bags and line used in experiments were deconstructed, and the internal fluid path surface area was measured and information for surface areas was manufacturer verified. The percent recovery was compared to QCM results.

Results

The significant results of the experiments are summarized in Figs. 3, 5, and 7. The average percent of the total mass that is estimated to be protein adsorbed at all concentrations when the adlayer and sample period solution was made up of both PS20 and protein drug was 25.54% [95% CI ±14.6%] of the mass for PP and 23.10% [95% CI ±11.8%] of the mass for PVC. A large drop in masses adsorbed when PS20 and protein were exposed to the hydrophobic surfaces was observed between the 0.001 mg/mL and 0.0001 mg/mL sample IP concentrations.

The masses adsorbed to each surface are shown in Fig. 4. These masses were used for their corresponding variable in Eqs. (3) and (4) to estimate mass fractions of PS20 and protein in an adlayer. The average mass recorded when PS20 and protein were in the NS-diluted formulation solution was at most within 300 ng/cm^2 to the average mass recorded when just protein in the NS-diluted formulation solution was sampled, and on average within 100–150 ng/cm^2. When PS20 and protein were sampled, the average mass was dependent on the protein concentration in solution, and while the 0.1 mg/mL and 0.0001 mg/mL solutions had a 5 to >10x difference depending on material in measured average adsorbed mass. The 0.01 mg/mL and 0.001 mg/mL measured average adsorbed masses were more similar, within 100 ng/cm^2. This means there is a saturation point at some higher concentration as well not studied where adsorption can no longer occur. Another significant result is the masses adsorbed at each corresponding concentration and condition between materials were not significantly different by two sample *t*-test and followed a pattern (i.e. if the PS20 only mass was lesser than the PS20 and protein mass and lesser than the protein only mass at 0.1 mg/mL PVC, the same pattern was observed at the PP 0.1 mg/mL level, and the same goes for other concentrations). This creates concentration and material mass adsorption levels for each condition and material.

The estimated adsorbed protein is observed to be dependent on the concentration of protein drug in the sample solution, which can be seen in Fig. 5. A natural log-linear fit yields a R^2 greater than 0.9 for both PVC and PP, indicating the protein concentration in solution explains the variation in adsorbed amounts. Again, the PP adsorbed amounts are estimated to be higher, but statistically insignificant (Fig. 5). Extrapolation into lower concentrations yields a point estimate intersection of the best fit functions at some low concentration that is the same estimated adsorbed amounts of protein. This is seen in the lowest concentration level adsorbed mass estimates being within one nanogram of each other (Fig. 5).

The estimated amounts adsorbed as they relate to ECLIA assayed infusion study results are shown in Figs. 6 and 7. The negative relationship in Fig. 6(A) between estimated mass of protein left on the IV set via QCM and ECLIA-estimated percent of dose left on the IV set shows the result of the hypothesis of the effect dose size had when the formulated NS-diluted IPs at different concentrations were all exposed to the same theoretical square centimeters of fluid path in the IV line. The higher the dose, the less the fraction of drug estimated to be left on the IV set, which changed the overall dose. The higher dose concentration solutions sampled had, when compared to the lower concentrations, one or two orders of magnitude higher concentrations of PS20 and protein in solution. Fig. 6 (B & C) also allows estimation the lowest allowable in-bag concentrations of surfactant and protein. Fig. 6 accounts for PES filtering, and the adsorbed

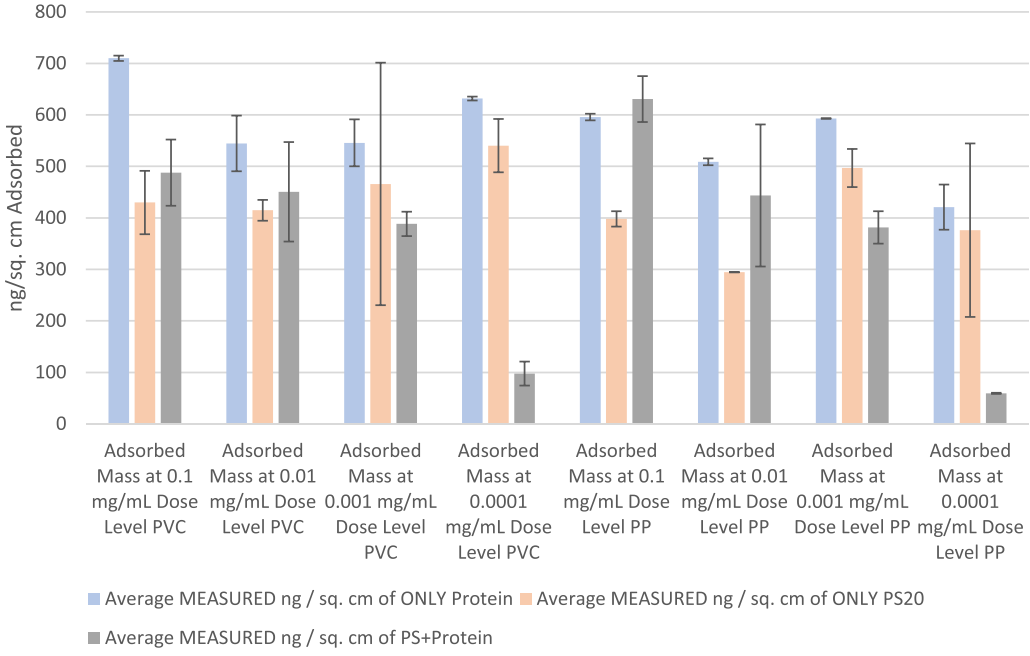

Figure 4. Measurements of Adsorbed Masses of Only Protein, Only PS20, and Protein+PS20 in Formulated Solution Diluted in NS – The average adsorbed amounts in each of the 12 conditions listed in Table 1 for the duplicate experiments accounting for the NS by subtracting it from the sample period mass is shown here. These measured average amounts were used in Eqs. (3) and (4) to estimate how much each substance contributed to the mixed adsorbed layer when the solution with both PS20 and protein in the formulated solution diluted in NS was exposed to the hydrophobic polymers. The left four bar groupings are for PVC, and the right four are for PP.

amounts when protein and polysorbate were exposed to a PES-coated sensor were insignificantly different from adsorbed amounts to sensors coated with PP and PVC, which allowed incorporation of the adsorbed protein fractions similarly to the filtered IV set model.

Fig. 6(A) is analogous to Figs. 6 and 7(B), as the percentages, the exact volumes of the NS IV bags, and the doses at the corresponding sample concentrations from Fig. 6 were used in Fig. 7. The percentages, infusion volumes, and conditions were used to estimate the nanograms per centimeter on a fluid path that would have been lost to the IV set in the ECLIA assayed infusion experiments. This yields the positive correlation between the QCM estimated adsorbed amounts of protein drug and the ECLIA assayed infusion masses drawn from the percent of dose lost, which corresponds with Fig. 6 (A). Finally, Fig. 6(B) allows a clinically relevant level to be determined for dosing. Both sets of data taken together, the larger the dose the less percent of dose lost to adsorption on the IV set, and the QCM estimates of this adsorption correlate well to infusion performance as measured by ECLIA. These two sets of data correlated with

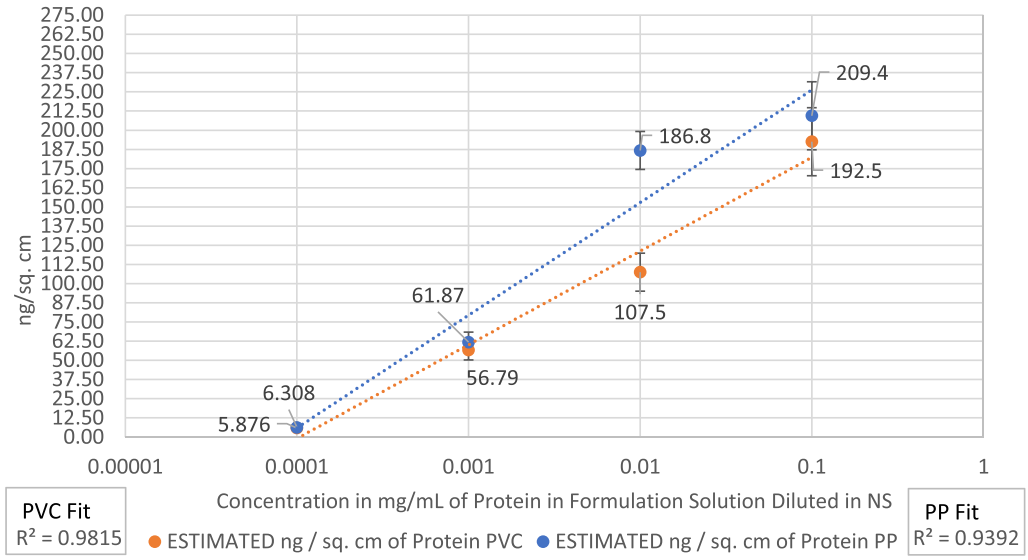

Figure 5. The concentration of Protein in Solution vs. QCM Average Adsorbed Estimates of Mass Contributions of Protein to Adsorbed Layer at PP and PVC Sensor Surface – Positive concentration relationship of protein drug in solution and adsorbed protein drug is shown here. A natural log-fitted function is plotted as a line of best fit for both materials. Points are labeled with the estimated adsorbed amounts. Error bars are constructed based on 95% CI for the fraction of protein adsorbed when PS20 and protein were exposed to the hydrophobic surfaces simultaneously.

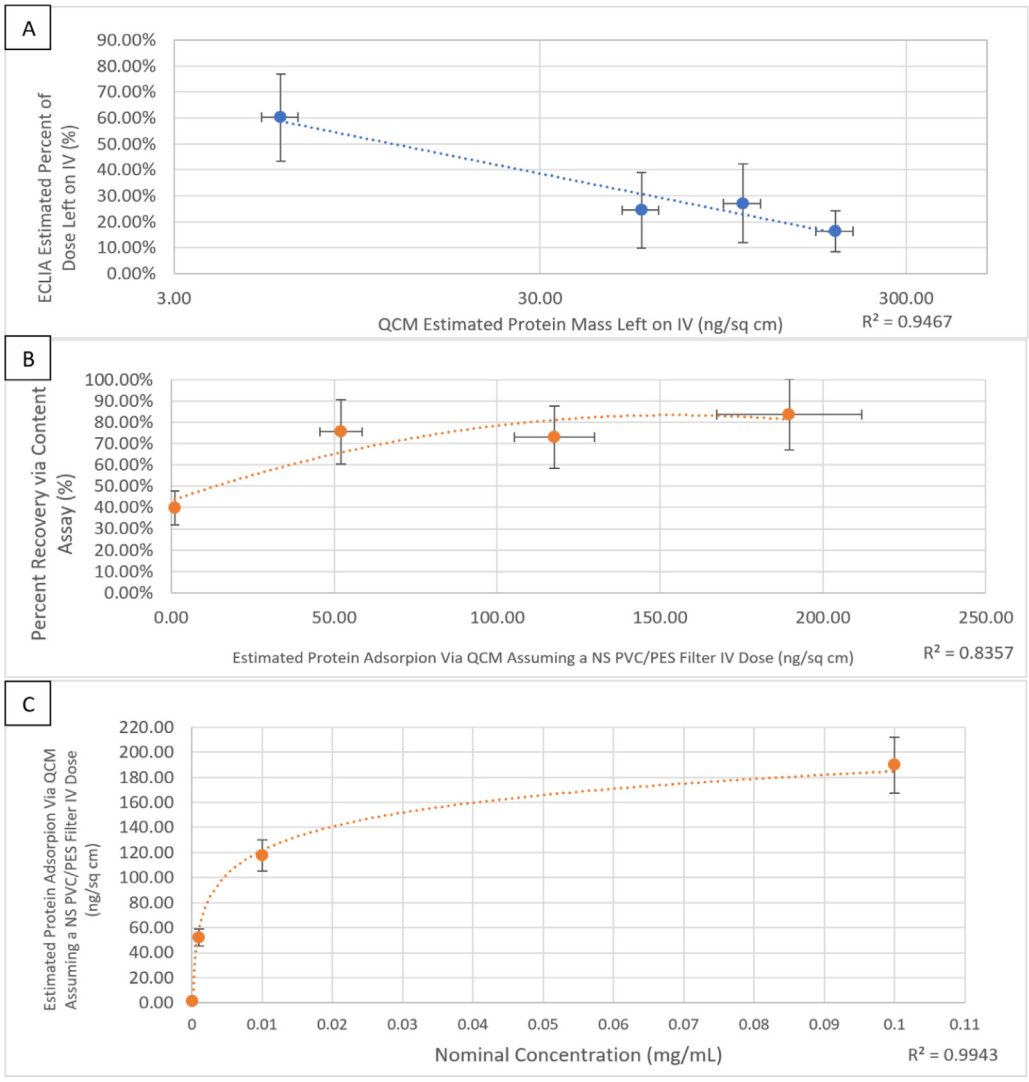

Figure 6. ECLIA Measured Percent of Dose Lost on IV Set vs QCM Estimated Mass Left on IV Set and Comparison to Concentrations – A negative relationship between the average amount of protein adsorbed per square centimeter via QCM experiments and percent of dose lost on IV set measured directly by ECLIA (A). A natural log-linear function is the line of best fit. These results indicate the larger the dose, the less fraction is lost on the IV set, highlighting the need for low-dose knowledge of adsorbed amounts. A polynomial relationship was fitted between the average adsorbed protein to the measured surface area of the IV set and percent recovery (B). The adsorbed protein and nominal concentration relationship are also shown (C). Setting the polynomial to equal 70% recovery (an industry recognized acceptability standard) yields an adsorbed amount of protein that, when used in the equation in B, yields the lowest useable concentration in a filtered IV setup of 0.00155 mg/mL protein and a corresponding 0.000066% PS20 given the original drug product's neat formulation.

QCM estimate data yield relationships with lines of best fit with high coefficients of determination.

A few experiments were done to verify whether the other excipients in the formulated solution diluted in NS were appreciably different from the normal saline solution. During the runs, the effect on frequency and by relation mass data was tested by running as a sample solution the formulation minus PS20 and protein, diluted to the same concentration as the corresponding surfactant and drug-containing solutions. The mass shift transformed from frequency of the NS only periods produced on average over all NS periods during and right before the sample period containing drug or PS20 or both was 63.59 ng/cm^2 [95% CI ±5.69 ng/cm^2] versus the formulated solution minus the protein and PS20 in NS at 82.25 ng/cm^2 [95% CI ±8.03 ng/cm^2]. There is only at most 32.37 ng/cm^2 separating these estimates when it comes to the confidence intervals, which is a negligibly small amount of frequency shift.

Particle concerns for dose accuracy are also of importance in protein drug delivery and give some estimation of possible aggregation, and the ECLIA experiments also included a portion where particles were measured in the IV infusions. Calculations were performed based on literature methods and data available from microfluidic imaging of the same samples from the IV ECLIA experiments (data not shown).[56] With an overestimation approach of assuming all particles are proteinaceous and that all protein monomers are effectively a sphere, which are both assumptions overestimating protein volume within particles, there were negligibly small amounts on the microgram scale lost based on measured particles in the preparations. The amounts of protein, even with this overly cautious approach, that were present in particles were not an issue with dose accuracy as they were below 1 ng, and were within acceptable limits based on USP ⟨788⟩ standards.[55,57]

Discussion

The experiments performed on this mAb in its formulation with NS must establish measurement reproducibility to the clinical

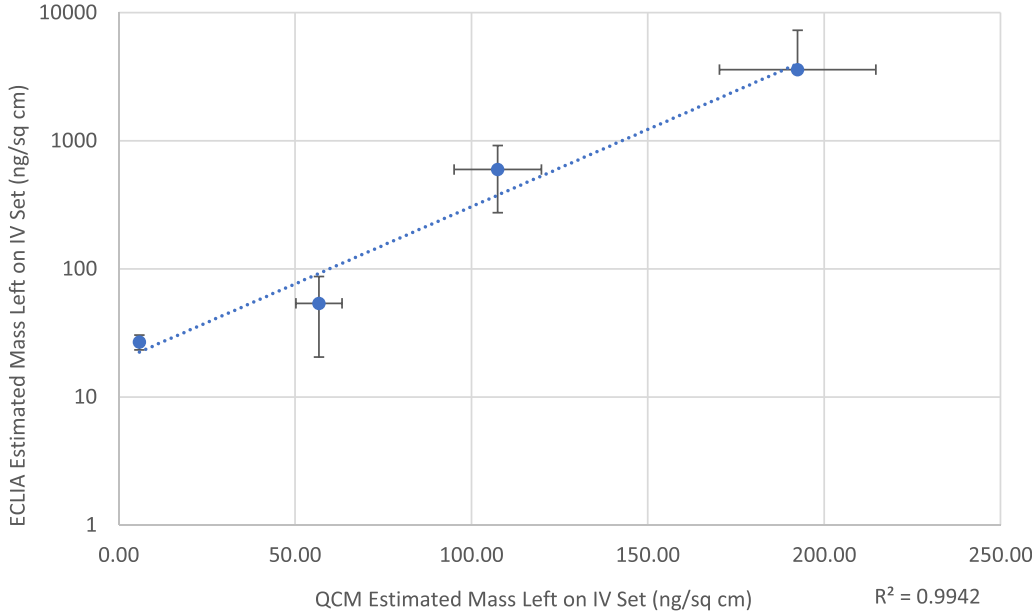

Figure 7. ECLIA Estimated Mass Left on PVC IV Set vs. QCM Average Estimated Mass Left on the IV Set – A positive relationship between the estimated amount lost on the IV set and the ECLIA estimated amount lost on IV set is shown here. A natural log linear-fitted function is plotted as a line of best fit. The estimate is based on percent recovery results by ECLIA. Percent lost is calculated by comparing the concentration submitted for ECLIA testing, and the ECLIA assay result, that percentage is used to estimate how many ng of protein was left on the IV set. The negative Y-axis error bar for the rightmost point is not shown because it is below 0.

conditions it tests, exhibit causality between formulation components and adsorption, and become clinically relevant to administering large molecular entities to be helpful in formulation design. Also, with IV sets composed of the polymers tested, with little differences between hydrophobic polymers tested, adsorption becomes generalizable from these experiments among sets with similar materials. Nevertheless, these results show a great deal of promise.

The first element of the above results essential to discuss of any method is the QCM's ability to accurately measure what it is supposed to measure—adsorption and resulting drug loss—over multiple experiments conducted in the same sequence or reproducibility. The measurements of the different solutions' adsorbed masses were all performed in duplicate or more, and the pooled standard deviation between all like run sets calculated from variance was 76.05 ng/cm^2 which speaks to the low variability from sensor to sensor on adsorbed mass measurements. Furthermore, measurements and calculations directly derived from measurements like Eq. (3)'s important results deviated on average less than 0.0001 mg/cm^2. The randomization of sensors used per material also eliminates sensor roughness as a significant confounder because a somewhat precise range of measurements were obtained in each given concentration and condition regardless of the sensor used. The reproducibility of this test for QCM is logically sound as well and has content validity, as the flow over the polymer sensor, which was cleaned before every use appropriately, mimics the flow seen in IV sets used in infusion experiments.[53] QCM can be a surrogate method to infusion studies to predict and compare mAbs because of the presented reproducible results and current thoughts on the method's robustness and applicability.[19,48,49,58]

Another aspect that the above results must be viewed in is the causality of the formulation components on adsorption and adsorption's causality associated with dose changes. Causality is made up of non-spuriousness, association, and temporal relationships.[59] The other substances in solution, as possible confounders besides the protein drug and PS20, could be adsorbing during IV administration, but this was not the case. It was proven by the experiments comparing the diluted formulation sans protein and PS in NS to NS alone that the other excipients do not adsorb and affect mass estimated at the sensor surface by any appreciably large amount, as evidenced by the slight difference of 63.59 ng/cm^2 [95% CI ±5.69 ng/cm^2] versus 82.25 ng/cm^2 [95% CI ±8.03 ng/cm^2] between these two periods. Furthermore, the sensors were cleaned before each experiment immediately before placing the sensors in the instrument, as was the instrument according to the instructions available from the instrument and sensor vendor, Biolin Scientific, so no other contaminants could have contributed to the masses.[53] On top of this non-spuriousness as to where adsorbed mass could have come from, there was both a dose-response relationship in the QCM data as well as association to ECLIA assay results. All coefficients of determination for functions best fitted between ECLIA experiments and QCM data were greater than 0.9, showing a strong association. Dose-response relationships are considered strong relationships for causality in the classical sense.[59,60] Here, dose level, QCM data, and infusion conditions inputs predict reasonably well adsorbed drug loss using the correlations found. Correlation was used because QCM adsorption amounts did not exactly correspond to amounts seen as lost in ECLIA studies, but instead were seen more as a small experimental replicate of ECLIA IV administration studies which resulted in strong relationships between dependent and independent variables. Finally, the QCM experiments were performed at corresponding concentrations to the ECLIA assayed infusion experiments without prior knowledge of drug loss results, meaning there was a blinding to the QCM's results' relationship to the ECLIA results and a proper temporal association can be thus established. The preliminary causality and relationships explored by these results is promising.

Finally, the most practical element of the results is their clinical relevance. There are many challenges to biologics compatibility studies, and the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), FDA, and USP mandate testing at different concentrations, with different materials, and different IV administration setups and diluents for pharmaceuticals.[12,55,57,61,62] There are even elements not addressed by the ICH guidelines for instance, which require testing in many cases.[12,63] QCM tests conditions at clinically relevant concentrations and polymers widely used. The issue with current conventional method application is once the infusion studies like ECLIA assays are

presented, there is no way to determine how or where drug loss occurred in the administration set. QCM alleviates these issues and is more clinically relevant by providing fast results in many cases within an hour, efficiently uses a small amount of expensive drug material, does not use any ancillary supplies, and tests by polymer material to pinpoint the portion of the materials where adsorption is occurring.

Although QCM has many upsides, there are some drawbacks to using QCM, such as specialized equipment, not immediately apparent applicability or acceptability, and required technical knowledge not needed for simple infusion simulation. Conventional experiments are intuitive, use developed methods, and are standard in the industry. Simple infusion simulation experiments are not as intensive in specialization or complexity. In the presented method, there are not large amounts of physiochemical, materials science-related expertise needed like in more recent QCM or other instruments' methods.[19,38] What standard methods lack is efficiency, and QCM estimation can make up for this, and relates to major detractors to conventional methods as they compare to QCM—inefficient resource use, and ambiguity regarding adsorption. The cost of the ancillary supplies, scientist experimental time, needle stick risks, and material to perform a compatibility study for parenteral formulations are high—often in the thousands to hundreds of thousands of dollars range—and can contribute to the already high cost of formulation development and come with technical challenges.[41] Though not immediately apparent, upon close inspection, QCM's costs are lower in both time and money and provide an even more detailed picture of clinical administration.

Conclusion and Future Directions

Estimates of adsorption can be made using QCM preliminary compatibility experiments to a degree useful enough to predict administration performance in the clinic. There is growing evidence here and in literature QCM can practically test regulatory mandated compatibility of formulations, and thus this speaks towards QCM playing a role in formulation development.[19,48] The mass of an adsorbed substance when NS-diluted drug and PS full drug product, NS-diluted drug in formulation in the absence of PS, and the NS-diluted formulation in the absence of only drug can be determined in repeatable measurements that deviate 0.0001 mg/cm^2 from each other. There is concentration dependent mass adsorption of drug and PS when full drug product diluted in NS is run as sample over PP and PVC surfaces. Because two different materials were tested, differences or lack thereof can be evaluated between adsorption of different sample solutions to PP and PVC surfaces and even more surfaces. Early estimation methods of mass fractions of drug and PS that are adsorbed when NS-diluted full drug product is run as a sample over PP and PVC surfaces can be constructed using simple equations albeit that are estimations in nature, but still provide valuable information. Finally, correlation of ECLIA-measured amount of drug lost on IV set in ng/cm^2 is leading to unique early estimation models applicable over many different concentrations not studied here to predict drug formulation performance when exposed to administration materials.

The present method may be applicable to different biologic drugs, formulations, and materials. A comparison between the formulations and different large molecule drugs on each material, between excipient or surfactant variant, and on physiochemical properties of each drug molecule could be made. A library of drug and formulation behavior in administration can be built so that QCM-led compatibility studies can be even more accurate in the face of conventional studies, and drugs with similar characteristics may be predictive of new molecules' behavior being discovered. Subcutaneous administration syringe polymers could also be tested, and that route of administration could be explored. Aggregation can also be compared with

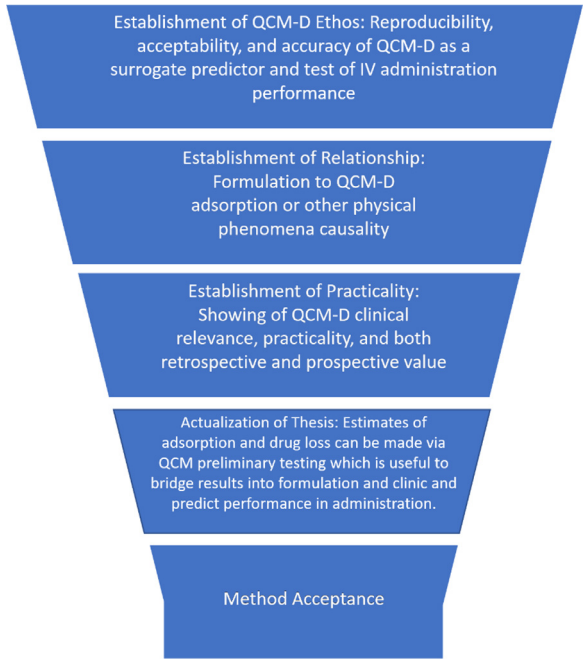

Figure 8. Logical building of QCM method use in formulation development.

adsorption results, and a holistic administration drug loss approach can be constructed. This holistic approach will make drug loss and QCM estimations even more clinically relevant, since the present study focusses on adsorption as a principal mode of drug loss, but also recognizes it is not the sole factor in administration loss. New formulations, administration challenges, combination products, as well as other drugs besides large molecule antibodies still face SC and IV administration challenges and are trying to enter the world of at-home administration. QCM can test these new biotherapeutics for adsorption and dose accuracy for a detailed understanding of administration (Fig. 8).[1,10,11] New ways of using technology like QCM to predict drug administration should help to understand large biotherapeutic administration better moving forward.

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