Desorption Strategies in Peptide Formulation Preparation: Insights from *In Vitro* Adsorption (Non-Specific Binding) Assessment Experiments

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Introduction

Compared to small molecules, peptides demonstrate high specificity and selectivity towards biological targets, minimizing off-target effects and toxicity. However, the larger size and inherent complexity of peptides present their unique formulating challenges Peptides are physicochemically unstable and exhibit susceptibility to oxidation and hydrolysis. A critical formulation challenge is the deviation between theoretical and measured peptide concentrations, primarily driven by non-specific adsorption onto contact surfaces (e.g., filter membranes, glass vials, syringe barrels). This multifactorial discrepancy – potentially involving peptide aggregation or interfacial denaturation – complicates root-cause identification and requires systematic investigation of material compatibility, solution stability, and process parameters.

Methods

Instruments and Materials

Figure 1. UPLC-UV

Table 1. UPLC-UV Method for Quantification of Peptides

Parameter	Specification
LC-UV System	Waters(H-Class)
Detector Type	ACQ-PDA
Column	ACQUITY UPLC BEH C18 1.7 μm 2.1 × 50 mm
Mobile Phase A	0.1% FA in water/ACN (v:v, 95:5)
Mobile Phase B	0.1% FA in water/ACN (v:v, 5:95)

Figure 2. Materials validated for peptide adsorption studies

Table 2. Properties of Common Materials in Peptide Adsorption Assessment

Material Type	Composition	Manufacturer
Borosilicate Glass Vials	Type III Borosilicate Glass	German DURAN
Filter Membranes 0.22μm	PES (Polyethersulfone)	Merk Millipore Ltd
Filter Membranes 0.22μm	PVDF (Polyvinylidene fluoride)	Merk Millipore Ltd
Syringe	PE (Polyethylene)	BD Syringe and Jiangsu Changjiang Medical Devices
Syringe	PVC (Polyvinyl chloride)	Jiangsu Huaxia Medical Equipment

In-vitro design

To quantify and mitigate peptide adsorption losses during bioprocessing, we have developed an *in vitro* adsorption assessment model. Peptide solutions were prepared in isotonic physiological saline or buffer solutions. The dynamic adsorption system simulated three key processing steps: primary incubation, filtration and syringe transit (Figure 2, Table 2).

Three critical sampling points were established(Figure 3):

- C_0 : Pre-filtration solution in **pre-saturated glass** vials (baseline control for adsorption)
- C_1 : Pre-filtration solution in **non-pre-saturated glass** vials (to assess glass adsorption)
- C_2 : Filtrate collected through a **pre-saturated filter** (to assess membrane adsorption)
- C_3 : Filtrate collected through a **non-pre-saturated filter** (to assess total membrane adsorption)
- C_4 : Syringe effluent collected using a **pre-saturated syringe** (to evaluate plunger/barrel adsorption)
- C_5 : Syringe effluent collected using a **non-pre-saturated syringe** (to quantify total syringe adsorption)

Peptide quantification was performed by UPLC-UV (Figure 1, Table 1).

Adsorption Calculation: Adsorption % = $[1-(C_n/C_0)]\times 100$

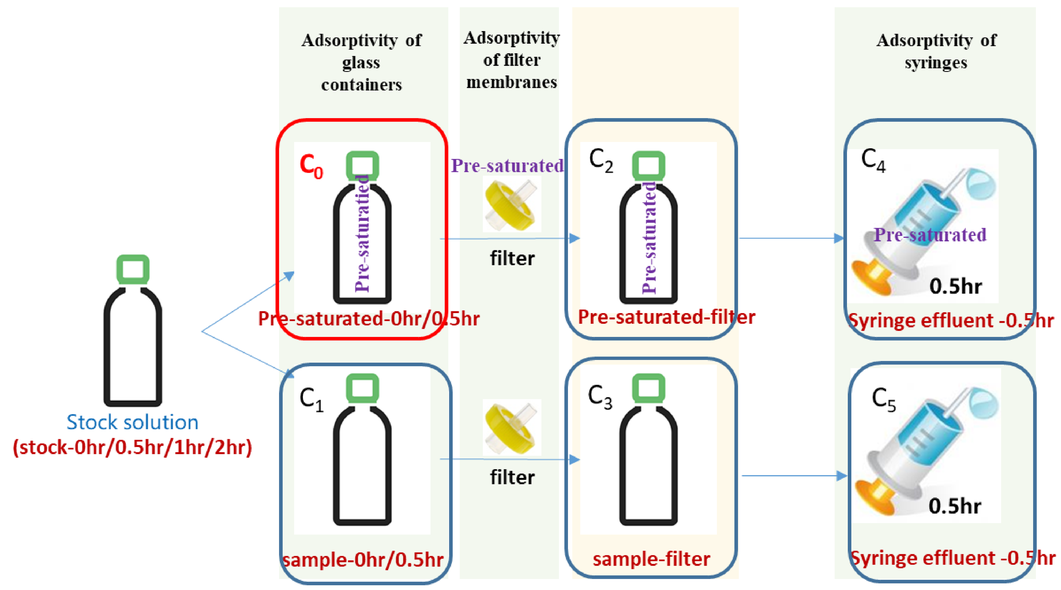

Figure 3. *In Vitro* Adsorption Assessment Pipeline for Peptide Formulations

Results

Analysis of adsorption data from over 100 peptide compounds revealed significant adsorption in 32.8 % of peptides tested. Table 3 provides the Adsorption Rates for Different Medium. The data indicated that adsorption was highest on filter membranes, ranging from 10-20%, followed by plastic syringes with approximately 5-10% adsorption, while glass containers demonstrated the lowest levels, at less than 2%.

Table 3. The Adsorption Rates for Different Medium

Adsorption Medium	Adsorption Rate Range (Low Concentration)	Adsorption Rate Range (High Concentration)	Risk Level
Filter Membranes	10-20%	2%-5%	⚠⚠⚠ High
Plastic Syringes	5-10%	No adsorption	⚠⚠ Medium
Glass Containers	<2%	No adsorption	⚠ Low

Furthermore, adsorption ratio was higher when peptide concentrations were low compared to high concentrations. Notably, adsorption to PES or PVDF filter membranes could be minimized through pre-saturation techniques. While PE syringes demonstrated some level of adsorption, PVC syringes displayed negligible adsorption.

Critical Concentration Dependency

- Low-concentration peptides (≤ 100 μg/mL):
  Adsorption loss 15-25% → Clinically significant PK distortion
- High-concentration peptides (≥ 100 μg/mL):
  Adsorption loss <5% → Negligible PK impact

Validated Desorption Strategies

- Optimize Container Materials: Use low-adsorption containers to reduce peptide adsorption to container surfaces.
- Add Stabilizers: Include stabilizers (e.g., surfactants) in the solution to reduce peptide adsorption loss.
- Pre-Saturation Treatment: Treat containers to make them less prone to peptide adsorption.

Conclusion

Our *in vitro* adsorption assessment model and desorption strategies, specifically designed with a focused emphasis on addressing absorption challenges, could enhance the reliability and precision of evaluating peptide-based drug candidates. Consequently, this could pave the way for more effective and stable peptide formulations and strengthen the validity of their preclinical *in vivo* studies.

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References

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