Adsorption Phenomenon and Development of Low Adsorption Vials for LC and LC/MS

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1. Introduction

In recent years, LC/MS systems capable of high-sensitivity and high-selectivity analysis have become an essential means of analysis in many fields. As the concentration of samples used for LC/MS analysis have become increasingly lower, there is concern that sample adsorption to containers could seriously compromise the reliability of analytical results. For glass containers, ionic adsorption, due to silanol groups on the glass surface, and hydrophobic adsorption, due to siloxane, both occur at the same time. In contrast, only material-based hydrophobic adsorption occurs for polypropylene (PP) containers. Therefore, we verified the phenomenon of adsorption to containers using various types of basic compounds and peptides (trypsin digested myoglobin and BSA) as models. The results showed that the adsorption phenomenon was different for each type of containers (glass versus PP vials) and confirmed that the vial adsorption phenomenon was one of factors that compromised the reliability of analytical results obtained. This article describes the adsorption behavior of newly developed low-adsorption HPLC vials with respect to basic compounds and peptides.

2. Current Status of Adsorption to Vials and Methods for Inhibiting Adsorption

Basic compounds and other substances with a high acid dissociation constant (pK_a) adsorb readily to glass containers by ionic adsorption, whereas compounds with a large octanol-water partition coefficient (logP) readily adsorb to both glass and PP containers by hydrophobic adsorption. Therefore, ionic adsorption to silanol groups in the glass containers can be effectively inhibited by adding a salt to sample solutions, and similarly hydrophobic adsorption to glass or PP containers can be effectively inhibited by adding an organic solvent or surfactant. However, in the case of high-sensitivity quantitative analysis using HPLC or LC/MS systems, the effects of the additives on separation and ionization in the MS unit must be considered, which can make it quite complicated to set sample preparation conditions optimized for inhibiting container adsorption .

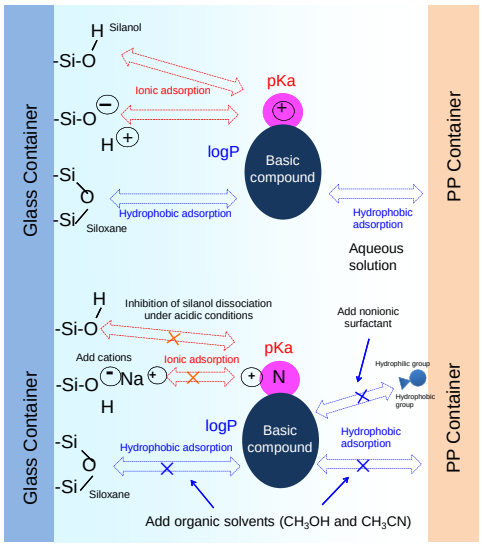
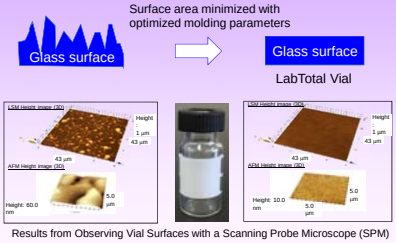


Fig. 1 Mechanism of Adsorption to Vials and Methods for Inhibiting Adsorption

3. Development of Low-Adsorption Vials

⦿ Glass Vial (LabTotal Vial)

Due to the roughness of typical glass vial surfaces, there is a large surface area in contact with samples. Therefore, the surface area was minimized by optimizing the molding parameters during manufacture.



Results from Observing Vial Surfaces with a Scanning Probe Microscope (SPM)

Fig. 2 Overview of Low-Adsorption Glass Vials

⦿ PP Vials (TORAST-H™ Bio Vials)

A non-ionic superhydrophilic base was chemically bonded to the PP vial surfaces.

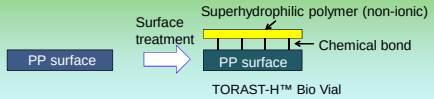


Fig. 3 Overview of Low-Adsorption PP Vials

4. Effectiveness of LabTotal Vials in Inhibiting Basic Compound Adsorption

Four types of basic compounds were analyzed to compare the adsorption inhibition effects using LabTotal vials with commercial LCMS vials and LC/GC vials. The resulting chromatograms are shown in Fig. 4, with corresponding area values (recovery rates) listed in Table 1. The results confirmed that the LabTotal vials inhibited adsorption significantly compared to competing vials.

Table 1 Comparison of Basic Compound Recovery Rates for Three Types of Vials

	Amitriptyline	Atenolol	Imipramine	Propranolol
LabTotal Vial	51,376 (100 %)	8,638 (100 %)	64,990 (100 %)	32,249 (100 %)
Competitor Vial A (for LCMS)	45,376 (88 %)	7,620 (88 %)	55,531 (85 %)	31,496 (97 %)
Competitor Vial B (for LC/GC)	21,788 (42 %)	6,137 (71 %)	24,131 (37 %)	27,327 (84 %)

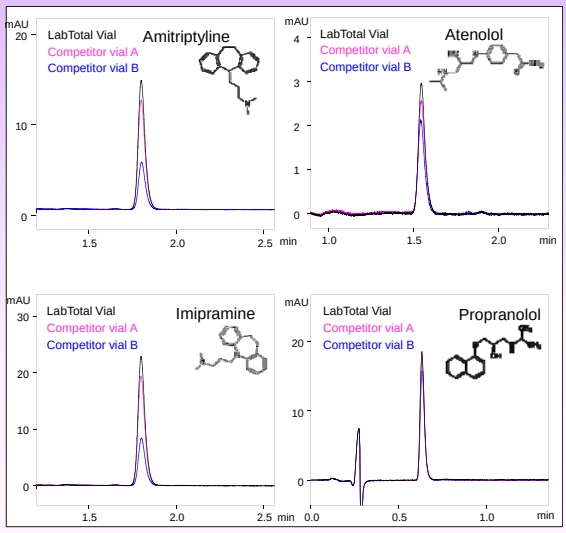


Fig. 4 Chromatograms for Four Types of Basic Compounds (1 mg/L)

5. Effectiveness of TORAST-H™ Bio Vials in Inhibiting Peptide Adsorption

We confirmed that adsorption of highly polar peptides (with retention times between about 7 and 8 minutes) was most prominent on glass vials, whereas adsorption of highly hydrophobic peptides (with retention times between about 12 and 16 minutes) was most prominent on PP vials.

TORAST-H™ Bio vials showed effectiveness in inhibiting adsorption for both types of peptides.

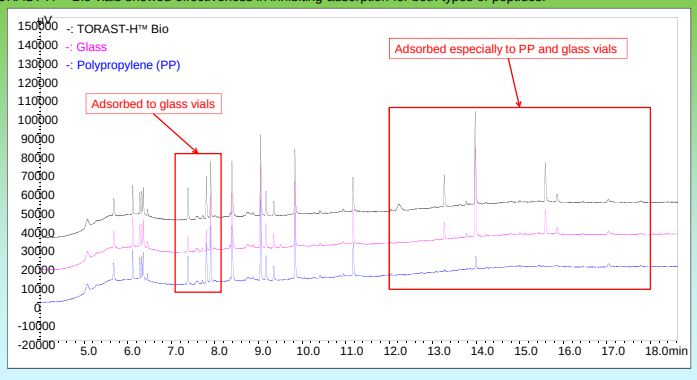


Fig. 5 Chromatograms of Trypsin Digested Myoglobin (1.9 pmol/mL)

6. Summary and Discussion

We developed vials (LabTotal and TORAST-H™ Bio vials) with low adsorption characteristics required for high-sensitivity LC and LC/MS analysis.

1) The low-adsorption glass vials (LabTotal vials) inhibit the adsorption of basic compounds.
2) The low-adsorption PP vials (TORAST-H™ Bio vials) inhibit the adsorption of peptides.

These low-adsorption vials are expected to help ensure the reliability of high-sensitivity analysis.