**Sampling ionized pharmaceuticals in protein binding assays using C18-based SPME coatings**

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Commonly used extraction phases in solid-phase microextraction include neutral coatings such as polyacrylate (PA) or polydimethylsiloxane (PDMS). However, these coatings only show high sorption coefficients for neutral analytes, with ionizable compounds only sorbing after matrix-modifying steps to ensure a large neutral fraction. Previously, we showed that the C18/SCX fiber (containing C18 and strong cation exchange (SCX) groups) showed limited pH-dependent sorption for ionizable compounds, indicating that these coatings could be beneficial for charged species especially.

We compared sorption of pharmaceuticals to two C18-based SPME coatings. Sorption of four model compounds was studied for both the analogous C18 fiber and the C18/SCX fiber. The chosen model compounds are all bases: amitriptyline (log Kow 4.81, pKa 9.7), amphetamine (log Kow 1.80, pKa 9.9), diazepam (log Kow 3.08, pKa 2.9) and tramadol (log Kow 2.45, pKa 9.2). All log Kow values are for the non-ionized compound.

Our results indicate that sorption to both C18 and C18/SCX coatings is only slightly pH-dependent. Furthermore, with sorption coefficients for cationic compounds still high at very low pH, this indicates that it is not the remaining neutral fraction that has high affinity for C18-based coatings but that also the charged species has similar affinity for these fibers. The C18/SCX coating showed somewhat higher sorption affinities for cationic compounds compared to C18 coating alone. However, sorption of charged species to the C18/SCX fiber decreases with increasing ionic strength, whereas the C18 coating might be less affected by the competitive effects of salts.

To prove that sorption of cationic compounds to the C18 coating was not driven by available silanol groups, anionic diclofenac (log Kow 4.26, pKa 4.0) was used to test this. Sorption of diclofenac to the C18 fiber was also only slightly pH-dependent. This proves that completely ionized compounds (albeit cationic or anionic) are able to sorb to C18-based coatings. Furthermore, diclofenac sorption to the C18/SCX coating is comparable to sorption to the C18 fiber at pH 7.4, showing the large contribution of C18 in the C18/SCX fiber to the sorption of diclofenac.

The developed SPME method was used to sample cationic compounds in serum albumin solutions, plasma and whole blood. This showed that SPME can be easily applied to determine free and bound fraction without significant interferences from endogenous compounds present. Furthermore, free and bound concentrations can then be used to calculate binding affinity of these compounds. Binding affinities calculated using SPME sampling were very comparable to reported literature values.

It is clear from our data that C18-based SPME coatings show improved sensitivity over conventional SPME coatings, can be applied without matrix-modifying steps, and are able to sorb both neutral and charged drug species. These insights highly contribute to the use of SPME in the measurement of sorption processes, including protein binding studies.