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Optimization of Extraction Conditions and Fiber Selection for Low-Molecular Weight Analytes and Semi-volatile Analytes Using SPME

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INTRODUCTION

Developing an application using SPME can be an intimidating task when looking at the many extraction variables and fiber coating options. This seminar will provide a logical approach to selecting the appropriate fiber coating and how to optimize the extraction conditions.

To do this, two studies were investigated. One study looked at the extraction of low-molecular weight analytes with varying functionalities. Six different fibers were evaluated for the extraction of these analytes. The extraction conditions such as pH of the solution and the effects of salt were studied.

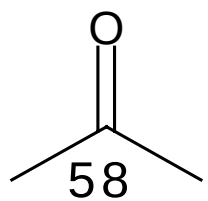
The second study looked at semi-volatile analytes using similar parameters as in the first study. For this study nine fibers were evaluated.

Types of SPME Fibers

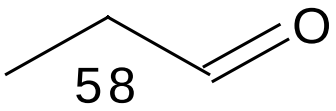
Bare fused silica	**Adsorbent**	**Unknown**
7µm Polydimethylsiloxane (PDMS)	**Absorbent**	**Nonpolar**
30µm PDMS	**Absorbent**	**Nonpolar**
100µm PDMS	**Absorbent**	**Nonpolar**
85µm Polyacrylate (PA)	**Absorbent**	**Polar**
65µm PDMS-DVB, StableFlex™	**Adsorbent**	**Bipolar**
65µm CW-DVB, StableFlex	**Adsorbent**	**Polar**
55µm/30µm DVB/Carboxen™-PDMS, StableFlex	**Adsorbent**	**Bipolar**
85µm Carboxen-PDMS, StableFlex	**Adsorbent**	**Bipolar**

The fibers can be classified by polarity or extraction type mechanism. The polar fibers are the polyacrylate coated fibers and the Carbowax-Divinylbenzene (CW-DVB) coated fibers. The other remaining fibers are nonpolar or bi-polar. The nonpolar fibers have a polydimethyl-siloxane (PDMS) coating, and the bi-polar fibers are primarily nonpolar, but will extract some polar analytes efficiently.
The other means for classifying fibers are by extraction mechanism. Absorbent fibers extract by partitioning into a liquid type coating. The analytes are retained by the thickness of the coating. Adsorbent type fibers contain porous particles suspended in a liquid phase. The particles retain analytes in the pores or on the surface. DVB contains primarily mesopores that extract larger analytes while Carboxen contains more micropores which are ideal for extracting smaller analytes. To expand the analyte range that could be extracted with one fiber, on fiber has DVB-PDMS coated over a layer of Carboxen-PDMS. The fibers are listed by retention strength increasing from top to bottom. All of the adsorbent type fibers are on a StableFlex core to reduce breakage and increase bonding strength.

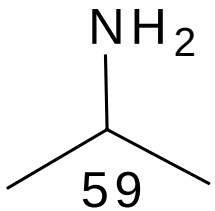
Fig 1 - Analytes in Volatile Study



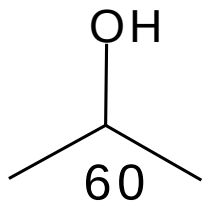
Acetone



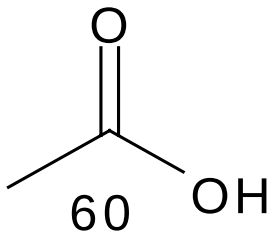
Propanal



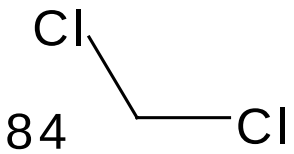
Isopropylamine



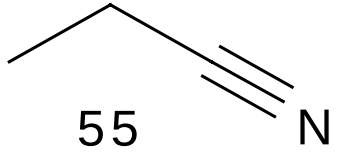
Isopropanol



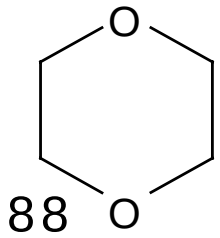
Acetic acid



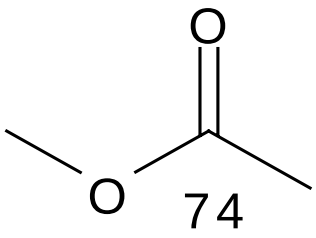
Dichloromethane



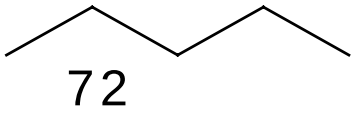
Propionitrile



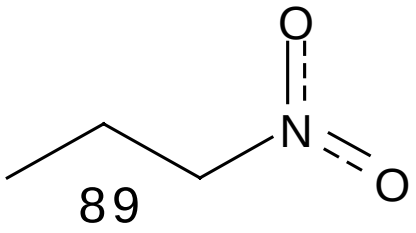
1,4-Dioxane



Methylacetate



Pentane



Nitropropane

Figure 1 contains the analytes in the mixture. Most of the analytes are similar in structure but vary by functionality. All of the analytes have a molecular weight between 58 and 89 AMU. There are 11 organic classes of analytes represented by this mixture of analytes. The primary purpose of this study was to determine the relationship between analyte polarity and fiber coating.

Analytical Conditions for Evaluation

Sample:	**Water containing 25% NaCl and appropriate 0.05M phosphate buffer, spiked with analytes to a final concentration of 2 ppm. No NaCl in DI water samples.**
Extraction:	**15 min with agitation, using Varian 8200autosampler, Heated headspace done manually at 50°C**
Desorption:	**2 min, temperature varies, depending on fiber**
Column:	**30m x 0.32mm x 4.0µm SPB™-1 SULFUR**
Oven:	**40°C (2 min) to 140°C at 8°C/min (1 min)**
Inlet:	**Split/splitless, closed 0.5min, 0.75mm ID liner**
Detector:	**FID**

Fig 2 - Effects of pH and Ionic Strength

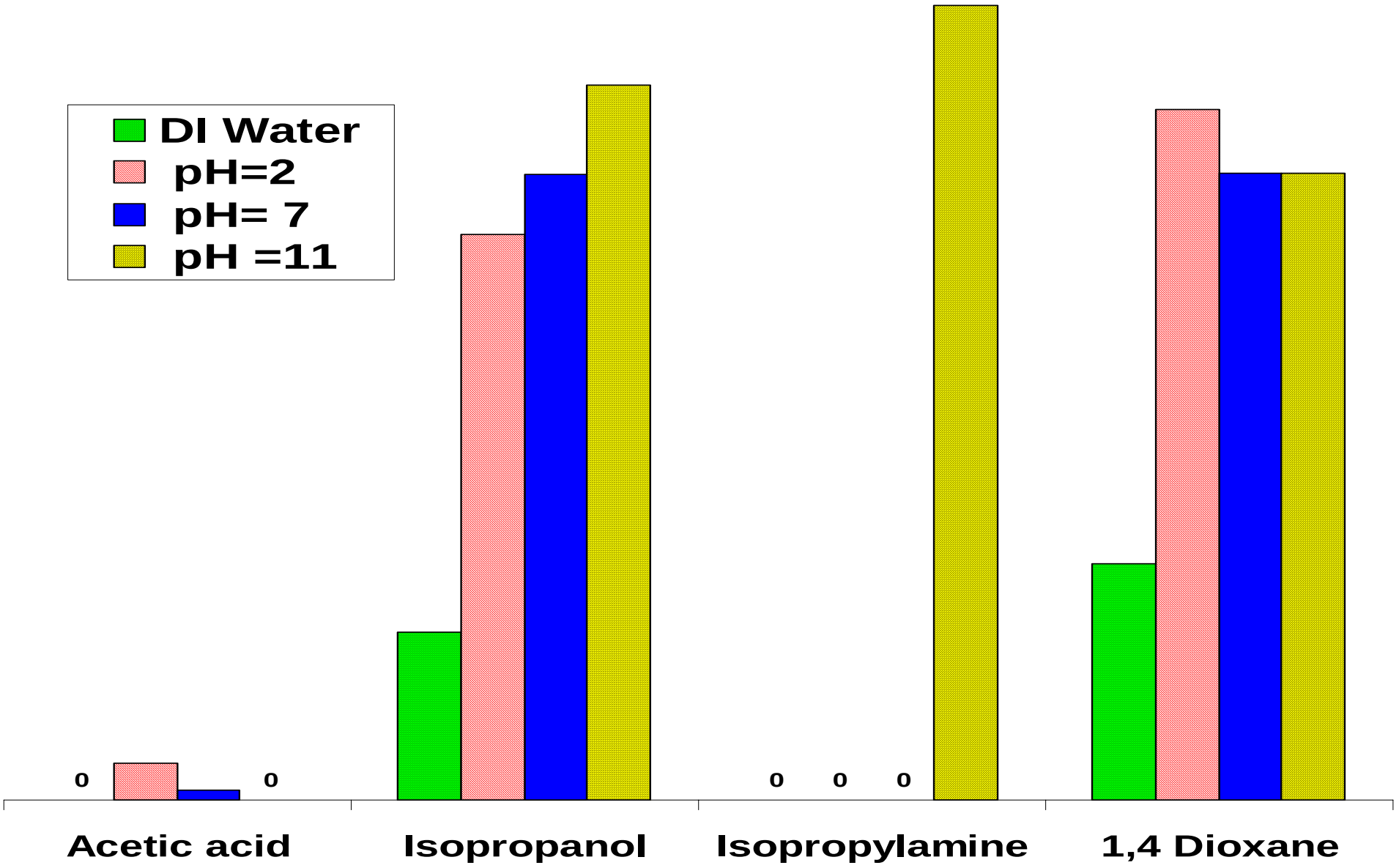


Fig 2 - Effects of pH and Ionic Strength Cont..

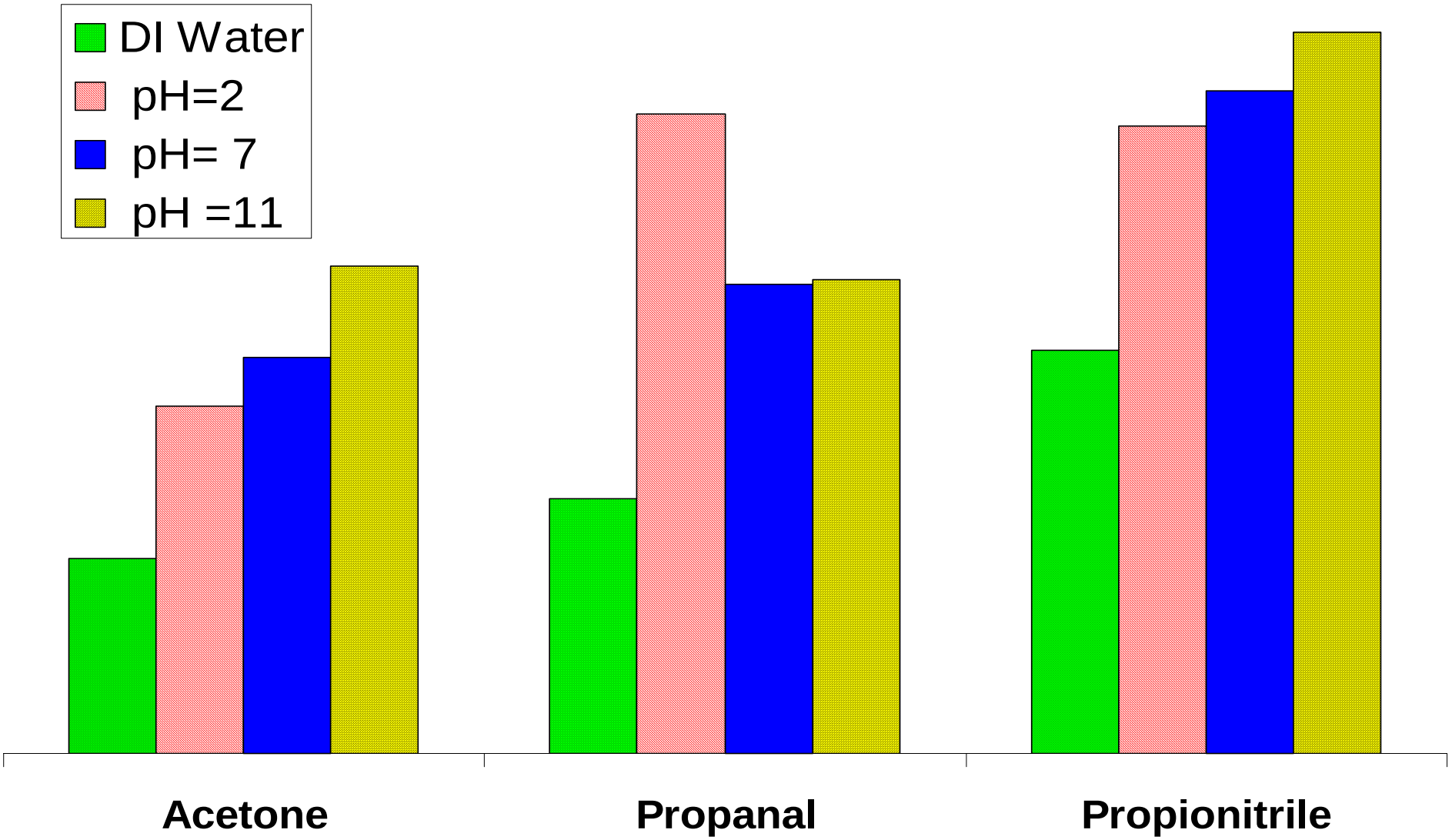


Fig 2 - Effects of pH and Ionic Strength Cot.

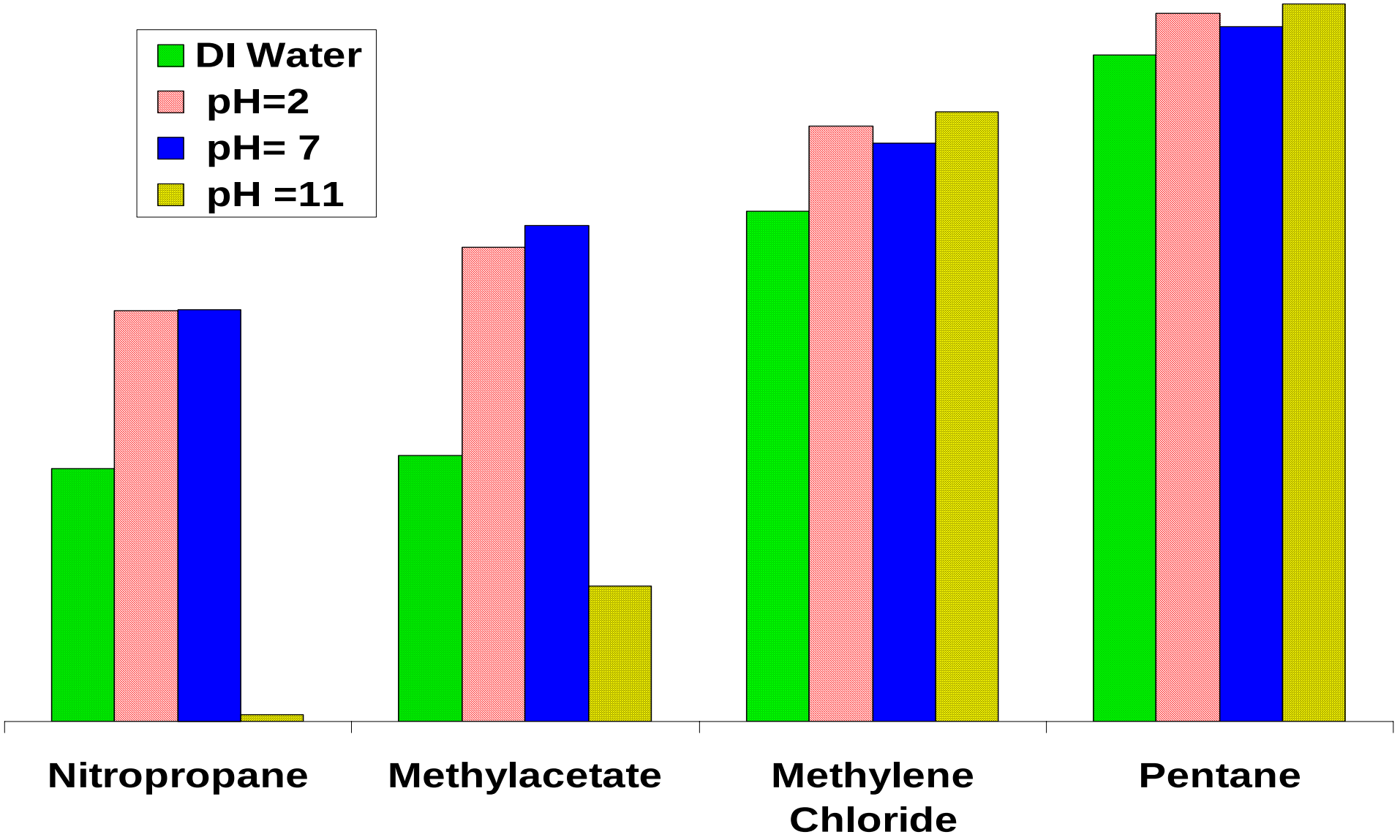


Figure 2 shows the comparison of the area counts from the analytes extracted at different pH levels. To obtain the values, the area counts from all of the fibers were averaged at each pH level. As expected acidic analytes (acetic acid) were extracted most efficiently from an acidic solution, pH 2, whereas bases (isopropylamine, propionitrile) were best extracted from basic solutions, pH 11. It was unexpected that acetone and isopropanol o would be most efficiently extracted at pH 11. Nitropropane and methylacetate were hydrolyzed in basic solutions which accounted for the poor recovery at pH 11. The other analytes were not highly affected by pH. The addition of NaCl improved the recovery of all of the analytes especially the polar analytes.

Fig. 3 - Comparison of Area Responses by Fiber Type

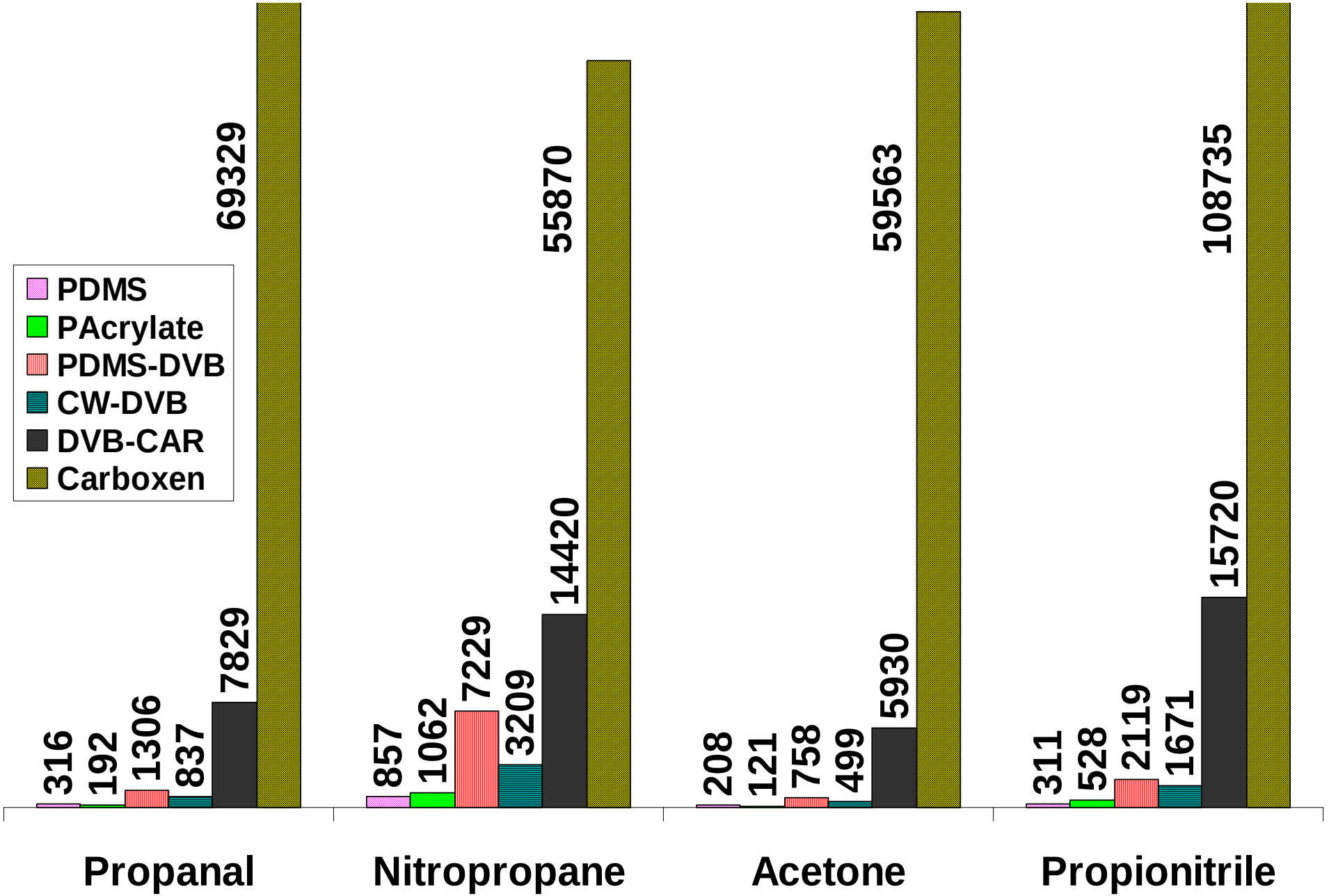


Fig 3 - Comparison of Area Responses by Fiber Type Cont.

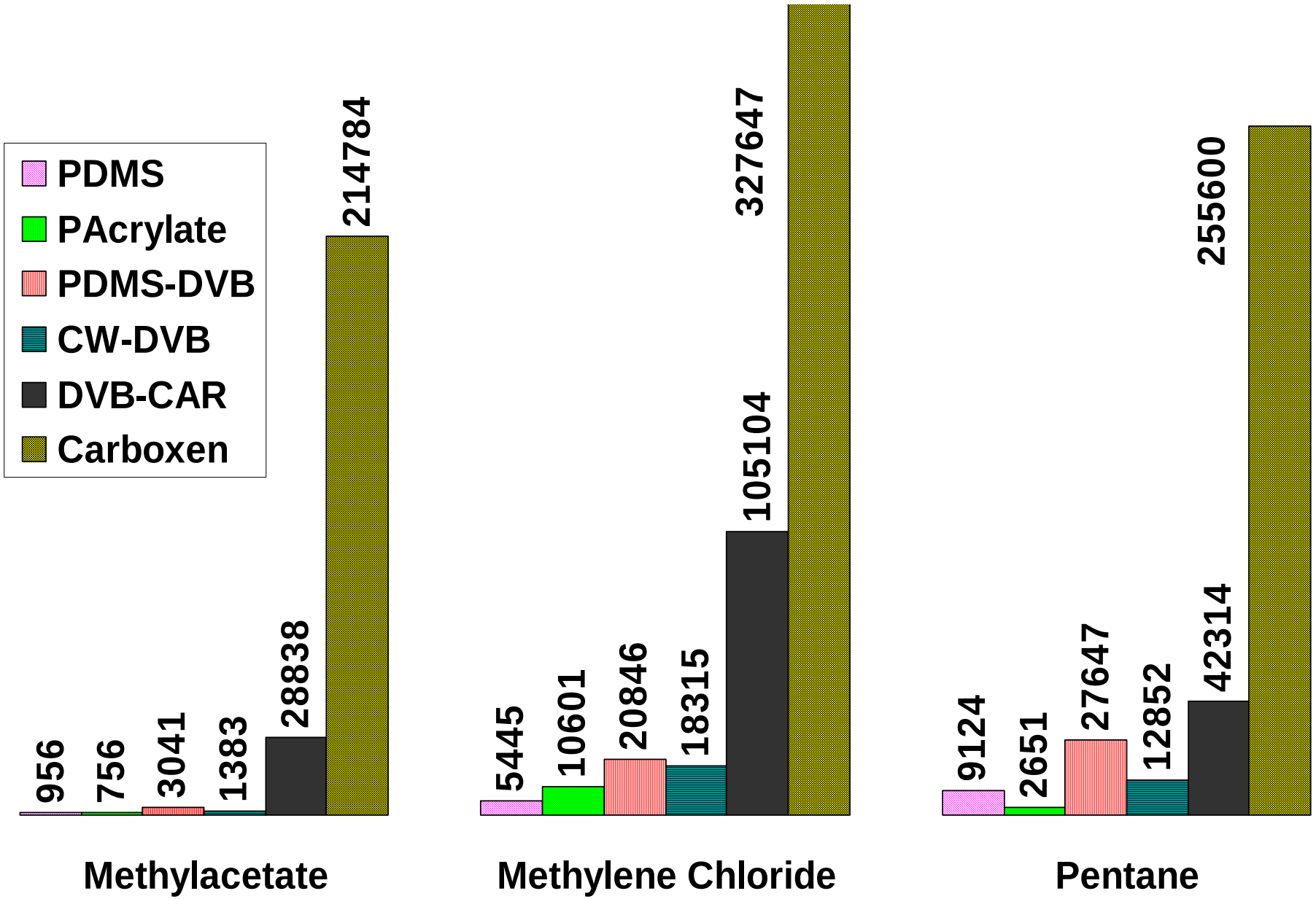


Fig. 3 - Comparison of Area Responses by Fiber Type Cont.

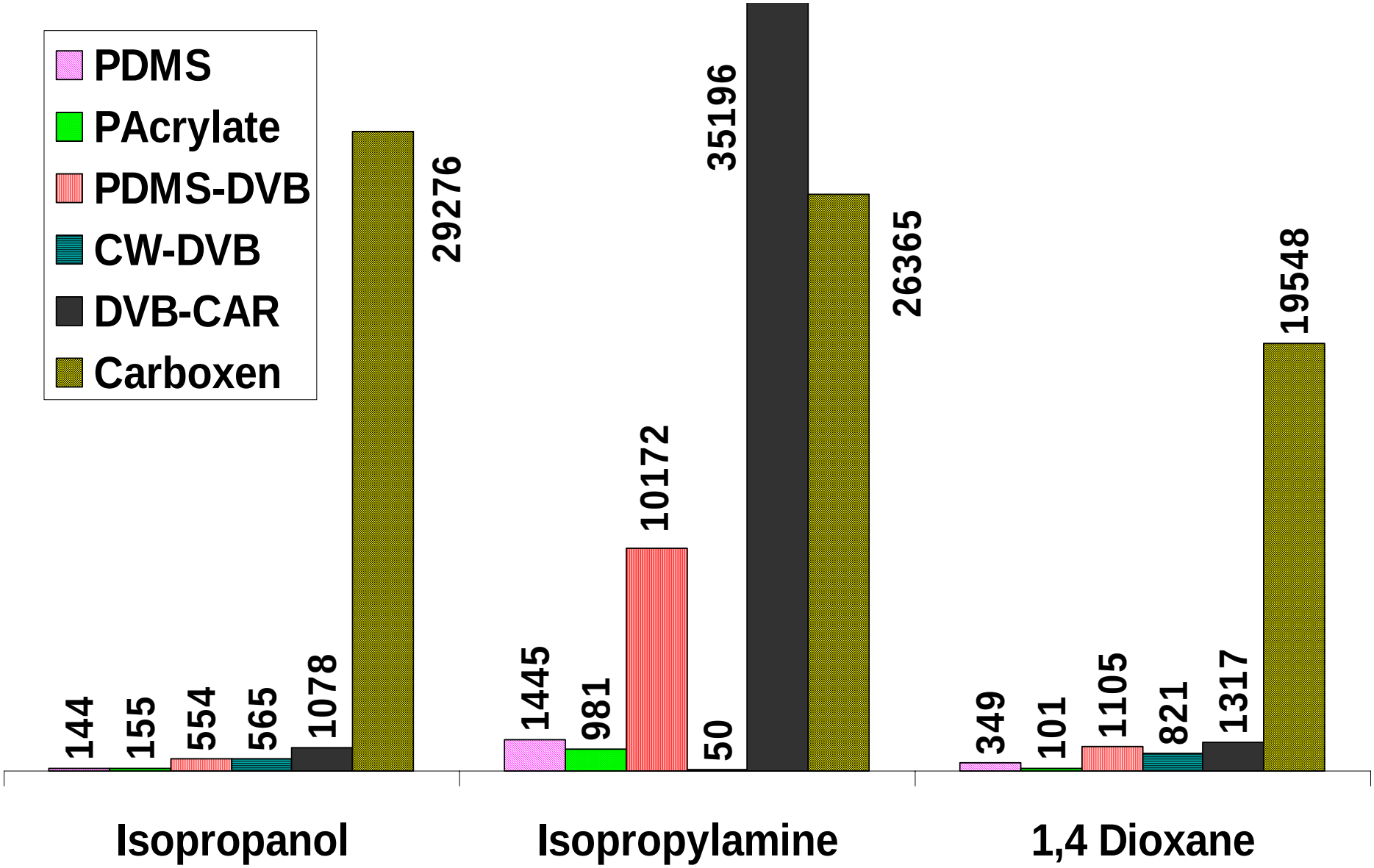


Figure 3 shows the comparison of the area responses for the analytes extracted by the various fibers. All of the area counts recorded were obtained from extraction at the optimum pH level for each analyte. The Carboxen-PDMS fiber is superior to the other fibers for extracting these low-molecular weight analytes. This fiber extracted more than 200 times as much of the polar analytes than the 100µm PDMS fiber. For the nonpolar analytes the advantage was not as great, but it was still significantly better than the other SPME fibers. Isopropylamine was the only analyte that was not most efficiently extracted by the Carboxen-PDMS fiber. The dual layered PDMS-DVB over Carboxen-PDMS was best for this analyte and second best for the other analytes. The PDMS-DVB coating has a high affinity for amines. The combination of the high affinity of PDMS-DVB for amines coupled with the microporosity of Carboxen, makes the DVB-Carboxen fiber the best choice for small amines.

Fig 4A - Analyte Polarity vs. Area Response

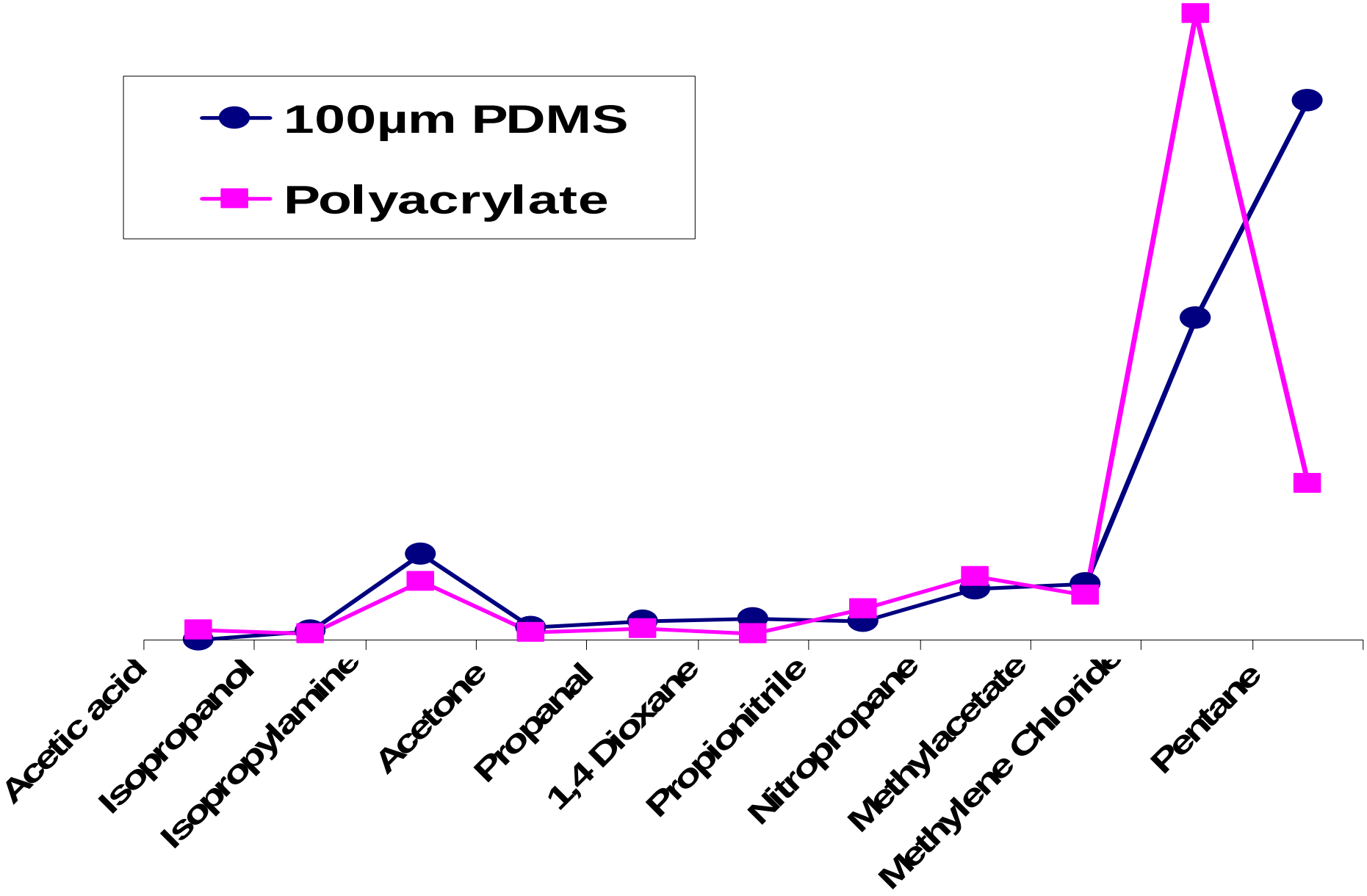


Fig 4B - Fiber Polarity vs. Area Response

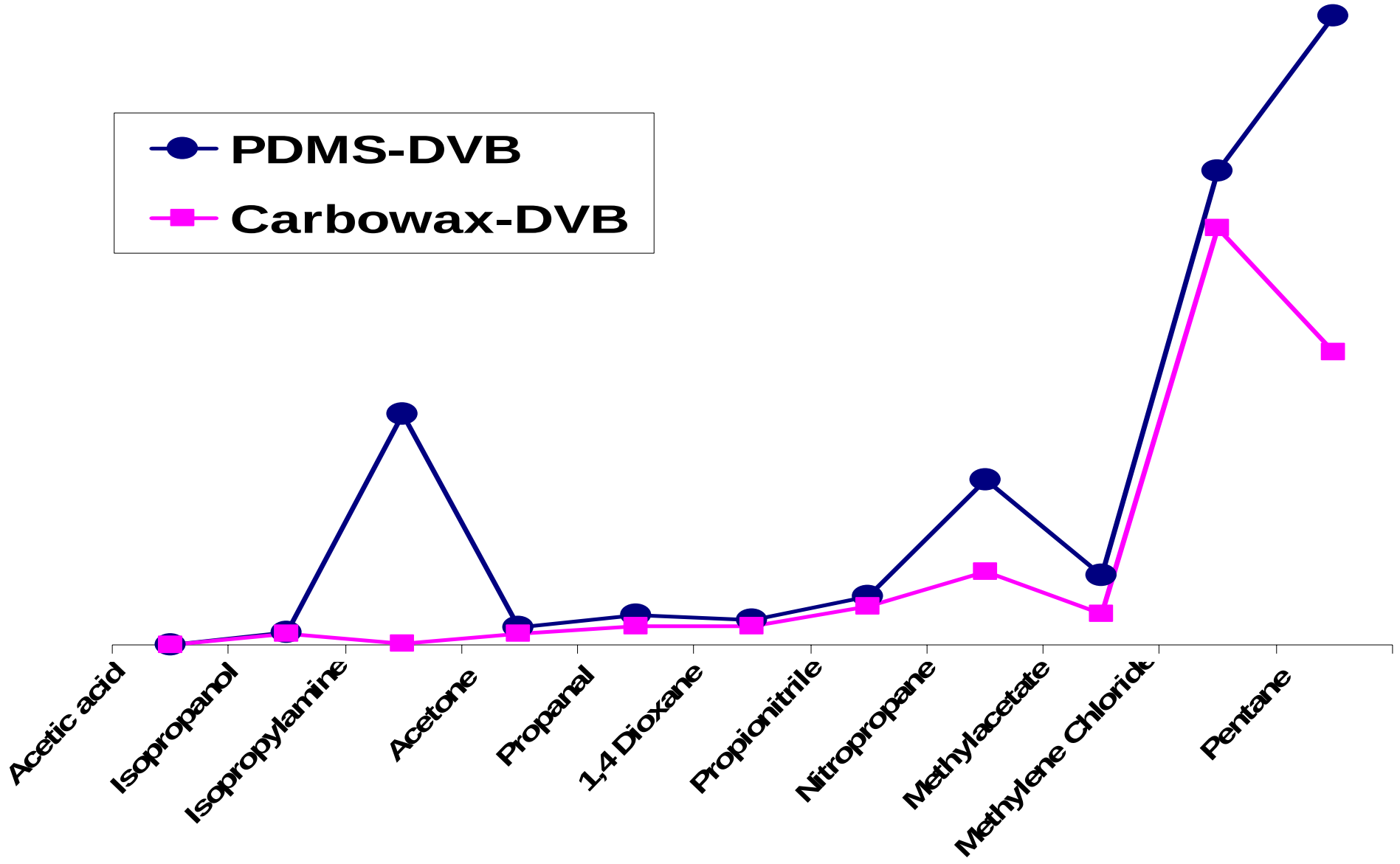


Figure 4 shows analyte polarity decreasing from left to right with respect to fiber polarity. **Figure 4A** contains a polar (polyacrylate) and nonpolar (100µm PDMS) absorbent type fibers, whereas, **Figure 4B** contains the adsorbent type fibers, the polar CW-DVB and the less polar PDMS-DVB. For both types of fibers, the more polar fibers did not extract the polar analytes better than the nonpolar analytes. Because of the small size of these analytes, fiber polarity had little or no influence on the extraction of the polar analytes. In both cases, the less polar fibers were better for the extraction of the more polar analytes. The only relationship that was seen between fiber and analyte polarity was that the polar fibers extracted less of the nonpolar analytes. This would provide some selectivity for the polar fibers.

Fig.5 - Semi-volatile Analytes Used in Study

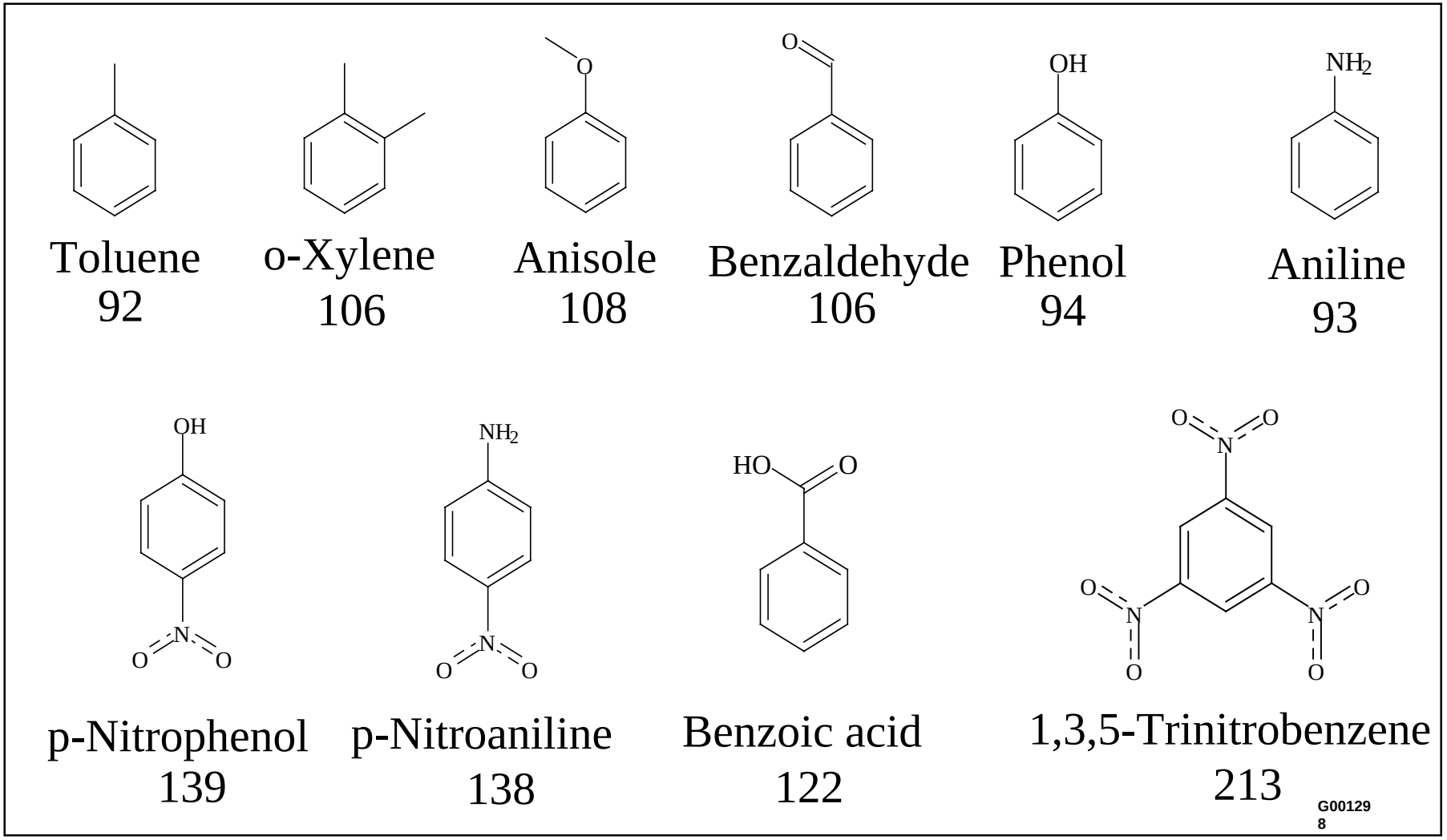
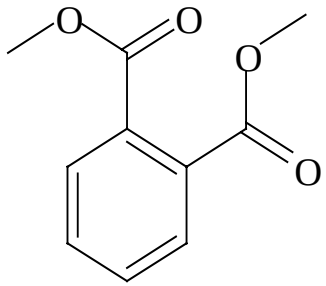
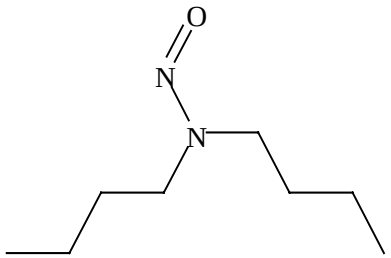


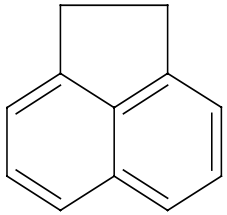
Fig. 5 - Semi-volatile Analytes Used in Study Cont.



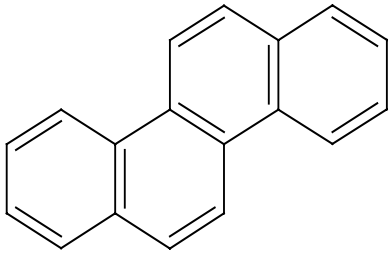
Dimethyl phthalate
194



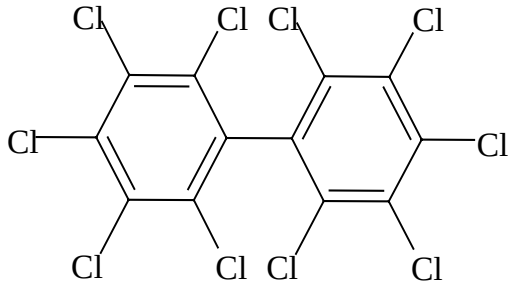
N,N-Nitrosodibutylamine
158



Acenaphthene
154



Chrysene
228



Decachlorobiphenyl
499

Figure 5 shows the analytes chosen for the semi-volatile study. Most of the analytes selected contained aromatic rings with varying functionalities. Some had one functional group while others had two or three functional groups to vary the polarity of the analytes. The effects of molecular size of the analytes was a desired input for this study. To vary the size, two PAHs and decaclorobiphenyl were added to the mixture.

Analytical Conditions for Evaluation of Fibers with Semi-volatile Analytes

Sample:	**Water containing 25% NaCl and appropriate 0.05M phosphate buffer, spiked with analytes to a final concentration of 75 ppb**
Extraction:	**Directly immersed for 30 min with agitation** **Heated headspace, 65°C for 30 min with agitation**
Desorption:	**3 min, temperature varies, depending on fiber**
Column:	**30m x 0.25mm x 0.25µm PTE™-5**
Oven:	**45°C (2 min) to 210°C at 10°C/min, then to 320°C at 20°C/min (10 min)**
Inlet:	**Split/splitless, closed 1 min, 0.75mm ID liner**
Detector:	**MS ion trap, m/z = 50-515 at 0.6 sec/scan**

Fig. 6 - Effects of pH

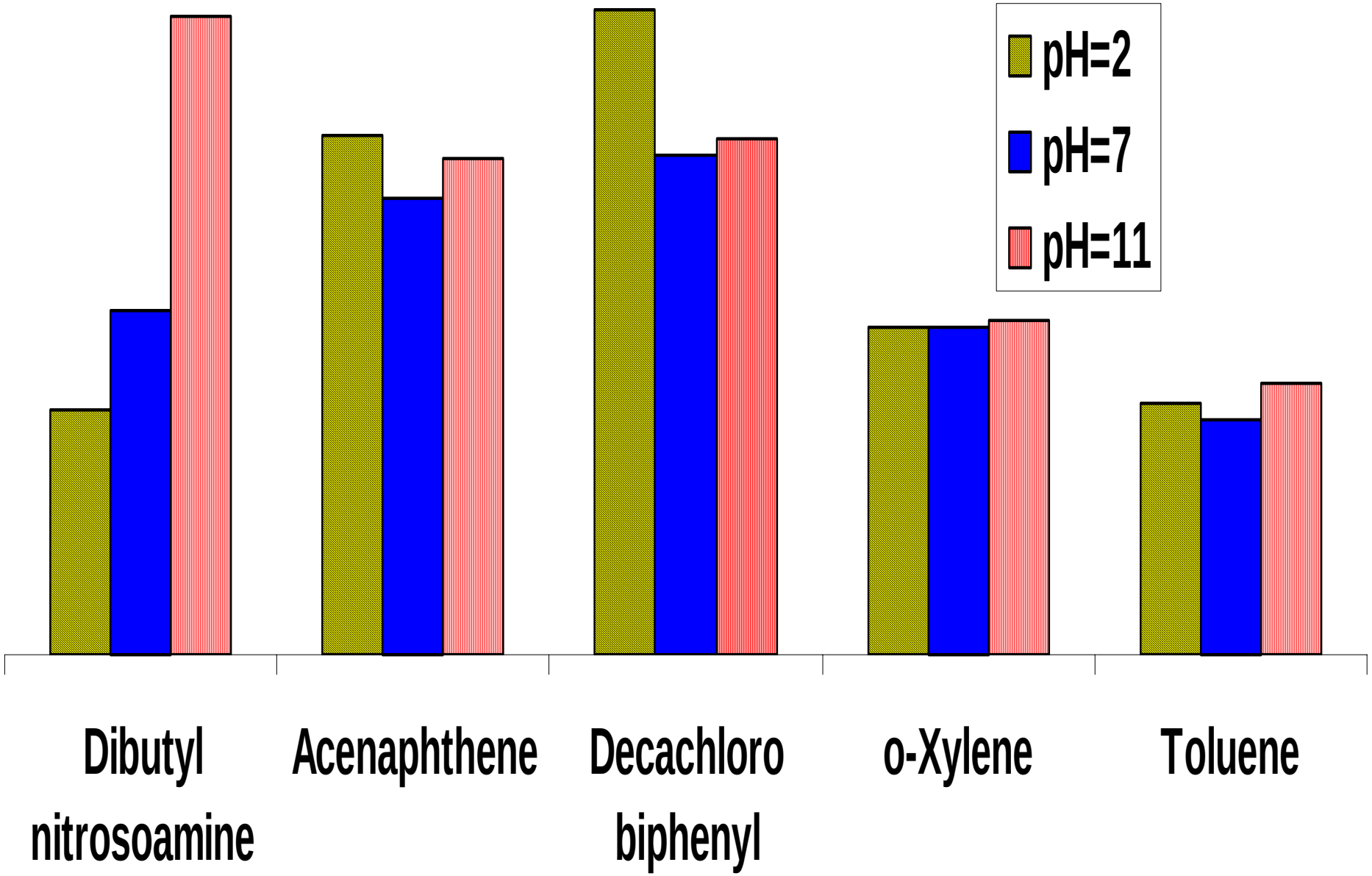


Fig. 6 - Effects of pH (cont.)

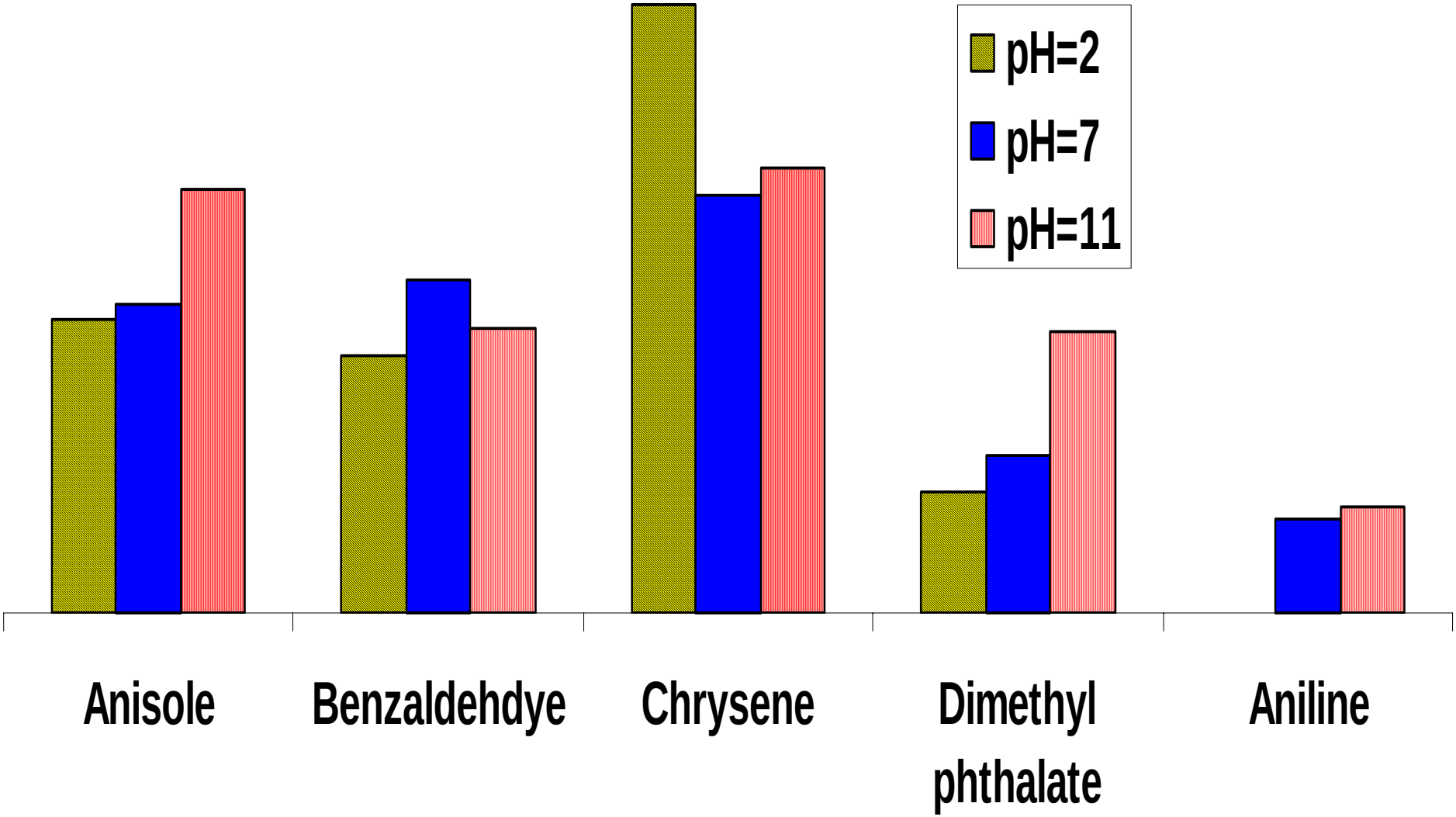


Fig. 6 - Effects of pH (cont.)

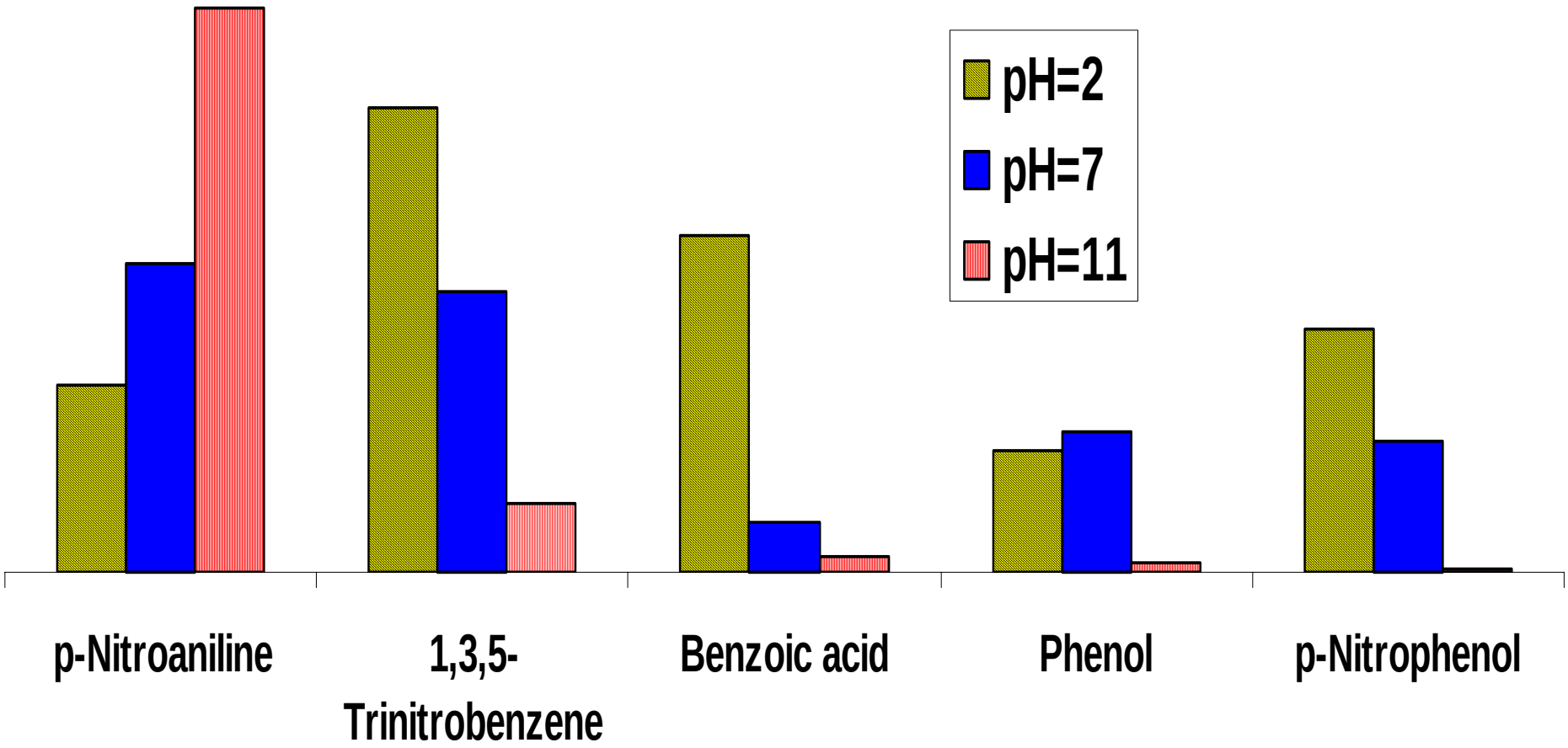


Figure 6 shows that the results from extraction of the analytes at 3 pH levels were for the most part as expected. The more basic analytes such as aniline, p-nitroaniline and nitrosodibutylamine were best extracted at pH 11. The acidic analytes, p-nitrophenol and benzoic acid were best extracted at pH 2. Moderately acidic phenol, was better extracted at a neutral pH than at pH 2. This has been shown in previous studies. Trinitrobenzene was best extracted at pH 2. This analyte is not stable in highly basic solutions. Dimethylphthalate extracted most efficiently from basic solutions. This result was not expected. It appears that anisole and chrysene are best extracted at pH 2, but these results are probably not significant to make that conclusion. The remaining neutral analytes were not affected by pH.

Fig 7A - Comparison of Area Responses by Fiber Type

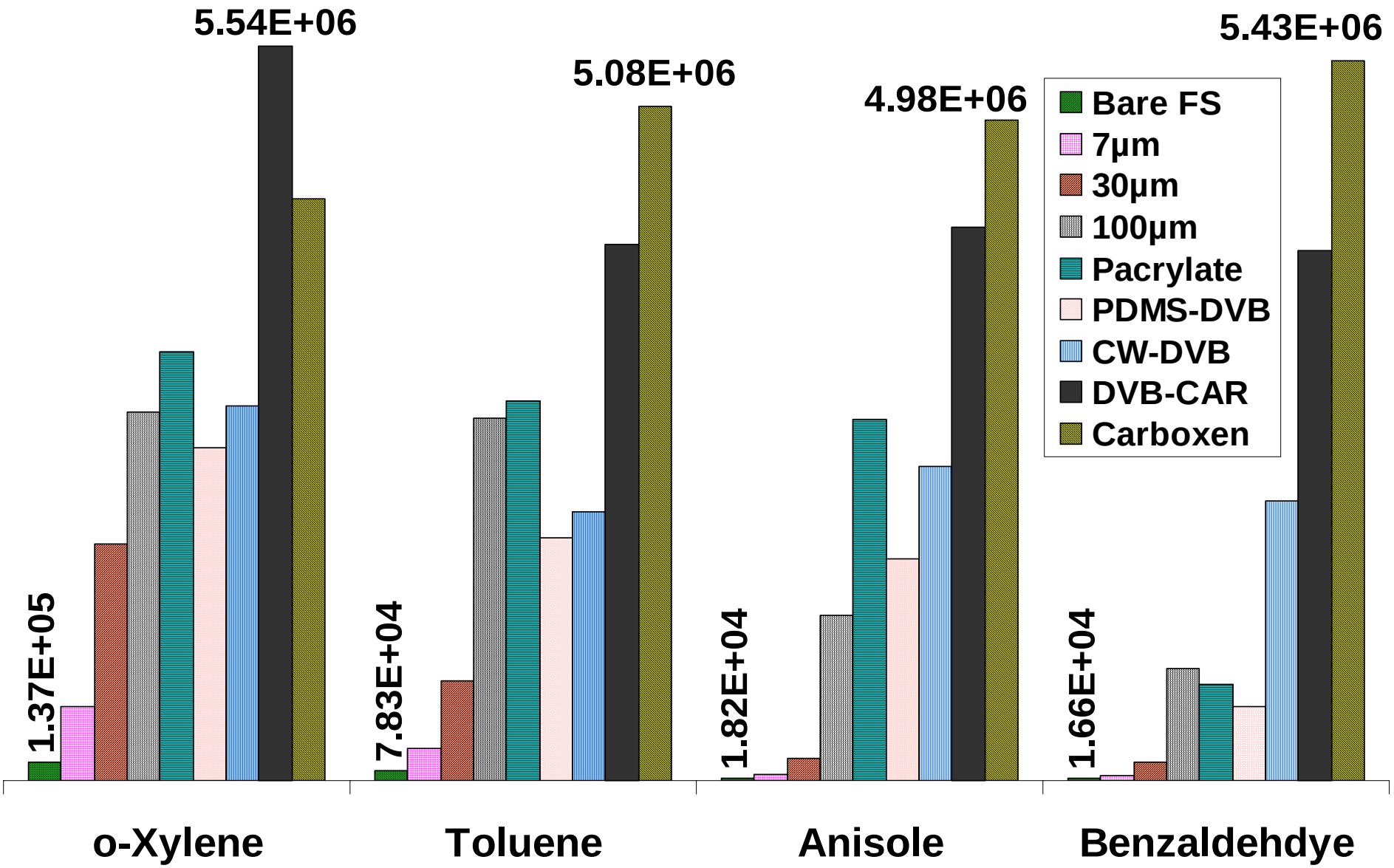


Fig 7B - Area Response vs. Fiber Type

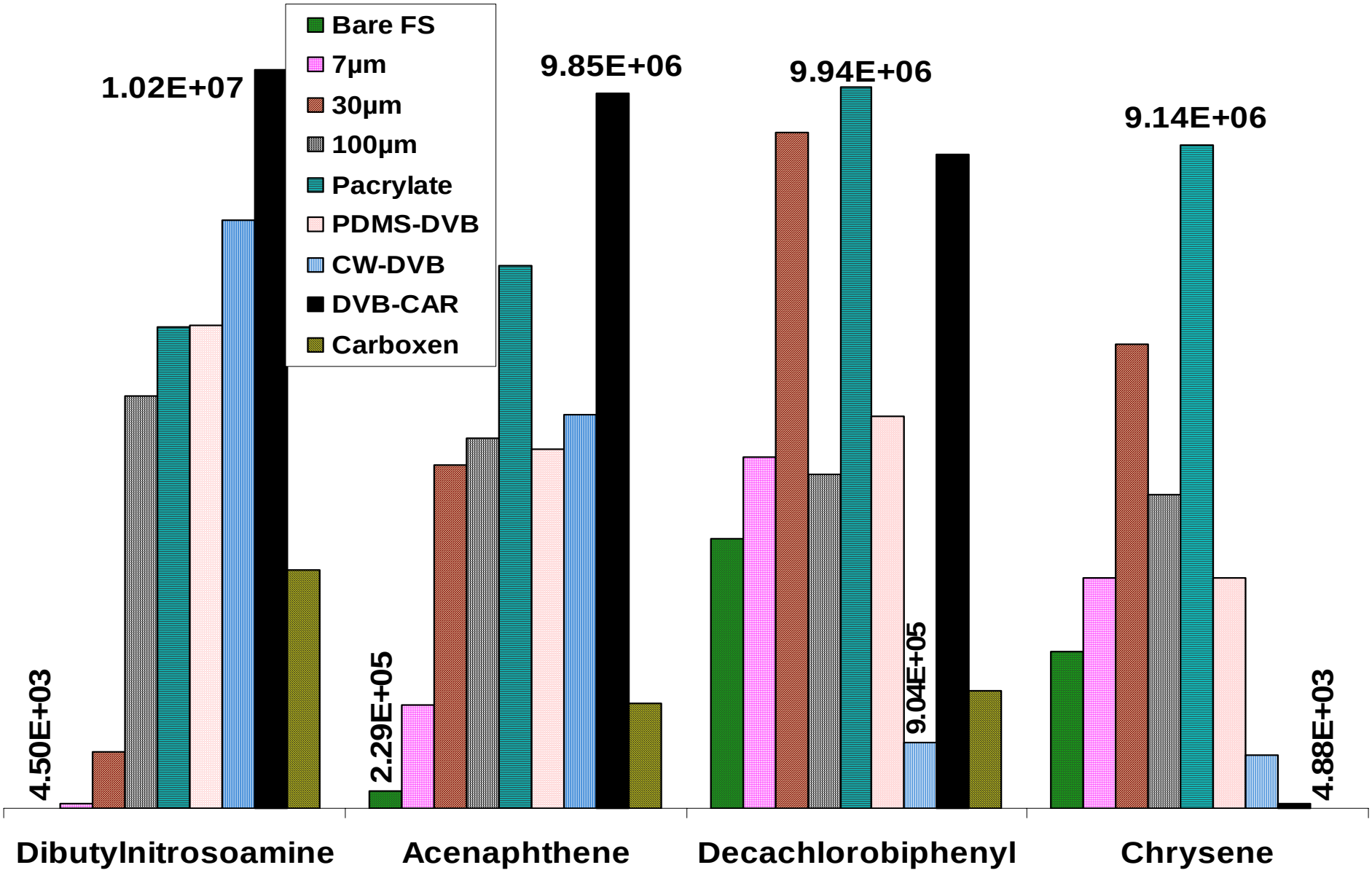


Fig. 7C - Area Response vs. Fiber Type

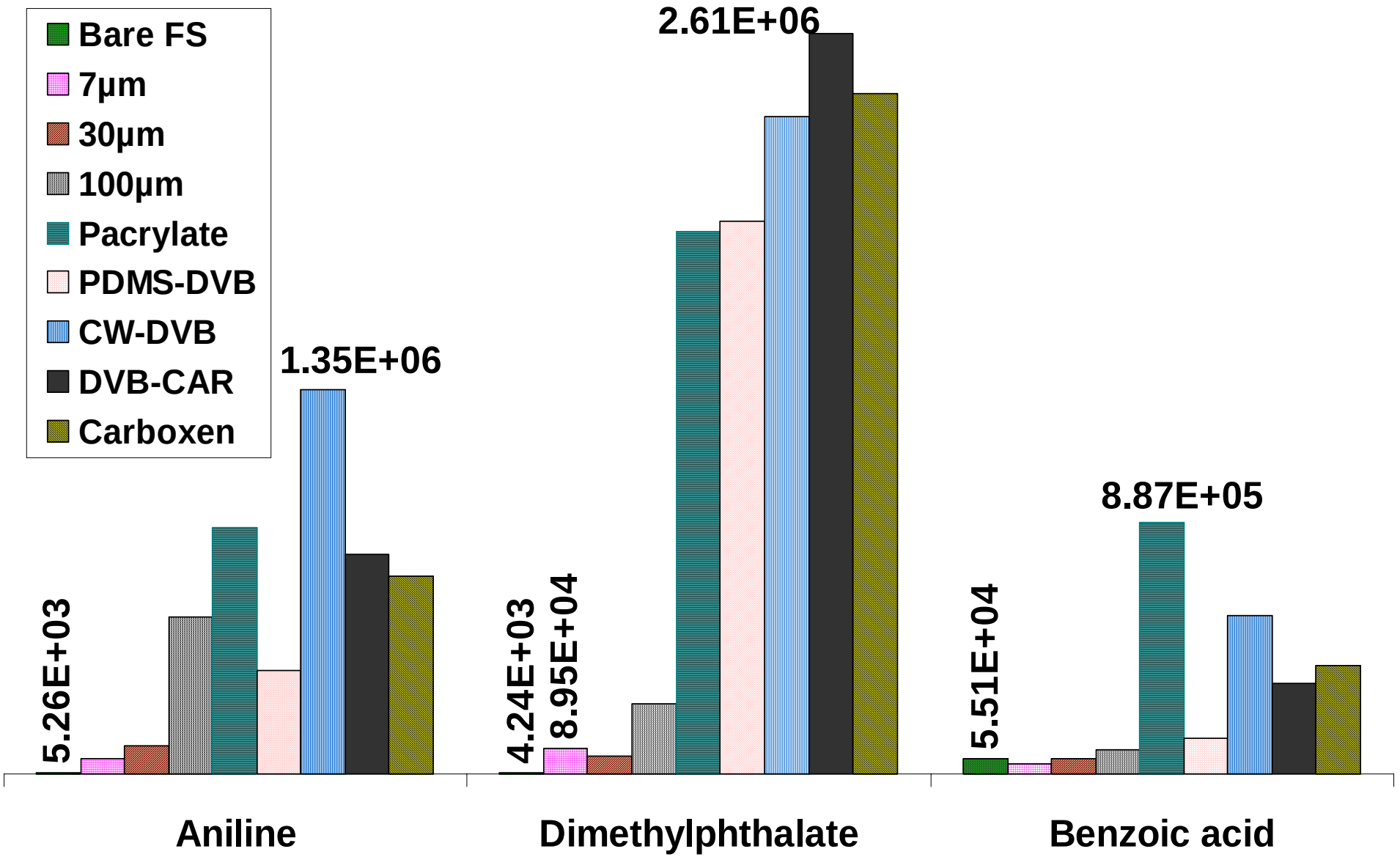


Fig. 7D - Area Response vs. Fiber Type

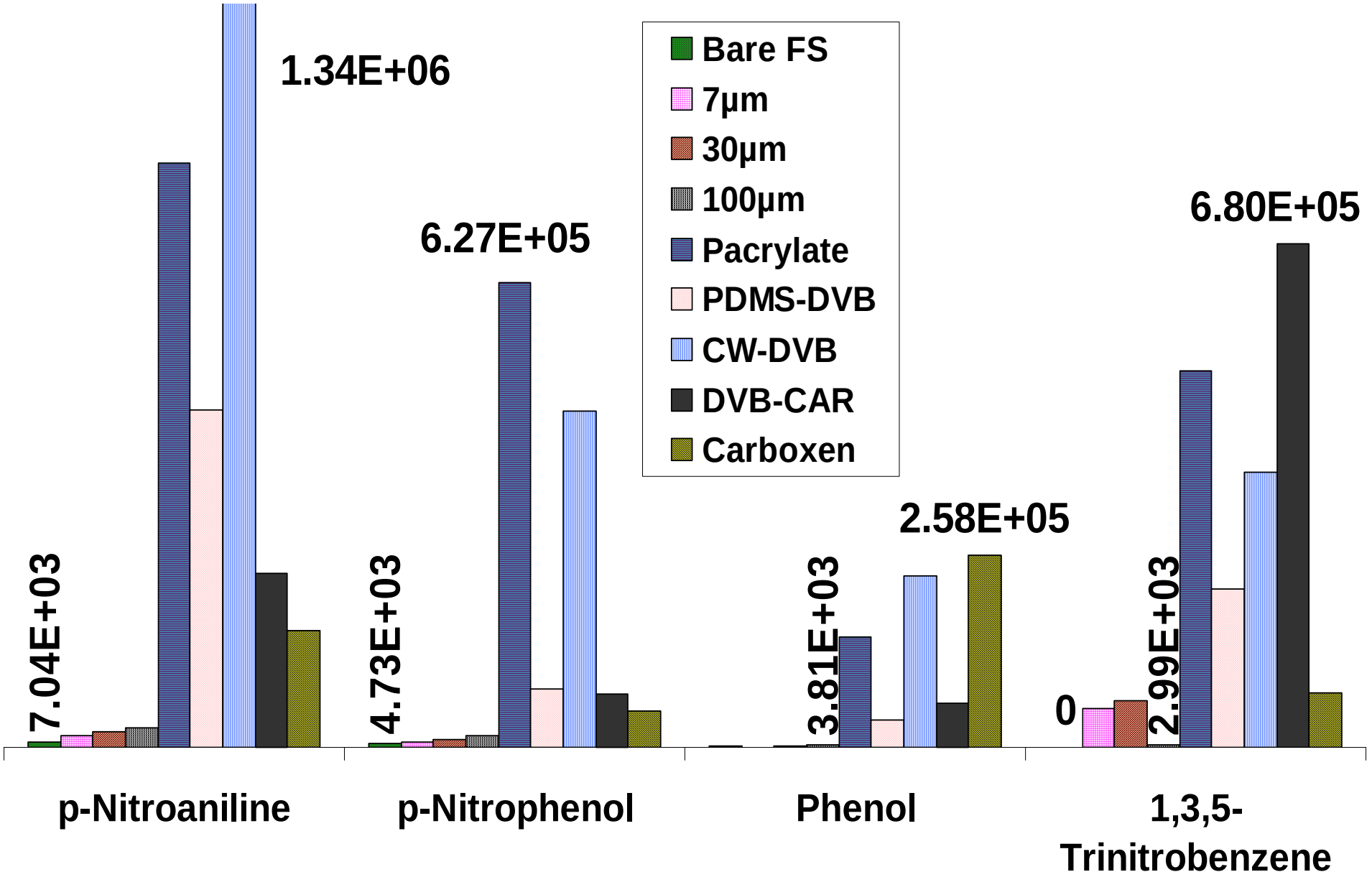


Figure 7 shows the results from the extraction of the analytes with the nine SPME fibers.

The smaller, nonpolar analytes shown in **Figure 7A** are extracted best with the Carboxen containing fibers. The porosity of Carboxen enables it to retain these smaller analytes.

Figure 7B contains the larger nonpolar analytes and nitrosodibutylamine. Chrysene and decachlorbiphenyl are poorly extracted by Carboxen. These larger analytes are efficiently extracted by PDMS fibers and polyacrylate. Bare fused silica can also extract these analytes but not reproducibly.

Figure 7C contains more polar analytes that are best extracted with the polar fibers CW-DVB and polyacrylate. The affect of fiber polarity is more significant with larger analytes. Dimethylphthalate is extracted most efficiently by the adsorbent containing fibers. It was not extracted well by PDMS fibers.

Figure 7D contains polar analytes that are best extracted with polar fibers. The effect of fiber polarity is significant. TNB was best extracted by DVB-Carboxen fiber.

Fig. 8A - Analyte Polarity vs. Area Response

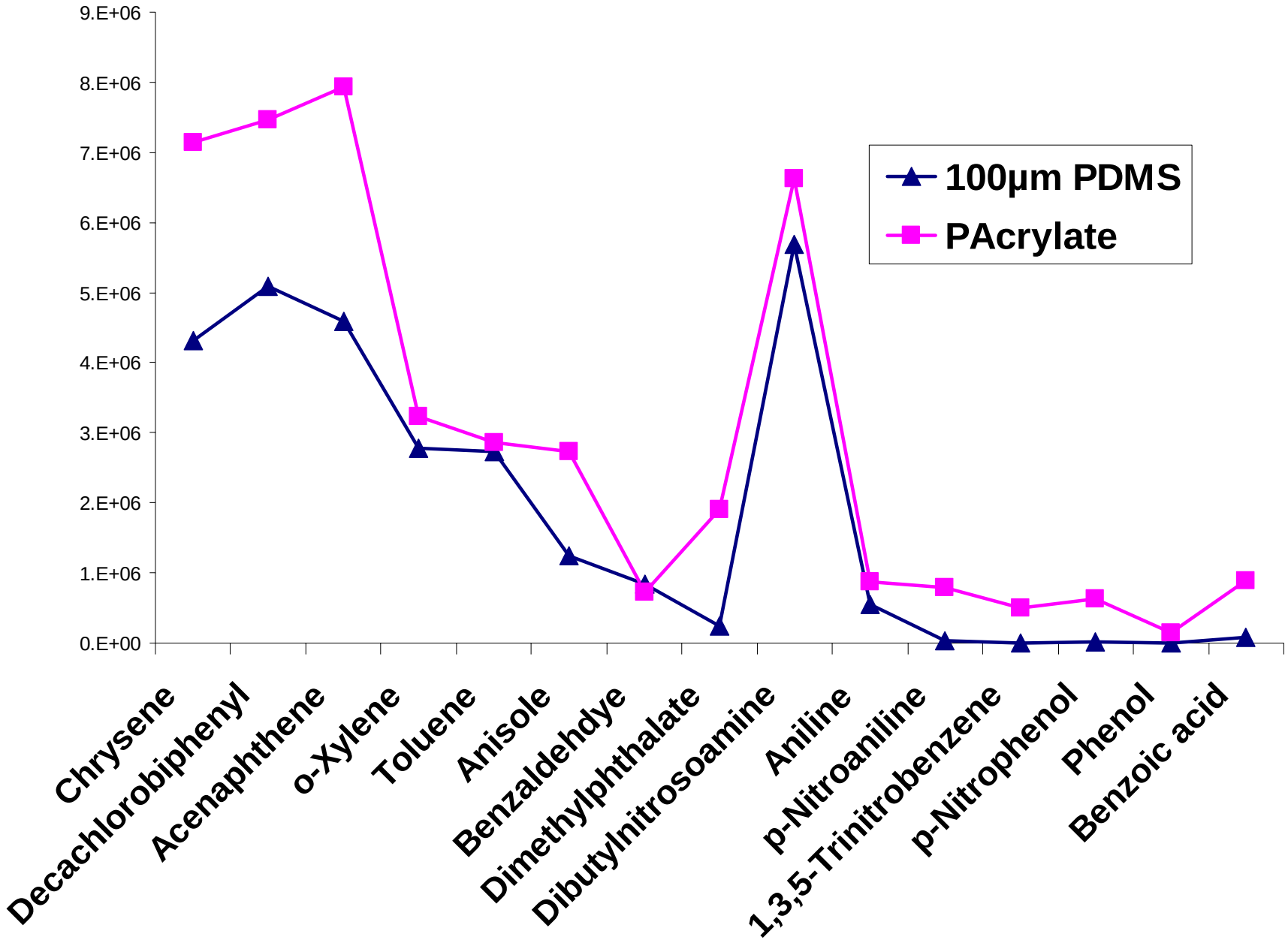


Fig. 8B - Analyte Polarity vs. Area Response

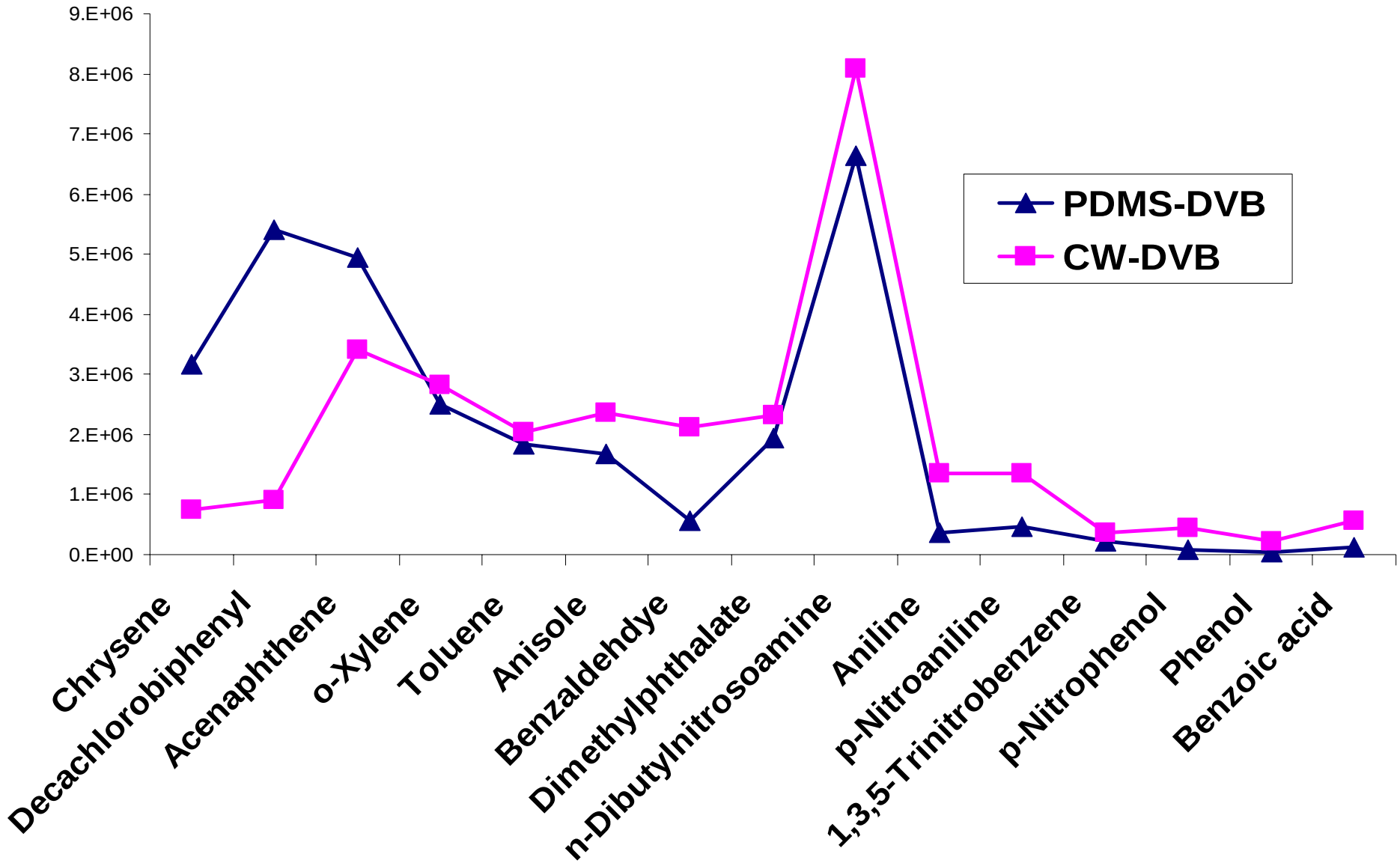


Figure 8 contains plots of analyte polarity, increasing from left to right vs. fiber polarity.

Figure 8A shows the absorbent fibers,100µm PDMS and the 85µm polyacrylate. The polar polyacrylate fiber not only extracts the polar analytes better, in some cases 3 orders of magnitude better, but it also extracts the nonpolar analytes better. However, the response is only 1.5-2 times greater. The affinity that polyacrylate has for aromatic compounds is the reason for the high extraction affinity for these nonpolar analytes.

Figure 8B shows the adsorption type fibers. In this case the less polar PDMS-DVB fiber extracted the nonpolar analytes better than the more polar CW-DVB fiber. The CW-DVB fiber was more efficient at extracting the more polar analytes than the PDMS-DVB fiber. These results were as expected.

Fig 9- Effects of Coating Thickness on Analyte

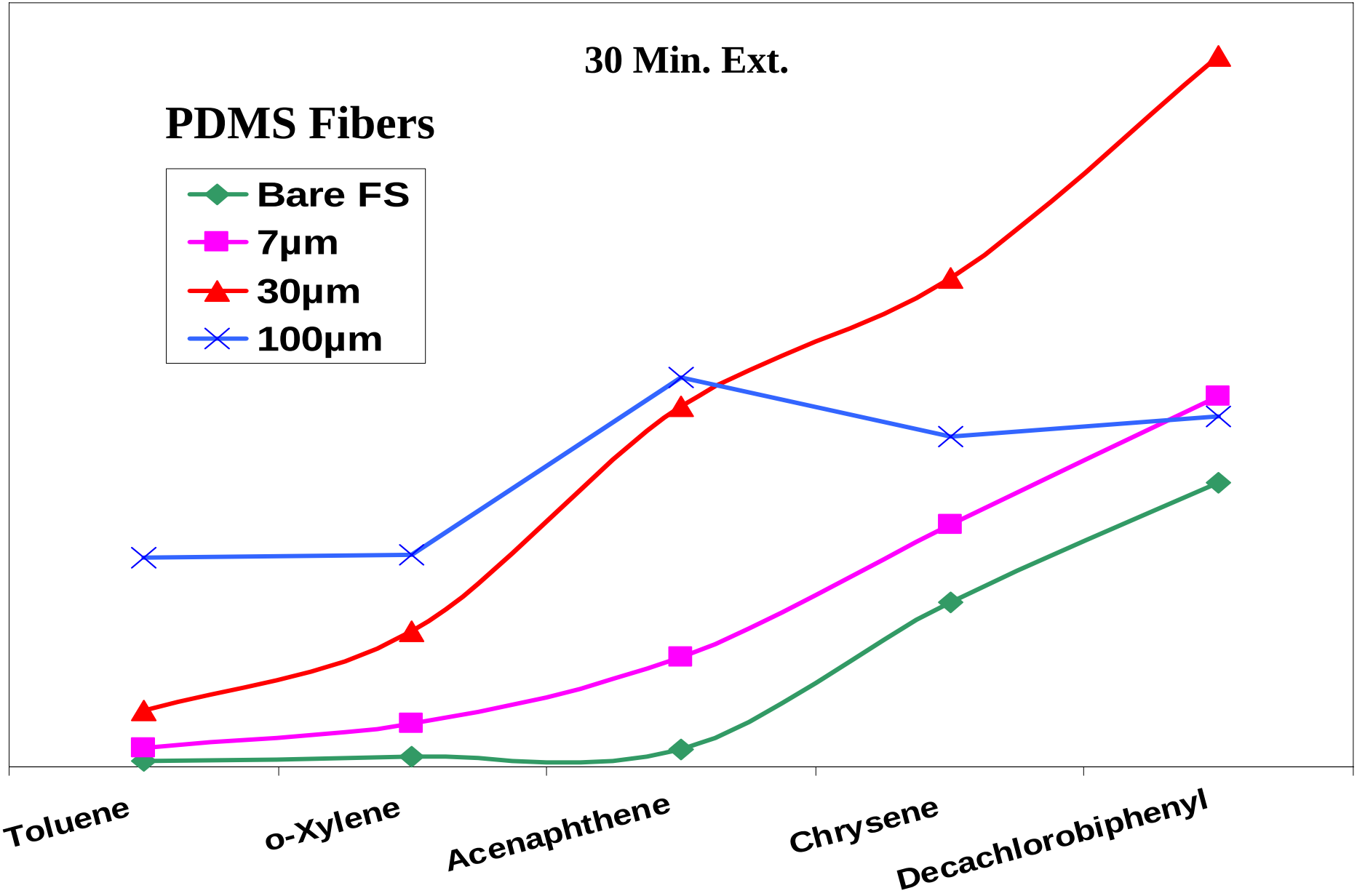


Figure 9 shows the results from a 30 min. extraction of the analytes with the 3 PDMS and bare fused silica fibers. The 100µm PDMS fiber extracts the lower molecular weight analytes efficiently, but the efficiency of the larger analytes is not as good. An extraction time of 30 min.is not a sufficient time to allow the larger analytes to migrate into the coating. The amount of analyte extracted would increase with a longer extraction time.

The 30µm PDMS is a suitable fiber for extracting both lower and higher molecular weight analytes within a reasonable amount of time. This is a good fiber choice for PAHs and PCBs.

The 7µm PDMS has less capacity and poorly extracts the lower molecular weight analytes, but it is suitable for higher molecular weight analytes. Bare fused silica and the 7µm produced parallel lines indicating the the extraction mechanism is similar. Most likely the 7µm PDMS fiber extracts by both adsorption and absorption.

Fig. 10 - Analyte Size vs. Area Response

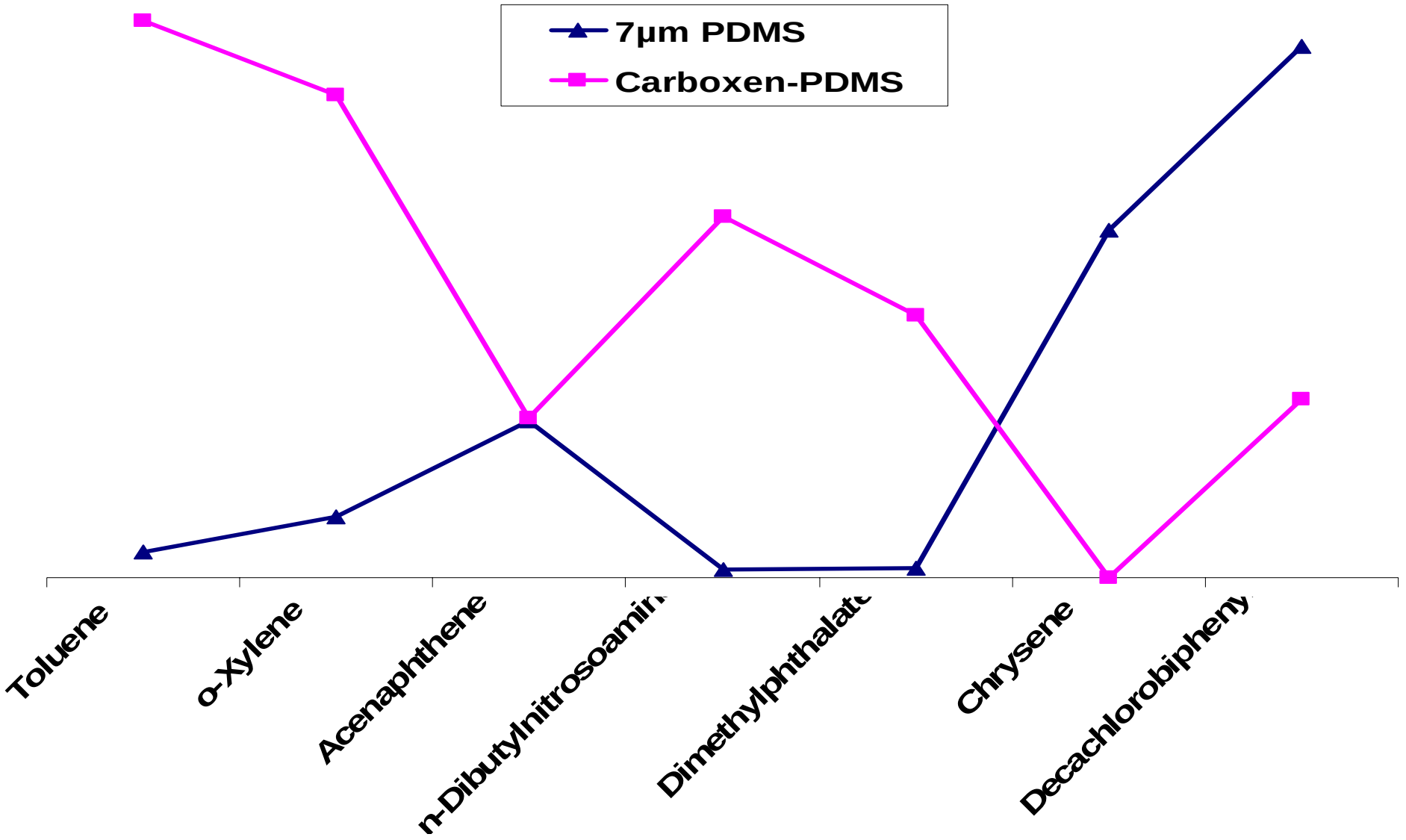


Figure 10 compares the extraction of analytes with the Carboxen-PDMS fiber and the 7µm PDMS fiber. As expected, as the molecular weight of the analyte increases, the response drops when using the Carboxen-PDMS fiber. This is particularly true for PAHs. Either the analytes are not being desorbed off the fiber or are too large to be extracted. It is most likely the former, but it could be a combination.

The responses for the analytes extracted with the 7µm PDMS fiber increase as the molecular weight increases. The more polar analytes are not efficiently extracted by the PDMS fiber.

CONCLUSIONS

- The pH of the extraction solution affects recovery of polar analytes.
- The addition of 25% NaCl improves analyte recovery
- Analytes with a molecular weight of < 90 AMU are most efficiently extracted by the Carboxen-PDMS fiber.
- analyte polarity has little impact on fiber polarity for low molecular weight analytes.
- Analyte polarity and fiber polarity are directly related for analytes with molecular weights over 90AMU.
- Larger analytes >150 AMU and PAHs are poorly extracted by Carboxen- PDMS fibers. Layering DVB over Carboxen expands the range.
- Thin absorbent type fibers are ideal for extracting nonpolar, high molecular weight analytes