Standard Operating Procedures for Analysis of Naphthenic Acids from Oil Sands Process-Affected Water

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REPORT SUMMARY

Naphthenic acids (NAs) are considered the main source of chronic and acute toxicity in oil sands process-affected water (OSPW). The purpose of this investigation is to establish elements of a standard procedure to determine and minimize the amount of NA loss during storage, sample preparation and pretreatment, and radiation emission such as sunlight, microwave and ultraviolet. In addition, efficiency of solid phase extraction (SPE) and industrial resins for NA separation from aqueous phase were studied.

For quantification of NAs, fluorescence spectroscopy was used, which requires minimum sample manipulation. Two different types of NAs, OSPW-associated (O-NAs) in oil sands process-affected water as well as Merichem NAs (M-NAs) dissolved in phosphate buffer (0.05M, pH = 8.5), were tested and compared in this research. The lowest NA loss among the tested filter membranes was observed in the PVDF filter membrane (Durapore 0.45 µm, Millipore), and among the tested filter syringes was observed in the Target GL microfiber (0.7 µm, National Scientific). Pre-wetting PTFE membranes with methanol may increase the NA loss. According to our observations, no significant difference was detected between the PVDF filter membrane (Durapore 0.45 µm, Millipore) and centrifugation in glass tubes (30 min, 3500 ×g) for solid separation (P-value>0.05).

For storage containers, the best performance (smallest NA loss) was observed in lime-soda and borosilicate glass; however, glass silanization may increase the adsorption of M-NAs on the glass surface. Significant M-NA loss was observed in all three plastic bottles (HDPE, LDPE, and PP). Despite that no significant reduction in concentration of O-NAs was observed, long term storage in plastic bottles is not suggested. Three types of centrifuge tubes (polypropylene ultra-high-performance (PP-UHP), polypropylene high-performance (PP-HP), and polystyrene) were tested and high M-NA loss was observed, especially in polystyrene centrifuge tubes. Similar to the plastic bottles, no considerable O-NA loss was detected, but long term storage in centrifuge tubes is not suggested because it is expected that those NA molecules with similar structure to M-NAs are suspected to be adsorbed on the surface of any plastic made containers.

The best storage condition was storage in the fridge (4^{o}C). Addition of methanol (50% v/v), pH increase to 11.1, or pH reduction to 2.1 caused false-positive and false-negative errors in NA concentration measured by fluorescent instrument. Freezing did not influence the NA concentration, however, possible NA loss due to storage in a plastic bottle or centrifuge tube should be taken into consideration. In the cap liner material test, the best performance was seen for PTFE and Tinfoil cap liners. The leakage of contaminants, interfering with NA measurement, from white rubber and polyethylene cap liners was seen. For long term storage of water samples, the PTFE cap liner is suggested.

Small reduction in M-NA concentration was observed after UV exposure, but the microwave did not influence either M-NAs or O-NAs. In rotavapor experiment with O-NAs, it was found that O-NA loss increases at low pH (2); however for M-NAs, no considerable difference in NA loss was seen at high (9) or low pH (2). The highest NA loss was observed in DCM solvent.

The C18 SPE laboratory cartridge and L493 industrial resins displayed the best performance (in terms of NA adsorption from aqueous solution and subsequent NA release into the eluent solution) among the tested SPE cartridges and industrial resins.

The results from this investigation elucidated the unknown aspects of sampling, storage conditions, and processing of NA containing water samples. Still, more investigations are required to optimize the performance of SPE laboratory cartridges and industrial resins. For future research, the amount of recovered NAs from industrial resins can be optimized by using various eluent solutions. The type of eluent solution is crucially important for further treatment of NAs on an industrial scale.

In addition, in this research only one type of OSPW sample was tested. It is hypothesized that if the OSPW sample is fresh or derived from a refinery, there is a chance that such a sample may behave in a similar fashion to M-NAs. Long-term storage and consecutive use of storage containers may also result in serious losses.

Measurement of NA using fluorescence instrument requires a minimum sample preparation and manipulation which reduce the error from NA loss. However, this method comes with some inherent issues in terms of accuracy. For future research we suggest that a high resolution instrument is used for quantification, fingerprinting and characterizing of NA molecules.

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1 INTRODUCTION

Despite a lack of pipeline availability, total Canadian oil production has risen 48% since 2005 (from 2.49 Mbbl/day to 3.68 Mbbl/day projected in 2014). The Canadian Association of Petroleum Producers (CAPP) predicts that this value will increase by 4% to 5% annually over the next 15 years (Canadian Association of Petroleum Producers 2014). In 2012, $28 billion in taxes and royalties were collected from the industry, and $15 billion went to the federal government (representing 6.1% of total revenues). Considering that this booming industry contributes to jobs in other industries including (but not exclusive to) construction, engineering, geology, finance, manufacturing, environmental analysis, and hospitality, its survival is imperative for the sake of Canada's economic prosperity (IHS 2014). That said, with the increasing public attention given to environmental protection, the challenges that come with extracting bitumen from the oil sands need to be addressed properly to keep Canada's environmental reputation in high regard in the world.

Despite the Government of Alberta releasing their proposal for a *World Class Monitoring, Evaluation, and Reporting System for Alberta* in June, 2011 (Alberta Environmental Monitoring Working Group 2011), there has yet to exist a clear standard procedure for storage, handling, and processing of oil sands process-affected water (OSPW) containing naphthenic acids (NAs). These acids, which are toxic to wildlife and corrosive to extraction equipment (Corinne 2010), are dissolved in caustic water during the bitumen extraction process.

Mined oil sands (which typically contain 10% to 12% bitumen by mass) are processed using hot water extraction in combination with hydrotransport pipelines (Papavinasem et al. 2012), and approximately 10 to15 bbl of water is required to process every bbl of bitumen (Romanova et al. 2006). Though 70% to 90% of the water is recycled, about 2.2 to 5 bbl of water is lost per barrel of bitumen (mostly to the tailings – which can be recovered over time). Most importantly, the NA concentration in OSPW cumulatively increases each time water is recycled.

Classical naphthenic acids are "water soluble weak acids, with the general formula $C_nH_{2n+z}O_2$"; where "n" represents the number of carbons in the acids, and "z" represents the hydrogen deficiency (a negative even number that increases as the number of rings in the acid structure increases). Non-classical NAs allow for more than two oxygen atoms (Dalmia 2013). While NAs contain mostly carbon and hydrogen (with oxygen atoms in the -COOH groups), they can also contain a wide range of heteroatomic species that can feature S, N, and O aromatic components (Birks et al. 2013, Gibson et al. 2011, Zhao et al. 2012). They "occur naturally in crude oils and bitumen and are the primary toxicants in wastewaters associated with oil refineries and oil sands extraction" (Dalmia 2013). The concentration of NAs can range from 20 mg/L (Dalmia 2013) to 130 mg/L in fresh oil sands process-affected water (OSPW) (Allen 2008), while the concentration in the North Saskatchewan and Athabasca Rivers and nearby lakes is usually below 1 mg/L (Scott et al. 2008).

It is vital that naphthenic acid concentrations in the tailings ponds be continuously monitored and accurately measured. Naphthenic acids can be lost via a variety of routes including adsorption to storage materials. The scope of this project includes four important factors which influence NAs loss before characterization: (1) container materials for NA storage, (2) storage conditions, (3) pretreatment, and (4) resins used for SPE extraction. To understand the differences in behavior between oil sands NAs and commercial NAs, a stock solution with comparable NA concentration was prepared using a commercial standard (Merichem: Houston, Texas, USA) and used as a reference for comparison.

A variety of different storage materials were tested in this study including: polypropylene and polyethylene (low/high density) bottles, polypropylene (high/ultra-high performance) and polystyrene centrifuge tubes, and finally borosilicate glass vials and amber/clear soda glass bottles. Naphthenic acid samples (from OSPW and commercial sources) were stored in containers with different materials, and the supernatant solutions were scanned and compared with standards. The NAs adsorbed on the walls of the containers were determined and performance of each container was evaluated and compared.

Naphthenic acids loss can also occur due to storage conditions, i.e., temperature, UV exposure, microwave exposure, etc. Each storage material underwent different conditions to determine the loss. Sample pretreatment is another cause of naphthenic acid loss. Filtration is usually needed to remove the suspended solids from OSPW before the naphthenic acids can be extracted. However, the filters have the ability to adsorb naphthenic acids (not to mention the suspended solids). Different types of filter membrane and syringe filter were evaluated and NA adsorption was studied.

Though laboratory procedures exist for extracting NAs from small quantities of OSPW with efficiencies varying from 40% to 80%, these liquid phase extractions (LPE) are costly and involve using vast amounts of toxic organic solvents such as dichloromethane (DCM) (Ross et al. 2012). The Solid-Phase Extraction (SPE) proposed in this study requires lower volumes of solvent; also the solvent used in SPE, methanol, has considerably lower toxicity than DCM, commonly used in LPE. The SPE method has been used previously for NA isolation (Pereira et al. 2014). Although naphthenic acids can be characterized with a high degree of accuracy using HPLC-MS (Dalmia 2013), the instrument is costly and training for an experienced analyst is time-consuming.

We also tested the viability of industrial resins to extract NAs from OSPW. Three industrial resins (OPTIPORE, AMBERLITE, and TAN-1 from DOW Chemical) and PES powder were examined. For each resin, both its ability to extract NAs from OSPW and the ease to release the NAs in eluent solution were studied.

This study employed fluorescence spectroscopy, a cost-effective analytical method, to quickly identify/quantify NAs in OSPW samples (Martin et al. 2014). Authors of this article are aware of inherent inaccuracy of fluorescence spectroscopy for NA quantification. In fact, this method targets fluorescent compounds (such as aromatic rings) to be NA markers. Despite these issues, fluorescence spectroscopy requires a small sample preparation and manipulation, which results in a minimize NA loss during measurement.

2 MATERIAL, REAGENTS, AND MEASUREMENT

All glassware was trace cleaned with detergent (Sparkleen1, Fisher brand), rinsed with tap water, pure methanol (Fisher Brand), and Milli-Q water (three times each rinse). All chemicals and solvents were certified by the A.C.S.

2.1 Apparatus

Apparatus used in this project are listed in Table 1.

Table 1. List of material and apparatus used for each experiment.

Apparatus	Manufacturer
General	
Safetypette™ pipetter (with filter)	Jencons (Lutterworth, Leicestershire)
Reusable borosilicate glass pipets (1, 2, 4, 5, 10, 50, 100 mL)	Fisher Scientific and Kimble Chase
Potassium phosphate dibasic anhydrous, A.C.S. Grade	AMRESCO® (Solon, OH)
Methanol Certified ACS (20L)	Fisher Scientific (Fair Lawn, NJ)
Quantitation of NAs in All Experiments	
Varian fluorescence spectrophotometer model Cary Eclipse (Scan Software Version: 1.1(132))	Agilent Technologies (Mississauga, ON)
Quarts cuvette (10 mm ×10 mm) with a PTFE stopper	VWR® (Chine)
Filter Membrane Adsorption Test	
Amber, Wide Mouth Type III soda-lime glass bottle with black phenolic caps and PTFE/LDPE liner (125 mL)	VWR® (USA)
PYREX® Filtering Funnels, 60° Angle (used for rinsing the filters)	Corning
KIMAX® Educational Grade Graduated Cylinder, Plastic Base (10 mL)	Kimble Chase
ME 25 ST (Membra-Fil® membrane), made of mixed esters of cellulose nitrate and cellulose acetate (47 mm, 0.45 µm)	Whatman™ (Germany)
Nylon Membrane, Type WNYL (47 mm, 0.45 µm)	Whatman™ (UK)
Cellulose Acetate Membrane Filters (47 mm, 0.45 µm)	Whatman™ (Germany)
Polycarbonate Membrane Filter	Whatman™

Apparatus	Manufacturer
Durapore Polyvinylidene Difluoride (PVDF) Membrane, hydrophilic (47 mm, 0.45 µm)	EMD Millipore (Ireland)
Glass Fiber Filters without Binders (47 mm, 0.7 µm)	EMD Millipore
Zefluor™ PTFE membrane (47 mm, 0.5 µm)	Pall Life Scientific
Syringe Filter Adsorption Test	
PTFE syringe filter (30 mm, 0.2 µm)	Thermo Scientific (Germany)
PTFE syringe filter with polypropylene housing (30mm, 0.45 µm)	Whatman™ (UK)
Target GL microfiber (30 mm, 0.7 µm)	National Scientific Company
Acrodisc® GHP (hydrophilic polypropylene) syringe filter (25 mm, 0.45 µm)	Pall Life Scientific
Glass syringe with luer slip tip (10 mL)	Cadence Science™
Comparison of Centrifuge with Filtration	
Durapore PVDF Membrane, hydrophilic (47 mm, 0.45 µm)	EMD Millipore (Irland)
Graduated PYREX® borosilicate glass centrifuge tube (100 mL) for petroleum experiments	Corning
47 mm glass filter holders 300ml graduated glass funnel, anodized aluminum clamp, and a no. 8 silicone stopper) and fritted glass support (40 mm to 60 µm)	VWR®
Graduated KIMAX® filtering flasks (1,000 mL)	Kimble Chase
Avanti J-26XP Centrifuge	Beckman Coulter
Storage Bottle Condition Test	
Clear borosilicate screw thread vials with pp hole cap & PTFE/silicone septa (60 mL)	Qorpak (USA)
Clear Boston round bottles (type III soda-lime glass) with PTFE faced foamed polyethylene lined white polypropylene cap (60 mL)	Wheaton (USA)
Amber Boston round bottles (type III soda-lime glass) with PTFE faced foamed polyethylene-lined white polypropylene cap (60 mL)	Wheaton (USA)

Apparatus	**Manufacturer**
Laboratory bottles, polypropylene (PP), wide mouth (60 mL)	VWR®
Laboratory bottles, high-density polyethylene (HDPE), wide mouth (60 mL)	VWR®
Laboratory bottles, low-density polyethylene (LDPE), wide mouth (60 mL)	VWR®
Polypropylene high-performance centrifuge tubes (PP-HP) with flat caps, sterile (15 mL)	VWR® (USA)
Polypropylene ultra-high performance centrifuge tubes (PP-UHP) light sensitive, sterile (15 mL)	VWR®(USA)
Falcon® centrifuge tubes, polystyrene, sterile	Corning (Mexico)
TraceClean® soda-lime glass Boston Round Bottles, Glass (250mL). Cleaned according to EPA procedures listed in the OSWER Directive 9240.0-05A “Specifications and Guidance for Contaminant-Free Sample Containers”.	VWR®
Cap Liner Test	
Amber, Wide Mouth Type III soda-lime glass bottle (125 mL)	VWR®(USA)
Phenolic cap with PTFE liner (cap size: 38 to 400)	VWR®(USA)
Phenolic cap with polyvinyl liner (cap size: 38 to 400)	VWR®(USA)
Phenolic cap with solid polyethylene (PE) liner (cap size: 38 to 400)	VWR®(USA)
Phenolic cap with tinfoil liner (cap size: 38 to 400)	VWR®(USA)
Phenolic cap with 14B white rubber liner (cap size: 38 to 400)	VWR®(USA)
Microwave Test	
Panasonic inverter microwave (1200W), frequency: 2,450 MHz	Panasonic
Amber, Wide Mouth Type III soda-lime glass bottle with black phenolic caps and PTFE/LDPE liner (125 mL)	VWR® (USA)
Rotavapor Test	
IKA RV 10 basic rotavapor	IKA Co. (Germany)

Apparatus	Manufacturer
Lab companion refrigeration circulator (model RW-0525G, 5 L)	Jeio Tech Co. (Korea)
UV light Test	
Entela Compact and Handheld Ultraviolet Lamps (UVGL-58), 254nm UV, 6 Watt, 115V ~ 60Hz, 0.12 Amps	UVP, LLC (Upland, CA)
Chromato-Vue® Mini Viewing Cabinets, Models C-10, UVP®	UVP, LLC (USA)
Freezing Test	
Whirlpool Freezer, Temperature -18°C, MOD: W8RXEGFWS00	Whirlpool Corporation
Polypropylene high-performance (PP-HP) centrifuge tubes with flat caps, sterile	VWR®(USA)
KIMAX® Educational Grade Graduated Cylinder, Plastic Base (10 mL)	Kimble Chase
Solid Phase Extraction (SPE) Cartridge	
Oasis® HLB 6 mL (500 mg) LP extraction cartridge	Waters Corporation (Ireland)
Oasis® MAX 6 mL (500 mg) LP extraction cartridge	Waters Corporation (Ireland)
ISOLUTE® C18 6 mL (500 mg) extraction cartridge	Biotage
ISOLUTE® SAX 6 mL (500 mg) extraction cartridge	Biotage
ISOLUTE® ENV+ 6 mL (500 mg) extraction cartridge	Biotage
Supelclean™ LC-Diol 3 mL (500 mg) extraction cartridge	Supelco Analytical (Bellefonte, PA)
Extraction Manifold, 20-position, 13 x 100 mm tubes	Waters Corporation (Milford, MA)
Industrial Resins and Adsorbent	
DOWEX TAN-1, a strong base anion exchange resin for the removal of large natural organic compounds such as tannins	Dow Chemical Co. (Midland, MI)
AMBERLITE™ PWA9, a strong base anion exchange resin for the removal of Natural Organic Compounds (NOM)	Rohm and Hass France S.A.S (a subsidiary of the Dow Chemical Co.) (Chauny, France)

Apparatus	Manufacturer
DOWEX OPTIPORE L493, Polymeric Adsorbent for the removal of aromatic and relatively non-polar organic compounds especially BTEX	Dow Chemical Co. (Midland, MI)
Veradel 3000P, polyethersulfone (PES) powders	Solvay Plastics

2.2 NA Measurement and Fluorescence Spectrophotometer Settings

To measure NAs, fluorescence spectroscopy was employed since it provides quick and easy quantification with minimal sample manipulation. For quantification of NAs, OSPW and pure methanol were mixed 1:1 by volume and scanned by the fluorescence spectrometer in synchronous mode at the following settings: start and stop Wavelength (nm): 250 and 400 respectively, delta wavelength: 18 nm, emission and excitation slit: 5 nm; scan control: medium, and excitation-emission filters were in automatic. The fluorescence intensity (a.u.) at 280 nm was used for quantification of NAs.

2.3 Master Stock Solutions

2.3.1 *OSPW NAs (O-NAs) Stock Solution*

5 L of OSPW were filtered (Durapore 0.45 µm PVDF filter membrane, Millipore) using a vacuum Erlenmeyer flask and filter holder. The pH was raised to >10 by adding a concentrated NaOH solution, followed by a 3x extraction using 50 mL of dichloromethane (DCM HPLC grade, BDH, Radnor, PA) per litre of sample in consecutive steps using a 2-L separatory glass funnel to remove the non-polar organic fraction from OSPW. Afterwards, the pH was reduced to pH<2 using a concentrated HCl solution. Another 3 x 50 mL DCM extraction per litre of sample was performed to extract the NAs this time. The weight of extracted NAs was measured in a pre-weighed glass bottle after overnight evaporation of DCM was accomplished under the fume hood. The stock solution of 2,500 mg/L of NAs in methanol was prepared and stored in a 125 mL amber soda-lime glass bottle with PTFE cap liner at 4^{o}C.

2.3.2 *Merichem NAs (M-NAs) Stock Solution*

0.1 g M-NAs was mixed with methanol to the final volume of 10 mL and stored at 4^{o}C.

2.4 Standard Curve Preparation

A 50% phosphate buffer-methanol solution (0.05M phosphate buffer in DI Water, pH = 8.5) was used to make standard solutions in low concentrations (0, 0.2, 0.5, 1, 2.5, and 5 mg L^{-1}) and high concentrations (0, 5, 12.5, 25, and 50 mg L^{-1}). A low or high concentration standard curve was used for quantification of NAs.

2.5 Water Sample Preparation

2.5.1 *OSPW Sample*

Unless stated otherwise, filtered OSPW samples (Durapore 0.45 µm PVDF filter membrane, Millipore) were used for the experiments. The OSPW sample, used in this research, was collected from the surface of an oil sands tailings pond with total suspended solids (TSS) of 40.53 ± 3.56 mg L^{-1} and pH of 8.6.

2.5.2 *Merichem-Phosphate Buffer Sample*

To prepare the Merichem-phosphate buffer solution, 4 mL of M-NA stock solution was added to the phosphate buffer solution (0.05M potassium phosphate dibasic, pH=8.5) to a final volume of 1 L (final NA concentration 40 mg NA L^{-1}).

3 METHODS FOR EACH EXPERIMENT

3.1 Filter Membrane Adsorption Test

Seven filter membranes (along with negative control – no filter added) were tested in triplicate for NA adsorption. 50 mL of filtered OSPW was added to 3 × 8 wide mouth glass bottles (125 mL). Filter membranes from each membrane type: Polycarbonate, Nylon, Cellulose Acetate, PVDF, ME 25 ST, GL FBR, and PTFE, were submerged into each bottle containing OSPW for 5 hours (see Figure A6).

O-NA content in OSPW (as described in section 2.2) and NAs adsorbed on the surface of the filter membrane were measured. To measure O-NAs on the surface of the filter membrane, each filter was transferred to the glass funnel by forceps and rinsed with 5 mL of 50% phosphate-methanol solution, which was collected in a 5 mL volumetric flask (see Figure A7) and scanned for NA content.

In this experiment, all filter membranes were used as they were received from the manufacturer, and without any pre-washing or pretreatment. Three bottles containing OSPW were assigned as the negative control (with no filter added). The same experiment was conducted for the Merichem-phosphate buffer solution.

3.2 Syringe Filter Adsorption Test

14 mL of filtered OSPW (0.45µm, Durapore PVDF Membrane) was filtered through each of the four types of syringe filters (Acrodisc® GHP, Target GL microfiber, PTFE syringe filter with polypropylene housing, and PTFE syringe filter). A glass syringe was used and experiments were triplicated for each filter syringe (see Figure A9). The PTFE syringe filter was soaked by passing 2 mL methanol through the filter before the experiment according to the manufacturer's instructions. The OSPW samples before and after filtration were scanned for NA concentration. Then, 50% phosphate-methanol solution was passed through the syringe filter (to a final volume of 5 mL) to quantify the NAs adsorbed inside the filter. The losses to the glass syringe were neglected.

The same experiment was conducted for the Merichem-phosphate buffer solution.

3.3 Influence of Suspended Solids

50 mL of the OSPW sample containing suspended solids was added to each of six glass centrifuge tubes (100 mL, Pyrex) and centrifuged at 3,500×g for 5 min. NA concentration in the supernatant from three centrifuge tubes was measured (see section 2.2) and the supernatant of the three remaining centrifuge tubes was filtered (0.45 µm, Durapore PVDF Membrane, Millipore), and the filtrate was scanned for NA content. Suspended solids on the surface of the filters were rinsed by 5 mL of 50% phosphate buffer-methanol solution and the solution was scanned for NA content. The same procedure was repeated at 15 and 30 min centrifugation times.

To repeat the experiment in 0 min centrifugation time, 3×50mL of OSPW was filtered (without centrifugation) and the NA content of the filtrate and suspended solids on the surface of the filter was determined.

For total suspended solids (TSS) measurement, three PVDF membranes (0.45 µm, Durapore, Millipore) were left in the oven at 75°C for 4 hours, cooled down in a desiccator (10 min), and weighed. 3×100 mL of OSPW were filtered and membranes containing suspended solids were dried in the oven and weighed as before. Subtraction of the weight of the membranes before and after OSPW filtration indicated the total suspended solids.

3.4 Containers for Storage

All containers, except centrifuge tubes, were trace cleaned (see section 2). 50 mL of filtered OSPW was added to each 60 mL bottle. 10 mL of filtered OSPW was added to each 15 mL centrifuge tube. The cap liner materials for all glass bottles were PTFE, while for the plastic bottles, the caps were made of the same materials as the bottles.

All containers were kept in the fridge for 8 days, and the concentration of O-NAs in OSPW was then measured using fluorescence. To measure the adsorbed O-NAs, the OSPW was discarded and the internal surface of each container was rinsed three times with 50% phosphate-methanol solution to a final volume of 5 mL, and then scanned for NA content. The blank (negative control) in this experiment was a filtered OSPW sample stored in a 250 mL soda-lime glass bottle with PTFE liner cap with no headspace (stored in fridge for 8 days).

The same experiment was conducted for the Merichem-phosphate buffer solution.

3.4.1 *Silanization Protocol*

Pre-cleaned glass bottles were heated at 80°C for two hours in an oven and cooled down in a desiccator. Each bottle was filled to the top with silanization[1] solution (5% dichloromethylsilane in toluene) and left under the fume hood overnight with closed cap. Then, the glass bottles were washed with pure toluene, followed by rinsing three times with methanol (Molnar et al. 2012). Each bottle was filled to the top with pure methanol and left under the fume hood for 30 min.

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[1] See http://en.wikipedia.org/wiki/Silanization

All bottles were heated again in the oven at 80^{o}C for two hours, followed by cooling in the desiccator.

3.5 Storage Conditions

Trace cleaned borosilicate glass bottles[2] with PTFE liner caps (60 mL) were used to evaluate the impact of temperature, pH and addition of methanol on NA losses during 8 days storage of OSPW. Each storage condition experiment was conducted in triplicate. 50 mL of filtered OSPW was added to each glass bottle.

Three bottles were stored in the fridge (≈4 ^{o}C), and three were left in a glass window sill receiving natural light at room temperature (≈22 ^{o}C). To evaluate the effect of pH, two different pH solutions (as low as 2.1 and as high as 11.1) of OSPW were prepared by adding concentrated HCl and NaOH solutions and stored for 8 days. To study the impact of methanol addition to OSPW (to potentially reduce NA losses), a 50% v/v methanol-OSPW solution was prepared and 5 mL was added to each glass bottle.

The blank (negative control) in this experiment was a filtered OSPW sample stored in a 250 mL soda-lime glass bottle with PTFE liner cap with no headspace (stored in fridge for 8 days). For NA measurement, 10 mL of OSPW was mixed with 10 mL of methanol and scanned (see section 2.2). In the "effect of pH" experiment, the pH of the OSPW samples was adjusted to ≈8.6 (the original pH of OSPW) before O-NAs measurement (see section 2.2) and compared to the blank sample.

The same experiment was conducted for the Merichem-phosphate buffer solution.

3.6 Influence of Bottle Cap Liners

Four different types of cap liners (cap size 38 to 400): PTFE, Polyvinyl, Polyethylene, and White Rubber were tested for their influence on OSPW storage. Twelve glass bottles (125 mL, type III soda-lime glass) were trace washed (see section 2) and filled with 50 mL filtered OSPW. Three caps for each liner were used to fasten the bottles. Bottles were stored in a fridge at ≈4^{o}C upside-down for 7 days to expose the cap liner to OSPW.

After 7 days of exposure, the OSPW was scanned by fluorescence to determine the NA content. To measure the adsorbed NAs, the inner surface of each bottle and its cap liner were rinsed three times with the phosphate-methanol solution to a final volume of 5 mL. NA concentration in the 50% phosphate-methanol solution was measured using the fluorescence spectrophotometer.

The same experiment was conducted for the Merichem-phosphate buffer solution.

3.7 Influence of Microwaves

50 mL of filtered OSPW was added to each of six wide mouth type III soda-lime glass bottles with black phenolic caps and PTFE/LDPE liners (125 mL). Three bottles with loose caps were

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[2] See http://en.wikipedia.org/wiki/Borosilicate_glass

microwaved until the formation of the first bubbles, then cooled down and stored in the fridge. Three remaining bottle samples were used as blanks. The NA concentration was determined by fluorescence instrument.

The same experiment was conducted for the Merichem-phosphate buffer solution.

3.8 Influence of UV Light

50 mL of filtered OSPW was added to each of six borosilicate glass beakers (50 mL) and three samples were exposed to UV light (254 nm) for 3 hours. UV exposure was conducted in UV Mini Viewing Cabinets with lamp distance of 20 cm to the uncovered beakers containing OSPW samples. Three UV-exposed samples and blanks were scanned for NA content.

The same experiment was conducted for Merichem-phosphate buffer solution.

3.9 Influence of Rotavapor in Different Solvents

Unless stated otherwise, rotavapor[3] conditions were 37 ^{o}C under vacuum (17 to 25 in. Hg).

3.9.1 *Experiments with O-NAs from OSPW*

By adding OSPW NAs stock solution (see section 2.3.1), 40 mg NAs L^{-1} solutions were prepared in dichloromethane (DCM), methanol, and phosphate buffer (0.05M potassium phosphate dibasic, pH = 8.5).

1. 50 mL of DCM solution (containing 40 mg NAs L^{-1}) was added into a 250 mL rotavapor flask and the test was conducted until all DCM evaporated (7 min). The remaining O-NAs were quantitatively transferred using pure methanol into a 50 mL volumetric flask and scanned for NA content.
2. 2 × 50 mL of methanol solution (containing 40 mg NAs L^{-1}) was added into 2 × 250 mL rotavapor flasks and the test was conducted for 7 and 30 min. The solution from each flask was quantitatively transferred to a 50 mL volumetric flask using methanol and scanned for NA content.
3. 2 × 50 mL of phosphate buffer solution (containing 40 mg NAs L^{-1}) – the first with pH of 2 (by adding concentrated HCl) and the second with pH 8.5 – was added into 2 × 250 mL rotavapor flasks and the test was conducted for 30 min. The solution from each flask was quantitatively transferred to a 50 mL volumetric flask using pure phosphate buffer (0.05M, pH = 8.5), and the final pH was adjusted to 8.5 (by adding NaOH), and the samples were scanned for NA content.
4. 50 mL of filtered OSPW was used for the rotavapor test for 30 min, and similar to section (3), pure phosphate buffer was used to adjust the final pH and volume to 8.5 and 50 mL, respectively.

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[3] See http://en.wikipedia.org/wiki/Rotary_evaporator

NA concentrations in blank solutions (without rotavapor) were measured and compared with solutions after rotavapor. To generate the comparable concentrations, the final volume of all samples (after rotavapor) was adjusted to 50 mL.

3.9.2 *Experiments with M-NAs*

40 mg NA L^{-1} solutions were prepared by adding M-NA Stock Solution into dichloromethane (DCM), methanol, and phosphate buffer (0.05M potassium phosphate dibasic, pH = 8.5). The same experiments as explained previously for OSPW NAs (1, 2, and 3) were conducted for solutions containing M-NAs, and the results from blanks and samples after rotavapor were compared.

3.10 Freezing Test

10 mL of filtered OSPW was added to each of 10 polypropylene centrifuge tubes, followed by freezing in a laboratory freezer at -18°C. With the starting of ice formation, centrifuge tubes were taken out of the freezer one by one at different freezing times and the liquid (non-frozen) fraction was determined. To determine the liquid fraction, the liquid was transferred to a 10 mL graduated cylinder and its volume was measured. The ice fraction was discarded. NA concentration in the liquid fraction was measured using the fluorescence spectrophotometer.

The same experiment was conducted for the Merichem-phosphate buffer solution.

3.11 Solid Phase Extraction (SPE) Cartridge

All SPE tests were triplicated along with duplicated blanks. The blanks experienced all procedures similar to other SPE cartridges, except the adding of OSPW. An extraction Manifold was used for the SPE experiment (see Figure A10). A protocol from the manufacturer was followed for the preparation of cartridges as follows: SPE cartridges were pretreated with 5 mL of methanol, filtered through methanol (by gravity and without vacuum), and scanned to evaluate the presence of any contaminants. In addition, HLB and MAX cartridges were rinsed with 5mL DI water. The pH of filtered OSPW (8.5) was adjusted to pH≈2.5 for HLB, ENV+, and C18, and to pH≈9.5 for LC-Diol, SAX, and MAX. 4 mL of OSPW sample was applied to each SPE cartridge at a flow rate of 1 mL per minute. The NA concentration in OSPW before and after SPE was measured using the fluorescence spectrophotometer.

For HLB only, 2 mL of DI water was applied after OSPW (as a wash). 9 mL of eluent solution was applied for each SPE cartridge at a flow rate of 1 mL per minute. The final volume of collected eluent was brought to 10 mL and scanned for NA content. The eluent solution for all cartridges was 5% acetic acid in methanol, except for HLB when pure methanol was used.

The same experiment was conducted for the Merichem-phosphate buffer solution.

3.12 Industrial Resins and Adsorbents

Three industrial resins from Dow Chemical Co. (TAN-1, PWA9, and L493) and polyethersulfone (PES) powder from Solvay Plastics were assessed to determine NA adsorption and desorption capability. Previous studies reported that the PES membrane has a high capacity for adsorption of NAs (Martin 2014). The SPE cartridge body from the previous experiment (see section 3.11) was used to conduct the industrial resins evaluation test. To do so, SPE cartridges were disassembled, sorbents were discarded, and cartridge bodies and porous glass stoppers were trace cleaned according to the procedure previously explained. ≈0.5 g of each resin was dried overnight at 60 ^{o}C, added into each clean cartridge, and then assembled for the experiment. Each resin was triplicated along with duplicated blanks (negative control). The blank was a cartridge containing resins, which experienced procedures similar to other cartridges, except the adding of OSPW. All resins were pretreated by applying 5 mL methanol. Then, two resins of TAN-1 and PWA9 were rinsed using 10% NaCl and 0.5% NaOH in DI water solution. All resins were washed using 3 × 5 mL DI water. 4 mL of filtered OSPW (0.45µm, Durapore PVDF Membrane) was applied to each resin at a flow rate of 1 mL per minute. Then, 9 mL of eluent solution was added at the same flow rate, and the final volume of collected eluent was brought to 10 mL and scanned for NA content. 10% NaCl and 0.5% NaOH in DI water solution was used as eluent for TAN-1 and PWA9, and methanol was the eluent for L493 and PES as recommended by the manufacturer.

The same experiment was conducted for the Merichem-phosphate buffer solution.

3.13 Statistical Analysis

To determine the significance of difference in NA concentrations for more than two samples, one way analysis of variance (ANOVA) was used, and for two samples the simple student T-test was performed ($P\text{-value} < 0.05$) using MS Excel. To determine the P-values among the different treatments, Tukey HSD (Honestly Significant Difference) analysis was conducted by SPSS Software (Version 22). The error of the mean is indicated using plus and minus one standard deviation in graphs and text. For calculation of percentage adsorbed NA, the mass of adsorbed NAs (mg) was divided by total NAs in stock solution (mg NAs), and was multiplied by 100. For better comparison, adsorbed NA percentages for all tests are given in Appendix 2.

4 RESULTS AND DISCUSSION

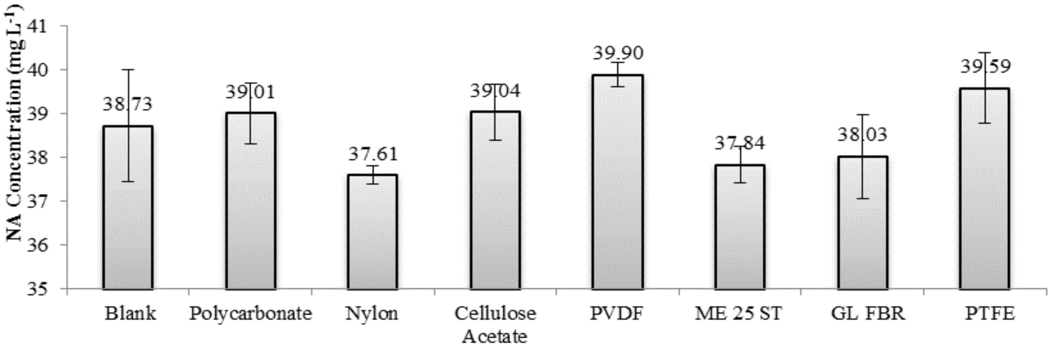
4.1 Filter Membrane Adsorption Test

4.1.1 *O-NAs in OSPW*

Figure 1(a) illustrates the concentration of O-NAs in OSPW after conducting the stationary adsorption test on filter membranes. The greatest reduction in O-NA concentration was observed in the Nylon membrane, followed by ME 25 ST and GL FBR membranes. The best performance (lowest NA loss) was observed for PTFE and PVDF, followed by Cellulose Acetate and Polycarbonate membranes. However, Tukey HSD P-values indicated no significant difference in O-NA concentration between the blank (original OSPW with no exposure to membrane) and OSPW samples after exposure to various membranes.

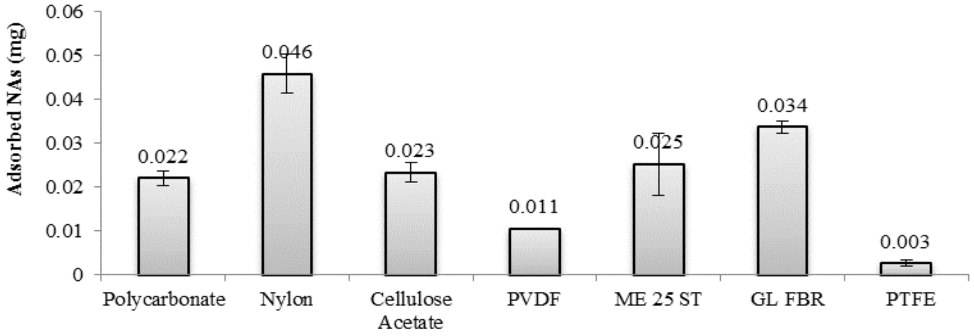
Based on Figure 1(b), the NA adsorption test demonstrated small O-NA adsorbed on the PTFE, PVDF, Cellulose Acetate and Polycarbonate membranes, which corresponds to O-NA concentrations (see Figure 1(a)). The highest O-NA adsorption was 2.37%, which was observed in Nylon membrane.

According to our observations, the reduction of O-NA concentration due to adsorption on the surface of membrane is statistically negligible; however, to minimize the possible O-NA loss, we suggest using PTFE or PVDF. When using PTFE membranes, it must be considered that a small amount of methanol must be applied to the surface of membrane before OSPW filtration. To see the impact of applied methanol (on the surface of PTFE membrane) on NA loss, please refer to the syringe filtration section.



Membrane	Polycarbonate	Nylon	Cellulose Acetate	PVDF	ME 25 ST	GL FBR	PTFE
P-value	1.000	0.599	0.999	0.551	0.817	0.933	0.836

(a)



Membrane	Polycarbonate	Nylon	Cellulose Acetate	PVDF	ME 25 ST	GL FBR	PTFE
Adsorbed O-NAs (%)	1.14	2.37	1.21	0.55	1.31	1.75	0.14

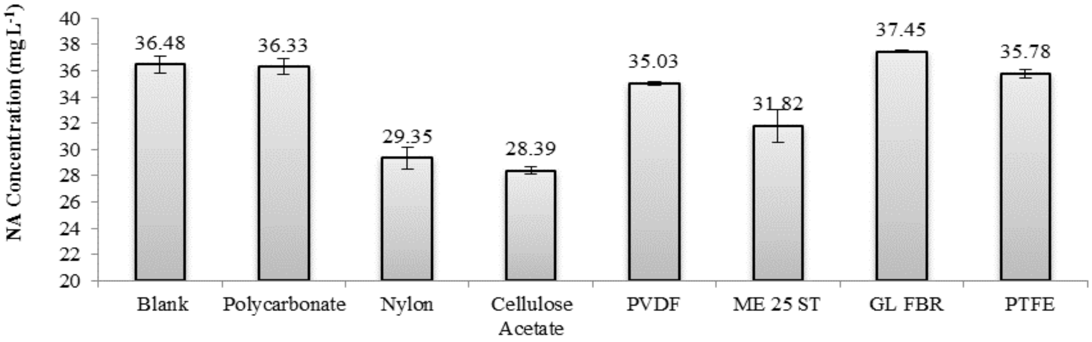
(b)

Figure 1. Partitioning of O-NAs on the surface of filter membranes and OSPW.
(a) O-NAs concentration in OSPW sample after membrane adsorption test. P-values (Tukey HSD, multi comparison method) for each membrane are shown in the table (α=0.05).
(b) Percentage and mg O-NAs adsorbed on the surface of filter membranes.

4.1.2 *M-NAs in Phosphate Buffer*

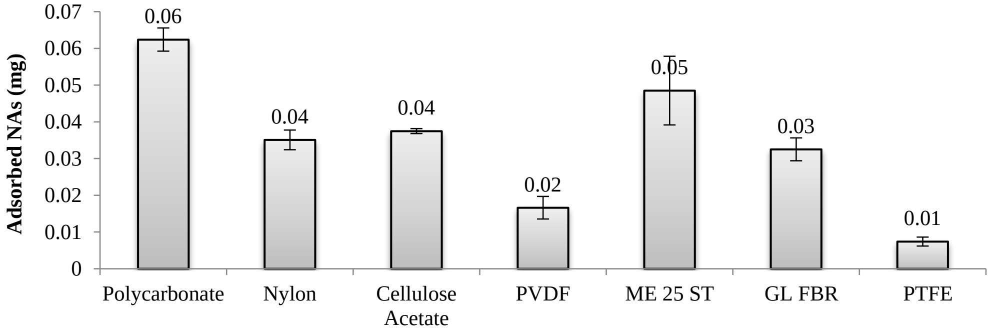
Figure 2(a) illustrates the concentration of M-NAs in phosphate buffer after the membrane adsorption test. The greatest NA concentration reduction was observed in Nylon, Cellulose Acetate, and ME 25 ST membranes.

Figure 2(b) verifies the adsorption of M-NAs on the surface of Nylon, Cellulose Acetate, and ME 25 ST membranes. However, the high adsorbed M-NAs on the polycarbonate membrane indicate the difference in adsorption behavior between M-NAs and O-NAs. Since the concentration of M-NAs in phosphate buffer did not change considerably, the accuracy of the fluorescence instrument might be a limiting factor for the contradictory result for the polycarbonate membrane.



Membrane	Polycarbonate	Nylon	Cellulose Acetate	PVDF	ME 25 ST	GL FBR	PTFE
P-value	1.000	0.000	0.000	0.172	0.000	0.589	0.872

(a)



Membrane	Polycarbonate	Nylon	Cellulose Acetate	PVDF	ME 25 ST	GL FBR	PTFE
Adsorbed M-NAs (%)	3.42	1.92	2.05	0.91	2.66	1.78	0.41

(b)

Figure 2. Partitioning of M-NAs on the surface of filter membranes and in phosphate buffer. (a) M-NA concentration in phosphate buffer samples after membrane adsorption test. P-values (Tukey HSD, multi comparison method) for each membrane are shown in the table (α=0.05).
(b) percentage and mg M-NAs adsorbed on the surface of filter membranes.

The thickness of the GL FBR membrane may contribute to the high NA content observed in Figure 2(b). The Tukey HSD comparison method indicated a significant reduction in the M-NA concentration for Nylon, Cellulose Acetate, and ME 25 ST membranes. This significant reduction in M-NA concentration indicates that M-NAs have a higher adsorption capability than O-NAs from OSPW. Generally, the best performance (lower NA loss) was observed for PTFE and PVDF membranes.

4.2 Syringe Filter Adsorption Test

A syringe filter comprises a membrane and a cartridge, which holds the membrane and is attached to a syringe from one side (see Figure A8).

The cartridge is made of various plastic materials. The NA adsorption data reported in this study cover the adsorption to the membrane as well as cartridge. According to the manufacturer's instructions, a small amount of methanol was applied to the PTFE syringe filter before usage.

4.2.1 *O-NAs in OSPW*

Figure 3(a) and P-values indicate that O-NA loss in syringe filtration is more than with a filter membrane (see section 4.1). O-NA concentration reduction in all of the syringe filters was significantly lower than the blank ($P\text{-value}<0.05$). However, the highest O-NA loss was observed in the PTFE syringe filter. Despite the fact that the PTFE membrane provides outstanding chemical compatibility, the surface of this type of PTFE membrane is hydrophobic and applied methanol might be the reason for higher O-NA adsorption.

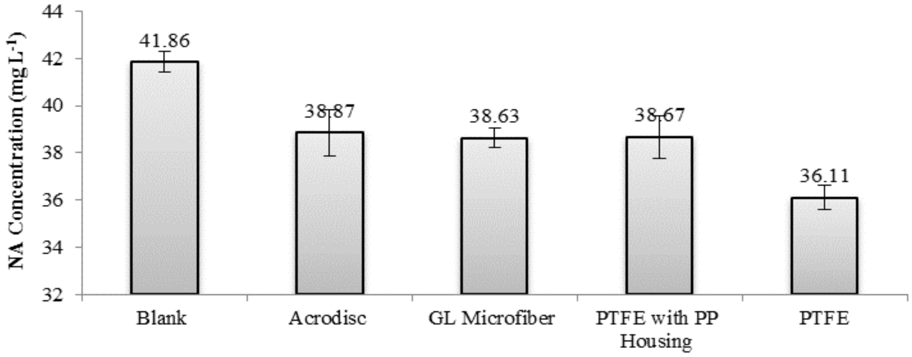
It is believed that this layer of organic phase on the membrane surface may work as a trap for naphthenic acids, and consequently this increases the NA accumulation. Figure 3(b) confirms the high adsorption of O-NAs (12.56%) in the PTFE filter cartridge in comparison to other filters.

There was no considerable difference in O-NA concentrations for Acrodisc, GL microfiber, PTFE with PP Housing in Figure 3(a); however, lower O-NA adsorption was observed for the GL microfiber syringe filter.

4.2.2 *M-NAs in Phosphate Buffer*

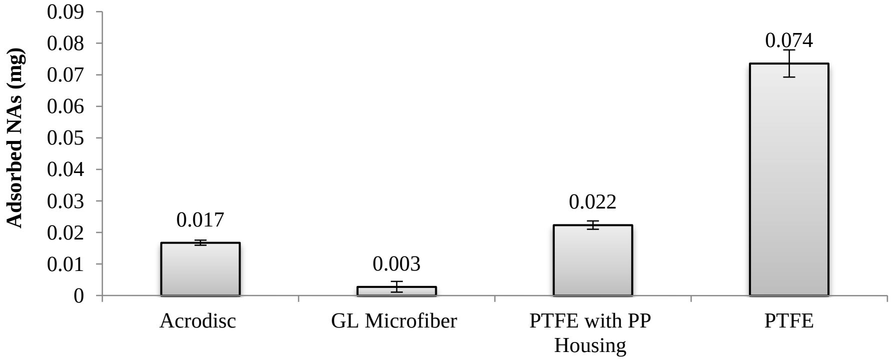
Unlike the syringe filtration results for OSPW, the results for M-NA concentration do not conform to the adsorption test (see Figures 4(a) and (b)). While the concentration of M-NAs in Figure 4(a) shows no significant difference for the PTFE syringe filter ($P\text{-value} = 0.225$), the highest adsorbed M-NAs was detected for this filter (Figure 4(b)). Since the adsorbed M-NAs for PTFE syringe filter exceeded 100%, it is assumed that an experimental error has been happened during test.

The M-NA concentration for Acrodic, GL Microfiber, and PTFE with PP Housing hovered around the M-NA concentration in the blank. We believe that the accuracy of the measurement method (fluorescence spectroscopy) may have impacted the results. However, in both experiments, considerably higher M-NA adsorption in PTFE syringe filters indicates the risk associated with pre-wetting PTFE with methanol (see Figures 3(b) and 4(b)). Membranes made of PTFE are made specifically for non-aqueous based samples and are suitable for aggressive conditions. The manufacturer claims that PTFE filter syringes exhibit minimum interference with extractable compounds in the solution, and with low non-specific binding (www.vwr.com). Therefore, it is assumed that pre-wetting with methanol might be the reason for the higher NA adsorption.



Membrane	Acrodisc	GL Microfiber	PTFE with PP Housing	PTFE
P-value	0.002	0.001	0.001	0.000

(a)

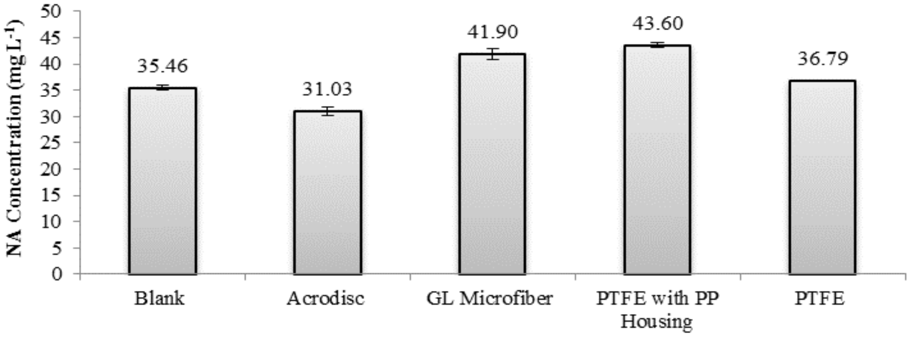


Syringe Filter	Acrodisc	GL Microfiber	PTFE with PP Housing	PTFE
Adsorbed O-NAs (%)	2.86	0.47	3.81	12.56

(b)

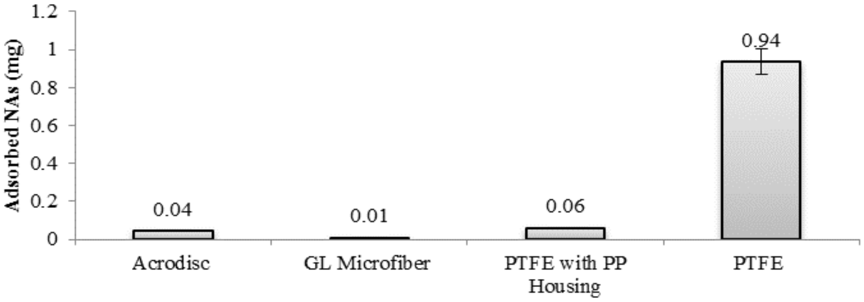
Figure 3. Partitioning of O-NAs on the surface of syringe filters and in OSPW.
(a) O-NA concentration in OSPW samples after syringe filtration test. P-values (Tukey HSD, multi comparison method) for each filter syringe are shown in the table (α=0.05).
(b) percentage and mg O-NAs adsorbed inside the filter cartridge and membrane surface.

For future research and for validating the impact of pre-wet methanol, we suggest that the PTFE filter syringe (with pre-wet methanol) is thoroughly rinsed with DI water (to minimize the interference of methanol on the NA adsorption) and NA adsorption results are compared to results from the PTFE filter syringe without DI water rinse. In addition, since the M-NA concentration in the blank is higher than three tested filter syringes (see Figure 4(a)), further research is required.



Syringe Filter	Acrodisc	GL Microfiber	PTFE with PP Housing	PTFE
P-value	0.000	0.000	0.000	0.225

(a)



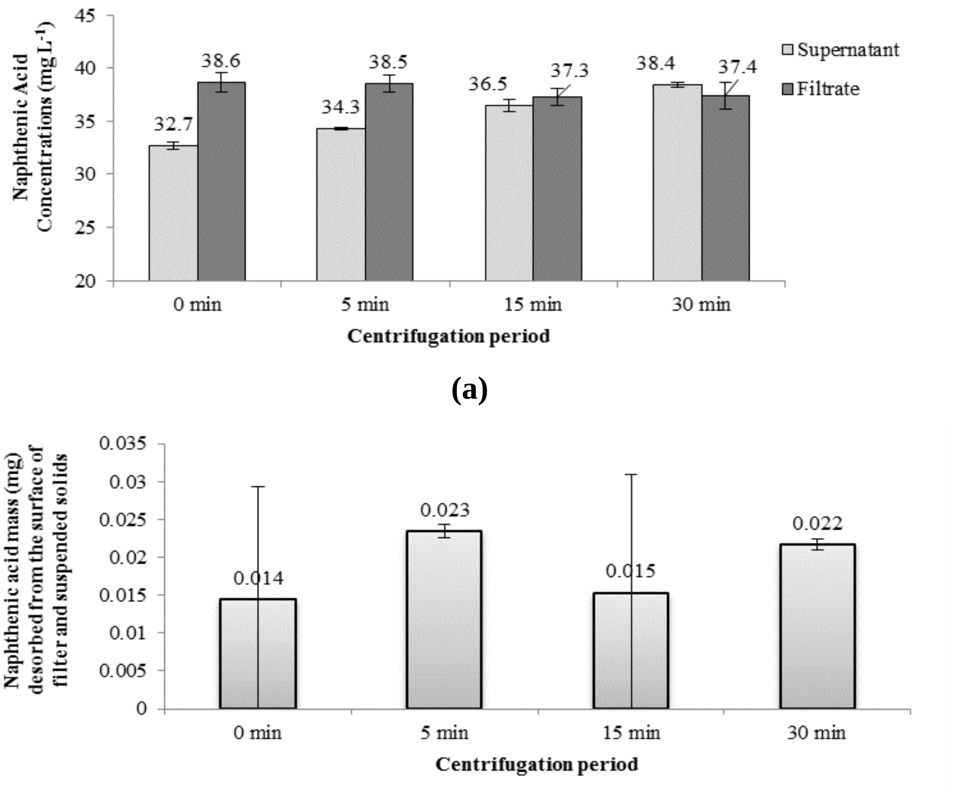
Syringe Filter	Acrodisc	GL Microfiber	PTFE with PP Housing	PTFE
Adsorbed M-NAs (%)	8.54	1.90	12.14	188.41

(b)

Figure 4. Partitioning of M-NAs on the surface of syringe filters and in phosphate buffer.
(a) M-NA concentration in phosphate buffer samples after syringe filtration test. P-values (Tukey HSD, multi comparison method) for each filter syringe are shown in the table ($\alpha=0.05$).
(b) percentage and mg M-NAs adsorbed inside the filter cartridge and membrane surface.

4.3 Influence of Suspended Solids

Figure 5 shows NA partitioning in supernatant, filtrate and filter (with solids) after 0 min, 5 min, 15 min, and 30 min centrifugation at 3,500xg. In Figure 5(a), the concentration of NAs in the OSPW supernatant apparently increases with centrifugation time. This might be a result of TSS presence in OSPW, which may interfere with fluorescence spectroscopy and cause a falsely lower O-NA concentration reading.



(a)

(b)

Figure 5. O-NA concentrations, partitioned in 50mL of OSPW sample.
(a) Supernatant (before filtration) and filtrate (filtered supernatant) at different centrifugation times at 3,500×g.
(b) The weight of detected O-NAs on the surface of suspended solids.

One-way ANOVA statistical analysis for filtrates after different centrifugation times (0, 5, 15, and 30 min) indicates that there is no significant difference (P-value 0.25). In other words, the centrifugation combined with filtration time did not impact O-NA concentration readings. To figure out the contribution of filtration to O-NA losses, a simple student t-Test (with equal variance validated using the F-test) was conducted between filtrate (0 min) and supernatant (30 min). This analysis indicates that there is no significant difference (P-value 0.71) between filtrate from the blank (0 min) and supernatant (30 min); in terms of O-NA losses, there is not a significant difference between the two methods of filtration and centrifugation. The PVDF filter (0.45 µm, Durapore PVDF Membrane, Millipore) was used, however, for other types of filters there might be some NA adsorption (losses) on the surface of membrane (see section 4.1).

Our observations indicated the small partitioning of O-NAs on the surface of suspended solids compared with dissolved O-NAs content. Based on our results, most of the NAs in OSPW are present in a soluble form, and only 1.4% of total O-NAs exist on the surface of suspended solids. Figure 5(b), which is NA adsorbed on the filter and suspended solids at different centrifugation times, shows that there is not a clear increasing or decreasing trend in O-NA content on the surface of the filter after various centrifugation times. This assumption was verified by running one-way ANOVA statistical analysis (no significant difference (P-value 0.68)) among O-NA content on the filter at different centrifugation times (0, 5, 15, and 30 min).

Total suspended solids (TSS) in OSPW used in this experiment was 40.53 ± 3.56 mg L^{-1} which is lower than reported by Allen (2008). OSPW, used in this experiment, was sampled from the surface of the tailings pond, which generally contains lower TSS concentration than samples taken from depth.

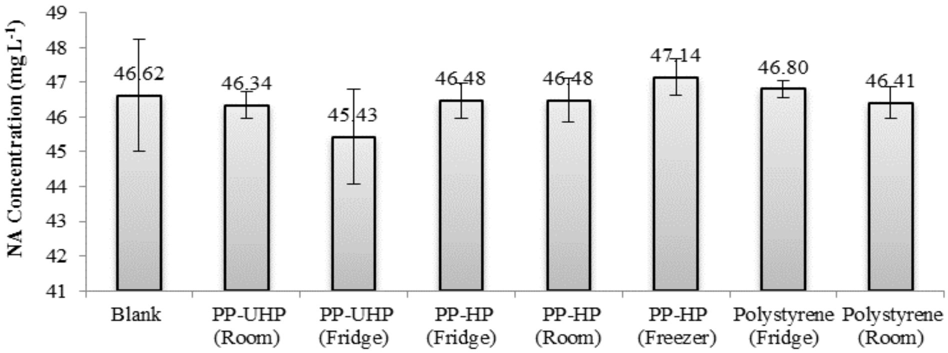
4.4 NA Losses in Containers for Storage

4.4.1 *Centrifuge Tubes*

Centrifuge tubes are extensively used in various OSPW experiments. Especially for biological studies, using sterile centrifuge tubes is an easy and reliable method for preservation of sterile samples, axenic cultures, and samples containing microorganisms. In addition, the plastic body of centrifuge tubes makes it possible to freeze samples with a lower risk of breakage. In this experiment, the adsorption of NAs from OSPW or Merichem and NA loss during 8 days storage in the fridge and at room temperature was studied for three types of sterile centrifuge tubes, including polypropylene ultra-high-performance (PP-UHP), polypropylene high-performance (PP-HP), and polystyrene. In addition, NA dissipation resulting from freezing for 8 days was assessed in PP-HP tubes after defrosting at room temperature.

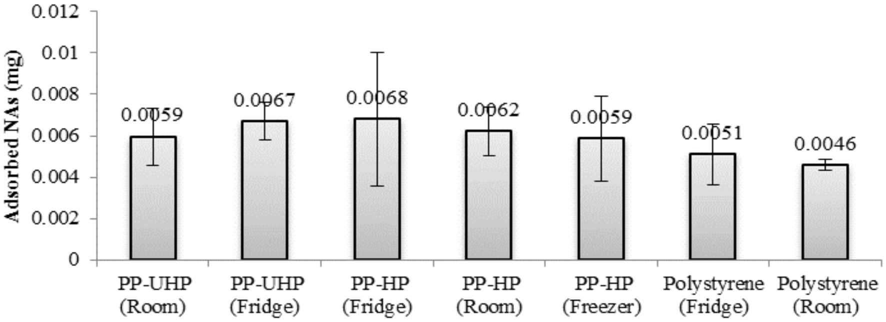
4.4.1.1 O-NAs in OSPW

Figure 6(a) illustrates NA concentrations after 8 days storage in various centrifuge tubes. Despite a small reduction in PP-UHP fridge (4 ^{o}C), the concentration of O-NAs in OSPW samples did not change considerably. The P-values from Tukey HSD analysis demonstrated no significant difference for all types of centrifuge tubes. The smallest P-value (0.681) was observed for PP-UHP (Fridge), which is still larger than 0.05. In addition, Figure 6(b) displays a fairly similar O-NA adsorption among centrifuge tubes with lowest NA adsorption of 0.98% in Polystyrene Room (22 ^{o}C) and highest NA adsorption of 1.46% in PP-HP Room. Storage temperature (room temperature or fridge) did not influence the NA loss. However, the OSPW used in this experiment was filtered (0.45 µm), and therefore bacterial population in the OSPW sample was probably negligible. Preservation of non-filtered OSPW (with high indigenous microbial population) may impact the O-NA concentration through bacterial biodegradation (Clemente et al. 2004, Corinne 2010, Toor et al. 2013a, Videla et al. 2009). In addition, freezing did not impact the O-NA concentration in this experiment.



Centrifuge tube	PP-UHP (Room)	PP-UHP (Fridge)	PP-HP (Fridge)	PP-HP (Room)	PP-HP (Freezer)	Polystyrene (Fridge)	Polystyrene (Room)
P-value	1.000	0.681	1.000	1.000	0.994	1.000	1.000

(a)



Centrifuge tube	PP-UHP (Room)	PP-UHP (Fridge)	PP-HP (Fridge)	PP-HP (Room)	PP-HP (Freezer)	Polystyrene (Fridge)	Polystyrene (Room)
Adsorbed O-NAs (%)	1.27	1.43	1.46	1.33	1.26	1.09	0.98

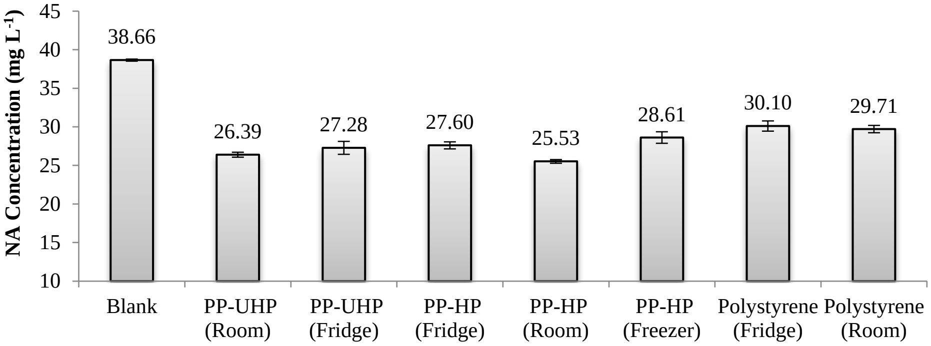
(b)

Figure 6. Partitioning of O-NAs on the surface of centrifuge tubes and OSPW
(a) O-NA concentration in OSPW samples after 8 days storage time. P-value (Tukey HSD, multi comparison method) for each centrifuge tube is shown in the table (α=0.05).
(b) percentage and mg O-NAs adsorbed on the surface of centrifuge tubes.

4.4.1.2 M-NAs in Phosphate Buffer

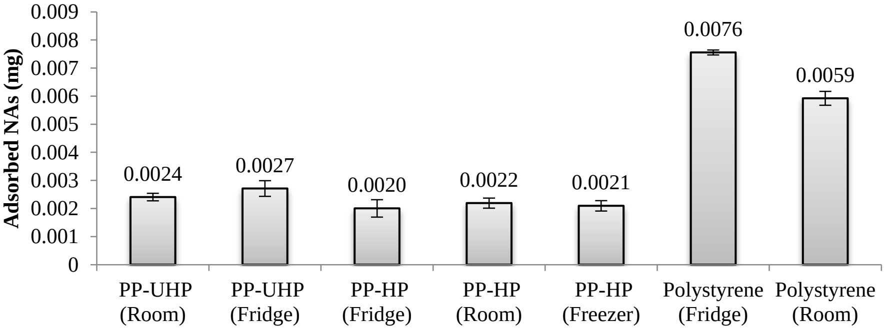
Unlike OSPW results, the concentration of M-NAs in phosphate buffer was reduced in all centrifuge tubes (Figure 7(a)). The M-NA concentration decreased from 38.66 mg L^{-1} to between 25.53 and 30.10 mg L^{-1}. P-values (0.000) also verify the significant reduction in M-NA concentrations in all centrifuge tubes. However, Figure 7(b) indicates a higher M-NA adsorption for Polystyrene centrifuge tubes. Pure methanol-phosphate buffer solution was utilized to validate the M-NA adsorption in Figure 7(b), and therefore small changes in M-NA content can be detected with the fluorescence instrument. The high NA adsorbed in Polystyrene centrifuge tubes (1.95% and 1.53% for fridge and room conditions, respectively) verifies the significant reduction in M-NA concentration in phosphate buffer. However, the NA adsorbed in PP-HP and PP-UHP was not detected.

For future research, other solvents can be used to increase the amount of desorption of NAs from the bottle surface. In addition, the accuracy of fluorescence spectroscopy method might be a limiting factor for the exact quantification of NA concentration. To better quantify the low concentration of NAs in 50% v/v methanol-OSPW solution, more accurate instrument is required.



Centrifuge tube	PP-UHP (Room)	PP-UHP (Fridge)	PP-HP (Fridge)	PP-HP (Room)	PP-HP (Freezer)	Polystyrene (Fridge)	Polystyrene (Room)
P-value	0.000	0.000	0.000	0.000	0.000	0.000	0.000

(a)



Centrifuge tube	PP-UHP (Room)	PP-UHP (Fridge)	PP-HP (Fridge)	PP-HP (Room)	PP-HP (Freezer)	Polystyrene (Fridge)	Polystyrene (Room)
Adsorbed M-NAs (%)	0.62	0.70	0.52	0.57	0.54	1.95	1.53

(b)

Figure 7. Partitioning of M-NAs on the surface of centrifuge tubes and phosphate buffer solution.
(a) M-NA concentration in phosphate buffer solution after 8 days storage time. P-value (Tukey HSD, multi comparison method) for each centrifuge tube is shown in the table (α=0.05).
(b) percentage and mg M-NAs adsorbed on the surface of centrifuge tubes.

According to our observations, the freezing effect on M-NA loss was not different from other treatments over the course of 8 day storage.

In future research, the NA loss in centrifuge tubes can be determined during longer storage times (several months); and also, different eluent solutions can be employed to measure the adsorbed NAs on the surface of centrifuge tubes.

4.4.2 *Soda-Lime Glass Bottles*

Soda-lime glass bottles[4] are commonly used for many oil sands related sampling and experiments. Despite the wide application of soda-lime glassware, the possible NA loss due to adsorption on the surface of this type of glass is unknown. In this experiment two types of soda-lime glass bottles (clear and amber) with the same shape and volume were used. In addition, the impact of glass silanization on the NA adsorption was studied.

4.4.2.1 O-NAs in OSPW

Figure 8(a) illustrates the O-NA concentrations before and after adsorption tests in various soda-lime glass bottles. No significant change (P-values >0.05) in O-NA concentration was observed for all types of bottles. A small deviation in NA concentration might be a result of the experimental error or low accuracy of the quantification method.

According to our results, the NA adsorption on the surface of soda-lime glass was small, however, Figure 8(b) demonstrates that silanization may lower the O-NA adsorption capacity of the soda-lime glass surface (0.18% and 0.20% of NAs adsorbed on clear and amber soda-lime glass bottle respectively).

In addition, the temperature (Room or Fridge) and the color of the glass (Clear or Amber) did not influence the O-NA concentration.

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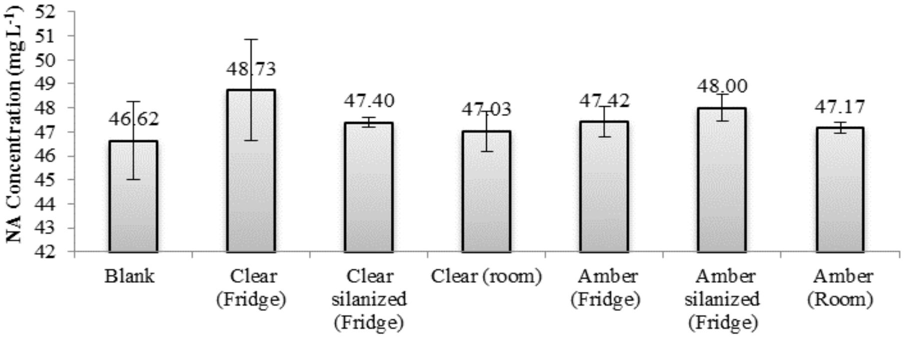
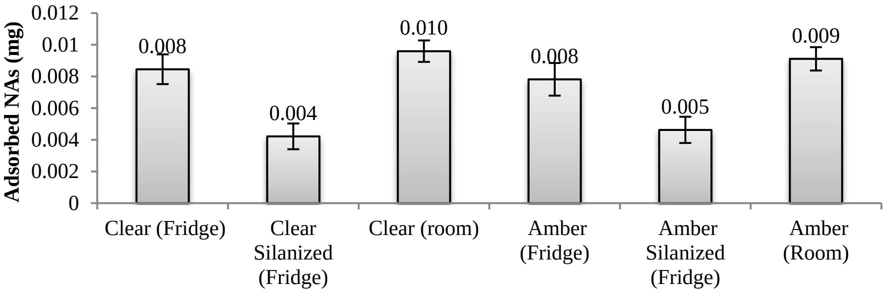
[4] See http://en.wikipedia.org/wiki/Soda-lime_glass



Soda-Lime glass bottles	Clear (Fridge)	Clear Silanized (Fridge)	Clear (Room)	Amber (Fridge)	Amber Silanized (Fridge)	Amber (Room)
P-value	0.290	0.973	0.999	0.969	0.723	0.996

(a)



Soda-Lime glass bottles	Clear (Fridge)	Clear Silanized (Fridge)	Clear (Room)	Amber (Fridge)	Amber Silanized (Fridge)	Amber (Room)
Adsorbed O-NAs (%)	0.36	0.18	0.41	0.34	0.20	0.39

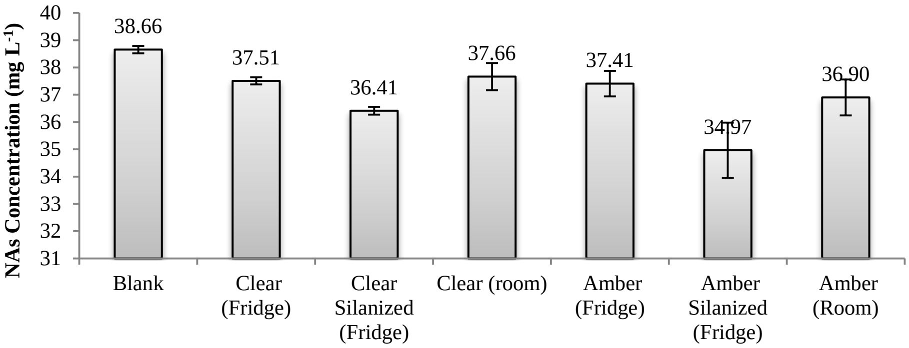
(b)

Figure 8. Partitioning of O-NAs on the surface of soda-lime glass bottles and OSPW.
(a) O-NA concentration in OSPW samples after 8 days storage time. P-value (Tukey HSD, multi comparison method) for each bottle is shown in the table (α=0.05).
(b) percentage and mg O-NAs adsorbed on the surface of soda-lime glass bottles.

4.4.2.2 M-NAs in Phosphate Buffer

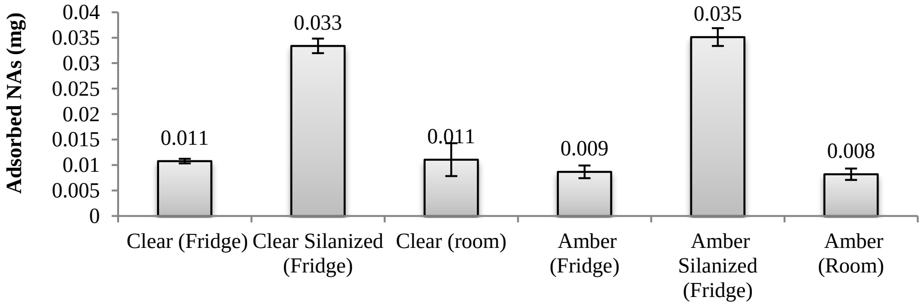
M-NAs displayed higher capacity to adhere and bind to the soda-lime glass surface in comparison with O-NAs in OSPW. The higher adhesion capacity was even more obvious for silanized soda-lime glass bottles for both Clear and Amber types (1.73% and 1.82% M-NAs adsorbed). The lowest M-NA concentration was observed for silanized Clear and Amber glass bottles (Figure 9(a)), equal to 36.41 and 34.97 mg L^{-1}, with P-values equal to 0.002 and 0.000, respectively.

Figure 9(b) confirms that silanization increases the NA adsorption capacity on the surface of soda-lime glass. This result does not conform to OSPW observations, when silanization slightly reduced the O-NA adsorption on the surface of soda-lime glass bottles. This implies a different adsorption property for O-NAs and M-NAs, which might be a result of differences in their molecular structures. It has been reported that commercial NAs such as Merichem possess smaller molecular masses than O-NAs (Scott et al. 2005). Based on our results, the storage temperature did not influence the NA concentration.



Soda-Lime glass bottles	Clear (Fridge)	Clear Silanized (Fridge)	Clear (Room)	Amber (Fridge)	Amber Silanized (Fridge)	Amber (Room)
P-value	0.186	0.002	0.316	0.127	0.000	0.016

(a)



Soda-Lime glass bottles	Clear (Fridge)	Clear Silanized (Fridge)	Clear (Room)	Amber (Fridge)	Amber Silanized (Fridge)	Amber (Room)
Adsorbed M-NAs (%)	0.56	1.73	0.57	0.45	1.82	0.42

(b)

Figure 9. Partitioning of M-NAs on the surface of soda-lime glass bottles and phosphate buffer solution.
(a) M-NA concentration in phosphate buffer solution after 8 days storage time.
P-value (Tukey HSD, multi comparison method) for each bottle is shown in the table (α=0.05).
(b) percentage and mg M-NAs adsorbed on the surface of soda-lime glass bottles.

4.4.3 *Plastic Bottles*

Compared to centrifuge tubes and soda-lime glass bottles, plastic bottles have not been employed extensively for monitoring, sampling, and experiments. However, OSPW samples are sometimes shipped from operation sites to laboratories in plastic buckets (Rogers et al. 2002). Also in some cases, such as sampling for heavy metal measurement, the plastic containers are preferred. In this experiment, the NA loss due to storage (8 days) in three types of plastic bottles, including high density polyethylene (HDPE), low density polyethylene (LDPE), and polypropylene (PP), was studied.

4.4.3.1 O-NAs in OSPW

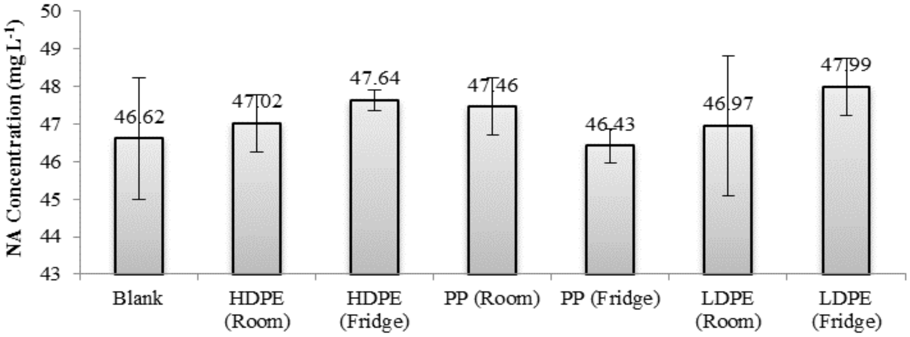
Figure 10(a) illustrates that storage of OSPW in various plastic bottles does not impact the O-NA concentration over the course of 8 days. The minimum observed P-value was 0.706 for the LDPE bottle at 4^{o}C (fridge) which indicates no significant change in O-NA concentration. Figure 10(b) also indicates a similar O-NA adsorption observed among all bottles, except LDPE (Room). The large error bar for LDPE (Room) bottles indicates an experimental error during test.

For future research, we suggest conducting the experiment at a longer exposure time to verify the impact of plastic materials during long-time storage.

4.4.3.2 M-NAs in Phosphate Buffer

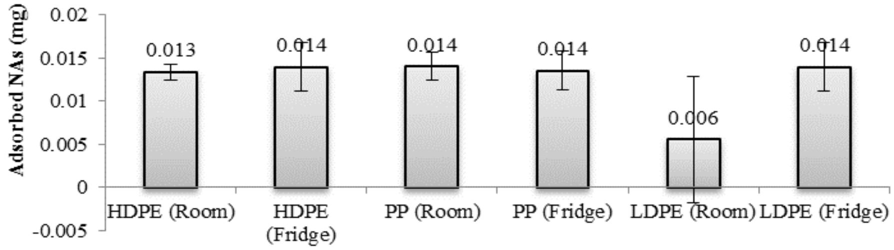
Figure 11(a) displays a significant reduction in the M-NA concentration in all plastic bottles (P-value = 0.000). However, similar to the previous experiments, the fluorescence instrument did not quantify the amount of M-NA loss and the amount of adsorbed M-NAs was small. Measurement using more accurate method with higher resolution is suggested for future investigations.

Figure 11(b) also demonstrates that there is not a considerable difference among plastic bottle types in terms of performance. In addition, it seems that storage in the fridge has slightly increased the adsorption of M-NA on the surface of all plastic bottles.



Plastic Bottles	HDPE (Room)	HDPE (Fridge)	PP (Room)	PP (Fridge)	LDPE (Room)	LDPE (Fridge)
P-value	0.999	0.899	0.955	1.000	1.000	0.706

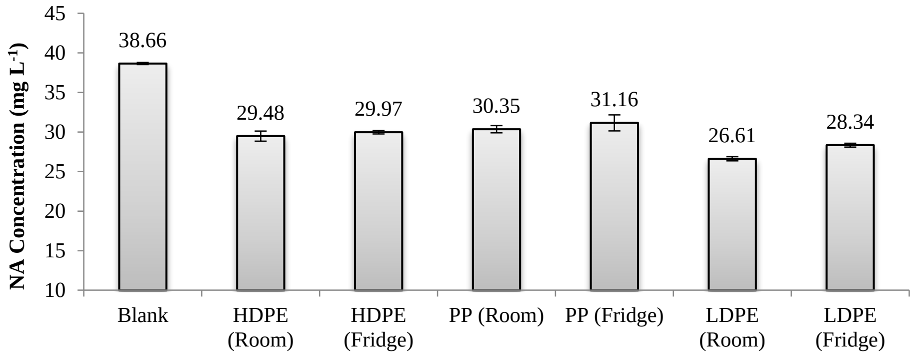
(a)



Plastic Bottles	HDPE (Room)	HDPE (Fridge)	PP (Room)	PP (Fridge)	LDPE (Room)	LDPE (Fridge)
Adsorbed O-NAs (%)	0.57	0.60	0.60	0.58	0.24	0.60

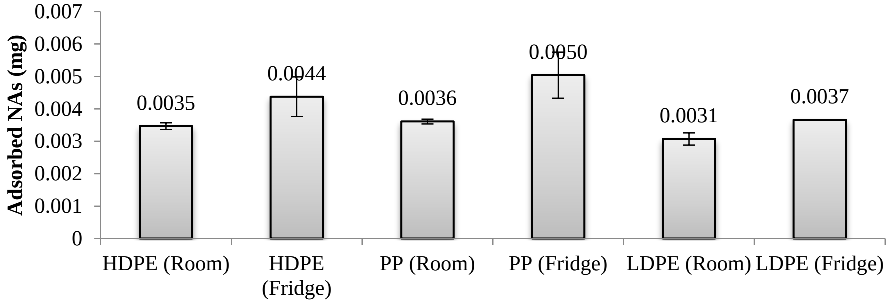
(b)

Figure 10. Partitioning of NAs on the surface of plastic bottles and OSPW.
(a) NA concentration in OSPW samples after 8 days storage time. P-value (Tukey HSD, multi comparison method) for each plastic bottle is shown in the table (α=0.05).
(b) percentage and mg NAs adsorbed on the surface of plastic bottles.



Plastic Bottles	HDPE (Room)	HDPE (Fridge)	PP (Room)	PP (Fridge)	LDPE (Room)	LDPE (Fridge)
P-value	0.000	0.000	0.000	0.000	0.000	0.000

(a)



Plastic Bottles	HDPE (Room)	HDPE (Fridge)	PP (Room)	PP (Fridge)	LDPE (Room)	LDPE (Fridge)
Adsorbed M-NAs (%)	0.18	0.23	0.19	0.26	0.16	0.19

(b)

Figure 11. Partitioning of M-NAs on the surface of plastic bottles and phosphate buffer solution.
(a) M-NA concentration in phosphate buffer solution after 8 days storage time. P-value (Tukey HSD, multi comparison method) for each plastic bottle is shown in the table (α=0.05).
(b) percentage and mg M-NAs adsorbed on the surface of plastic bottles.

4.5 Storage Conditions

In this section, the best preservation conditions in terms of temperature, pH, and addition of methanol were determined. To conduct this experiment, trace cleaned borosilicate glass bottles were employed and the impact of glass silanization was studied.

4.5.1 *O-NAs in OSPW*

Storage in the fridge (4 ^{o}C) provided the best conditions for OSPW preservation (Figure 12(a)), resulting in the closest NA concentration compared with the original OSPW. The presence of light caused a small increase in O-NA concentration readings over the course of 8 days. In this test, the filtered OSPW sample was used, and therefore the microbial population and consequently microbiological activity was considerably reduced. However, for the storage of non-filtered OSPW, room temperature might impact the O-NA concentration through microbial biodegradation (Toor et al. 2013b). Also in the presence of light, algae growth may cause a false-higher NA concentration in samples (Mahdavi 2014).

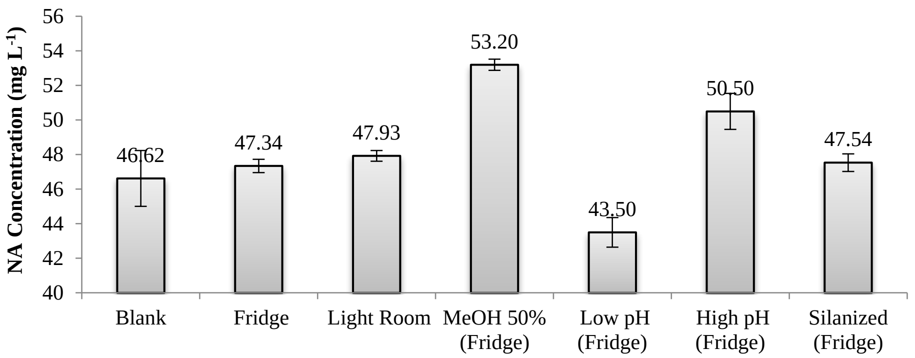
An addition of methanol curbs the biological activities, and this may result in a better preservation condition, preventing NA biodegradation. However, our observations indicated a significant increase (P-value = 0.000) in O-NA concentration from 46.6 ± 1.6 to 53.2 ± 0.3 mg NA L^{-1} (Figure 12(a)).

At low pH (pH = 2.1), the NA molecules turn into non-polar form and become less soluble in water. Over the course of 8 days, the insoluble NAs will aggregate and attach to the surface of the glass bottle. This resulted in a significant reduction in NA concentration readings (P-value = 0.007). In Figure 12(b), a high mass of NA detected on the surface of the glass bottle validates the aggregation and partitioning of NAs at low pH. Since low pH insolubilizes NAs in OSPW and creates a non-homogeneous solution, results should be interpreted with caution if O-NAs are to be measured in the same solution. This situation could be exacerbated if plastic bottles were used.

At high pH (pH = 11.1), NA molecules should completely dissociate to the ionized form and fully dissolve in water (Headley and McMartin 2004). Although OSPW at high pH creates a homogeneous solution, the high pH and complete dissociation of NAs resulted in false-higher O-NA concentration readings (P-value = 0.001).

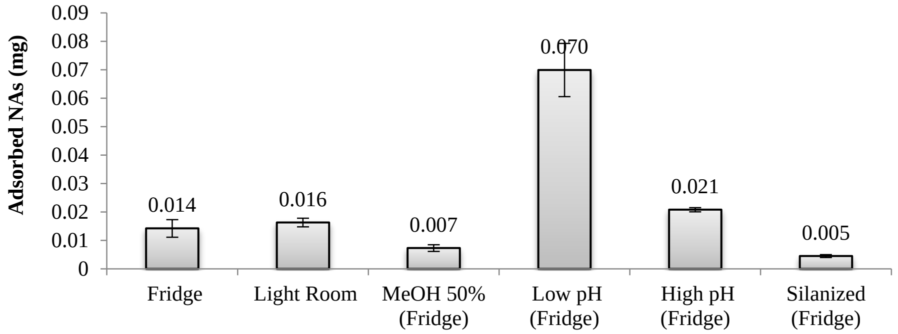
The exact reasons for false-higher NA concentration for methanol added samples and high pH samples are unknown at this time. We hypothesize that the addition of methanol or increase in pH may result in separation of dimers, polymers or complete dissolution of micelles over the course of 8 days and this might be responsible for higher intensity on fluorescence spectra. As well, the influence of solvent on fluorescence readings cannot be precluded as shown in Martin et al. (2014).

Silanization of borosilicate glass did not impact on the O-NA concentration (P-value = 0.833<0.05) in this experiment and Figure 12(b) indicates a slight decrease in the NA adsorption resulting from silanization of the glass surface as noted previously.



Storage Conditions	Fridge	Light Room	MeOH 50% (Fridge)	Low pH (Fridge)	High pH (Fridge)	Silanized (Fridge)
P-value	0.935	0.521	0.000	0.007	0.001	0.833

(a)



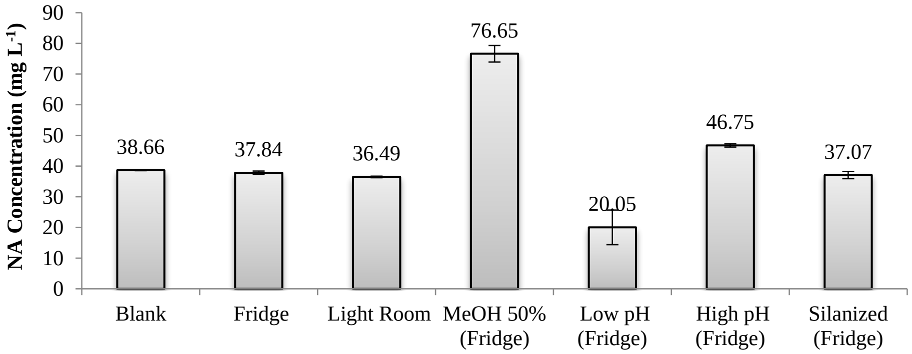
Storage Conditions	Fridge	Light Room	MeOH 50% (Fridge)	Low pH (Fridge)	High pH (Fridge)	Silanized (Fridge)
Adsorbed O-NAs (%)	0.61	0.70	0.31	3.00	0.89	0.19

(b)

Figure 12. OSPW storage tests: fridge versus room temperature with presence of light, addition of methanol (50% v/v), low pH (2.1), and high pH (11.1), and usage of silanized borosilicate glass.
(a) Concentration of O-NAs in OSPW samples after 8 days. P-value (Tukey HSD, multi comparison method) for each condition is shown in the table (α=0.05).
(b) percentage and mg O-NAs adsorbed on the inner surface of borosilicate glass bottles.

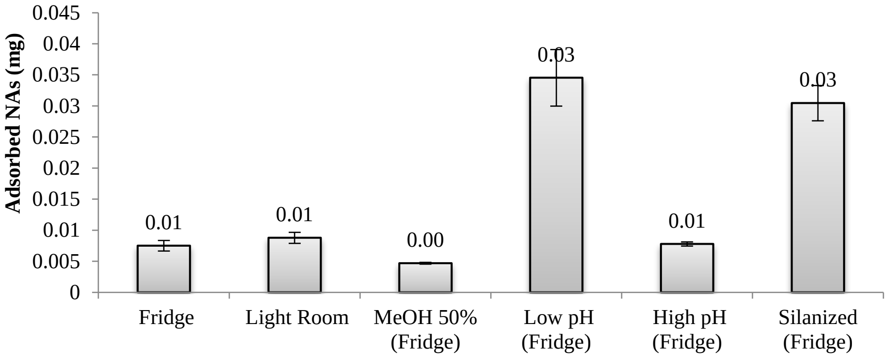
4.5.2 *M-NAs in Phosphate Buffer*

Based on Figure 13(a), addition of methanol caused a false-higher result in the M-NA concentration in phosphate buffer (P-value = 0.000), which is similar to OSPW results. At high pH (11.1), an increase in M-NA concentration from 38.66 to 46.75 mg L^{-1} was observed. At low pH (2.1), aggregation of M-NA was visible to the naked eye, in the form of small droplets on the surface of the phosphate buffer solution. This explains the apparent reduction of 48.1% in the M-NA concentration at low pH (Figure 13(a)), as well as high NA adsorbed in Figure 13(b).



Storage Conditions	Fridge	Light Room	MeOH 50% (Fridge)	Low pH (Fridge)	High pH (Fridge)	Silanized (Fridge)
P-value	0.999	0.916	0.000	0.000	0.14	0.996

(a)



Storage Conditions	Fridge	Light Room	MeOH 50% (Fridge)	Low pH (Fridge)	High pH (Fridge)	Silanized (Fridge)
Adsorbed M-NAs (%)	0.39	0.45	0.24	1.79	0.40	1.58

(b)

Figure 13. M-NAs storage tests: fridge versus room temperature with presence of light, addition of methanol (50% v/v), low pH (2.1), and high pH (11.1), and usage of silanized borosilicate glass.
(a) Concentration of M-NAs in phosphate buffer samples after 8 days. P-value (Tukey HSD, multi comparison method) for each condition is shown in the table (α=0.05).
(b) percentage and mg M-NAs adsorbed on the inner surface of borosilicate glass bottles.

In Figure 13(b), considerable M-NA adsorption was observed for silanized borosilicate glass. This observation is in agreement with results from silanized soda-lime glass bottles (section

4.4.2). For the silanized glass bottle, a small reduction in M-NA concentration was detected in Figure 13(a). However in Figure 13(b), 1.58% of the M-NAs were adsorbed onto silanized glass which is considerable comparing with other bottles. Due to huge changes in M-NA concentration in MeOH 50% samples, the Tukey HSD method did not statistically identify a significant change at high pH and silanized glass conditions (Figure 12(a)). However, the change in M-NA concentrations for these samples is obvious.

4.6 Influence of Bottle Cap Liners

One of the factors that could be important for storage of OSPW is selection of materials for the cap liner. Cap liner material should not affect liquids stored in bottles through physical interaction or chemical reaction.

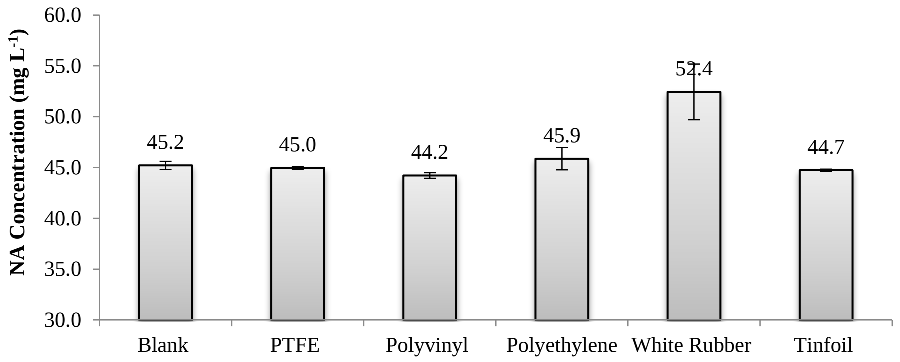
4.6.1 *O-NAs in OSPW*

Figure 14(a) shows that the PTFE cap liner, followed by polyvinyl, and tinfoil demonstrated small influence on OSPW within 7 day storage time. However, a higher apparent concentration of O-NAs in OSPW was observed for white rubber and polyethylene cap liners, which indicates the possibility of leaching of some organic compounds into the OSPW sample from the liner materials. However, the increase in O-NA concentration was statistically significant (P-value 0.000) only for the white rubber cap liner. Figure 14(b) also verifies the possibility of leakage from the white rubber and polyethylene cap liners. Since fluorescence spectroscopy mainly targets the hydrocarbons with aromatic rings, it can be concluded that the leached compounds from the white rubber and polyethylene cap liners might possess aromatic structures or simply be fluorescence sensitive.

4.6.2 *M-NAs in Phosphate Buffer*

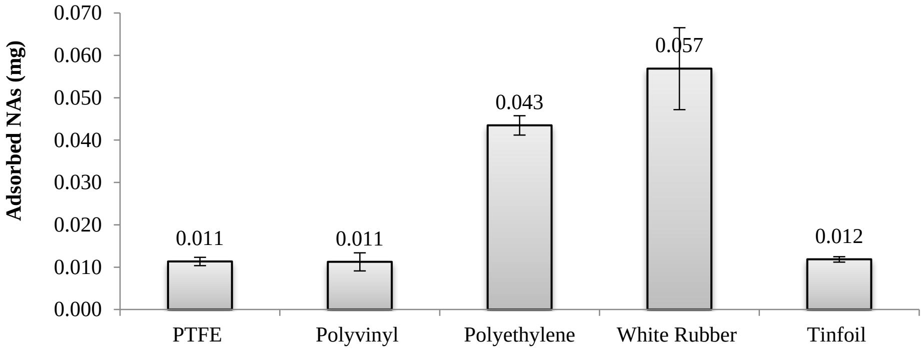
Figure 15(a) illustrates the M-NA concentration in phosphate buffer after conducting the cap liner experiment. Similar to the OSPW test, no significant reduction in NA concentration was observed; however, a leakage from the white rubber cap liner resulted in an increase in M-NA concentration. The leakage was verified through adsorbed NAs in Figure 15(b) which resulted in false-higher M-NA concentration. In Figure 15(b), the minimum M-NA adsorbed was observed for the PTFE cap liner.

Generally, the PTFE cap liner demonstrated the best performance for storage of water samples containing either M-NAs or O-NAs.



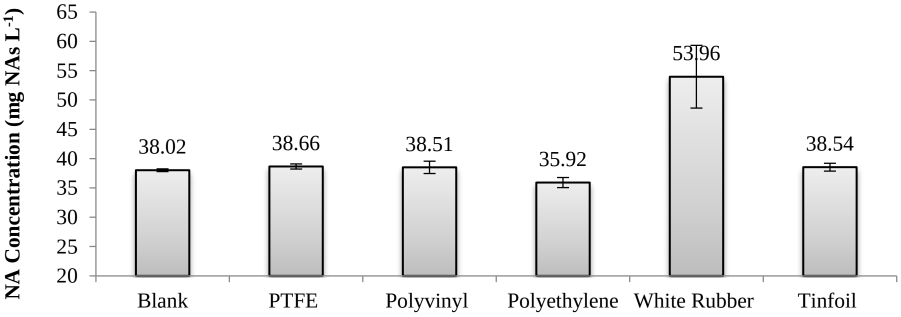
Cap Liner	PTFE	Polyvinyl	Polyethylene	White Rubber	Tinfoil
P-value	1.000	0.910	0.984	0.000	0.996

(a)



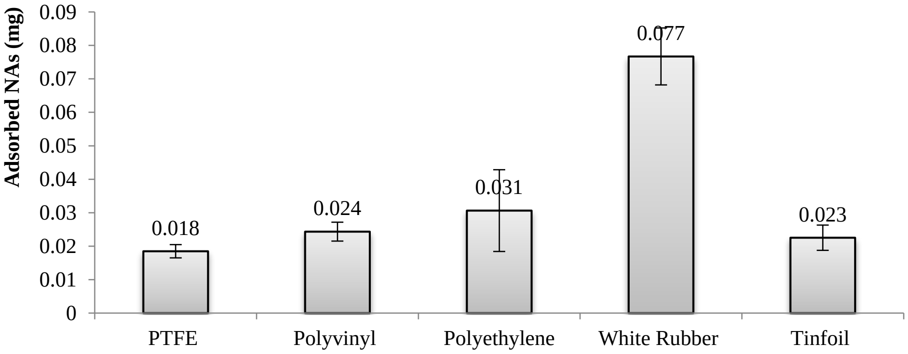
(b)

Figure 14. Cap liner adsorption test for OSPW.
(a) NA concentration in OSPW after 7 day exposure time, P-value (Tukey HSD, multi comparison method) for each cap is shown in the table (α=0.05).
(b) NA adsorption on the cap liner.



Cap Liners	PTFE	Polyvinyl	Polyethylene	White Rubber	Tinfoil
P-value	0.999	1.000	0.859	0.000	1.000

(a)



(b)

Figure 15. Cap liner adsorption test for M-NAs.
(a) M-NA concentration in phosphate buffer after 7 day exposure time, P-value (Tukey HSD, multi comparison method) for each cap is shown in the table (α=0.05).
(b) M-NA adsorption on the cap liner.

4.7 Influence of Microwaves

Microwaves are electromagnetic radiation with frequencies ranging between 300 MHz and 300 GHz and wavelengths between 1 mm and 1 m (Hitchcock 2004). Despite a wide application in industry, the influence of microwave radiations on NAs is unknown. Figure 16 shows no change in the concentration of NAs in OSPW and phosphate buffer solutions. The simple t-Test comparison analysis (equal variances were determined using the F-test) confirms that statistically there is no significant difference with P-values of 0.22 and 0.74 for O-NAs and M-NAs, respectively. For future research, we suggest that NA samples are exposed to microwave radiation for a longer time and the possible change in molecular structure of NAs is assessed by fingerprinting techniques.

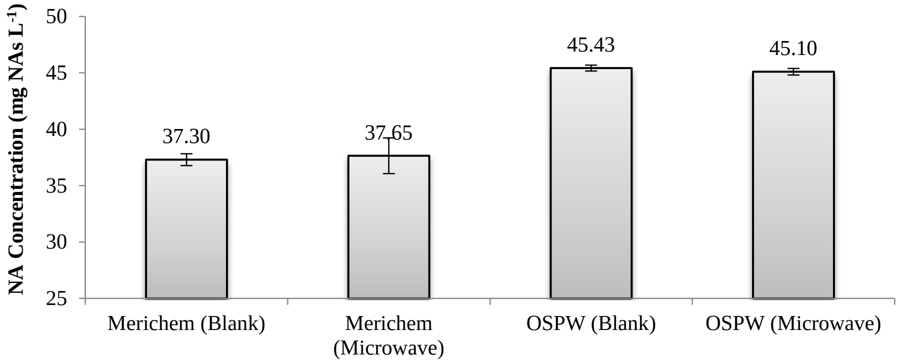


Figure 16. Concentrations of NAs (From OSPW and Merichem) before and after microwave radiation exposure.

4.8 Influence of UV Light

In this experiment, the impact of UV radiation (254 nm) on NA concentration is assessed. UV in combination with oxidants (such as hydrogen peroxide) has been used for advanced oxidation of NAs (Afzal et al. 2012, Liang et al. 2011). However, the short time exposure of NAs in solution to UV light and its influence on NA concentration is unknown.

Figure 17 shows a negligible reduction in O-NA concentration after exposure of the OSPW sample to UV radiation with a P-value (t-Test analysis with equal variances) equal to 0.13. However, the decrease in M-NA concentration was significant with a P-value of 0.000. We hypothesize that NA oxidization reactions may have taken place through UV-photolysis and advanced oxidation processes (Sonntag 2006). Conducting an advanced oxidation experiment was not in the scope of this research, however, it is assumed that due to the presence of scavengers such as bicarbonate species in OSPW, hydroxyl radicals would not react efficiently with O-NAs. Lack of scavengers in the phosphate buffer may be responsible for higher degradation in M-NAs.

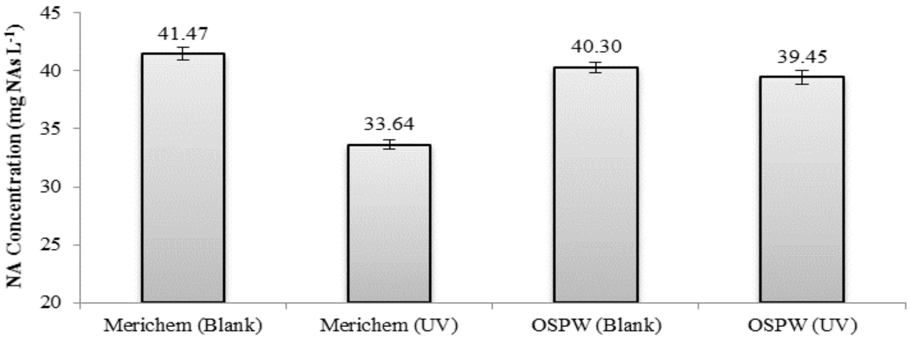


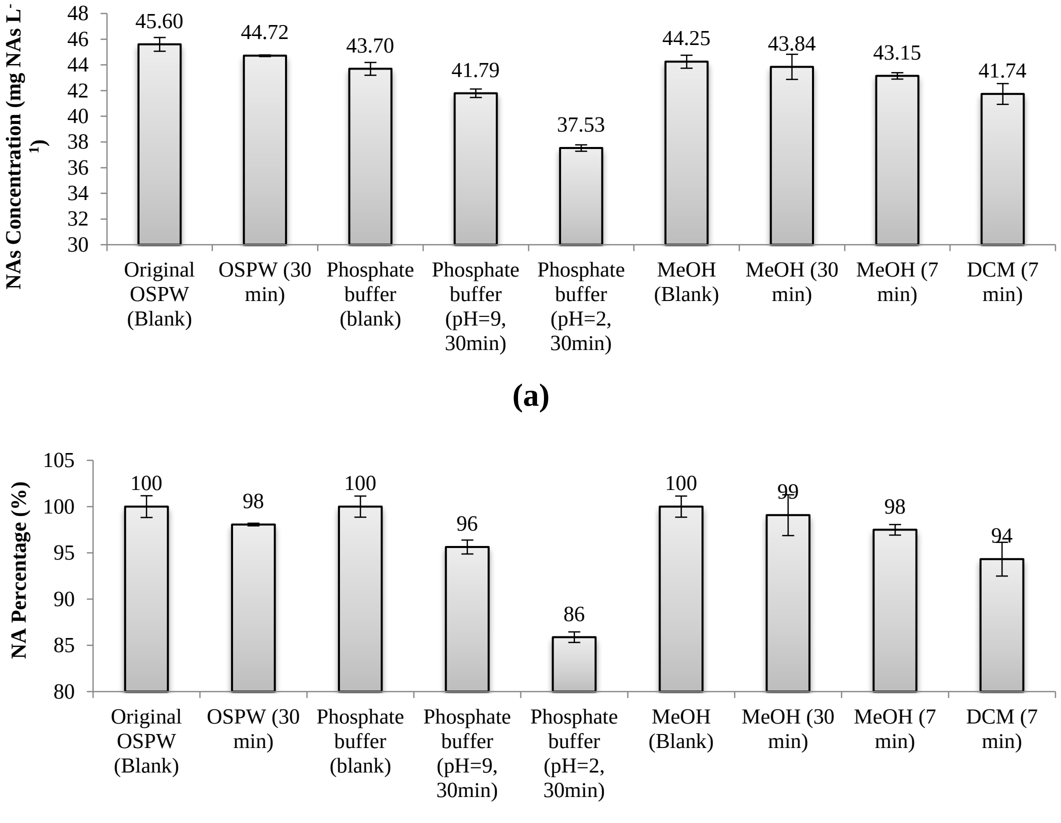
Figure 17. Concentration of NAs (from OSPW and Merichem) before and after UV radiation exposure.

4.9 Influence of Rotavapor with Different Solvents

In this section, the NA loss during rotavapor is studied. In some experiments, rotavapor was used to reduce the volume of the solvent containing NAs (Quesnel et al. 2011), however, the NA loss via solvent evaporation in rotavapor is unknown.

4.9.1 *O-NAs from OSPW*

Figure 18(a), shows the reduction in O-NA concentration resulting from rotavapor in different solutions and at different time periods. Since the final volume of solutions before NA measurement was the same (50 mL), the reduction in concentration of NAs represents the true NA loss due to rotavapor. A small reduction in O-NA concentration from 45.60 to 44.72 mg L^{-1} in the OSPW sample shows that the amount O-NA loss in OSPW is negligible. In phosphate buffer, O-NA loss at low pH (2) was significantly higher than at high pH (9). The complete dissociation of O-NA molecules may reduce the O-NA loss, however, at low pH, O-NAs will be insoluble in water and can be purged and evaporated from the aqueous phase due to vacuum (57.57 to 84.66 kPa) and temperature (37°C) in rotavapor.



(a)

(b)

Figure 18. Concentration of O-NAs after rotavapor in different solutions (OSPW, in phosphate buffer, methanol (MeOH), dichloromethane (DCM)) and at different rotavapor periods (minutes).
(a) O-NA concentrations, (b) O-NA percentages.

It may be hypothesized that at a pH lower than the pKa of organic acids, the amount of loss of organic compounds (especially the volatile fractions) will increase. Figure 18(b) illustrates that 96% and 86% of O-NAs will remain in the solution after rotavapor at high and low pH conditions, respectively. The amount of O-NA loss in methanol in 30 min rotavapor was slightly smaller than in the phosphate buffer (blank). In DCM solution, the NA loss reached 6% within the 7 min rotavapor period; and within this period the whole DCM (50 mL) was vaporized.

According to our observations, the amount of O-NA loss in aqueous solutions highly depends on the pH. In organic solvents, by increasing the evaporation of solvent, the O-NA loss will increase as well.

4.9.2 *M-NAs in Phosphate Buffer*

Figure 19(a) illustrates the M-NA loss in different solutions after rotavapor. The amount of M-NA loss in aqueous solutions is more than in organic solvents. Unlike O-NAs in OSPW, the pH did not influence the M-NA loss considerably. In Figure 19(b), the M-NA concentration reduced from 100% to 88% and 89% at high and low pH, respectively. M-NAs have lower molecular mass in comparison with O-NAs (Scott et al. 2005), which often results in higher volatility (Headley and McMartin 2004). It is assumed that the higher volatility of M-NAs resulted in losses independent of pH. Among organic solvents, the largest M-NA loss was observed in DCM (3% loss).

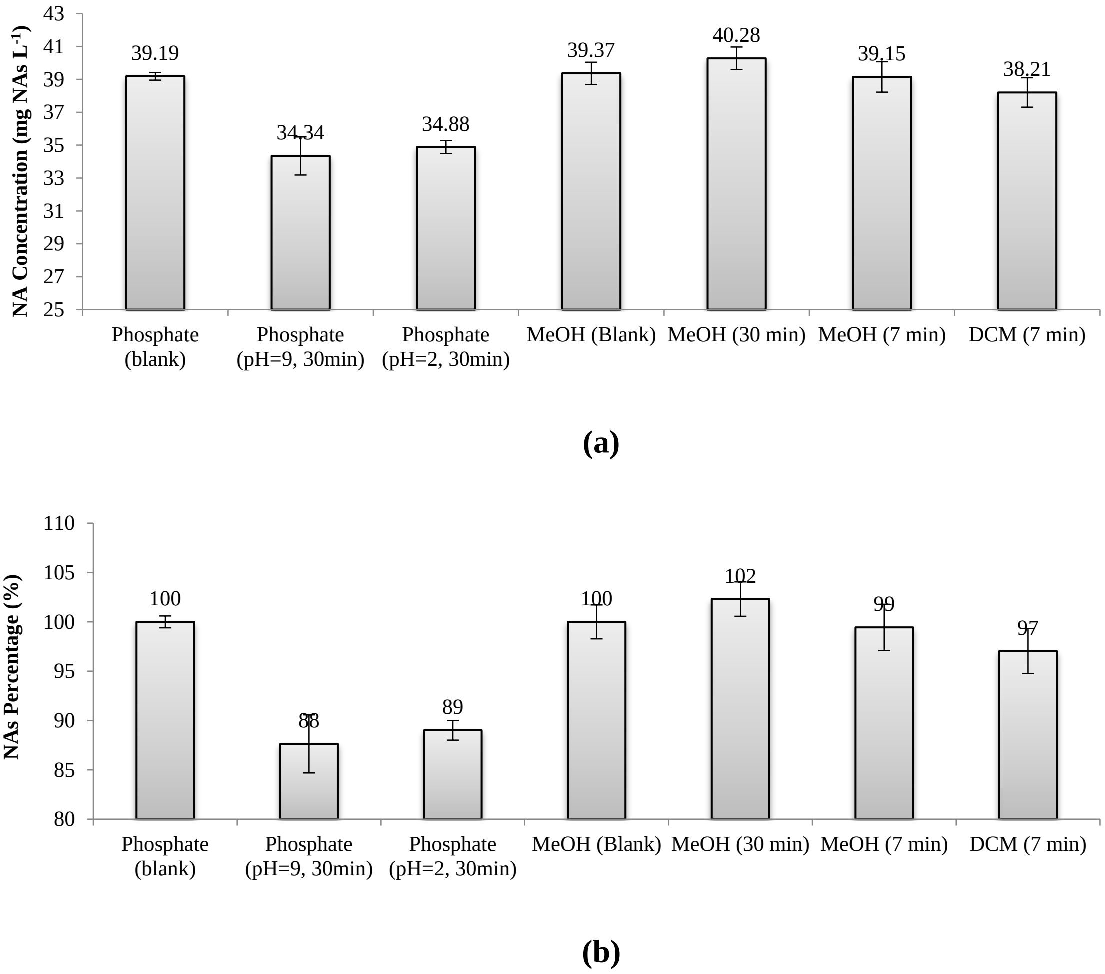


Figure 19. Concentrations of M-NAs in different solutions after rotavapor (in phosphate buffer, methanol (MeOH), dichloromethane (DCM)) and at different rotavapor periods (minutes).
(a) M-NA concentrations.
(b) M-NA percentages.

4.10 Freezing Test

Spray freezing has been used for treating wastewater in cold regions (Biggar et al. 2005, Gao et al. 2003). The water ice crystals have a specific symmetry structure that cannot accommodate other atoms (Gao et al. 2003). The freezing treatment technology relies on the rejection of contaminants from the ice structure, which is expected to result in the accumulation of impurities in the non-frozen phase. In this experiment, the impact of freezing on the accumulation of NAs in the non-frozen phase was studied.

4.10.1 *O-NAs in OSPW*

Figure 20 illustrates an increase in the concentration of O-NAs in the non-frozen phase. The slight increase starts when the non-frozen phase is less than 46% of total volume. The low concentration of NAs and fast freezing time (less than 5 min) might be the main reasons for low separation efficiency. It is reported that when using the spray freezing method (at -10^{o}C), the concentration of chemical oxygen demand (COD) in the non-frozen phase can be increased to 1.62 times the initial COD concentration (Gao et al. 2003).

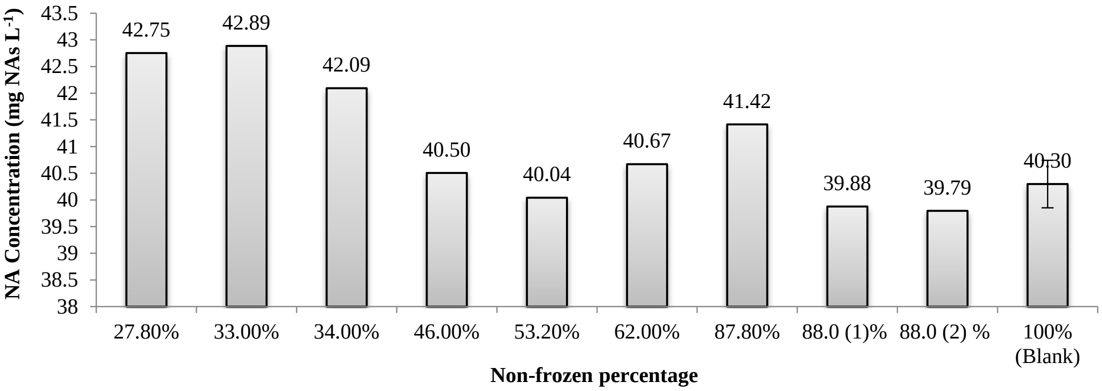


Figure 20. The O-NA concentrations in different percentages of non-frozen phase in OSPW.

Since the crystallization process requires enough time to form clean crystals, freezing at slow speed may increase the efficiency of NA separation. According to our results, the highest concentrated NAs in the non-frozen phase reached 42.89 mg L^{-1}, when 33% of total OSPW sample was still non-frozen.

For future research, freezing at different crystallization periods can be conducted to determine the influence of time on treatment efficiency. In addition, by measuring the NA concentration in the frozen phase (ice), the efficiency of the treatment can be validated.

4.10.2 *M-NAs in Phosphate Buffer*

Figure 21 shows the concentration of M-NAs which remained in the non-frozen phase of the phosphate buffer. This figure displays no increasing or decreasing trends in M-NA content in various percentages of non-frozen phase. M-NAs (and also Fluka and Kodak commercial NAs) have lower molecular mass than O-NAs from OSPW (Scott et al. 2005). It is assumed that compounds with smaller molecular size might embed into ice crystals more easily than the larger molecules of O-NA in OSPW. However, more research is required to verify this assumption, and to clarify unknown aspects of ice crystallization and rejection of impurities from the crystal lattice.

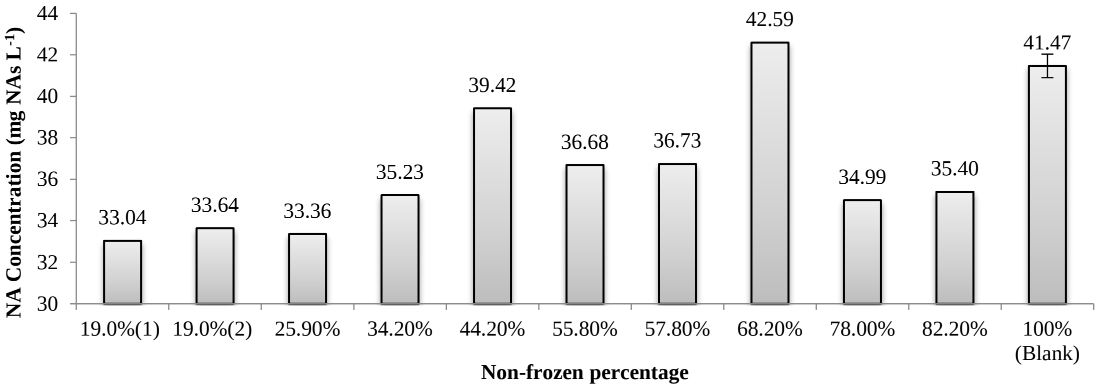


Figure 21. The M-NA concentrations in different percentages of non-frozen phase in phosphate buffer.

4.11 Solid Phase Extraction (SPE)

In most of the methods for quantification and characterization of NAs, the NAs need to be extracted from the aqueous phase and transferred to an organic solvent. NAs can be isolated from water samples via methods such as solid phase extraction (SPE) and liquid phase extraction (e.g., NA extraction using dichloromethane and a separatory funnel). However, when using the liquid phase extraction method there are concerns regarding NA loss during DCM evaporation (see section 4.9), extraction efficiency, time required for extraction, and health concerns. SPE provides a fast and reliable NA recovery method without the risk of excessive NA evaporation and loss.

Six different SPE cartridges were tested and evaluated in terms of NA adsorption by measuring the NA concentration in filtrates, and NA recovery by quantifying the NA content the in eluent. The properties of target SPE cartridges are:

- ISOLUTE® C18 is a retentive, non-polar sorbent for extraction of acidic, neutral and basic compounds from aqueous sample matrices. Silanol base interaction is exploited to improve the extraction efficiency in basic conditions (www.biotage.com).
- ISOLUTE® ENV+ is a hyper cross-linked hydroxylated polystyrene-divinylbenzene copolymer, which is a highly retentive, non-polar sorbent that is used for the extraction of polar analytes from large volume water samples. ISOLUTE® ENV+ is usually used when analytes are not retained by C18 or C8 sorbents (www.biotage.com).
- Oasis HLB is a strong polymeric adsorbent with a unique hydrophilic-lipophilic balance. This reversed-phase sorbent maintains high retention and capacity for acidic, basic and neutral analytes (www.waters.com).
- Supelclean™ LC-Diol consists of polypropylene hardware with a 2,3-dihydroxypropoxypropyl bonding matrix active group and a silica gel based

material matrix. This cartridge is categorized as Normal-Phase Supelclean SPE Products (http://www.sigmaaldrich.com/canada-english.html).

- ISOLUTE® SAX is a quaternary amine bonded sorbent (a strong anion exchange) employed for the isolation of acidic analytes from aqueous (or partially aqueous) samples. This sorbent maintains a permanent positive charge across the pH range; and also is not suitable for extraction of strong acids (since elution of analytes is done by eliminating the charge on the analyte (low pH)) (www.biotage.com).
- Oasis MAX is a mixed-mode, reversed-phase, strong anion polymeric sorbent. Oasis MAX is selective for acids and stable in organic solvents (www.waters.com).

4.11.1 *O-NAs in OSPW*

Figure 22 shows the efficiency of six tested SPE cartridges for extraction of O-NAs from OSPW. The average of the fluorescence intensities of the blanks (from eluents) was subtracted from the eluent for each type of SPE cartridge. Except LC-Diol, all SPE cartridges eliminated O-NAs almost completely (see O-NA concentration in filtrates). However, adsorbed O-NAs in Env+ cartridge were not fully recovered by the eluent. This fraction (less than 20%) remained in the cartridges and resulted in a lower mass of O-NAs in the eluent. A higher mass of O-NAs was detected in eluents from HLB, SAX, and MAX. It is believed that some compounds are released from sorbents and therefore caused false-higher NA concentration in the eluents. Release of impurities from HLB and SAX was also observed in eluents from blanks (negative controls, please refer to Appendix 6). The reason for false-higher NA concentration in eluents is unknown and more investigation is required to determine the type of impurities in the eluents. The best performance was observed for the C18 cartridge with full adsorption of O-NAs from OSPW and complete desorption of NAs in the eluent solution.

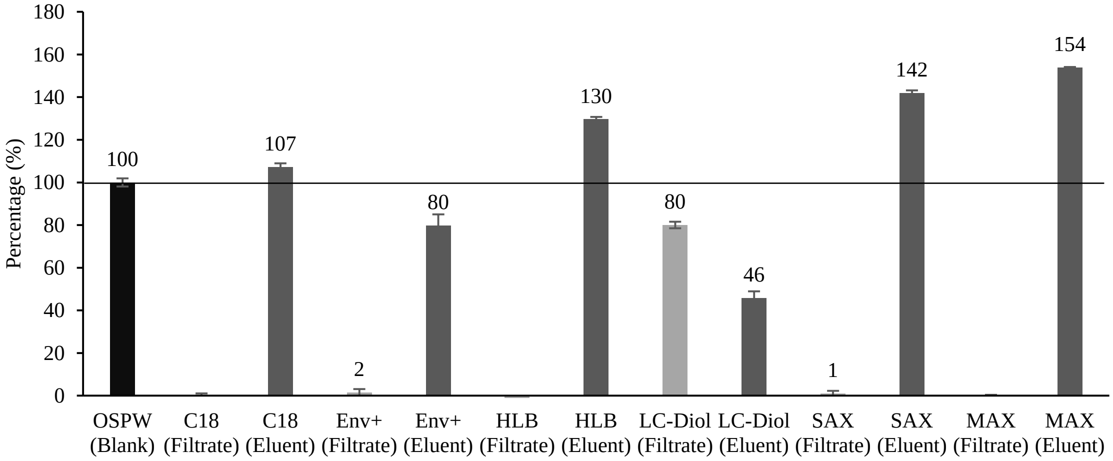


Figure 22.   Mass percentage of non-adsorbed O-NAs in OSPW (filtrate), and recovered O-NAs (eluent) for each SPE cartridge.
Horizontal line is 100% (control).

4.11.2 *M-NAs in Phosphate Buffer*

Figure 23 shows that most of the tested SPE cartridges, except LC-Diol and SAX, completely removed M-NAs from the phosphate buffer. The average of the fluorescence intensities of the blanks (from eluents) was subtracted from the eluent for each type of SPE cartridge. Similar to the OSPW results, low M-NA recovery was observed in the eluent for the Env+ cartridge. The best performance was observed with the C18 cartridge.

The percentage of M-NA recovery in eluents from HLB and MAX slightly exceeded 100%, which conforms to OSPW results. To detect and identify the unknown compounds released into the eluent, more research is needed, but it is believed that these compounds may possess aromatic or specific molecular structures which are detectable by fluorescence spectroscopy used for NA quantification.

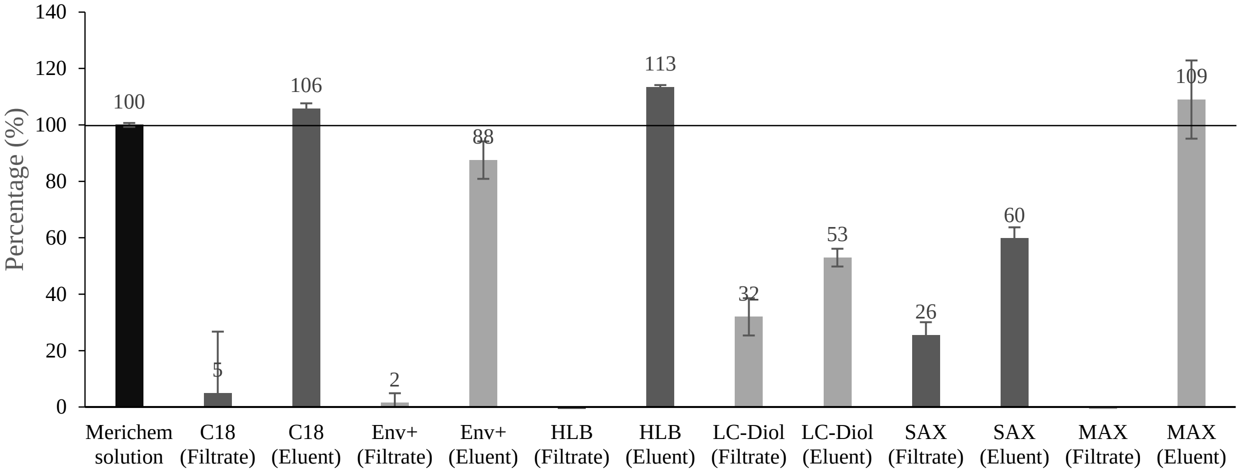


Figure 23. Mass percentage of non-adsorbed M-NAs from phosphate buffer (filtrate) and recovered M-NAs (eluent) for each SPE cartridge.
Horizontal line is 100% (control).

4.12 Industrial Resins and Adsorbent

One of the methods for NA isolation from OSPW and tailings pond water is adsorption by resins. NAs are responsible for extensive corrosion of equipment, they reduce the quality of bitumen recovered from oil sands ore, and they are toxic to the environment (Corinne 2010). In this research, three types of industrial resins along with polyethersulfone (PES) powder were assessed for NA adsorption. PES powder is not an industrial resin; however, it has been reported that membranes made of PES adsorb NAs efficiently (Martin 2014).

4.12.1 *O-NAs in OSPW*

Figure 24 shows the efficiency of O-NA removal from OSPW using tested industrial resins. The average of the fluorescence intensities of the blanks were subtracted from the Eluent for each type of resin. The lowest O-NA adsorption from OSPW was observed for the PES powder. Small particles of PES powder were seen in the filtrate and also the eluent, which may interfere with O-NA measurement. Since PES was a raw chemical power and was not designed to be used as a commercial resin for water and wastewater application, it is not suggested to be used in future investigations. All industrial resins demonstrated strong capacity for O-NA isolation, however, desorption of O-NAs in eluent was small for TAN-1 (7% recovered), and only 51% of adsorbed O-NAs was desorbed from PWA9. Similar to laboratory SPE cartridges, the amount of O-NA desorbed from L493 exceeded 100%, which indicates the possibility of release of compounds from this resin. To validate the quality of the eluent and presence of released compounds, more research is suggested to be conducted with L493 resins.

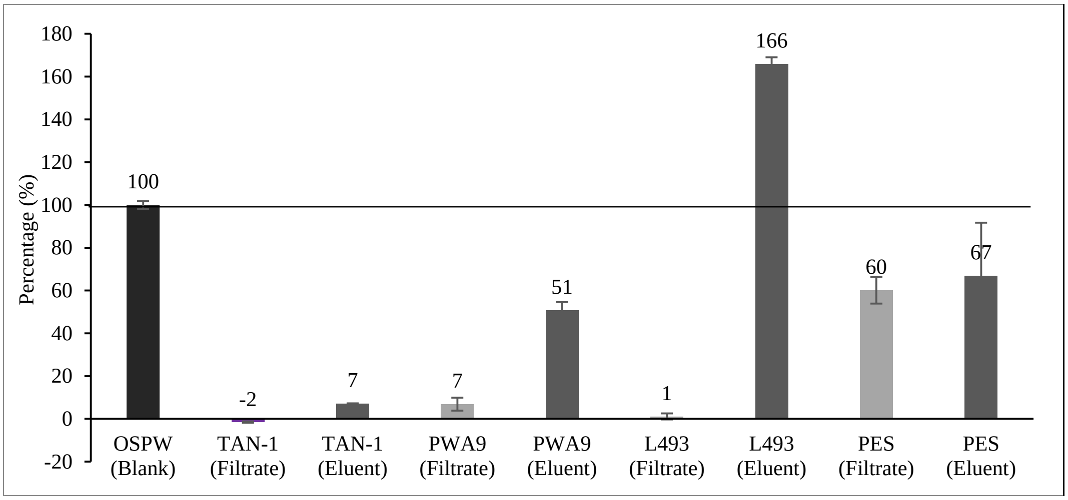


Figure 24. Mass percentage of non-adsorbed O-NAs in OSPW (filtrate), and recovered NAs (eluent) for each industrial resin.
Horizontal line is 100% (control).

4.12.2 *M-NAs in Phosphate Buffer*

Figure 25 illustrates that all industrial resins and PES powder adsorb M-NAs, however, the highest removal efficiency was observed for TAN-1 and L493 resins. Eluents from TAN-1 and PWA9 showed small desorption and recovery of M-NAs. The best performance in terms of M-NAs adsorption from phosphate buffer and desorption into eluent was found in L493. It is important to consider that the eluent for this resin was methanol, which might present other challenges when it comes to industrial applications.

For future investigations, we suggest that the efficiency of M-NA desorption from TAN-1 and PWA9 be optimized using various aqueous eluent solutions.

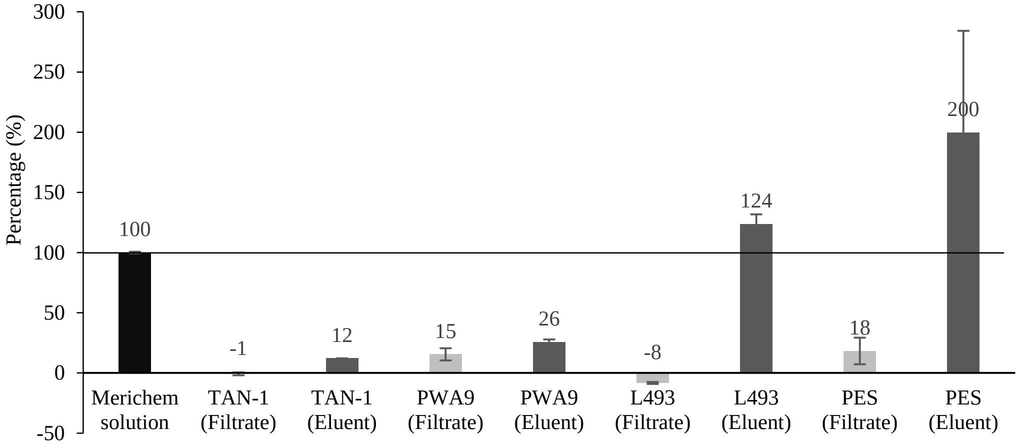


Figure 25. Mass percentage of non-adsorbed M-NAs in phosphate buffer (filtrate), and recovered M-NAs (eluent) for each industrial resin.
Horizontal line is 100% (control).

5 CONCLUSIONS

This set of investigations was conducted to elucidate the NA losses (from OSPW or Merichem) during sample handling and storage, preservation conditions, processing and sample preparation, and exposure to different types of radiation. Our observations indicated that 0.45 µm PVDF membrane resulted in minimum NA loss. In addition, methanol added prior to filtration with the PTFE membrane may increase the NA loss. The best syringe filter tested with minimum NA adsorption was GL microfiber. No significant difference between two methods of suspended solids separation, centrifugation (using glass tubes) and filtration (using 0.45 µm PVDF Membrane), was observed (P-value >0.05).

For storage and preservation of samples, various types of centrifuge tubes, soda-lime and borosilicate glass, and plastic bottles were assessed. Polystyrene centrifuge tubes performed slightly better than polypropylene for preservation of OSPW. However, for M-NAs in phosphate buffer, higher adsorption was observed in polystyrene centrifuge tubes compared with polypropylene. No significant difference was found among different types of soda-lime glass bottles; though, silanization slightly reduced the adsorption of O-NAs in OSPW, while silanization increased M-NA adsorption and adversely affected performance of this type of glass. In addition, no difference between amber and clear soda-lime glass bottles was observed for both NA types.

Storage in plastic bottles (HDPE, LDPE, and PP) showed no change in concentration of O-NAs in OSPW; however, adsorption of M-NAs was observed to some extent in all plastic bottles. The best preservation condition was storage in a fridge at 4^{o}C for both NAs. Addition of methanol, pH increase to 11.1 and reduction of pH to 2.1 adversely influenced the NA concentration and caused false-higher or lower results. PTFE and Tinfoil showed the best performance among four tested cap liners. White rubber and polyethylene liners are suspected to leach contaminants, which interfered with NA measurement and caused false-higher concentrations.

Microwaves did not influence NA concentration; however, a small reduction in M-NA concentration was observed after UV exposure.

In rotavapor experiments, the largest NA loss happened in DCM solvent. In aqueous samples, the highest O-NA loss was observed at low pH, but M-NA loss was independent of pH change.

Among tested SPE cartridges, C18 demonstrated the best performance in terms of NA adsorption from sample and NA desorption in eluent solution. In the industrial resins experiment, all three resins removed NA from the aqueous phase; however, the main challenge was desorption and recovery of NAs from resins. L493 showed the best performance, though more research is needed to determine the suitable eluent solution and to optimize the NA recovery.

All the results and conclusions in this investigation are based on fluorescence spectroscopy for the measurement of NAs, which mainly targets hydrocarbons with aromatic rings as an indicator of presence of NAs in solution. For future investigations, high resolution instruments for characterization and fingerprinting of NAs is suggested in order to have a better understanding of the partitioning of NAs.

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7 GLOSSARY

7.1 Terms

Analysis of Variance (ANOVA)

A statistical method to determine if there is significant difference among the means of treatments.

Axenic Culture

Axenic culture is a sample containing only and only one microorganism.

Blank

Blank, or a negative control, is a sample which experienced no treatment. This is the indicator of original sample.

Bottle Cap Liner

The liner is a layer of material which is located between cap and bottle, and inner-seals. The cap liner is the only part of the cap which is exposed to the stored solution, and therefore it plays an important role in the preservation.

Dimers

An oligomer consisting of two structurally similar monomers joined together.

Dissociation

The removal of a proton from a weak acid molecule, forming the conjugate base.

Eluent Solution

A solution which elute adsorbed compounds from a sorbent.

False-higher Detection

False-higher detection happens when the detected concentration of a compound is higher than its actual concentration. Instrumental error, experimental error, and methodological errors or interference of other materials with measurement may result in false-higher detection

Filtrate

The liquid sample after passing through a filter or SPE cartridge.

Micelles

The aggregation of surfactants (organic compounds with one side polar and another side non-polar) with the polar head in contact with surrounding water, and the non-polar side (or regions) in the micelle center.

Naphthenic Acids

International Union of Pure and Applied Chemistry (IUPAC) defines naphthenic acids as acids, chiefly monocarboxylic, derived from naphthenes. Naphthenes are primarily cycloalkanes especially cyclopentane, cyclohexane, and their alkyl derivatives.

Oligomer

A molecular complex that consists of a few monomer units, in contrast to a polymer, where the number of monomers is, in principle, not limited. Dimers and trimers are oligomers composed of two and three monomers, respectively.

pKa

A logarithmic constant of acid dissociation.

Polar Compound/Non-Polar Compound

A polar compound contains polar covalent bonds, i.e., electrons are not share equally between two bonding atoms. Polar compounds, including inorganic acids, bases, and salts, can ionize when dissolved or fused.

A non-polar compound occurs where there is an equal sharing of electrons between two different atoms. Non-polar compounds include fats, oil and petro/gasoline.

Silanization

Silanization is the process of covering of a glass surface with organofunctional alkoxysilane molecules.

Sorbent

A material (usually solid phase) which is able to adsorb a compound from fluid phase.

t-Test

A statistical method for comparison of the means of two treatments if the null hypothesis is supported. This method uses student's t distribution for analysis of data.

Tukey HSD (Honestly Significant Difference) analysis

A statistical method for comparison of the means of every treatment to the means of other treatments (multiple comparison procedure) using a studentized range distribution.

7.2 Acronyms

A.C.S grade	American Chemical Society grade
a.u.	Arbitrary Unit

COD	Chemical Oxygen Demand
DI water	Deionized Water
FTIR	Fourier-Transform Infrared
HPLC	High-Performance Liquid Chromatography
HPLC-MS	High-Performance Liquid Chromatography – Mass Spectrometry
LPE	Liquid Phase Extraction
OSPW	Oil Sands Process Water
OSRIN	Oil Sands Research and Information Network
SPE	Solid Phase Extraction
TSS	Total Suspended Solids
UV	Ultraviolet

7.3 Chemicals

DCM	Dichloromethane
HCl	Hydrochloric Acid
MeOH	Methanol
M-NAs	Merichem Naphthenic Acids
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide
NA / NAs	Naphthenic Acid / Naphthenic Acids
O-NAs	OSPW Naphthenic Acids

7.4 Container Materials

HDPE	High Density Polyethylene
LDPE	Low Density Polyethylene
PES	Polyethersulfone
PP	Polypropylene
PP-HP	Polypropylene High-Performance
PP-UHP	Polypropylene Ultra-High-Performance
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene Difluoride

APPENDIX 1: Pre-experiment to Validate 50% Phosphate-Methanol Solution

For many experiments in this research (such as paper membrane, syringe filter, bottle and cap adsorption tests, etc.), the adsorbed O-NAs needed to be quantified. To measure the O-NAs adsorbed, the surface needed to be rinsed using a suitable eluent solution. This eluent solution needed to be able to desorb and dissolve NAs adhered on the surface. In this experiment, two types of eluent solution were evaluated and compared: (1) 50% phosphate-methanol solution (containing 50% v/v of 0.05M phosphate buffer (pH = 8.5) and methanol), and (2) pure methanol. It is believed that a constant pH of 8.5 facilitates desorption of NAs from the surface, and it increases the solubility of O-NAs in 50% phosphate-methanol solution. In addition, 0.05M phosphate buffer provides a constant pH for O-NAs quantification, and this makes results from different samples comparable.

Experiment with Nylon Paper Membrane

Test procedure

1. The nylon filter was chosen for this experiment since it has a proven high capability of NA adsorption compared with other filters.
2. Nylon filters were rinsed with pure methanol and dried at room temperature (just for cleaning).
3. Each nylon filter was submerged in 50 mL filtered OSPW in a closed-cap 130 mL glass bottle with PTFE liner (similar to the paper membrane adsorption test, see section 4.1).
4. After 4 hours, three nylon filters were washed with pure methanol (5 mL) and three were washed with eluent solution. The whole spectra for each solution was plotted and compared.
5. NAs quantification in OSPW: The original filtered OSPW was mixed with Methanol (50% / 50%), scanned by fluorescence spectroscopy, and the spectra were plotted.
   Note: all the samples were triplicated.

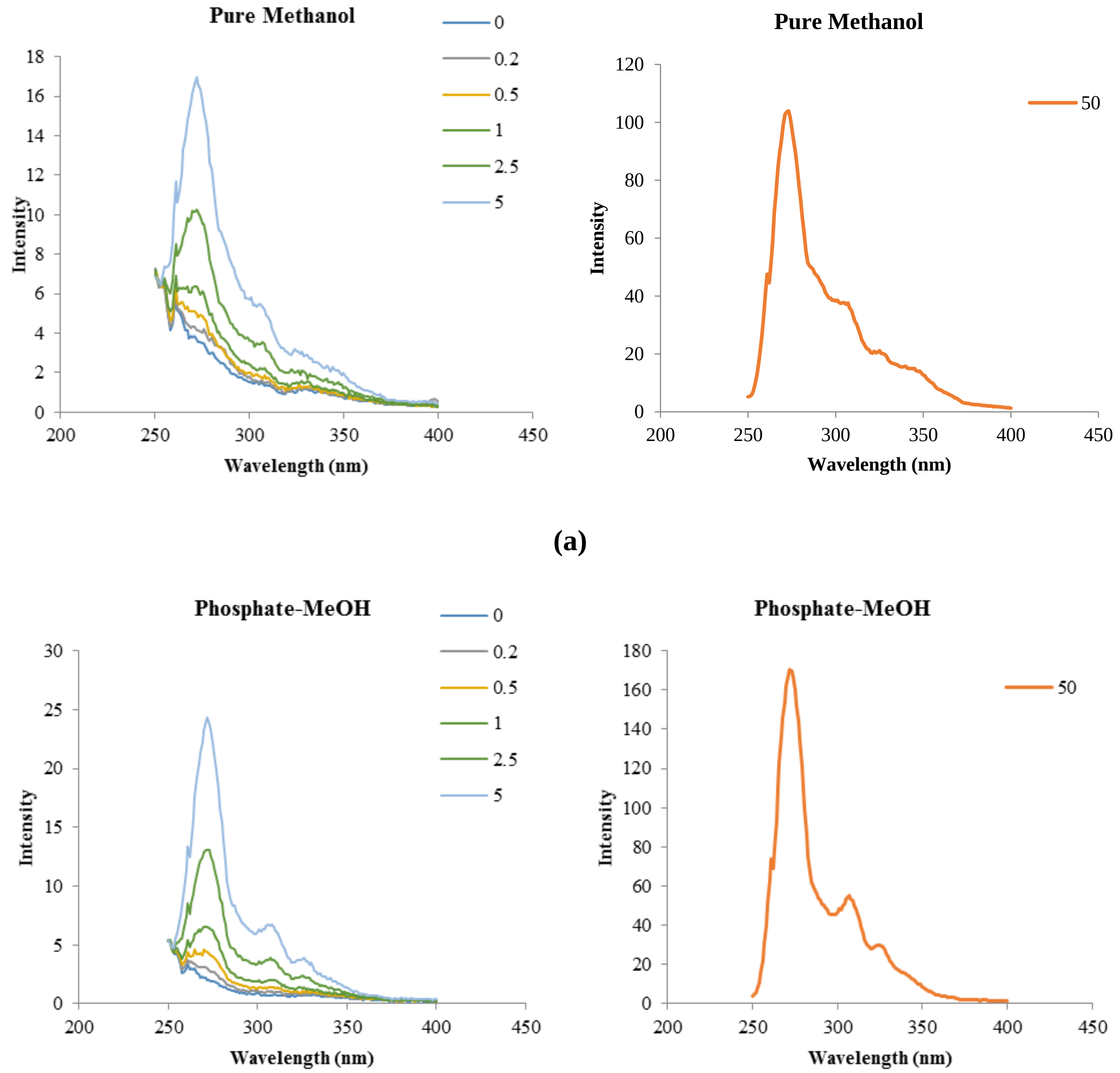
Low Concentration Standard Curve

Two standard curves for each eluent solution (pure methanol and 50% phosphate-methanol solutions) including the O-NA concentrations of 0, 0.2, 0.5, 1, 2.5, 5 mg/L and also a 50 mg/L (to see the graph at high concentration) were prepared.

Results and Discussion

Low Concentration Standard Curve

Figure A1 shows O-NA standard solutions in pure methanol and 50% phosphate-methanol solutions for low concentrations (0 to 5 mg/L) and high concentration 50 mg/L. In Figure A1(b), the O-NA peaks in 50% phosphate-methanol solution are sharper than methanol.



(a)

(b)

Figure A1. O-NA standard solutions (a) in pure methanol and (b) in 50% phosphate-methanol solution.

In addition, O-NAs in 50% phosphate-methanol solution have a higher maximum intensity than the methanol solution with the same O-NAs concentration. At a wavelength of 280 nm, the standard curve in 50% phosphate-methanol solution has a coefficient of determination (R^2) equal to 0.9999; but in pure methanol $R^2 = 0.9956$. From Figure A1, it can be concluded that a 50% phosphate-methanol solution containing various concentrations of O-NAs creates more consistent results and higher intensities which consequently gives better accuracy for O-NAs measurement.

Filter Adsorption Experiment

In this section, two eluent solutions of pure methanol and 50% phosphate-methanol solution are compared to ascertain their ability to desorb O-NAs from the surface of the nylon paper membrane. Figure A2 illustrates that the shape of the peaks from pure methanol differs from O-NAs in the 50% phosphate-methanol solution and OSPW. The common shape of O-NAs involves one large peak (around 280 nm) and one small peak (around 310 nm). The shape of peaks in pure methanol might be a result of the impact of methanol (which is a moderately strong solvent) on the surface of the nylon membrane and leakage of some compounds.

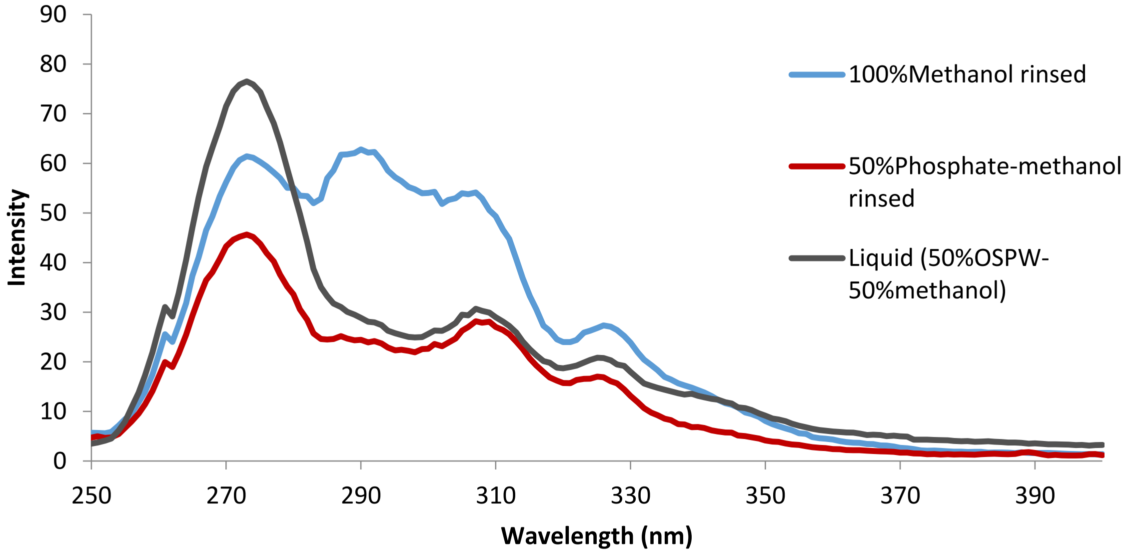


Figure A2. O-NA desorbed by pure methanol and 50% phosphate-methanol solutions, and comparison to O-NAs dissolved in OSPW.

According to Figure A2, there are several peaks in pure methanol, and choosing a clear wavelength as the indicator for NAs is not possible. However, unlike the methanol solution, 50% phosphate-methanol solution demonstrated a similar spectra shape to O-NAs in OSPW (50% OSPW / 50% methanol) as well as the standard curves. Since the O-NAs in the standard solution are relatively pure (the non-NA fraction was removed by dichloromethane at PH>10), it is believed that the phosphate buffer is more selective to desorption of O-NAs from the nylon filter. Methanol, which is a stronger solvent, is capable of removing more organic compounds; however, it may influence the surface of membranes, and some compounds may be released.

It needs to be understood that the fluorescence instrument is not targeting O-NAs (for example FTIR is targeting the carboxylic functional groups of O-NAs for measurement), but it just measuring mainly the aromatic rings, and all non-NA organic carbons with aromatic structures (like PAHs) may easily interfere with the fluorescence instrument. Therefore, it is desirable to use 50% phosphate-methanol solution which mostly desorbs O-NAs from the nylon filter. In addition, choosing a peak or wavelength as the indicator of O-NAs concentration is another challenge of using pure methanol.

Experiment with Polypropylene (PP) Bottles

Test Procedure

1. Six PP bottles (60 mL each) were washed according to the procedure explained in section 2.
2. 50 mL of filtered OSPW was added to each bottle.
3. After 3 hours of exposure time, OSPW was discarded.
4. The internal surface of each bottle (three out of six) was rinsed by pure methanol to a final volume of 5 mL.
5. Three remaining bottles were rinsed by 50% phosphate-methanol solution to a final volume of 5 mL.
6. Each eluent solution was scanned by fluorescence spectroscopy.

Results and Discussion

Figure A3 illustrates that the O-NA content in the 50% phosphate-methanol solution gives a peak larger than the O-NA content in methanol. Using the corresponding standard curves, the concentration of O-NAs was calculated to be 2.174 and 1.830 mg L^{-1} for 50% phosphate-methanol solution and methanol, respectively. The results indicate that both eluent solutions could desorb the O-NAs on the surface of the PP bottle, however, 50% phosphate-methanol solution performed slightly better. O-NAs will dissociate faster in the constant pH of 8.5 in the 50% phosphate-methanol solution, and this may improve the desorption capacity in this solution.

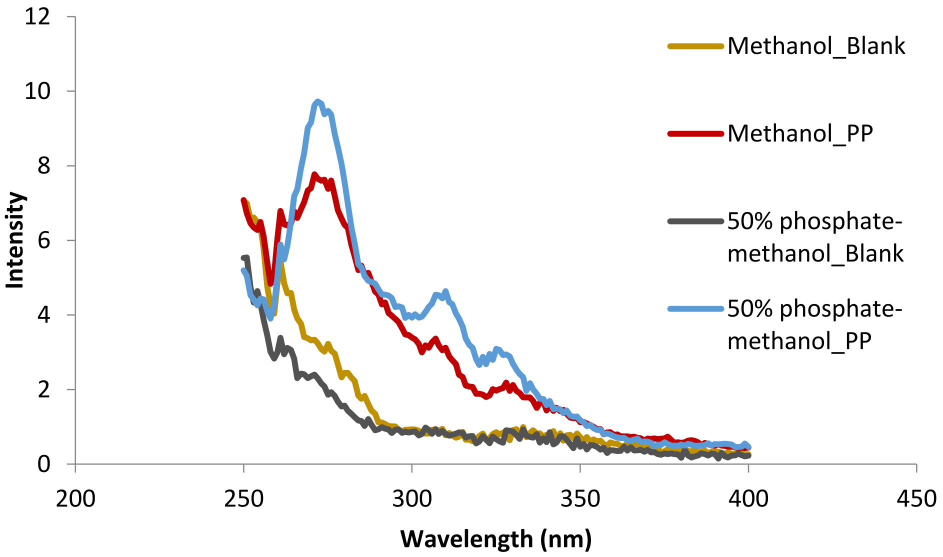


Figure A3. Desorption of O-NAs from the polypropylene bottles using two eluent solutions of 50% phosphate-methanol solution and pure methanol.

Conclusion

50% phosphate-methanol solution performed better and should be used for desorption of O-NAs from the surface.

APPENDIX 2: Adsorbed NA Percentages

Membrane	Polycarbonate	Nylon	Cellulose Acetate	PVDF	ME 25 ST	GL FBR	PTFE
Adsorbed O-NAs (%)	1.14	2.37	1.21	0.55	1.31	1.75	0.14
Adsorbed M-NAs (%)	3.42	1.92	2.05	0.91	2.66	1.78	0.41

Syringe Filter	Acrodisc	GL Microfiber	PTFE with PP Housing	PTFE
Adsorbed O-NAs (%)	2.86	0.47	3.81	12.56
Adsorbed M-NAs (%)	8.54	1.90	12.14	188.41

Centrifuge tube	PP-UHP (Room)	PP-UHP (Fridge)	PP-HP (Fridge)	PP-HP (Room)	PP-HP (Freezer)	Polystyrene (Fridge)	Polystyrene (Room)
Adsorbed O-NAs (%)	1.27	1.43	1.46	1.33	1.26	1.09	0.98
Adsorbed M-NAs (%)	0.62	0.70	0.52	0.57	0.54	1.95	1.53

Soda-Lime glass bottles	Clear (Fridge)	Clear silanized (Fridge)	Clear (Room)	Amber (Fridge)	Amber silanized (Fridge)	Amber (Room)
Adsorbed O-NAs (%)	0.36	0.18	0.41	0.34	0.20	0.39
Adsorbed M-NAs (%)	0.56	1.73	0.57	0.45	1.82	0.42

Plastic Bottles	HDPE (Room)	HDPE (Fridge)	PP (Room)	PP (Fridge)	LDPE (Room)	LDPE (Fridge)
Adsorbed O-NAs (%)	0.57	0.60	0.60	0.58	0.24	0.60
Adsorbed M-NAs (%)	0.18	0.23	0.19	0.26	0.16	0.19

Storage Conditions	Fridge	Light Room	MeOH 50% (Fridge)	Low pH (Fridge)	High pH (Fridge)	Silanized (Fridge)
Adsorbed O-NAs (%)	0.61	0.70	0.31	3.00	0.89	0.19
Adsorbed M-NAs (%)	0.39	0.45	0.24	1.79	0.40	1.58

APPENDIX 3: Filter Membrane Adsorption Test (images)

Figure A6. Filter membrane adsorption test (stationary phase experiment). Filter membranes were exposed to water sample in 125 mL glass (Type III soda-lime) bottles with black phenolic caps and PTFE/LDPE liner.

Figure A7. Filter membranes were rinsed by 50% phosphate-methanol solution (after NA adsorption) to the final volume of 5 mL. To do this, the filter membrane was transferred by forceps from glass bottles to glass funnel and rinsed. The solution was collected in 10 mL graduated cylinder and transferred quantitatively to 5 mL volumetric flask.

APPENDIX 4: Syringe Filter Adsorption Test (images)

Figure A8. A syringe filter.

Figure A9. Glass syringe and 5 mL volumetric flask used in syringe adsorption test.

APPENDIX 5: Solid Phase Extraction (SPE) Test (images)

Figure A10. Extraction Manifold for solid phase extraction (SPE) and industrial resins experiments.

APPENDIX 6: Negative Controls (blanks) in Solid Phase Extraction (SPE) and Industrial Resins Experiments

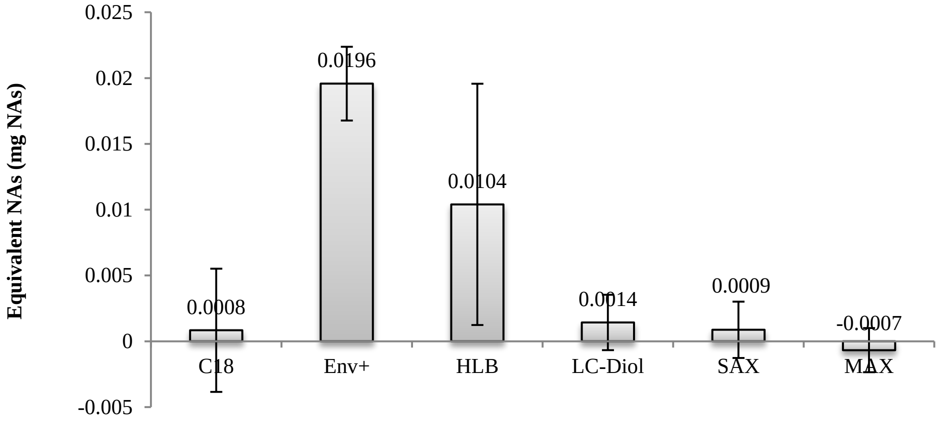


Figure A11. Negative controls in solid phase extraction (SPE) experiment.

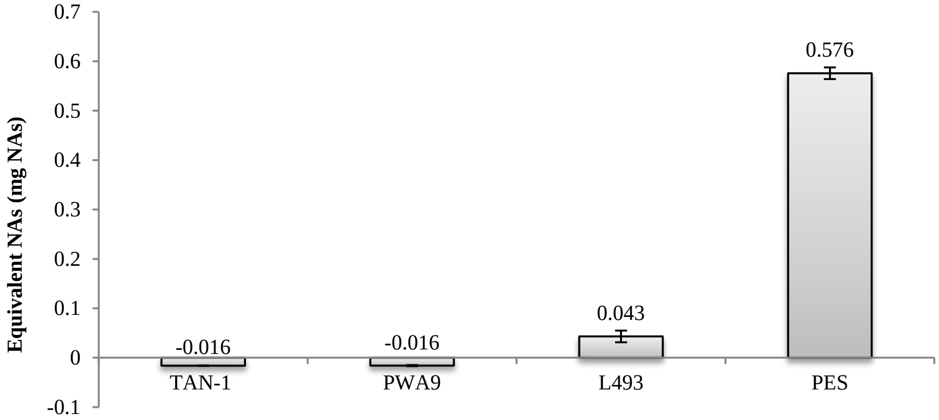


Figure A12. Negative controls in industrial resin experiment.

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