Impacts of pH on NSAID adsorption to microplastics in the North Pacific Gyre

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Abstract

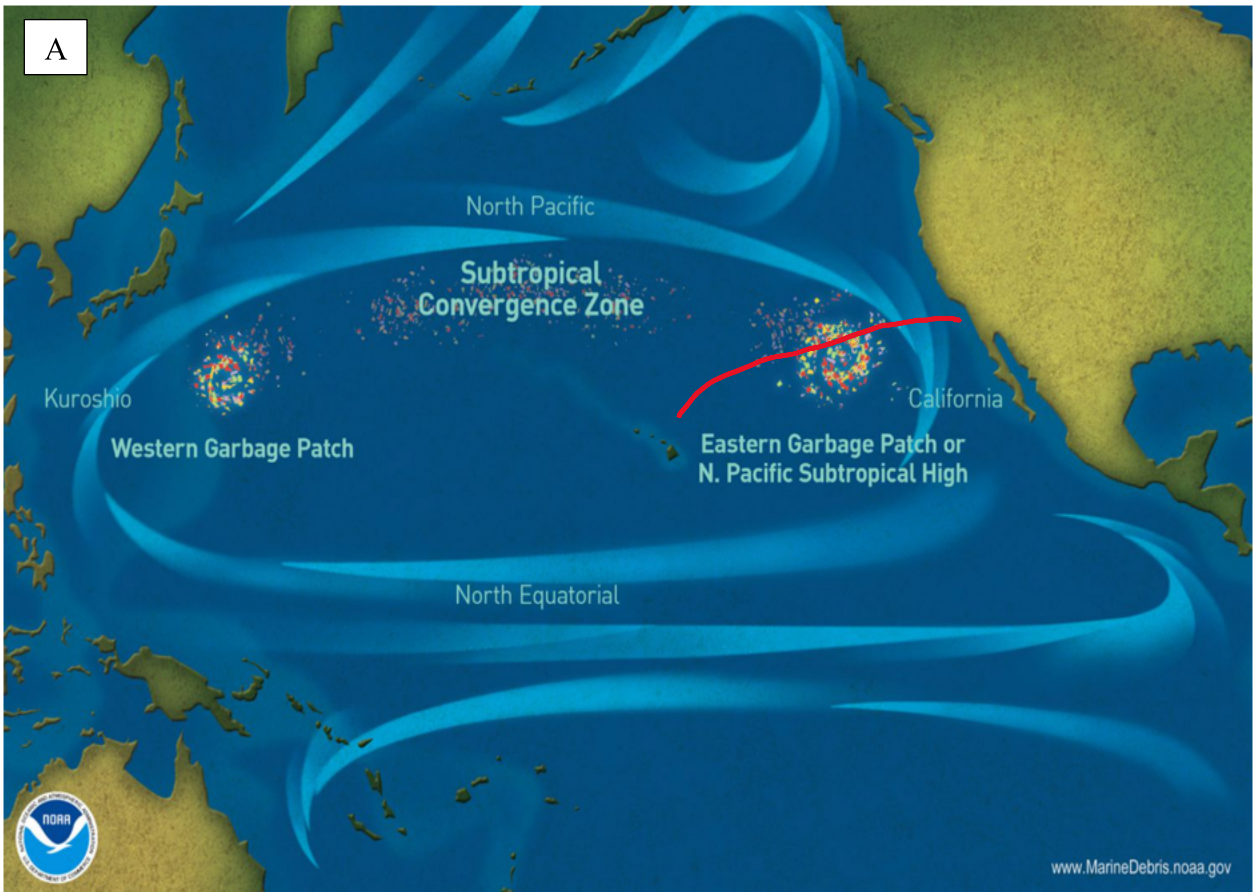
The adsorption of non-steroidal anti-inflammatory drugs (NSAIDs) to microplastics, and subsequent ingestion by marine organisms, has many detrimental impacts on the marine organisms, including behavioral changes, reproduction disruption, and mortality. In this study, I researched the impacts of pH on the capacity of NSAID adsorption to microplastics. I anticipated that lower pH levels would coincide with a higher adsorption capacity due to a more protonated microplastic surface area being present in low pH levels. To test this hypothesis, I added microfiber filters and NSAIDs to seawater samples from the North Pacific Gyre – a convergence zone of microplastics – allowed for adsorption to occur and analyzed the results via mass spectrometry. There was extremely strong and nearly irreversible adsorption between the NSAIDs and the plastics. Only the NSAIDs with the simplest chemical compounds, aspirin, Naproxen, and Ibuprofen, were found to desorb and only under highly acidic conditions (pH $\leq$4). This is important due to how, as NSAIDs travel through the food web, they may enter and desorb in the acidic (pH 1.5-3.5) digestive tract of large mammals.

Plain Language Summary

The relationship between pharmaceuticals, like aspirin or ibuprofen, and plastic pollution has become important because negative impacts on marine organisms have been discovered. Impacts such as changes in behavior and mating, or even death is known to occur when marine organisms ingest toxic chemicals. More acidic water (low pH) might lead to a stronger relationship between the pharmaceuticals and plastics, suggesting that as the ocean acidifies its pollutant load will change. To evaluate this, I mixed plastic and pharmaceutical pollution together in different seawater samples collected from the North Pacific Ocean, specifically in the region known as the Great Garbage Patch. After analyzing the samples using a mass spectrometer, it was discovered that the chemicals adsorbed virtually irreversibly to the plastics due to the strong chemical bonds between the pharmaceuticals and the plastics. This is important as is means the pharmaceutical pollution is extremely difficult to remove from the environment and could cause damage to mammals if they enter their digestive systems.

Introduction

The North Pacific Gyre, located between Hawaii and California, is a major convergence zone within the Pacific Ocean created by four currents flowing in a clockwise motion (National Geographic Society 2012). Within this convergence zone, plastics that are decades old have been discovered, creating the Great Garbage Patch (Egger et al. 2020). This study specifically takes place in the Eastern Pacific Garbage Patch (Figure 1).



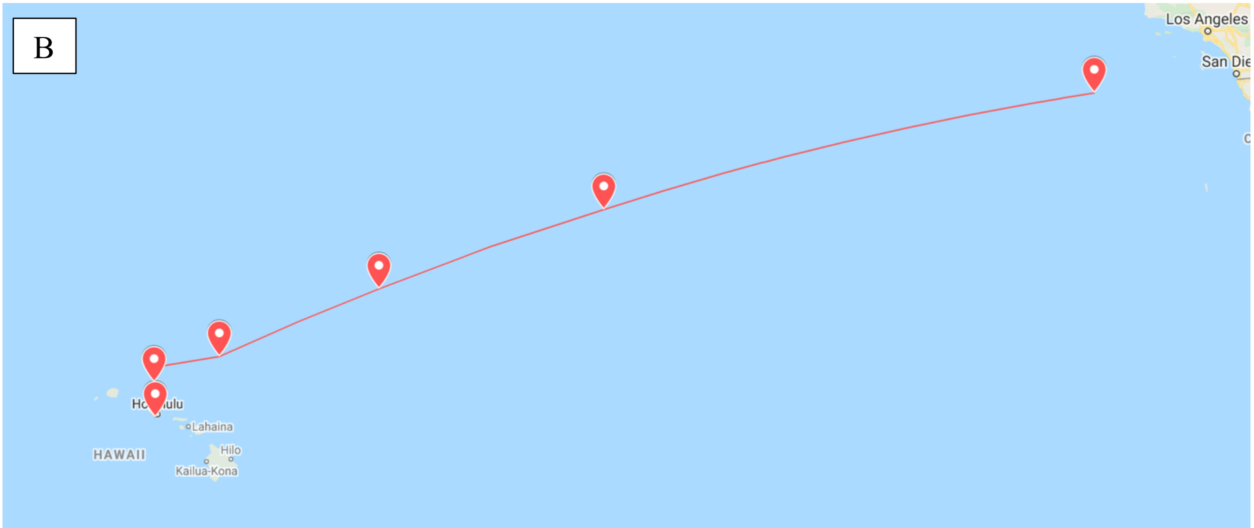


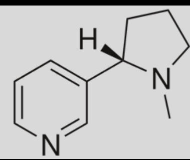
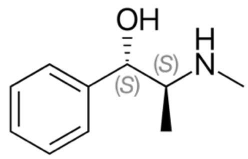
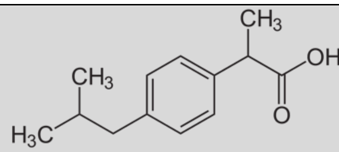
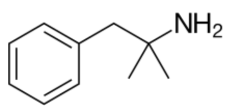
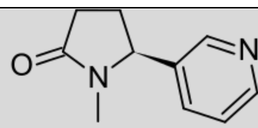
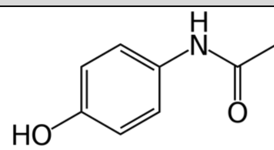
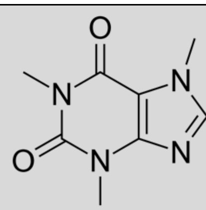
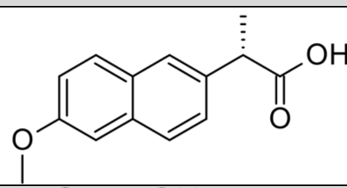
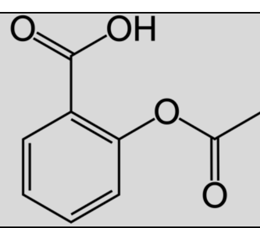
Figure 1A shows a map from the National Geographic Society (2012) displaying the largest garbage patches currently present in the Pacific Ocean. The red line in 1A represents the track the boat followed during the expedition. Figure 1B shows the stations data was collected from through the expedition between Hawaii and California.

The Eastern Pacific Garbage Patch is an ideal location for a study of microplastic pollution as the convergence zone has slowly been accumulating plastics as debris enters currents and becomes incorporated into the gyre (Boerger et al. 2010). In fact, an estimate by Eriksen et al. (2014) concluded that there are about 525 trillion plastic particles currently in the ocean, weighing about 268,940 tons – and 37.9% of this plastic is circulating within the North Pacific Gyre, or about 199 trillion plastic pieces weighing 96,400 tons.

Plastics have been discovered to be a major transporter of chemical pollutants into marine life due to the adsorption process of chemical pollutants onto the surface area of microplastics (Li, J et al. 2018, Li, S et al. 2018, Seidensticker et al. 2018, Wang et al. 2015, Wardrop et al. 2016, Wilkinson et al. 2017). This creates a problem as chemical pollutants have been discovered to negatively impact marine organisms in a variety of ways, including behavioral changes, reproduction disruption, metabolism disorders, tissue alterations, and mortality (Li, S et al. 2018, Ryan et al. 1988, Rzymski et al. 2017, Seidensticker et al. 2018, Wang et al. 2015, Wardrop et al. 2016, Yu et al. 2019). It has also been found that organisms at a higher trophic level are more exposed to risk as they ingest increased amounts of NSAIDs through food-chain transfer (Almeida et al. 2020). Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen, and naproxen, comprise 15% of the pharmaceutical pollution present in the ocean (Świacka et al. 2020), and commonly adsorb to microplastics (Elizalde-Velázquez et al. 2020, Li, J et al. 2018, Wilkinson et al. 2017). The NSAIDs focused on in this study, shown in Table 1, have similar chemical structures. Apart from caffeine, each comprise of a benzene ring and multiple other functional groups. Aspirin, naproxen, and ibuprofen are the simplest NSAIDs structures in this study, composed of only carbon (C), oxygen (O), and hydrogen (H) atoms.

Ocean acidification is a well-known issue in today's world. As CO_2 levels continue to rise, the ocean's assimilation of CO_2 also increases, resulting in the ocean's pH getting lower (Doney et al. 2008). In turn, this changes the chemical characteristics of the water, which has potential to impact many conditions. One change includes how, at low pH levels, plastic surfaces will tend to protonate in the water, leading to elevated adsorption capacities (Wang et al. 2015). According to Yu et al. 2019, "Compared with salinity, the effects of pH were seldom investigated. However, considering ocean acidification and sinking of microplastics, research on pH is necessary." All these factors are what led to the research question: Does the pH of seawater influence the adsorption capacity of NSAIDs onto microplastics?

Table 1 provides names, chemical formulas, and general uses / impacts of the NSAIDs studied in this experiment.

NSAID Name	Formula	Impacts on Human Body
Nicotine		Very addictive, causes increased blood pressure, narrow arteries, and increased heart rate
Pseudoephedrine		Used to relieve stuffy noses by narrowing the blood vessels in the nose. Can result in increased heart rate and blood pressure
Ibuprofen		Used to relieve inflammation, fever, and general pain by blocking body's production of substances that cause these.
Phentermine		Prescription drug used to lose weight by activating the body's fight or flight system and causing appetite loss.
Cotinine		Produced in body when exposed to nicotine. Currently being studied as possible treatment to depression and PTSD
Acetaminophen		Used to relieve pain and flu symptoms.
Caffeine		Stimulates the central nervous system to cause alertness and relieve drowsiness.
Naproxen		Used to relieve arthritis symptoms through hormone reduction.
Aspirin		Used to relieve fevers and mild pain by blocking body's production of substances that cause these.

Materials and Methods

Data for this experiment was collected on board the RV Thomas G. Thompson at five stations between Honolulu, Hawaii and San Diego, California between December 17th – 30th, 2021, shown in Figure 1B above. Prior to boarding the RV Thomas G. Thompson, necessary NSAIDs and microfiber filters were purchased from Millipore-Sigma and Fisher Scientific, respectively. A 1 mL interference mix (consisting of acetaminophen 1μg/mL, caffeine 1μg/mL, cotinine 1μg/mL, ibuprofen 1μg/mL, naproxen 1μg/mL, nicotine 1μg/mL, phentermine 100μg/mL, pseudoephedrine 100μg/mL) and 7.6mg of aspirin were purchased. Glass, nitrocellulose, Teflon, and polycarbonate microfiber filters, all with a 25mm diameter, were also purchased. Samples were created by threading one of each filter on a fishing line, which was then stored in combusted aluminum foil until use.

On the boat, the working standard was created. The inference mix and aspirin were mixed and measured together to create a 0.1μg/μL (equal to 1 part per thousand) working standard of NSAIDs. At each station, approximately 200mL of seawater was collected in sample jars via the CTD at each designated depth. The plastic samples were then hot glued to the lid of the sample jar. Next, 50μL of the working standard was injected into the seawater (creating a 2.5μg/L or 2.5 parts per million solution) and the jars were placed into a 9ºC fridge for one hour to allow for adsorption. After one hour, the samples were taken out and separated into 2mL tubes, one filter per tube. These tubes were placed in a -50ºC freezer until analysis in Seattle. This process was repeated at each station.

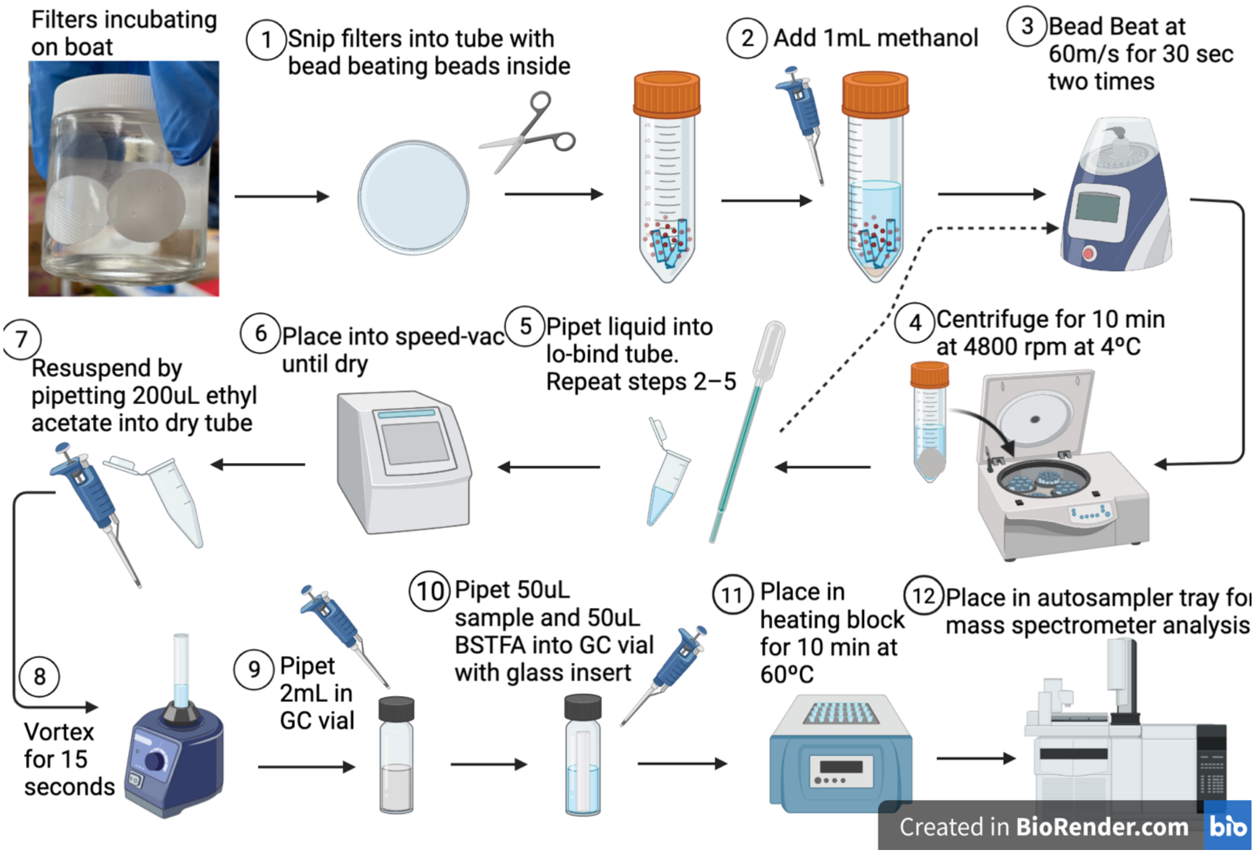


Figure 2 is a flowchart of the analysis steps taken in Seattle.

The analysis process is shown in Figure 2. It began with filling a 60mL syringe with ½ 100µm bead and ½ 400µm beads and filling 2mL tubes about 1/3 full of the beads, using one tube for each sample. The filters were then taken out of the freezer and allowed to thaw until malleable. Next, the filters were cut with scissors into small strips and placed into the bead tubes, using one filter per tube. The scissors were cleaned with methanol between each filter.

1 mL of Optima LCMS methanol was added to each bead beating tube before being placed into a MP Bio FastPrep homogenizer. The samples were run through the bead beater at 6m/s for 30 seconds twice before being placed into the centrifuge for 10 minutes at 4800 rpm at 4ºC. Using a glass pipet, the liquid was carefully removed from each tube and moved into a 2mL

lo-bind tube, careful to avoid extracting pieces of the filters. The samples were placed into the centrifuge a second time at the same settings and pipetted again.

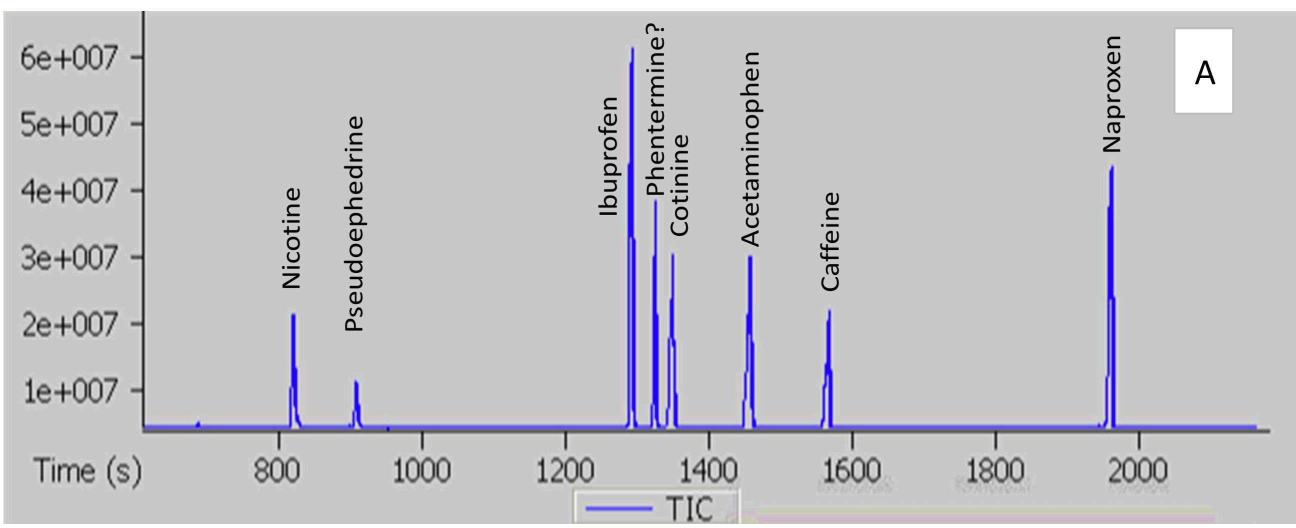
The lo-bind tubes were then placed into the speed-vac until dried, typically after 1-2 hours. Next, the samples were resuspended by pipetting 200µL of ethyl acetate into the dried sample tubes. The samples were vortexed for 10-15 seconds to ensure mixing before pipetting 2mL into a GC vial. In a different GC vial, a small tube was inserted before pipetting 50µL of BSTFA and 50µL of the sample. These vials were vortexed for a few seconds before being placed into a heating block for 10 minutes at 60ºC. Finally, the samples were placed into the autosampler tray and processed through the mass spectrometer.

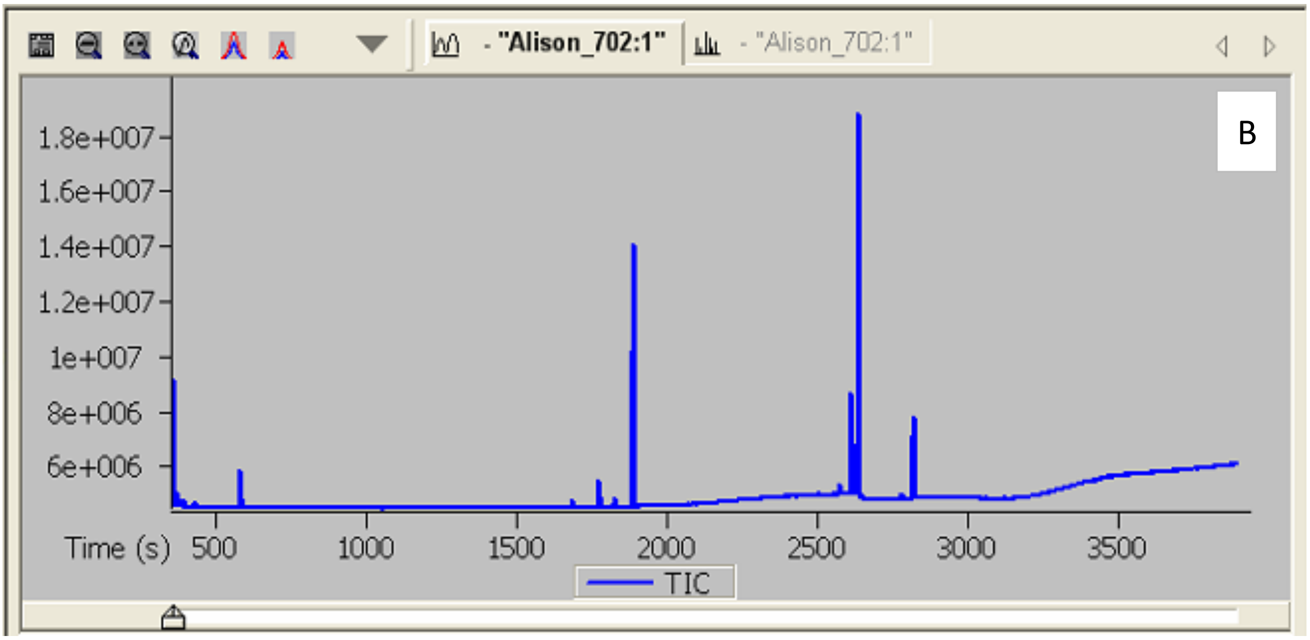
The mass spectrometry analytical methods performed were slightly modified from the methods used in Keil and Neibauer (2009). Samples were analyzed by gas chromatography mass spectrometry (GCMS) using an Agilent 6890 Gas Chromatograph fitted with a J&W DB-1MS analytical column (60 m length, 320 µm internal diameter, 0.25 µm film thickness). Helium was used as a carrier gas in the GC, and samples were detected using a LECO Time-of-Flight Mass Spectrometer. The NIST (National Institute of Standards and Technology) GCMS database and mass spectral output of the compounds were compared to identify the components in the samples.

A second experiment was conducted in Seattle to assist in understanding the data collected onboard the RV Thomas G. Thompson. For this experiment, 1/8 of a 25mm round glass, Teflon, and polycarbonate filter was added to five different 40mL Teflon vials. Pieces from a recovered buoy, rope, and large plastic piece from the research trip were also added, measured so that each element was the same surface area. 20mL of Eastern Tropical North Pacific seawater from 2000m, 40µL of aspirin, and 40µL of the interference mix were all added to each vial, creating a 2mg/L solution of NSAIDs in the water (1000x higher concentration than performed at sea). Acid was added to three of the samples, NaOH was added to one sample, and one sample was untouched, resulting in the following pH samples: 9.55, 7.88, 5.85, 3.28, 2.60. The samples were placed into a shaker and left at room temperature for five days (120x longer than the previous experiment, similar to the works of Elizalde-Velázquez (2020) and Wang (2015)). After the incubation period, samples were processed identically to previous samples, following the steps shown in Figure 2.

Results

The working standard of NSAIDs was processed in the gas chromatography - mass spectrometer (Figure 3A), confirming that each of the NSAIDs were injected into each sample. Aspirin was found to be desorbed from the glass and Teflon samples from Station 10 at 4,000m (Figure 3B and 3C). Data from the second experiment found that ibuprofen and naproxen were both desorbed from the recovered buoy from water samples with pH levels of 3 and 2 (Figure 3D and 3E, respectively).





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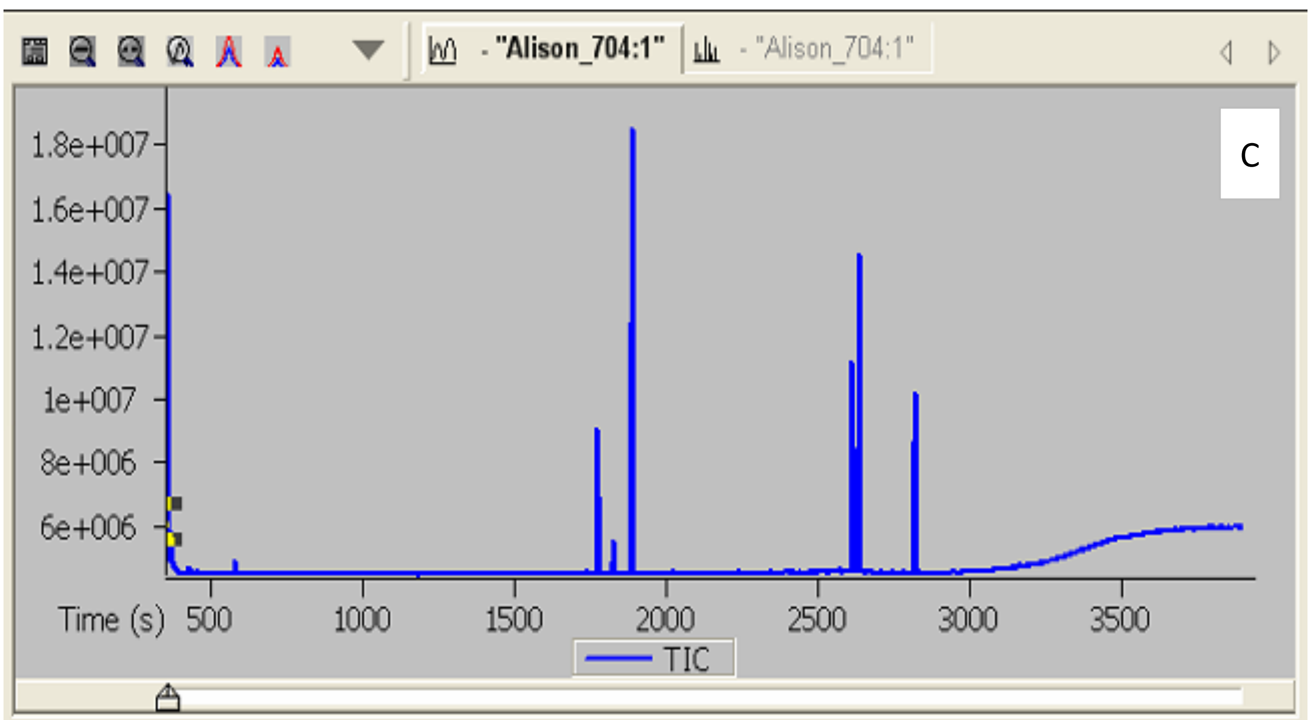

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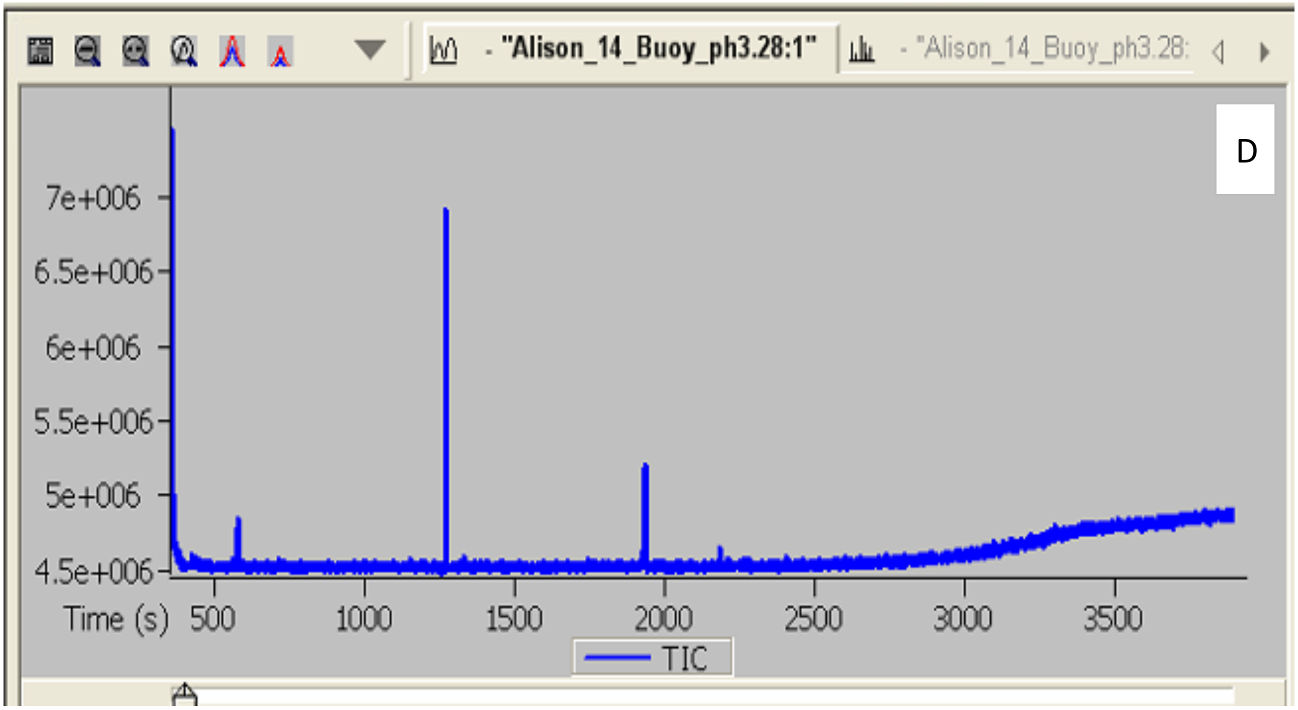

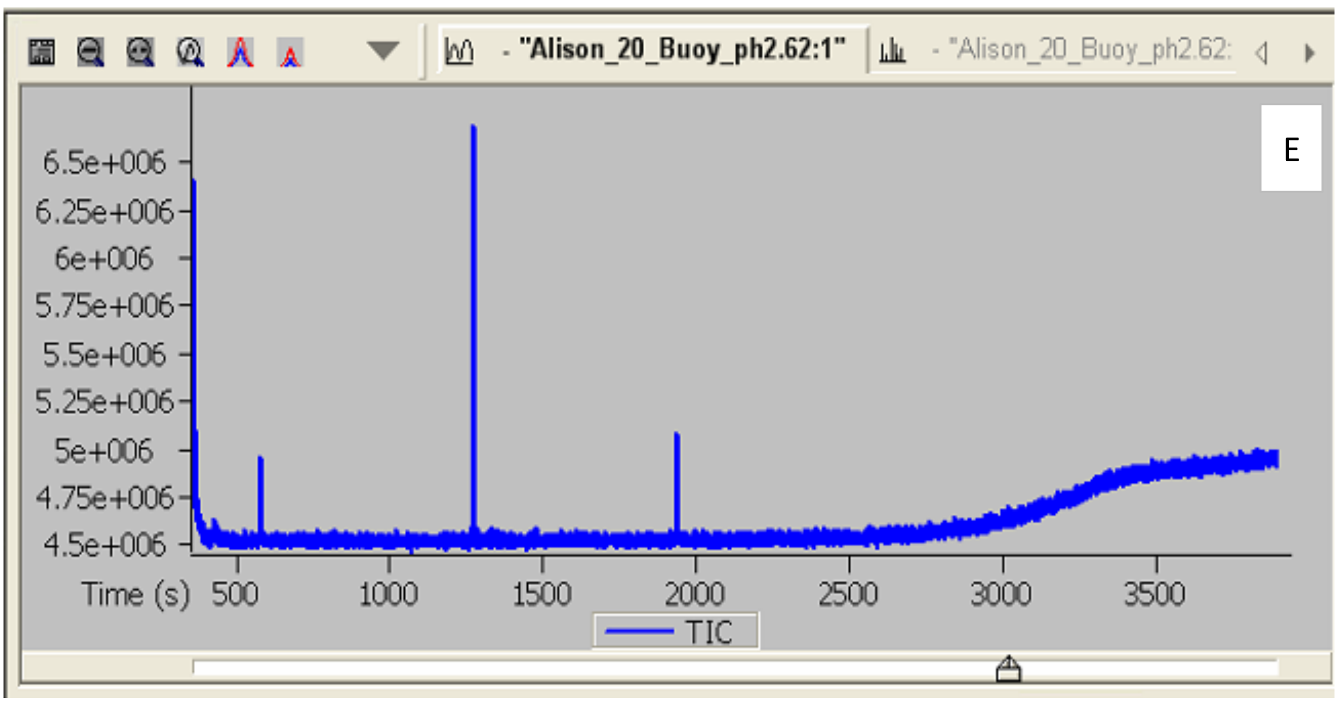

Figure 3 shows the chromatographs of the data where NSAID adsorption was observed. Panel A is the working standard that was injected into each sample, Panel B is glass collected at 4,000m from Station 10, Panel C is Teflon collected at 4,000m from Station 10, Panel D is the buoy at pH 3, and Panel E is the buoy at pH 2. A peak means that a non-steroidal was observed.

Table 2 provides the alternative steps that were taken after the first experiment yielded unexpected results. The results of these steps and their interpretations are also included in the table.

Steps	Result	Interpretation
Analyze NSAID working standard in GC	Standards were easily detected (Figure 3A)	NSAIDs were indeed added to the experiment.
Water samples from experiments analyzed by GC	No NSAIDs detected	NSAIDs are no longer in the solution, must have adsorbed to available surfaces. Experiment was conducted at a variety of natural pH's without a change in results, so adsorption was independent of ocean occurring pH's.
Desorbed in methanol and ran on GC	No NSAIDs detected, no desorption occurred	Adsorption to surfaces is stronger than desorption in methanol.
Reduced resuspension volume to better concentrate compounds and ran on GC	Virtually nothing observed – see discussion	Desorption was observed for aspirin on glass and Teflon at 4,000m and ibuprofen and naproxen at pH 2 and 3. Overall, adsorption is very strong.

Discussion

RV Thomas G. Thompson experiment

The results from data collected on the RV Thomas G. Thompson were unexpected and the apparent lack of data was concerning. The only NSAID desorption detected out of over 80 samples was aspirin from the glass and Teflon filters from Station 10 at 4,000m, discovered after reducing the resuspension volume of methanol (done to enhance detection limits to their maximum) or approximately parts-per-trillion levels. To ensure that the NSAID working standard was detectable by the GC, a sample was run, and it delivered the expected NSAID peaks (Figure 3A). At first, it simply seemed as if no adsorption occurred between the non-steroidal anti-inflammatory drugs and the plastic filters because no substrates were recovered. NSAIDs are known to be soluble in methanol (Sharifabadi et al. 2014), the solution used in the lab analysis steps, yet only very small amounts of the NSAIDs desorbed using this protocol (Figure 3A and 3B). This led to tests of the water samples from each station to evaluate if the NSAIDs simply did not adsorb to the plastics to being with. The water samples, however, found no trace of NSAIDs, leading to the conclusion that NSAIDs did adsorb to the surfaces in the container (Table 2), but did not desorb in methanol. This test also meant that adsorption was not dependent on the naturally occurring ocean pH's as each sample found no trace of NSAIDs in the water.

Seattle lab experiment

The second experiment was designed to induce desorption and deviated from the real-world environment of the previous study, by creating an altered environment for adsorption to occur, one similar in pH to the digestive system of a mammal (Elizalde-Velázquez et al. 2020, Wang et al. 2015). NSAIDs were injected at a concentration of 2 mg/L, a much higher concentration to the previous 0.1μg/μL injection. Samples were left to adsorb in varying pH solutions, with acidic options of pH levels 2 and 3 created to encourage adsorption and desorption. Samples were also left to incubate for five days opposed to one hour and were at room temperature instead of the 9ºC fridge. In this experiment, naproxen and ibuprofen were observed on the recovered buoy in both the pH 2 and pH 3 samples, corresponding with hypothesis of desorption occurring in more acidic environments, but still lower amounts of desorption than expected. Water samples from this experiment also did not indicate the presence of NSAIDs, proving that the adsorption of NSAIDs is strong and largely irreversible.

Further discussion of results

NSAIDs are known to be incredibly "sticky" and adsorb to objects quickly; however, it is difficult to separate them from the substance once adsorption has occurred (Zhang et al. 2017). In fact, loaded surfaces, such as a plastic filter covered in NSAIDs, desorbing the non-steroidals is nearly impossible (Oba et al. 2021). This is due to the chemical bonds that form between the NSAIDs and plastic, a connection that the presence of methanol cannot break. When comparing the chemical structures of the NSAIDs in Table 1, the three most simple compounds – and the only compounds made purely of C, O, and H – are aspirin, naproxen, and ibuprofen. These are also the only NSAIDs found to have desorbed in this experiment, meaning the relative simplicity of the structures resulted in weaker bonds that methanol was able to break.

The implications of NSAIDs adsorbing to substances and not desorbing are many. Since the adsorption bond of NSAIDs is so strong, it is incredibly difficult to remove NSAIDs from the environment once they have entered. While this means their immediate impact may not be visible, the substances the NSAIDs have adsorbed to are still present in the environment. As these substances move through the food web, the NSAIDs will eventually enter the digestive tract of larger mammals – the acidic environment in which desorption might occur and inflict the negative impacts of NSAIDs onto the organism (Almeida et al. 2020).

Future research about NSAID adsorption and desorption to plastic in the marine environment should focus on efficient ways to remove the pharmaceutical pollution from the environment, such as through stronger solutions than methanol. Additionally, research into which plastics NSAIDs adsorb the most should be encouraged as plastic pollution in the ocean undoubtedly increases.

Conclusion

Overall, the introduction of pharmaceutical pollution and its adsorption to plastics in the aquatic environment can induce many negative impacts on marine organisms such as mating and behavioral changes. This study found that NSAID adsorption is independent of pH in naturally occurring ocean pH’s and that the bond between NSAIDs and plastics is incredibly strong, making desorption difficult. Desorption may occur in extremely acidic conditions, creating a threat to mammals as NSAIDs travel through the food web and enter their digestive tracts. Future research should be done regarding efficient methods to desorb NSAIDs from substances and ultimately remove them from the environment.

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