The Effect of Extraction Methodology on the Recovery and Distribution of Naphthenic Acids of oilfield Produced Water

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Abstract

Comprehensive chemical characterization of naphthenic acids (NAs) in oilfield produced water is a challenging task due to the sample complexity. Additionally, the sample preparation steps may have a negative effect on the explored chemical space of NAs. In this study, we evaluated the effect of extraction method on the chemical space, relative recovery, and the distribution of NAs in a produced water sample. We employed three generic and pre-established extraction methods (i.e. liquid-liquid extraction (Lq), and solid phase extraction using HLB cartridges (HLB), and the combination of ENV+ and C8 (ENV) cartridges) for our evaluation. The ENV method produced the largest

number of detected NAs of 134 out of 181 in that sample whereas the HLB and Lq methods produced 108 and 91 positive detections, respectively. For the relative recoveries, the ENV performed better than the other two methods. The uni-variate and multi-variate statistical analysis of our results indicated that the ENV and Lq methods were explained most of the variance observed in our data. When looking at the distribution of NAs in our sample the ENV method appeared to provide a more complete picture of the chemical diversity of NAs in that sample. Finally, the results are further discussed.

Introduction

Naphthenic acids (NAs) are naturally occurring compounds in petroleum, with a highly variable composition depending on the source of the oil.[1] The concentration of NAs in petroleum can range from non-detect to 3% by weight.[8] NAs constitute a complex mixture of chemicals, due to the multiple possible chemical structures (i.e. structural isomers) for the same chemical formula. For example for an NA with the formula of $C_{10}H_{18}O_2$, assuming 6 component rings, there are more than 37 isomers. A lot of these isomers have similar structure and thus similar chemical and physical properties. Therefore, mixture of NAs becomes an extremely challenging matrix to resolve and characterize.[8] As a consequence, the composition of NAs in a complex matrix such as oilfield produced water (PW) is unknown.

Oil production PW is one of the largest streams of industrial treated wastewater in the world.[?] PW is an unresolved complex mixture and consists of a wide variety of chemicals from metals to organic pollutants, including NAs.[? ? ? ? ?] Moreover, multiple studies have reported that the NAs are one of the toxic components of the oilfield PW to a variety of organisms.[8,16? ?] However, little is known about their chemical composition as well as their environmental fate and behavior. Therefore, a better understanding of the chemical composition of the NAs in the oilfield PW is warranted.

The chemical characterization of NAs in the PW is typically performed on the acidic fraction of the total extract of PW.[8,16? ?] This approach is utilized to tackle the sample complexity provided by both the NAs and PW.[8] However, these sample manipulations may cause undesired effects on the final extracts, specially when dealing with such complex mixtures. For example, in our previous study we demonstrated that the choice of the extraction procedure changes the explored chemical space of the sample.[3] However, to our knowledge there has not been any published work that evaluated the effect of extraction procedure on the composition of NAs in the PW.

In this study, we evaluated the effect of extraction procedure on the explored chemical space, the recovery, and the distribution of NAs in the PW. We employed three generic extraction methods a liquid-liquid extraction method and two solid phase extraction (SPE) approaches. The extracts were analyzed as such (i.e. no fractionation) via liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS), which was essential to accurate identification of NAs in the PW samples.[?]

Methods

Sample Preparation and the Experimental Design

A sample of PW (total volume of 5 L) was obtained from an oil platform in the Halten bank off coast of mid-Norway in February 2017.[2] The sample was divided into 9 parts, each of 400 mL. These samples were extracted using three generic extraction methods: liquid-liquid extraction (Lq); HLB cartridges, here referred to as HLB; and the combination of C8 and ENV+ cartridges, which we refer to as ENV. The details of the extraction procedure for all three methods are provided elsewhere.[3] In short, the Lq method was the dichloromethane (DCM) extract of the acidified PW, repeated three times, with a final volume of 2 mL. For the

solid phase extraction methods (SPE), both cartridges were conditioned with a combination of methanol and water as recommended by the vendors. The preconditioned cartridges then were loaded with 400 mL of PW using a vacuum pump. These, then, were eluted with two times the volume of the cartridges employing a mixture of hexane, DCM, and 2-propanol. The final extracts of 2 mL were stored in freezer until the analysis. This combination of eluents was previously shown to be effective for extraction of analytes with a wide range of chemical and physical properties in complex samples.[3]

Three procedural blanks were generated for each extraction method. For Lq method, these blanks were the extract of the glassware using a mixture of DCM and a 1N solution of HCl. Regarding the SPE methods, the blanks were the extracts of the preconditioned cartridges with the same solvent mixture used for extraction of the samples.

The final extracts, including the blanks, were spiked with 100 ng of diazepam-D5 as the injection standard for monitoring the instrument performance during the analysis. The detailed list of chemicals and suppliers are provided in the Supporting Information, section S1.

Instrumental Conditions and Analysis

Seven μL of each extract was injected into a Waters Acquity UPLC system (Waters Milford, MA, USA) equipped with UPLC HSS C18 column (2.1×150 mm, particle size 1.8 mm) (Waters, Milford, MA, USA). More details regarding the chromatographic method is provided in the Supporting Information, section S2.

The UPLC system was coupled to an Xevo G2-S Q-TOF-MS (Waters Milford, MA, US) time of flight high resolution mass spectrometer. The Mass spectrometer was operated with a nominal mass resolution of 35,000 and a sampling frequency of 2.3 Hz. This system was

equipped with electron spray ionization source (ESI) operated in negative mode. During each cycle the mass spectrometer acquired a full-scan spectrum between 60 Da and 600 Da employing a collision energy of 6 eV.

All the samples including the blanks and quality control/assurance were analyzed using the above instrumental conditions.

Quality Control/Assurance (QC)

For the purpose of QC, all the glassware used in this study were baked at 450°C overnight. The samples were divided into sets of three extracts, which were followed by a solvent injection to avoid the carryover from previous injections. Additionally, the signal of the injection standard (i.e. diazepam-D5) was monitored in order to assess the stability of the instrument during the analyses. We observed less than 20% variability in the signal of the injection standard. Therefore, we interpreted that the chromatograms were adequate for our data processing workflow without any pre-processing.

Data Processing Workflow

All the chromatograms, including the samples and blanks, went through the following data processing steps sequentially. The acquired chromatograms were converted to an open MS format (i.e. netCDF) employing DataBridge provided via MassLynx (Waters, Milford, the US). The converted data were imported into the Matlab[4] environment (Matlab R2015b) for further processing. The imported data were mass calibrated prior to be evaluated for the NAs. The details of the mass calibration are reported elsewhere.[5–7] In short, for the mass calibration, the measured mass of the calibrant injected into the source in 20 S intervals were compared to the exact mass of the same compound. The observed mass errors were used to calculate the needed mass shift over the whole chromatogram using a third order polynomial. The estimated mass shift then was applied to the data in order to produce the

calibrated chromatograms. The mass calibrated data were used for the identification and signal extraction of NAs.

Identification and Signal Extraction

In order to identify the NAs in our samples, a list of NAs using their general formula (i.e. $C_nH_{2n-z}O_2$) was generated. In this list the number of carbons (i.e. n) ranged between 8 to 35 while the number of rings ranged from zero to 6 (i.e. z= 0 : -2 : -20). This range was selected based on the previously reported analyzable range of NAs via LC-HRMS.[8] In addition to these conventional NAs, we added several sulfur containing NAs based on the literature reports.[9] This resulted in a total of 181 NAs to be screened for in the samples (Table S1). For the identification of NAs, we generated the extracted ion chromatogram (XIC) of each NA in the list, employing a mass accuracy of $\pm$ 3 mDa. This mass window was selected based on the observed mass resolution measured using the signal of the calibrant. The generated XICs were integrated over the whole chromatogram to produce the signal specific to each NA in the list. This procedure was carried out for all the calibrated chromatograms including the blanks. The signal of each NA after the blank subtraction was used for the comparison of the performance of the three extraction methods employed in this study. During the identification, we performed a noise removal step which consisted of elimination of the NAs that produced a signal smaller than 500 counts and the NAs that were detected only in one out of three replicates. These eliminated NAs were considered non-detects for that method. This approach enabled us to accurately detect the tested NAs and compare the three extraction methods investigated in this study.

Relative Recovery Calculations

We calculated the relative recovery of each NA using the approach proposed by Samanipour et al.[3] Each NA, in this study, resulted in 9 cumulative signal values (i.e. the integrated XIC for each extract 3 methods $\times$ 3 replicates) generated via three different extraction methods.

The largest method averaged cumulative signal was considered the total extractable material for that NA. Therefore, the recovery of each NA was calculated based on its signal from each extract divided by the total extractable material for that NA. Using this approach we were able to evaluate the performance of different extraction methods for each NA.

Statistical Analysis

In order to further evaluate the performance of the three extraction methods, we performed both uni-variate and multi-variate statistical analysis. For the uni-variate test, we employed the non-parametric test Kruskal-Wallis.[10] A $\rho < 0.05$ was selected as the threshold for the rejection of null-hypothesis with 95% confidence interval. With regards to multi-variate test, principal component analysis (PCA) was used in this investigation.[11] Prior to our PCA analysis our data was scaled utilizing Pareto scaling.[12] This approach has shown to be effective in keeping the data structure intact while reducing the importance of large signals. For the PCA, the singular value decomposition (SVD) was employed in order to isolate the statistically relevant components.[13] This algorithm (i.e. SVD) is effective in dealing with datasets where the number of variables is larger than the number of observations. This procedure was previously shown to be effective in separating different extraction methods from each other while isolating the variables that were causing the separation.[14]

Results and Discussions

Detection of NAs

The ENV method with 134 positive detections out of 181 total tested NAs, performed the best, when looking at the number of positively detected NAs in the samples via different extraction methods. The HLB and Lq methods resulted in positive detection of 108 and 81 NAs, respectively (Fig. 1). We further examined the effect of the number of rings and the number of carbons on the detection frequency of NAs produced via each extraction method.

The ENV method systematically produced larger detection frequencies for all 7 z values when compared to the other two methods , Fig. 1. The largest detection frequency for both ENV and HLB was observed for NAs with a z value of -4 (i.e. 2 rings) with positive detection of 23 and 19 NAs, respectively. On the other hand, the Lq method showed to be unaffected by the number of rings in terms of the detection frequency resulting in an average of 11 NAs detected for all seven cases. The non-parametric Kruskal-Wallis test [10] results (i.e. $\rho < 0.05$) indicated that the differences observed in the detection frequencies versus the ring number were statistically significant. Further examination of these results suggested that the two SPE methods performed in a similar way whereas the Lq method appeared to be different from those two. Overall, all three methods covered a range of NAs from aliphatic chains (i.e. z=0) up to 6 rings (i.e. z=-12) while all three methods were unable to detect NAs with larger number of rings, thus z values between -14 and -20. Moreover, none of the methods detected the sulfur containing NAs, which may suggest their absence and/or lower than limit of detection concentrations in the analyzed sample.

For the effect of the number of carbons on the detection frequency of NAs, the ENV method covered all n values ranging from 8 to 35, Fig. 1. The HLB method produced zero positive detections for n values of 8 and 25 while the Lq method was limited in an n value range of 9-29. The ENV method resulted in the largest detection frequency of NAs for 20 out of 27 n values across the tested range. For cases where Lq method was the best performing approach with n values of 11, 12, 15, and 17, the mentioned NAs appeared to be aliphatic NAs. Moreover, they all were removed during the noise removal (i.e. their signal was smaller than 500 counts). For the remaining three cases with n values of 28, 29, and 34, HLB method performed better than ENV extraction method. For these cases, the missing NAs were: a one ring NA for the n value of 28, a two ring NA for the n value of 29, and finally, a five ring NA for the n of 34. Also for these cases, the noise removal step caused the elimination of these

NAs from the detection list of ENV. Based on the fact that all these discrepancy cases where generated during the noise removal step, we interpreted that the sample complexity/matrix effect was the main cause of these observations. Finally, we preformed the non-parametric Kruskal-Wallis test to evaluate the trend observed in the detection frequency versus the n values. The $\rho < 0.05$ of this test suggested a statistically significant difference between the methods. Further investigation in the outcome of this statistical test showed the similarity of the SPE methods when compared to the Lq method.

Overall, the ENV method appeared to perform the best by extracting the largest number of NAs across all the z values and n values. Additionally, this method showed a consistent performance when looking at the z and n values compared to the other two methods (i.e. HLB and Lq).

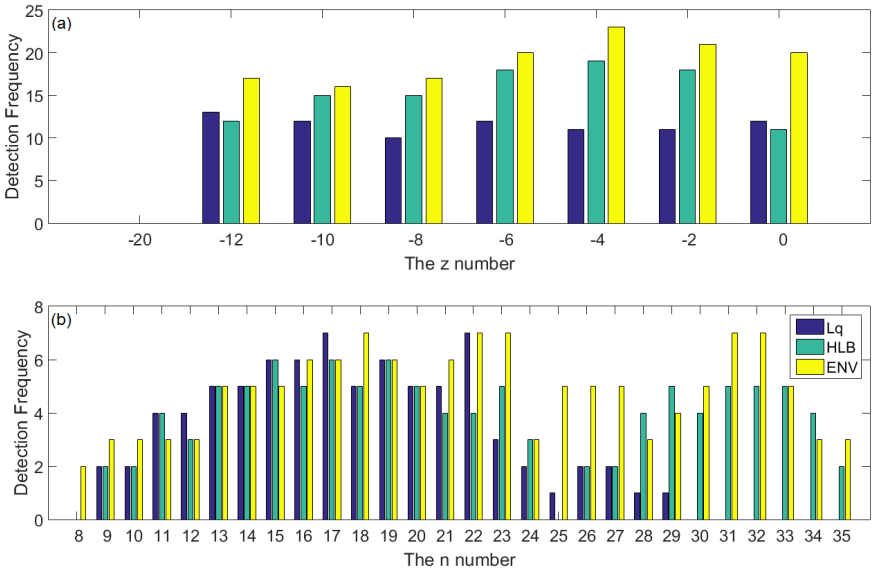

Figure 1: showing the detection frequency of NAs versus (a) the z value (i.e. the number of aliphatic rings) and (b) the n number (i.e. the number of carbons).

Extraction Recoveries

The ENV method resulted in an average relative recovery of 49.6 % across all the tested NAs whereas HLB and Lq produced average relative recoveries of 44.7% and 42.1%, respectively. We also evaluated the recoveries of the NAs for each method based on the number of carbons and the number of rings.

For the aliphatic NAs (i.e. z=0), the Lq method performed better than the other two methods resulting in 100% relative recoveries for 12 out of 27 NAs, Fig. 2. The other two methods (i.e. HLB and ENV) produced a larger level of variability in the relative extraction recoveries across the analyzed NAs, ranging from non-detect for n=12 and 17 to 100% for n larger than 29. However, the ENV method was the only method that extracted the largest number of NAs compared to the other two methods. Additionally, this method showed to be successful in capturing the smallest and the largest NAs in this group. For small NAs with n ranging from 8 to 10 both HLB and Lq resulted in zero recoveries, which was attributed to the low affinity of these NAs for HLB resin and DCM. However, further structural elucidation is necessary to confirm this hypothesis. On the other hand, for NAs having n values larger than 22, the two SPE methods were able to isolate those NAs while the Lq failed in this task. This trend was associated with the lower solubility of larger NAs in DCM. However, in this case also further structural elucidation is necessary to confirm this hypothesis. For NAs with z values between -2 and -10 (i.e. 1 to 5 rings), the ENV method systematically produced higher relative recoveries compared to the other two methods, Fig. 2, S1, S2, S3, and S4. Among these cases, for z values of -2, -4, and -6 both ENV and Lq preformed better than HLB in extracting smaller NAs. However, for NAs with n values larger than 22 the two SPE methods perform better both in terms of number of detected NAs and the relative recovery of individual NAs. Finally, for NAs with a z value of -12, thus 6 rings, the Lq performs better than the other two methods producing 100% relative extraction recoveries for 13 out of 17 NAs, Fig. 2. This method however was unable to isolate the NAs with

number of carbons larger than 31. Overall, none of the methods were able to extract all the tested NAs. However, the ENV method appeared to perform better than the other two methods when looking at the relative recoveries and the number of detected of NAs.

The PCA of the scaled and mean centered relative recoveries also showed the better performance of the ENV method compared to the other two methods based on its cluster location in the score plot and the density of the variable clusters in the loading plot (Fig. S5). The PCA was able to clearly separate different extraction methods from each other using the first two principal components, Fig. S5. Between these two PCs, we were able to explain ∼62% of variability in our dataset. Most of within group variability for ENV and HLB methods appeared to be explained in the PC2 dimension whereas for Lq method a larger variability alongside PC1 was observed. This implied a larger observed variability in the Lq method compared to the other two methods, which was in agreement with our previous observations[3] and also the fact that Lq method includes more manual steps.[7,15] Moreover, the results of the Kruskal-Wallis test ($\rho < 0.05$) indicated that the differences between the ENV and Lq is the most dominant one while HLB method appeared to be more difficult to be distinguished from the other methods individually.

Based on our results, the ENV method appeared to be the best performing method from both the extraction recovery point of view as well as the extraction method reproducibility.

The NA Distribution in Produced Water

We further evaluated the effect of the extraction method on the overall distribution of tested NAs in the analyzed produced water. The noise removed extracted signal of the NAs for each extraction method was utilized for these evaluations.

When looking at the distribution of NAs in the analyzed produced water via SPE meth-

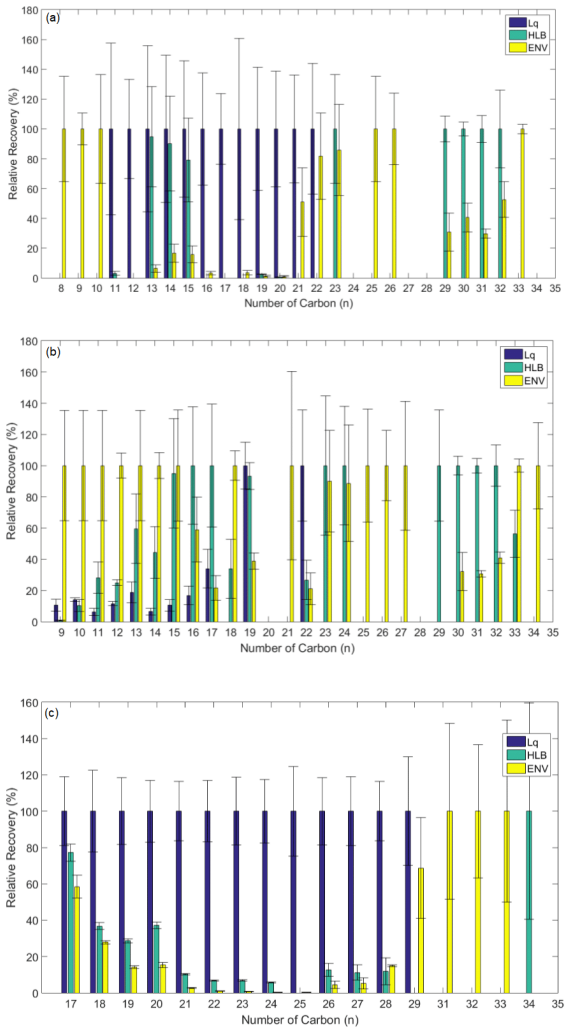

Figure 2: showing the relative recoveries of NAs versus the n value for (a) the z=0 (i.e. no ring), (b) the z=-4 (i.e. two rings), and (c) the z=-12 (i.e. six rings).

ods, the NAs with z values ranging from -4 to -12 appeared to be the most abundant ones. On the other hand, via Lq method the NAs with z value of -12 were the most abundant group while for other z values, this method produced relatively similar abundances, Fig. 3. All three extraction methods produced the smallest relative abundances for the aliphatic NAs. All the methods, for z values between -2 and -10, resulted in higher relative abundances for n values between 13 and 18, which was in agreement with previous reports regarding the distribution of NAs in produced water or similar matrices.[16–18] For z value of -12, the most

abundant NAs were those with n values between 16 and 20 for all three tested extraction methods.

The ENV method appeared to cover the largest NA chemical space compared to the other two methods, where the chemical space is defined as the total number of tested NAs, Fig. 3. The performance of the other SPE method, thus HLB, appeared to be more similar to the ENV rather than the Lq method. For Lq method the distribution of the NAs appeared to be affected mainly by their solubility in DCM. As a consequence, the boundaries of the explored chemical space via Lq method were dominated by the molecular size. In other words, the non-extracted NAs via the Lq were either too small or too large, therefore non soluble in DCM. For the two SPE methods, the explored chemical space appeared to be less concise when compared to the Lq method. We interpret that this observed trend was mainly caused by the interactions of individual compounds with the resin, sample complexity, and the matrix effects. Moreover, we observed that the HLB method, in particular, showed less affinity for the smaller NAs (i.e. n value of 8) compared to the ENV method. To further test this, we explored our chromatograms for NAs with z value of 0 and n values of 7 and 6, which were not included in our initial list of NAs. None of the three tested extraction methods detected the NA with z=0 and n=7. However, for NA with z=0 and n=6, the ENV method was the only one producing a positive detection for that particular NA, Fig. S6. This further indicated the difficulties that the Lq and HLB methods have in extracting smaller NAs.

The ENV method was able to explore the largest chemical space of NAs compared to HLB and Lq methods. Additionally, this method was the only one producing a positive signal for hexanoic acid, which is considered the marker for the presence of NAs in produced water according to the Norwegian Oil and Gas.[15] Even though this method (i.e. ENV) did not produce the highest recoveries for all the tested NAs, it resulted in 100% relative recoveries

for the largest number of NAs explored in this study. Our results in overall suggested that among the tested extraction procedures the ENV method is the most effective one for analysis of NAs in produced water.

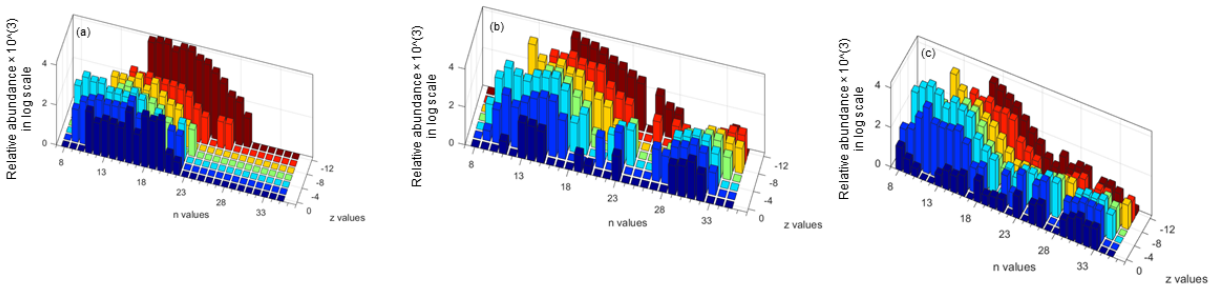

Figure 3: depicting the relative abundance of the analyzed NAs using (a) Lq, (b) HLB, and (c) ENV extraction methods. The relative abundances (i.e. "Z" axis) are multiplied to 1000 and are shown in log scale for ease of visual comparison among the three extraction methods.

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Supporting Information

The Supporting Information including details regarding the chemicals, the list of tested NAs, and figures related to the relative recoveries and statistical analysis is available free of charge on the ACS Publication website.

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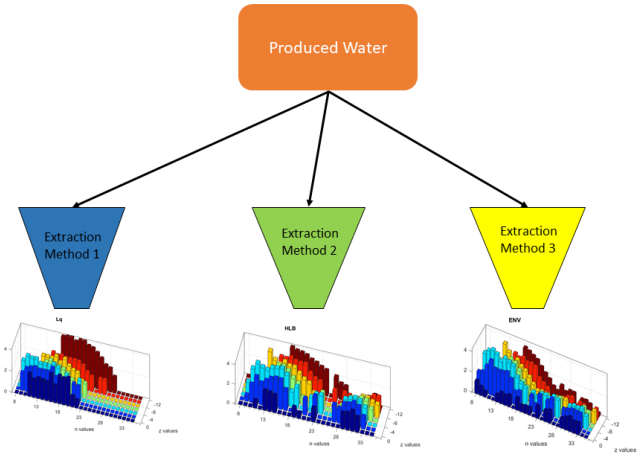
Produced Water
Extraction Method 1
Extraction Method 2
Extraction Method 3
Lq
HLB
ENV
n values
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TOC Art