공학석사학위논문

Quantification of Microplastics using UV-VIS Spectroscopy

UV-VIS 분광법을 이용한 마이크로플라스틱 정량 분석

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이 논문을 공학석사 학위논문으로 제출함

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이 하 경의 석사 학위논문을 인준함

2021년 2월

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Abstract

Quantification of Microplastics using UV-VIS Spectroscopy

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Microplastics are sorted as emerging contaminant which has possibility of human toxicity. Their large surface area occurs active surface reaction which can make them easily adsorb to other contaminants in the water. Also, they can be the media for the contaminant transport in the water. To deal with this issue, enough database about the amount of microplastics is essential for making proper solution. Hence, whole world is collecting microplastics quantification data from watershed, water treatment plants, and even from products with water. In line with this research trend, various quantification methods are studied but simple and convenient method is required. Therefore, this research selected UV-VIS spectroscopy which has quick and accurate analysis ability with easy operation. The objective of this research is to propose UV-VIS spectroscopy as a new and potential microplastics

quantification method.

First, proper suspension method investigation and surfactant determination for microplastics analysis were conducted. Among surfactants, TWEEN 80, a non-ionic surfactant could suspend microplastics in the water. Also, the absorbance change by microplastics themselves was not detectable alone. TWEEN 80 worked to detect microplastics as a base substance for UV-VIS analysis. It took the role of the analysis aid.

Second, the calibration curve method was employed to validate UV-VIS specstroscopy as the microplastics quantification method. It was validated with five validation parameters(LOD and LOQ, linearity, accuracy, precision, and robustness). Also, it was confirmed that this method is applicable to the density same or less than 1g/cm^3. Then, by comparison with (1)visual identification, (2)Q-(H)NMR, (3)gravimetric analysis as other potential microplastics quantification methods, its application possibility was investigated.

In conclusion, UV-VIS spectroscopy can be an alternative microplastics quantification method with its simplicity and convenience. The simplicity and accuracy will save the analysis period which will contribute to faster solution making for microplastics issues. This method can further be developed by developing an analysis kit or find additional proper pre-treatment methods to increase its linearity.

Keywords: microplastics, quantification, UV-VIS spectroscopy, emerging contaminants, environmental analysis

Student Number: 2019-20281

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1. Introduction

1.1 Background

1.1.1. Microplastics issues of water

Plastics are inseparable material for modern society. With lower density compared to metal or ceramic, plastic can be the source of light and a strong product. It does not easily degrade. Besides, plastic is a cheap material and can produce large quantities in a short time. Therefore, plastic has been widely utilized since it was discovered. The global plastics production rate is increasing continuously. Compared to the production in the 1980s, the plastic production rate is 7 times higher in 2015 (see Fig 1)(Geyer et al., 2017). It is difficult to find an alternative to this ubiquitous synthetic polymer.

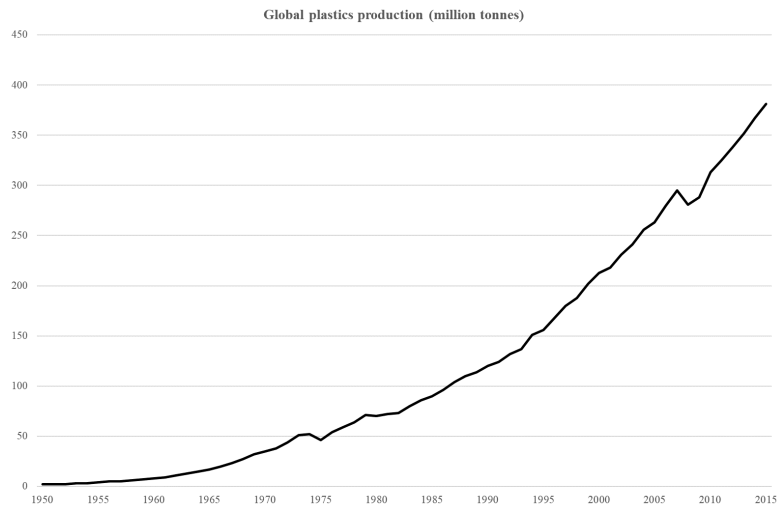


Fig 1. Global plastics production

Asia and the Pacific region are emitting a large number of plastics to the river and ocean(Jambeck et al., 2015). Among them, East Asia and the Pacific region do not manage plastics carefully with a ratio of 60% (see Fig 2). As many manufacturing plants are located in Asian countries, this statistic result appeared. Therefore, finding a proper solution for this region is required.

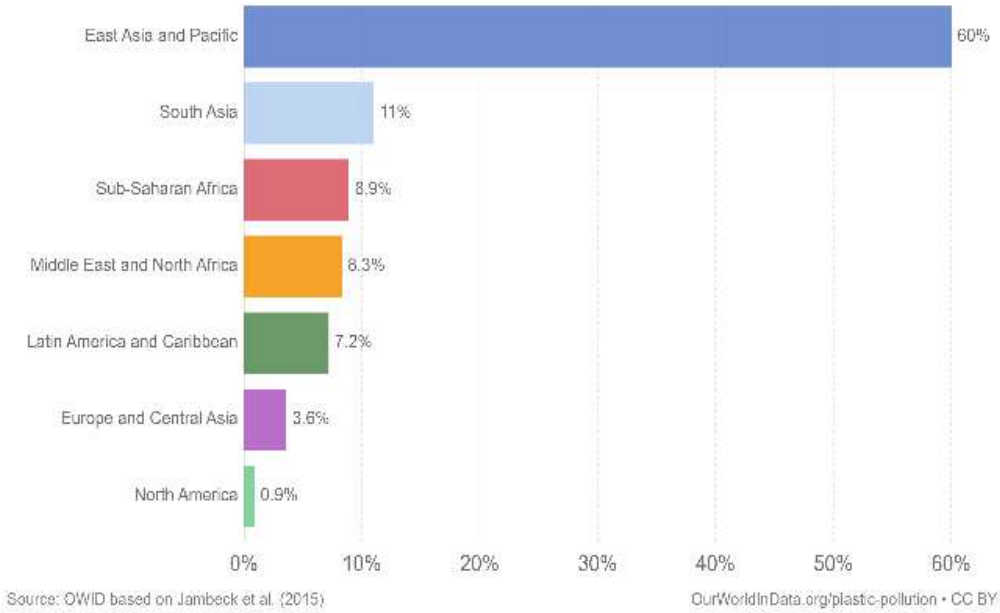


Fig 2. Global mismanaged plastics by region

Plastics are not completely treated at water treatment plants or directly dumped into the watershed by the drain of factories and households. These mismanaged plastics are not degradable and have a small size with a large surface area. These pollutants are called microplastics. Microplastics are sorted as emerging contaminants that have potential damage to the human body. Researchers are discussing

setting an official definition of micro/nano plastics. Microplastics are so far defined as all plastic particles less than 5mm in size. If the size is bigger than 5mm, it is called macroplastics. Plastics between 1 to 5mm are large microplastics. Smaller than 1mm are microplastics and smaller than 1μm are nanoplastics(Academies, 2019). These various sizes of plastics are exposed to our precious environment. Microplastic issues firstly rose with worries about pollution on the sea(Barboza et al., 2019). However, it was not only the matter of sea. Microplastics are detected at other watersheds either like the river and lake. Also, they exist in the soil and the air. It means that microplastics are everywhere. Among them, treating water which is the most common route of microplastics' movement should be preceded. Microplastics are well adsorbed to other contaminants in the water with their large surface area and surface characteristics. Then, they can act as the media of other contaminant's transportation(Nguyen et al., 2019). It means that it can also be attached or be taken by aquatic animals and plants whose top predator in the food chain is human beings. The Human can finally uptake microplastics and it can be accumulated in our body(Joana Correia Prata et al., 2020). That is why microplastics are under human toxicity research. Therefore, the removal of such microplastics is important for a stable water environment and human health.

In order to treat microplastics completely, accurate quantification and qualification of microplastics should be preceded. With these analysis results, research on the treatment technologies can be conducted. The widespread and qualified qualification of microplastics is

FT-IR(Fourier-transform infrared spectroscopy)(Thompson et al., 2004). There are already many databases of various plastics for the detection of the type of microplastics. However, not only the type of microplastics but the amount of contaminants is important to design a proper solution for the issue. Since microplastics have been recognized as emerging contaminants, their quantification methods researches started to be conducted. After bulk samples are collected from the watershed or water treatment plants, samples are rinsed and pre-treated to remove interfering substances for quantification. First, Visual identification with Micro-FT-IR (Micro-Fourier-transform infrared spectroscopy) is the most common quantification method(Corami et al., 2020). The principle is counting the total amount of microplastics by a person with the microscope. It is an easy method but not applicable for bulk samples of more than 4 liters. Also, this method is focused on qualification with FT-IR, and because human counts particles by the individual, there is the possibility of error occurrence. Secondly, thermal analysis is a rising method. It contains Pyrolysis-GC/MS (Gas chromatography/mass spectrometry) and TGA, TDS-GC/MS (thermogravimetric analysis, thermal desorption-gas chromatography/mass spectrometry). These methods can accurately detect microplastics and delicate separation of various microplastics is possible(Hermabessiere et al., 2018). Also, comparatively high concentration samples are acceptable for analysis. However, it takes a long time to get the result, and to set a stable database is difficult. Other than these methods, there are several potential microplastics quantification methods. At this point, a simple and comfortable microplastics quantification method is needed. Hence, simple but accurate microplastics quantification methods are required.

1.1.2. UV-VIS spectroscopy

UV-VIS spectroscopy(Ultraviolet-visible spectroscopy) is the quantification method by measurement of the attenuation of a beam of light after it passes through the sample(Muñoz Caro, 2018). The absorbance of light means the concentration of substances. The principle is the measurement of the electronic transition of atoms and molecules in the electromagnetic spectrum range set from ground state to excited state. It is simple and takes less time compared to other quantification methods if the experiment requires the same pre-treatment process. Besides, it has application possibility in a wide concentration range with high analysis accuracy. Even with these advantages, No research applied UV-VIS spectroscopy as a potential microplastics quantification method so far.

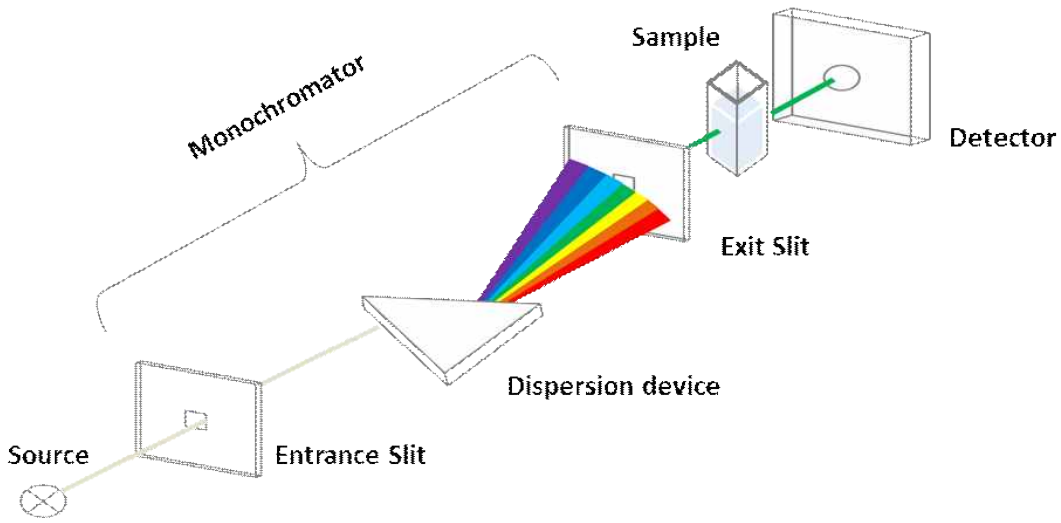


Fig 3. UV-VIS spectroscopy schematic diagram

1.2 Research objectives

The main objective of this research is to propose UV-VIS spectroscopy as an appropriate microplastics quantification method to figure out microplastics abundance in the environment. Detailed objectives of the research are as follows:

(1) To select proper pre-treatment (suspension method and surfactant determination) for microplastics analysis

(2) To apply calibration curve method and validate the method

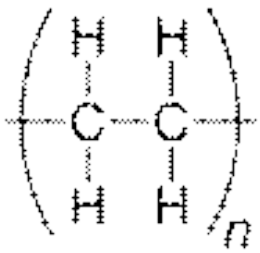
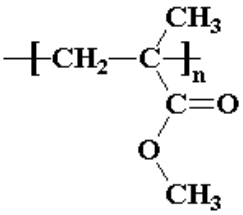
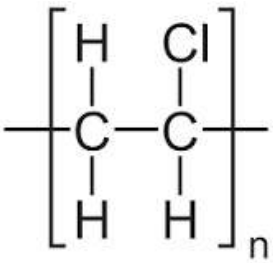
(3) to compare with other potential quantification methods and prove UV-VIS spectroscopy as a compatible method.

2. Materials

2.1 Microplastics

About 20mg/L~5g/L of microplastics are detected from watersheds and on average 80mg/L are detected from wastewater treatment plants(Lee et al., 2016) in South Korea. Among abundantly detected microplastics, PE(Polyethylene), PMMA(Poly Methyl Methacrylate), and PVC(Polyvinyl Chloride) of three plastics were chosen for this research.

Table 1. Characteristics of each plastic

	PE (Polyethylene)	PMMA (Poly Methyl Methacrylate)	PVC (Polyvinyl Chloride)
Structure			
Formula	$(C_2H_4)_n$	$(C_5O_2H_8)_n$	$(C_2H_3Cl)_n$
usage examples	general plastic containers	various acrylic products	water pipe
Density	0.88-0.96g/cm^3	1.18g/cm^3	1.45g/cm^3
Melting point	115° C	160° C	100° C
Boiling point	135° C	200° C	260° C

Each functional groups and chemical characteristics are different. PE and PVC were purchased from Sigma-Aldrich Korea Ltd., SIAL. PMMA was purchased from Goodfellow Korea.

PE is a hydrocarbon chain with molecular formula $(C_2H_4)_n$. molecular structures are featured in Table 1. It is one of thermoplastics and light and flexible. From industrial material to household goods, it is one of the most widely used plastic. Two representative types of PE are HDPE(high-density polyethylene) and LDPE(low-density polyethylene). Both have a density of less than 1g/cm^3.

PMMA is an acrylic resin whose main source is MMA(Methyl Methacrylate). With its transparency, weatherability, and high functionality, it is widely used for information, electrical, and various fields. Its molecular formula is $(C_5O_2H_8)_n$.

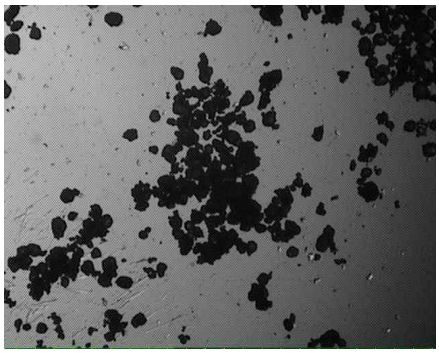
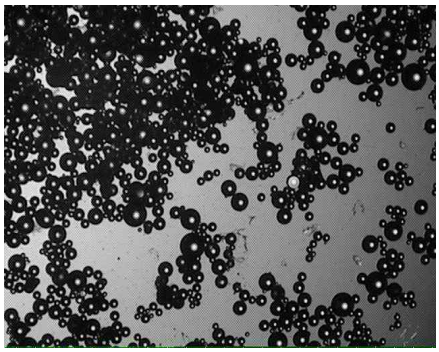
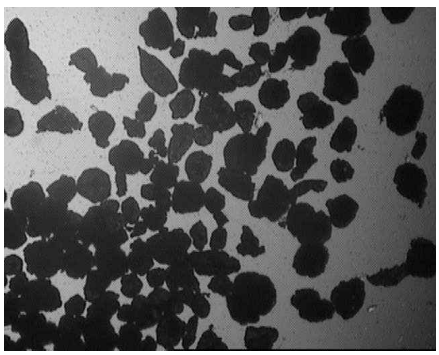
PVC is also one of thermoplastics and stable and not easily be worn. It is weak to heat so for the experiment, the temperature should be controlled carefully. It is well-known as the name of vinyl. From polyethylene, one of hydrogen is replaced by chlorine with the molecular structure of $(C_2H_3Cl)_n$.

The melting and boiling point of the three plastics is same or higher than 100° C. However, thermal degradation will occur at about over 70° C condition. Therefore, the temperature condition less than 70° C should be set.

Shapes were visualized with a microscope from Campscope and program iT Plus 4.0 (Sometech, South Korea) as Table 2. PE and PVC have an irregular shape. PMMA has a spherical shape. Microplastics detected from real water conditions have various shapes like irregular,

spherical, linear, plate types, etc(Choi et al., 2018). Likewise, plastics used for this research reflect real conditions.

Table 2. Shape of each microplastics

	Shape (x1)	Shape (x200)
PE		
PMMA		
PVC		

Also, particle size distribution was measured with Mastersizer 3000 (Malvern, United Kingdom) as graphs in Fig 4~6. It showed the plastics size range. PE is in about 25~78.7μm size range, PMMA is in 27.5~91.4μm range, and PVC is in 87.7~178μm range. All samples showed micro-size range so that they can be classified as microplastics. All these different characteristics of microplastics are applied for further research processes.

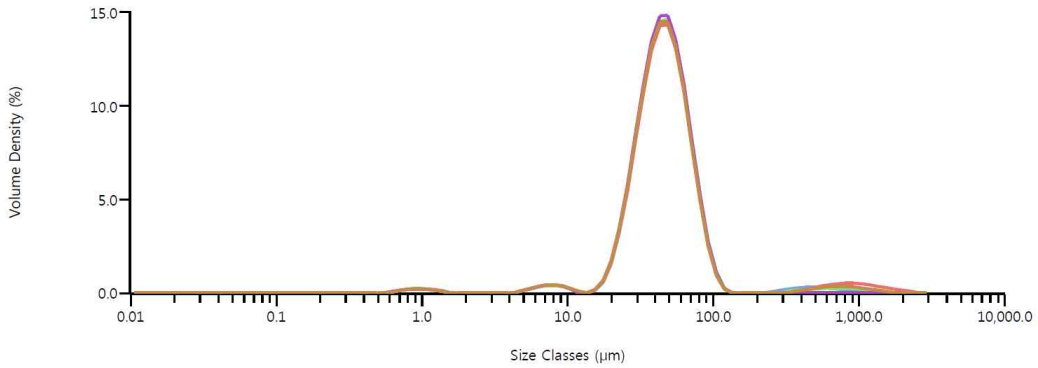


Fig 4. Size Distribution (PE)

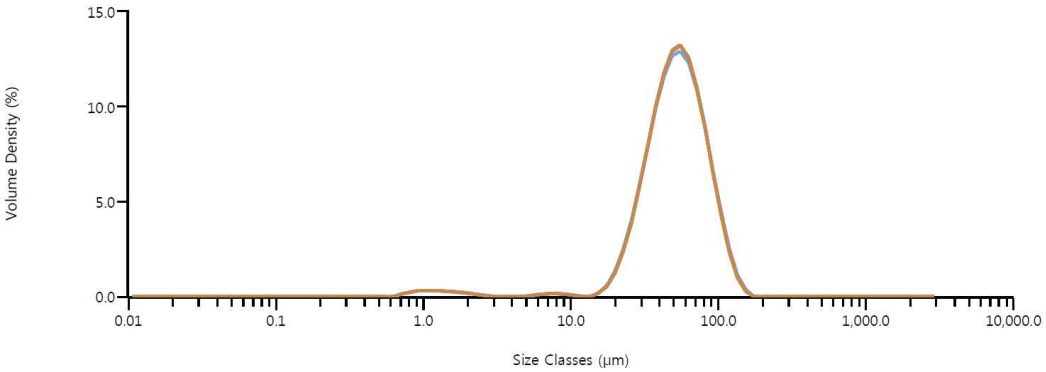


Fig 5. Size Distribution (PMMA)

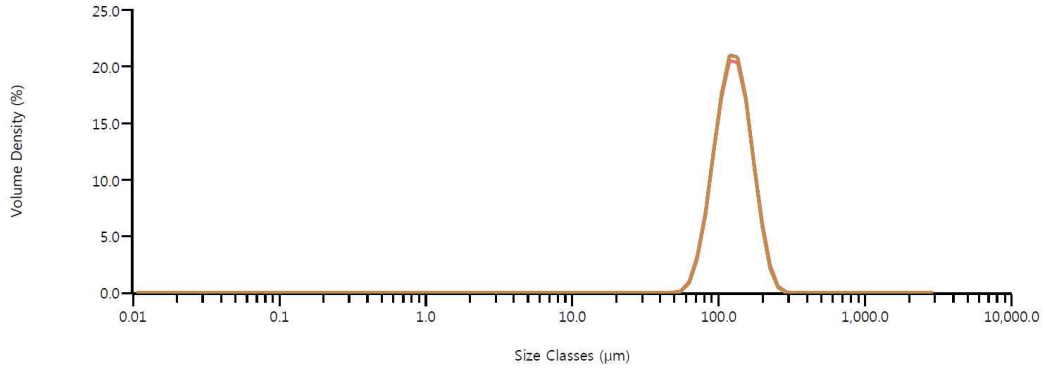


Fig 6. Size Distribution (PVC)

2.2. Microplastics staining

White-colored microplastics were stained for several experiments to enhance their visibility. Nile red (5H-Benzo[α]phenoxazin-5-one, 9-(diethylamino) was used to stain plastics(Maes et al., 2017). It is lipophilic and fluorescent. Plastics also have lipophilicity, which makes plastics easily be stained. Nile red stock solution was prepared. The reagent was purchased from Sigma Aldrich Korea Ltd., SIAL. 0.05g/L (Nile red/acetone) concentration of the solution was contained in an amber glass container. This was 10 times diluted with n-Hexane (CAS 110-54-3, LiChrosolv® for liquid chromatography, Supelco®, Merck, South Korea) which is a working solution. 200mg plastics are stained with 15ml Nile red working solution and dried in 60 degree Celsius oven for 2 hours. Then white-colored plastics turn to pink to violet color.

Table 3. Microplastics staining results

PE	PMMA	PVC
		

2.3 Zeta potential of microplastics

Each zeta potential of microplastic in the aquatic phase was analyzed to figure out the surface charge of microplastics in a wide pH range. ZC-2000, MICROTEC, Japan and ZEECOM(Zeta potential analyzer program) was used.

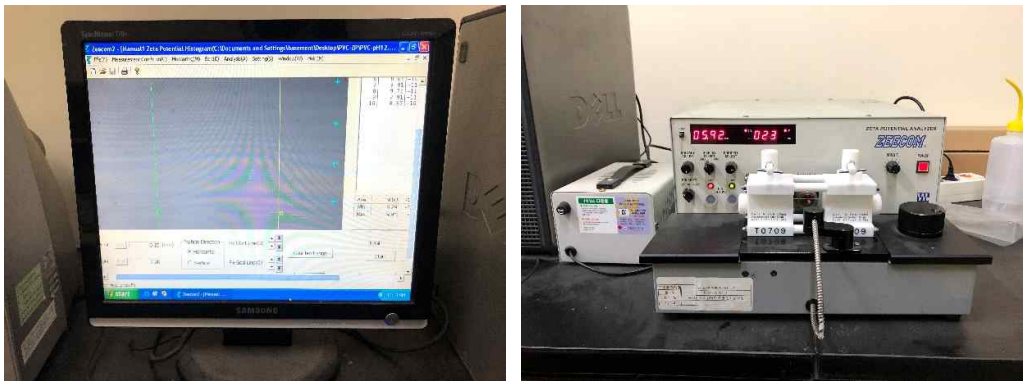


Fig 7. Zeta potential analyzer

More than 1%(w/w) concentration of microplastics are suspended in the electrolyte for the pH 2~12 range. The electrolyte used for this experiment as the background was 0.01M sodium chloride. pH was controlled with 0.1M hydrochloric acid and 0.1M sodium hydroxide. For suspension, stick sonication was conducted for one minute per each sample. The principle of zeta potential analysis is the speed of the particle (less than 100um) at a specific voltage and electric current condition. The higher speed means the higher absolute value of the zeta potential. pH of natural water and water from the water treatment plant is about 6-8 range normally. In this range, all

microplastics samples showed a negative surface charge. Microplastics have a positive charge only at extremely acidic conditions (less than pH 3).

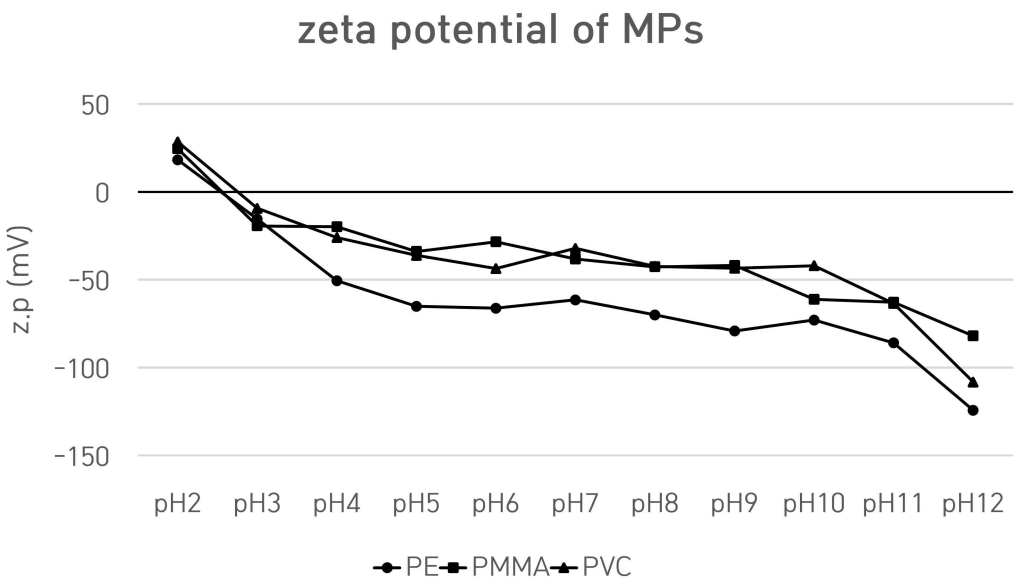


Fig 8. Zeta potential of microplastics

3. Pre-treatment for UV-VIS spectroscopy

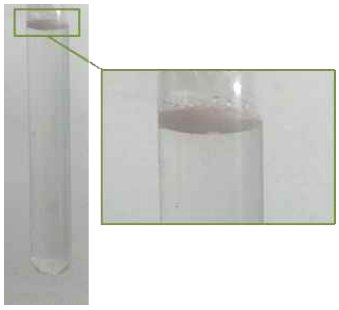
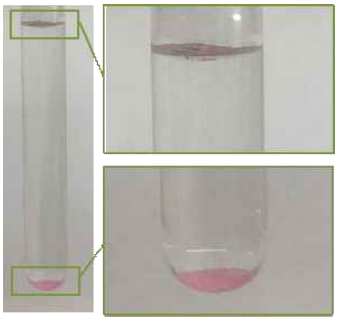
3.1 Requirement of suspension and pre-treatment method decision

To obtain uniformed analysis results, microplastics should be evenly suspended during the measurement. The distribution of microplastics without any treatment at the aqueous phase was checked. PE which has a density lower than 1g/㎤ floated on the surface of the water. For PMMA and PVC with a density higher than 1g/㎤ was partially floated and remainings were precipitated. Some of these high-density plastics were floated because of the surface tension of the water. For precipitated microplastics, the settling velocity of PMMA particles was 0.01m/s and PVC was 0.03cm/s. According to this result, microplastics are not well suspended without proper treatment. Therefore, suspension is required.

Besides, without proper solvent or treatment, microplastics are not detected by UV-VIS spectroscopy. For organic compounds, only conjugated substances having π electrons can be detected(Yin et al., 2016). However, microplastics do not have this structural characteristic. Therefore, a proper solution for the detection is required.

For economical and practical analysis, this research tried to discover simultaneous suspension and detection methods.

Table 4. Microplastics distribution at aqueous phase

Low density ($\rho \leq 1 g/cm^3$)	High density ($\rho > 1 g/cm^3$)	High density ($\rho \gg 1 g/cm^3$)
PE	PMMA	PVC
		
floated	floated, precipitated	floated, precipitated

3.2 Dispersant decision

3.2.1. Surfactants

Microplastics themselves were not suspended well in the water. Some surfactants are utilized for particle suspension. They can be used with their both hydrophobic and hydrophilic characteristic. In addition, some surfactants are detectable by UV-VIS spectroscopy. In this research, three representative surfactants are compared.

The success of the suspension is determined by the surface tension between the particle and the liquid. Therefore, surface tension reduction by the surfactant as the dispersant is required.

(1) Sodium Dodecyl Sulfate

Sodium Dodecyl Sulfate(SDS) is an anionic surfactant(Borode et al., 2019). It is assumed that it is hard to anticipate the electronic adsorption by anionic surfactant because the surface charge of plastics is negative. It was purchased at Sigma Aldrich Korea Ltd., SIAL.

(2) Cetyltrimethylammonium bromide

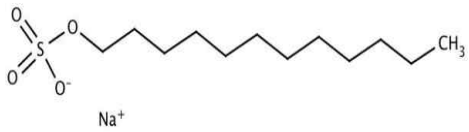
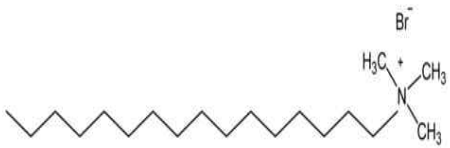
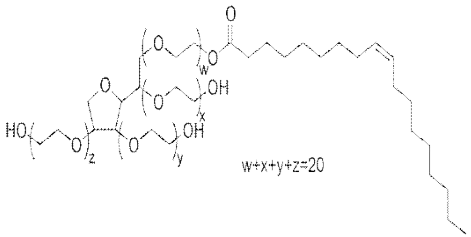
Cetyltrimethylammonium bromide(CTAB) is cationic surfactant(Amba Sankar et al., 2019). It is assumed that this surfactant will occur electro-adsorption between the positively charged surfactant and negatively charged microplastics in the aqueous phase. It was purchased

at Sigma Aldrich Korea Ltd., SIAL.

(3) Polysorbate 80

Polysorbate 80(TWEEN 80) is a non-ionic surfactant(Balakrishnan Nair et al., 2018)(Gerdes et al., 2019). Non-ionic surfactant may achieve stereoscopic stabilization by the particle surface adsorption. Also, the critical micelle concentration is lower than the ionic surfactant. Hence, it is expected that with less amount of surfactant the suspension can be achieved. The surfactant was purchased at Duksan, South Korea.

Table 5. Molecular structure of surfactants

surfactant	Anionic surfactant	Cationic surfactant	Nonionic surfactant
	SDS (SodiumDodecylSulfate)	CTAB (Cetyltrimethylammonium bromide)	TWEEN 80 (Polysorbate 80)
molecular structure			

3.2.2. Methods

The suspension and detection experiment was conducted. For each surfactants, 1, 5, 10, 50, 100, 150, 500 mg/L of surfactants were prepared. Microplastics with 80mg/L concentration were injected for each sample. After that, each sample were violently mixed manually and left for 5 minutes to see the suspension. The peak appearance was figured out at the wavelength range of 200~800nm using UV-VIS spectroscopy.

3.2.3. Results and discussion

After manual mixing, suspension was identified. For SDS and CTAB, none of the microplastics were suspended in the presence of the surfactant. With TWEEN 80 condition, PE and PMMA were suspended evenly. However, PVC did not suspended in any surfactant condition. The high density of PVC occurred the rapid precipitation of it. The suspension status was summarized in Table 6.

Table 6. Microplastics suspension with surfactants

	SDS	CTAB	TWEEN 80
PE	X	X	O
PMMA	X	X	O
PVC	X	X	X

Surfactant detection by UV-VIS spectroscopy was employed. First, as the results, SDS was not detectable for the whole ultraviolet and visible wavelength(200~800nm) range. CTAB did not show a meaningful peak at the same wavelength range. Therefore, both SDS and CTAB were not proper surfactants for UV-VIS spectroscopy.

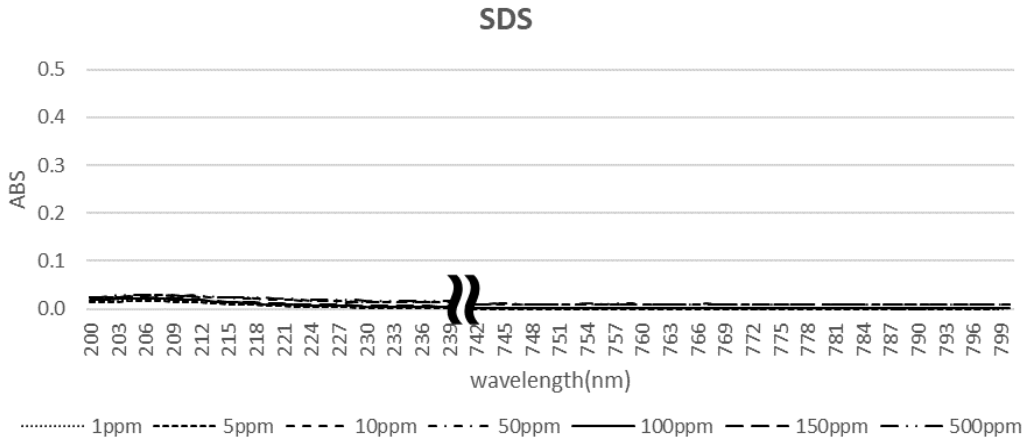


Fig 9. UV-VIS spectra of SDS

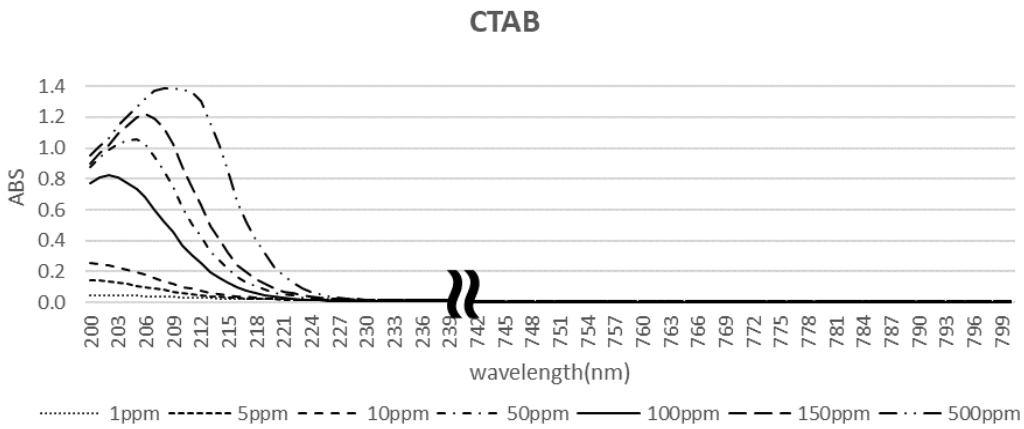


Fig 10. UV-VIS spectra of CTAB

For the second, TWEEN 80 was detectable at 234nm wavelength. Because of the effect of by-product during the TWEEN 80 manufacturing process, the peak appears at this wavelength(Mousset et al., 2013)(Zhang & Qi, 2017). Another peak appears at 195nm by the double bond of oleic acid. However, the peak at the range of less than 199nm is unstable. Therefore, that range is not used for the analysis. TWEEN 80 can be used as a surfactant for microplastics because it is detected by UV-VIS spectroscopy. The limit of detection(LOD) was 20.4mg/L and the limit of quantification(LOQ) was 61.81 mg/L. Hence, 100mg/L of TWEEN 80 was applied for further research steps as enough concentration.

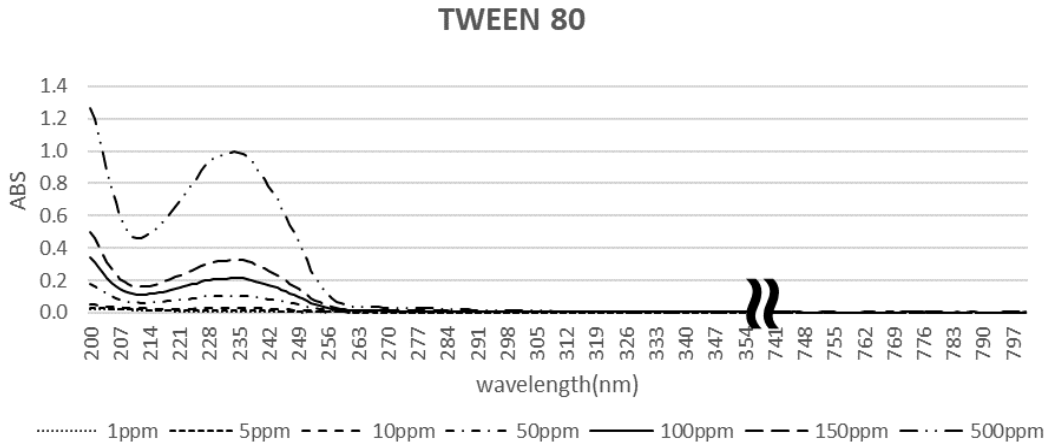
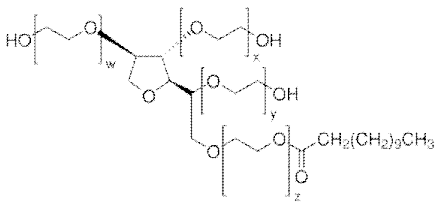
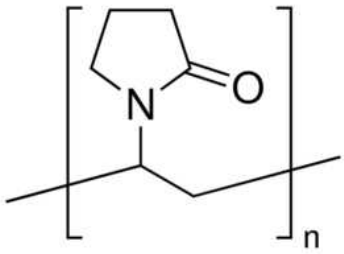


Fig 11. UV-VIS spectra of TWEEN 80

With this result, only TWEEN 80, the representative non-ionic surfactant was detected by UV-VIS spectroscopy. Therefore, additional non-ionic surfactants were employed to compare with TWEEN 80 and

identify whether they are detectable and applicable or not. Additional surfactants are Polysorbate 20(TWEEN 20) and Polyvinylpyrrolidone(PVP). Both are also representative non-ionic surfactants(Salaberria et al., 2020).

Table 7. Additional non-ionic surfactants

Additional surfactant	TWEEN 20 (Polysorbate20)	PVP (Polyvinylpyrrolidone)
molecular structure		

The result showed that TWEEN 20 did not make the peak. TWEEN 20 has a shorter aliphatic tail compared to TWEEN 80(Ortiz-Tafoya & Tecante, 2018). Therefore, comparatively, it has higher hydrophilicity which is the reason why a peak did not appear. Also, PVP made a significant peak neither for the whole ultraviolet and visible wavelength(200~800nm) range.

Hence, it is confirmed that TWEEN 80 is ultimately the most reasonable surfactant for UV-VIS analysis.

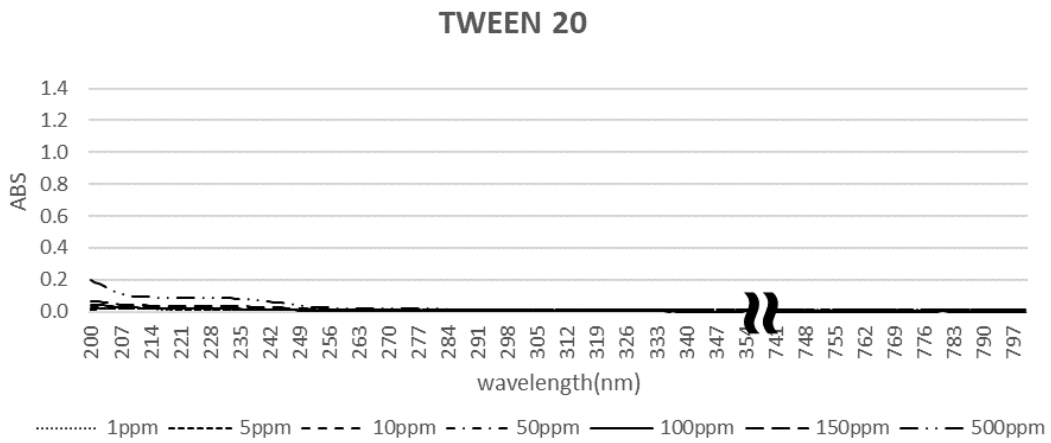


Fig 12. UV-VIS spectra of TWEEN 20

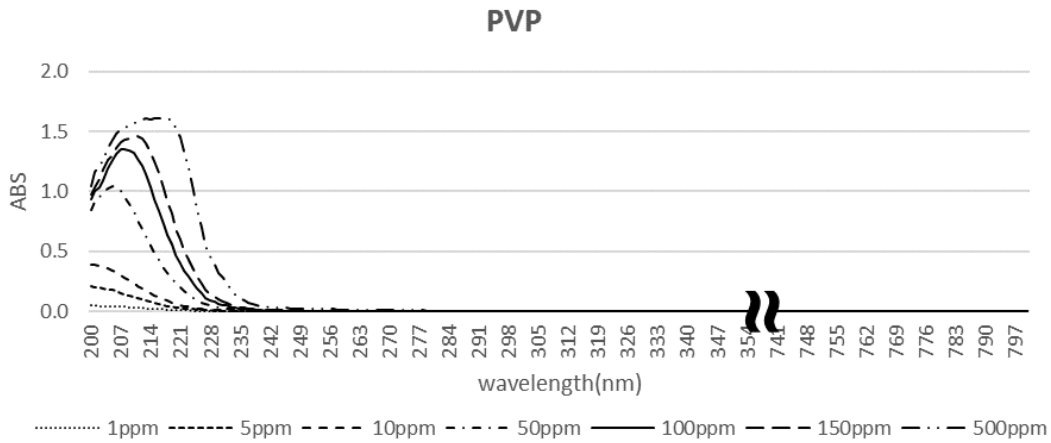


Fig 13. UV-VIS spectra of PVP

3.3 Surfactant adsorption and suspension

The mechanism of TWEEN 80 adsorption was investigated. A non-ionic surfactant has a long and weak hydrophilic head with low persistence length(Li et al., 2012). It makes a disordered layer on the surface of microplastics. In the aqueous phase, water penetrates the gap between surfactant and microplastics which makes the adsorption stronger. Also, the desorption is slower than other types of surfactants (Meconi et al., 2016). Fig 14 describes the adsorption of surfactants to the microplastic particle. Light blue and red-colored molecules are TWEEN80. Dark green and grey colored small molecules are water. This, TWEEN 80, can be the representative non-ionic surfactant to suspend microplastics in analysis samples.

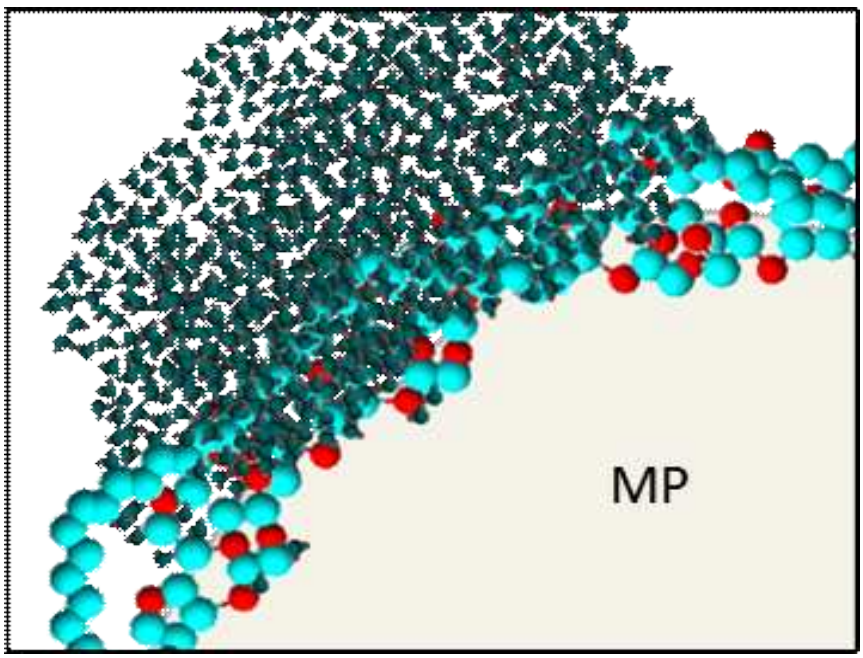
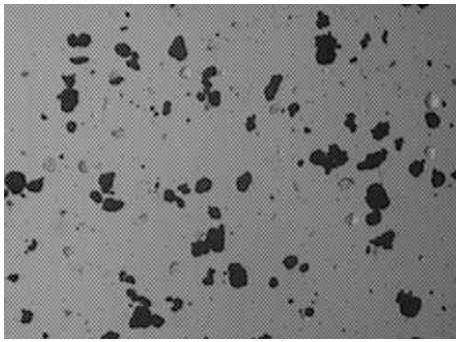
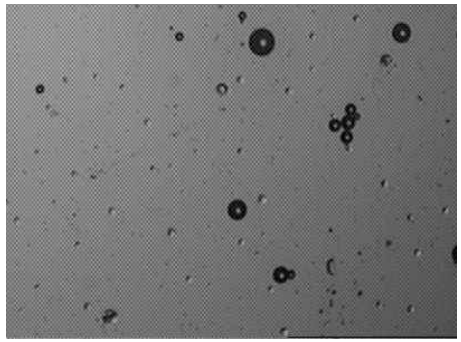
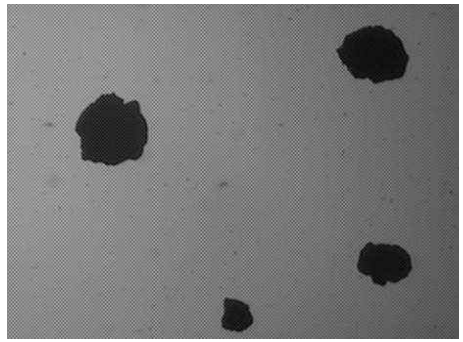
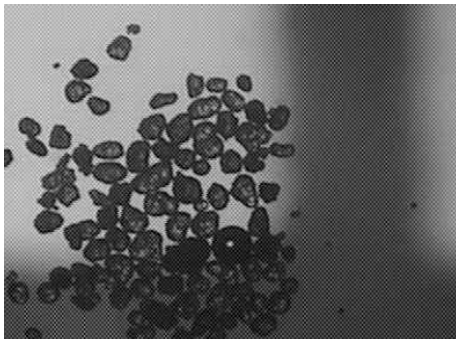
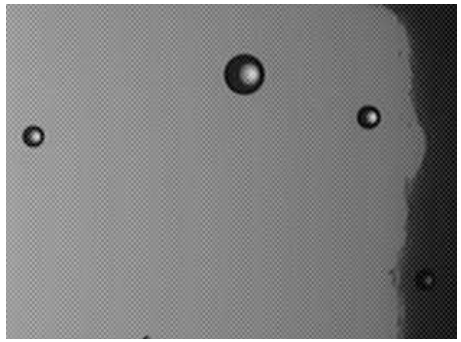
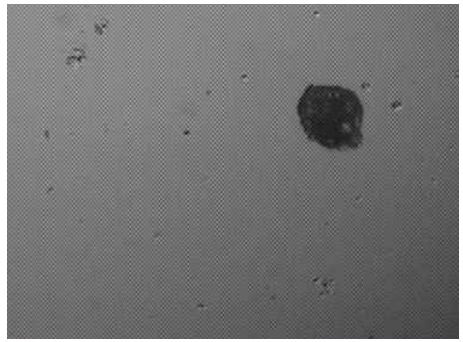


Fig 14. Absorption of surfactants to the microplastic particle

Surface shape change was identified with the microscope Campscope and program iT Plus 4.0 (Sometech, South Korea). Pure microplastics in the deionized water were controlled and it was compared with the compound of microplastics and TWEEN 80 in the deionized water. 100ppm of TWEEN 80 concentration condition was made. The magnification was 200 times for the microscopy.

Surface shapes were different with and without TWEEN 80. With tween 80, a heterogeneous shape appeared than the condition without TWEEN 80 as figures in Table 8.

Table 8. Surface shape change in presence of TWEEN 80

	PE	PMMA	PVC
Without TWEEN 80			
With TWEEN 80			

3.4 Microplastics suspension method

Previous surfactant decision experiments were conducted by manual mixing. To select the most appropriate suspension method, three methods were compared. Magnetic mixing (MSH-20D, WiseStir®, South Korea), vortexing (Vortex-Genie 2, Scientific Industries, Inc., United States), and sonication (VCX-750 Vibra-Cell processors, Sonics & Materials, Inc., United States) were conducted. The principle of magnetic mixing is mixing for 30 minutes with the rotation of the magnetic bar by magnetic force. Vortexing is the violent mixing of the sample by the vortex generation from the bottom to the top of the conical tube. Sonication is mixing and suspension by ultrasonic wave for a minute.

80mg/L of each stained microplastics were injected into the reaction tube. After that, each suspension test was conducted.

For microplastics suspension, PE and PMMA which have relatively low density could be suspended well but high-density plastics could not be suspended with any methods (see Table 9). Therefore, for further steps, PVC was vigorously mixed five times right before the measurement for suspension. For PE and PMMA, the suspension method was selected depending on the experimental condition like tube size and microplastics concentration.

Table 9. Microplastics suspension depending on the mixing method

Mixing method	magnetic mixing	Vortex mixing	sonication
PE	O	O	O
PMMA	O	O	O
PVC	X	X	X

3.5 Microplastics detection with UV-VIS spectroscopy in presence of surfactant

To implement simultaneous suspension and detection with UV-VIS spectroscopy, TWEEN 80 100mg/L was injected as the dispersant. Then, the peak appearance in different concentrations of microplastics contaminated water conditions (5, 10, 50, 100, 300, 500mg/L) was identified.

As microplastics dosage increased, the absorbance also increased for all microplastics (see Fig 15~17). This result shows that TWEEN 80 can be the base substance for microplastics absorbance.

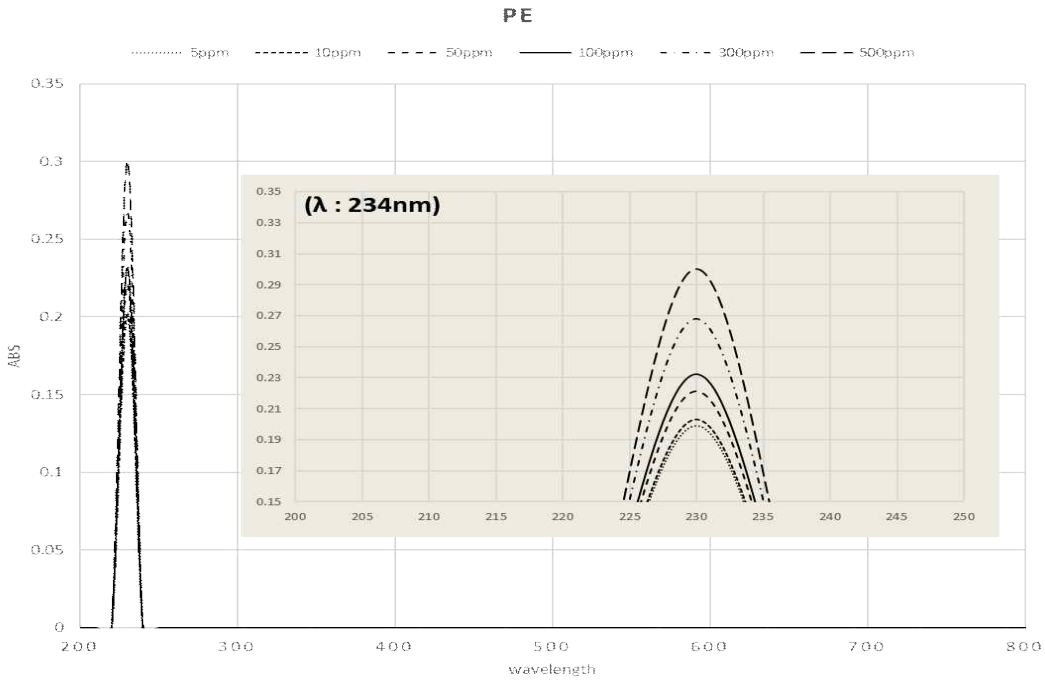


Fig 15. Peak appearance with TWEEN 80 and in different PE concentrations

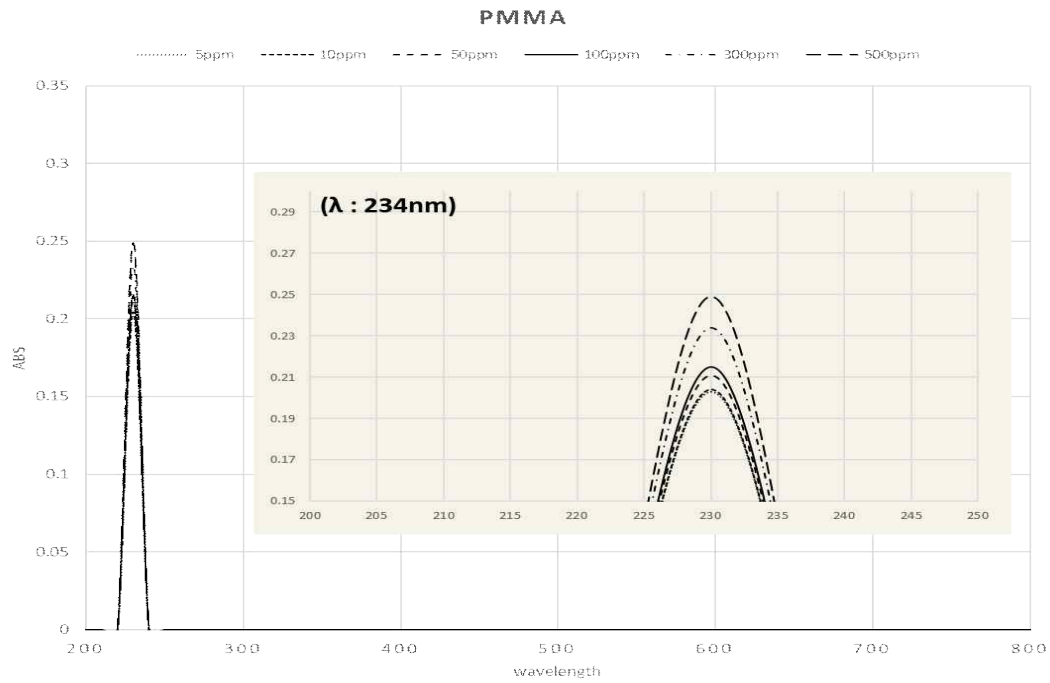


Fig 16. Peak appearance with TWEEN 80 and in different PMMA concentrations

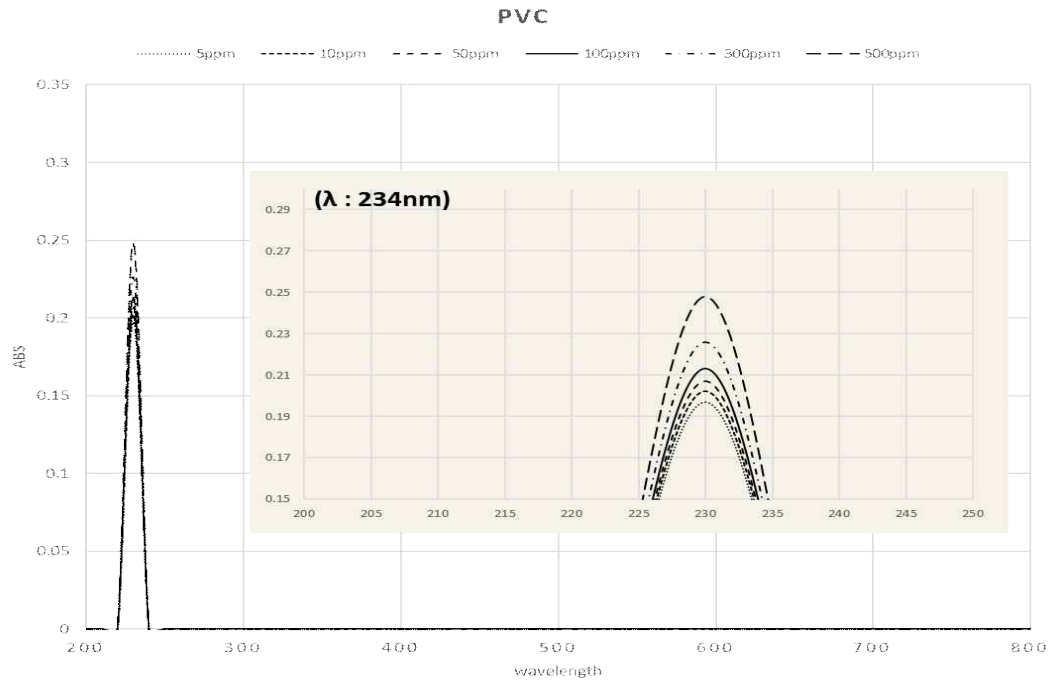


Fig 17. Peak appearance with TWEEN 80 and in different PVC concentrations

In summary, the simultaneous suspension and detection method was investigated with the requirement for pragmatic pre-treatment for microplastics quantification. Among potential microplastics dispersants, TWEEN 80 was the most viable substance with its physical and irregular absorption to the surface of microplastics. For suspension, density lower than 1g/cm^3 or similar to 1g/cm^3 was well suspended by any suspension methods in presence of TWEEN 80. However, high-density microplastics were precipitated even with suspension and the existence of surfactant.

4. Application of calibration curve method and its validation

4.1 Methods

The calibration curve method was applied for the microplastics quantification using UV-VIS spectroscopy. More than three standard solutions with different concentrations were prepared. Their absorbances were measured and a calibration curve was drawn based on the absorbance results. After that, samples in the detectable concentration range were prepared. The concentration can be evaluated by the calibration curve. This is the most accurate empirical way of figuring out the change of absorbance(Lee et al., 2013).

For, UV-VIS spectroscopy, it follows the Lamber-Beer law.

$$A = -\log T = \epsilon BC$$

$$(T = I_t / I_o)$$

A: absorbance

T: transmittance

I_t*: intensity of light exiting*

I_O*: intensity of light entering*

ε *: molar absorption coefficient (M-1cm-1)*

C: molar concentration (M)

B: optical path length (cm)

The analysis was conducted with UV-VIS spectrophotometer P330, IMPLEN. Quartz cuvette was used to contain samples. For the measurement at the ultraviolet wavelength region, the cuvette cell should be applied. The wavelength for drawing the calibration curve was determined by seeing spectra in 200-800nm range. Wavelength peak in this range is selected as the most proper point. 100mg/L TWEEN 80 dissolved deionized water is the reference solution. According to the previous part of this research, TWEEN 80 shows a peak at 234nm wavelength point. Therefore, the absorbance at 234nm might be applied. Six microplastics contaminated water with different concentrations are prepared for calibration curve drawing (20, 40, 60, 80, 100, 120mg/L) to meet the actual microplastics contaminated conditions of the wastewater treatment plant in South Korea. The pH of the experimental solution is about 6.5-7 range. Room temperature condition was set. The presence of electrolyte and interfering substances was inhibited for a stable experiment(Ames & Willard, 1953). The validation of UV-VIS spectroscopy was conducted with five parameters of LOD and LOQ, linearity, accuracy, precision, and robustness(Araujo, 2009).

4.2 Results

4.2.1. Calibration curve

Calibration curves of each microplastics were drawn. The absorbance of the control solution was not modified to zero. As the reference solution is the compound of TWEEN 80 and deionized water, the absorbance of TWEEN 80 can have significant meaning. Therefore, its absorbance was kept as the control(see Fig 18~20).

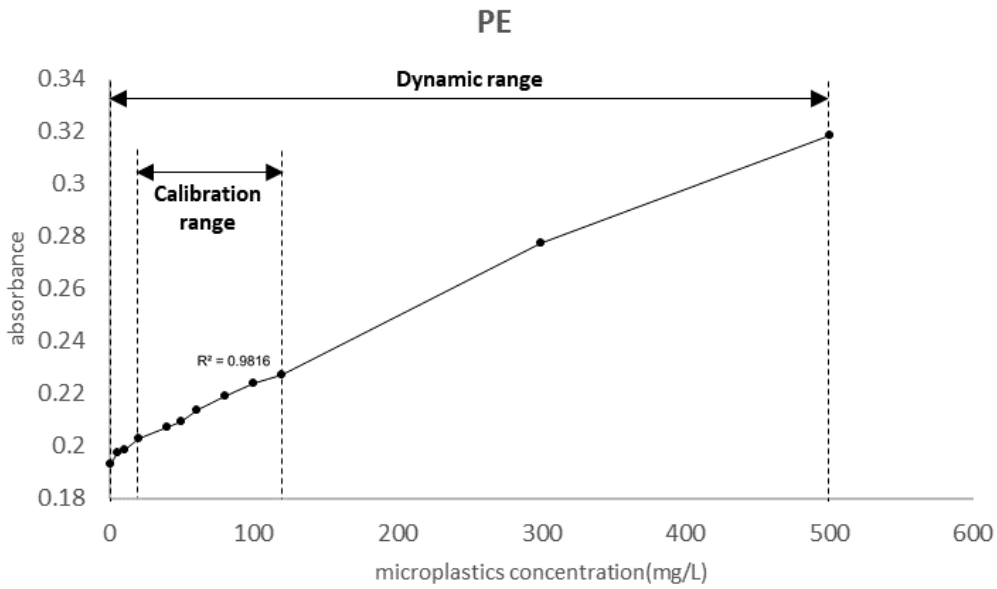


Fig 18. Calibration curve of PE

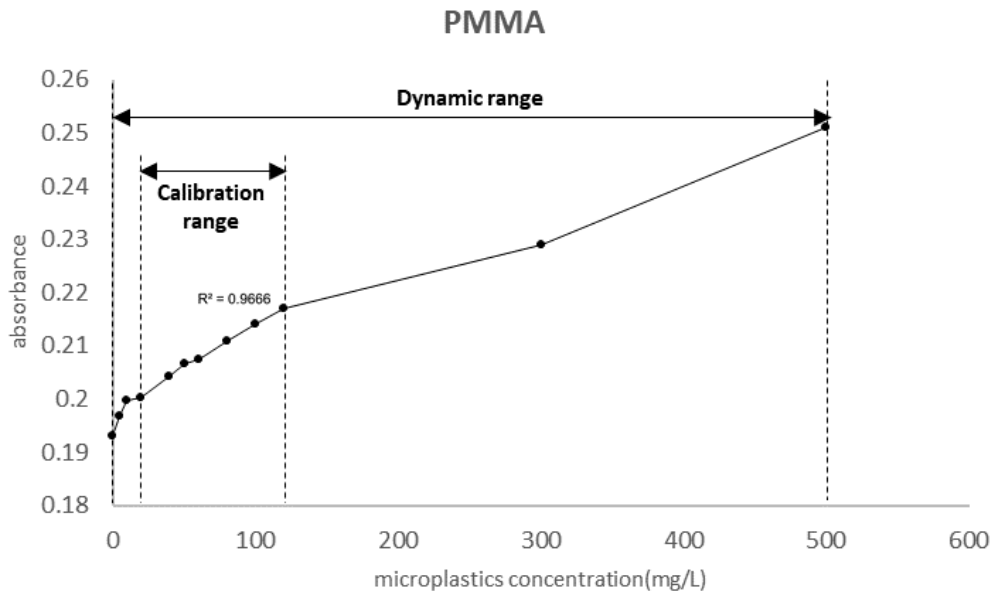


Fig 19. Calibration curve of PMMA

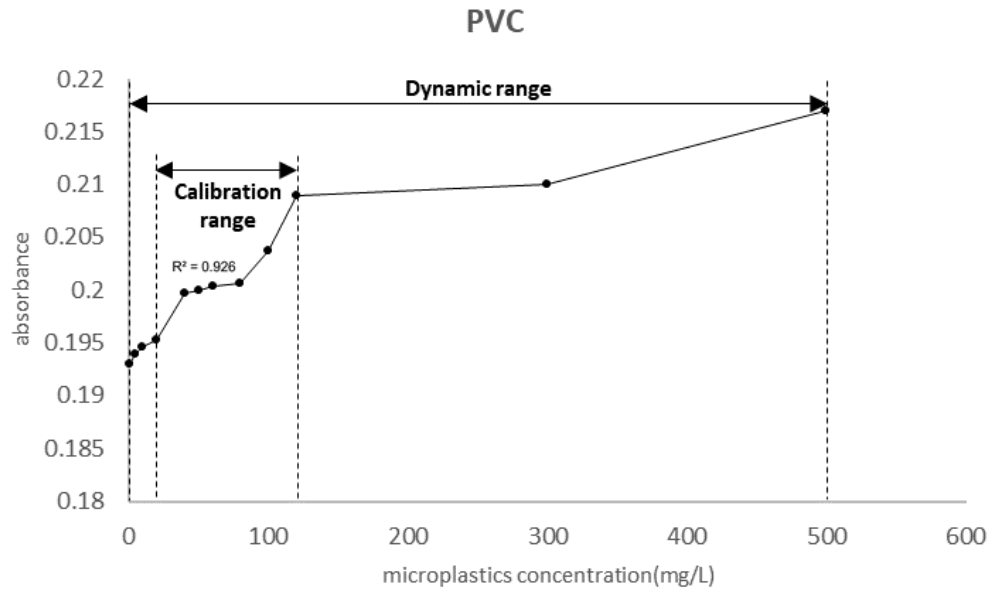


Fig 20. Calibration curve of PVC

4.2.2. LOD & LOQ

Limit of Detection(LOD) is the minimum amount of analyte, and it does not necessarily be quantified. Limit of Quantification(LOQ) is the minimum amount of analyte which can be expressed as the quantity having precision and accuracy. It is the validation parameter that is used for the quantification of micropollutants.

LOD and LOQ were determined by the regression test. The mathematical expressions are as below.

LOD=3.3*(standard Deviation of y-intercept/slope)

LOQ=10*(standard Deviation of y-intercept/slope)

The standard deviation can be derived from the calibration curve.

The results are shown in Table 10. The environmental microplastics concentration range is about 20mg/L~5g/L. The microplastics concentration of the first-sedimented water at the wastewater treatment plant is about 80mg/L. According to the result, every plastic can be detected and quantified regarding each LOD and LOQ for general environmental conditions. However, for the water condition of the wastewater treatment plant, only PE is in reasonable detection and quantification range. LOD of PE, PMMA can be reasonable for general environmental conditions. The detection of microplastics with a concentration less than LOD should be detected by further studies.

Table 10. LOD and LOQ result

	PE	PMMA	PVC
LOD	12.9712	38.5585	81.1387
LOQ	39.3067	116.8439	245.8749

4.2.3. Linearity

Linearity is the ability to obtain linear measurement value in a specific range in proportion to the concentration of analytes. To validate the linearity, at least five standards are required. It can be examined by the coefficient of determination(R^2) based on the linear regression. The closer the value of the coefficient of determination is to 1, the more linear it is.

The coefficient of determination is 0.9816 for PE, 0.9666 for PMMA, and 0.926 for PVC. Linearity was higher in the order of PE, PMMA, and PVC. The lower suspension stability because of the density might cause the lowest linearity of PVC (see Table 11).

Table 11. Linearity result

	PE	PMMA	PVC
R^2 (coefficient of determination)	0.9816	0.9666	0.926
y-intercept	0.1972	0.1995	0.1965
slope	0.0002	0.0001	0.00004

4.2.4. Accuracy

Accuracy is the degree to which a measurement is close to the standard value. To determine the accuracy, every experiment was conducted in triplicate for every concentration condition. Also, the recovery test was conducted. Additional substances can interfere with the detection of the main constituent and affect the recovery rate.

For the recovery test, the simulation with the actual wastewater condition was employed(Joana C. Prata et al., 2019)(Estahbanati & Fahrenfeld, 2016). Primary treated domestic wastewater was collected at Tancheon water re-use center, South Korea. For one cycle of the experiment, 300ml wastewater was prepared. It was filtered with sieves to remove particles of more than 106㎛. Then only dissolved matters and colloids exist in the sample. 87*3mg of specific microplastic is spiked to the filtered wastewater and magnetically mixed for 30 mins. These are separated into three 100ml beakers and dried at 70℃ dry oven for 24 hours. To digest organic matter wet peroxide oxidation (WPO) was used. 80ml 30%(v/v) hydrogen peroxide and 40ml 0.05M Iron(Ⅱ) sulfate were added to the beaker. With constant stirring, sodium chloride was put and heated at 70℃ for 30 minutes. For better digestion, additional hydrogen peroxide can be added. This sample is rinsed with deionized water and vacuum filtration was conducted. This filtered sample was moved to the 100ml flask with surfactant and magnetically mixed for 30 minutes. Then, quantification was performed using UV-VIS spectrophotometer.

The results showed a recovery rate of 109% for PE, 98% for PMMA, and 134% for PVC (see Table 12). Because the linearity of the calibration curve was less than 1, there could be some errors. Also, indigested substances of wastewater might hinder the ideal analysis condition and occurred interference. For PVC, the recovery rate was more fluctuating than the other two microplastics. PVC might have higher interference because of the calibration curve linearity.

Table 12. Accuracy result

	PE	PMMA	PVC
Recovery rate(%)	109	98	134

4.2.5. Precision

Precision can be validated when the error is less than 1% within the triplicate measurement. This is evaluated by the standard deviation or relative standard deviation.

For every concentration condition of each microplastics, all results showed an error of less than 1%. Therefore, its precision was validated (see Table 13).

Table 13. Precision result

	error (%)						
MPs conc. (mg/L)	0	20	40	60	80	100	120
PE	0.12	0.12	0.12	0.12	0.06	0.09	0.18
PMMA	0.12	0.13	0.12	0.12	0.10	0.20	0.15
PVC	0.12	0.03	0.20	0.20	0.20	0.28	0.29

4.2.6. Robustness

Robustness is the parameter of whether the measurement value is affected or not by condition changes. If the value is susceptible to condition changes, the analysis condition should be properly controlled, and or cautionary phrases should be included for the analysis method.

First, analysis results had no significant difference in room temperature and refrigeration conditions. Therefore, the analysis can be conducted in a normal room temperature condition.

Second, after about 48 hours, the decomposition rate of TWEEN 80 increased. Therefore, diluted TWEEN 80 solutions and samples should be prepared right at the time for analysis. Also, it is better to prepare with the amber vial to inhibit the effect of the light.

Lastly, in the case of density higher than 1.2g/cm^3, the sample should be violently stirred five times and analyzed as quickly as possible.

The validity of the UV-VIS spectroscopy was examined with five parameters. Results showed a reasonable extent for validation in comparison to other analytical methods. Therefore, the possibility of UV-VIS spectroscopy for the microplastics quantification method was confirmed.

5. Comparison with other potential microplastics quantification methods

5.1 Visual identification

5.1.1. Principle and methods

Visual identification of microplastics is the most widely applied quantification method so far. It is a quantification method by manual counting of the number of microplastics using a microscope.

Samples with the same concentration condition with previous parts (0, 20, 40, 60, 80, 100, 120mg/L) were prepared. 1ml of the suspended sample was spiked to the grid plate for microscopy. Campscope microscope and program iT Plus 4.0 (Sometech, South Korea) was used for the counting. 50 grids were counted for each sample. The concentration was calculated by dividing the average number of microplastics in a grid by the volume of the sample and multiply it with the number of grids the 1ml spiked sample covers.

$$concentration(ea/L) = \frac{average\ number\ of\ MPs\ in\ a\ grid}{volume\ of\ the\ sample} * number\ of\ grids$$

5.1.2. Results and discussions

The number of microplastics increased as the microplastics concentration increased for all three microplastics (see Fig 21~23).

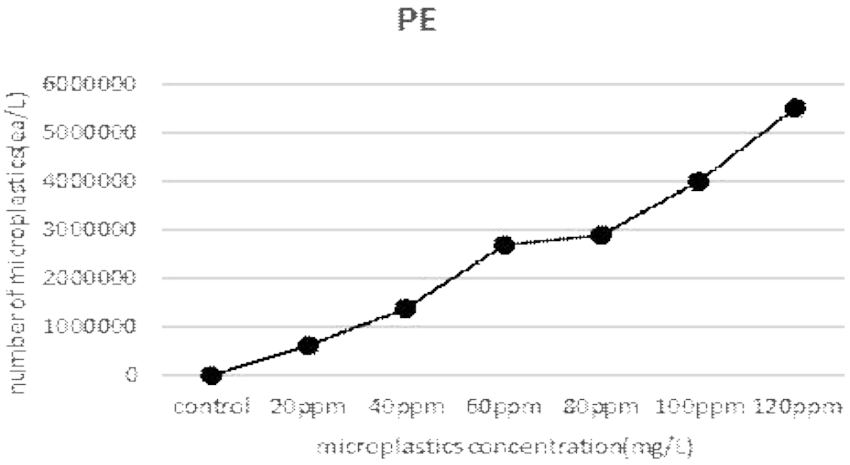


Fig 21. microplastics quantification with visual identification: PE

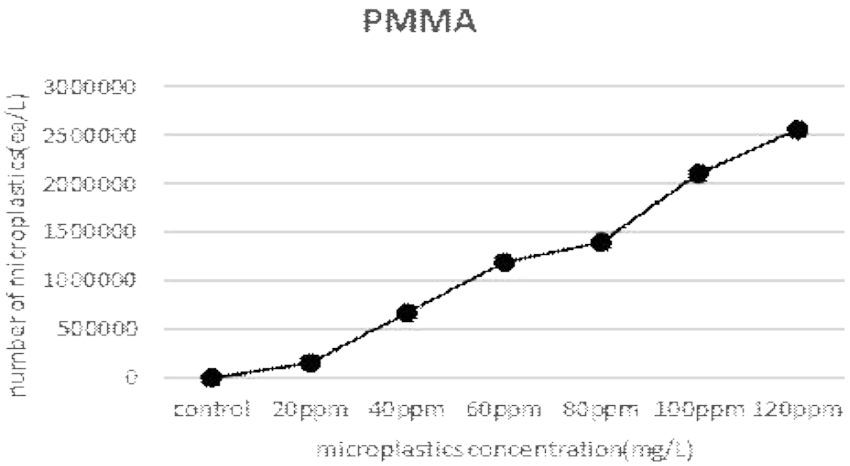


Fig 22. microplastics quantification with visual identification: PMMA

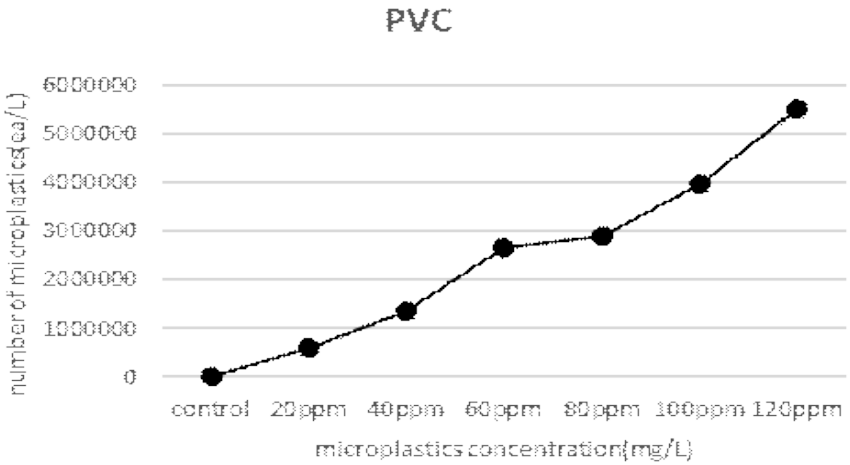


Fig 23. microplastics quantification with visual identification: PVC

However, because of the heterogeneity of microplastics, particle sizes and weight can be different in similar abundance conditions(Rivers et al., 2019). Therefore, visual identification has its limitation for accuracy, the method to comprehend more precise condition with concentration utilizing the quantification method.

5.2 Q-(H)NMR

5.2.1. Principle and methods

Nuclear Magnetic Resonance(NMR) is to analyze certain samples using RF(Radio Frequency) which occurs during the rotational phase transition of the nuclear(Peez et al., 2019)(Peez & Imhof, 2020). This method is normally used for the structure analysis of chemicals. Its utilization for quantification is a new approach. Every plastic has an H atom. Therefore, the resonance of hydrogen is used. The signal intensity is proportional to the number of proton atoms contributing to the resonance. Hence, the signal intensity, which is the peak area is used for the quantification. This method is in the development process as a potential microplastics quantification method.

600Mz high-resolution NMR spectrometer, AVANCE 600, Bruker was employed for the analysis. Samples were put into the magnetic field. 5mm BBO-H&F-D CryoProbeProdigy was used. After the sample was injected into the instrument, the transistor irradiates the radio wave. Then it went to the receiver shim coil and spin works. Finally, the console got the signal and converted it into a spectrum. Before the analysis, proper pre-treatment is required. Sample with proper solvent should be prepared. Deuterated solvents are used for selective signal acquisition and secondary referencing. These solvents should have high solubility but not be reactive. Also, low volatility at high temperatures is required. If the solubility of the sample is low, higher sensitivity equipment is required.

The calibration curve method was applied to this research. Peaks were fit and signals were overlapped. Integrated peak area was used as the quantity of microplastics. Experimental conditions are as Table 14 based on literature. PMMA analysis was omitted because research for PMMA is not conducted based on other NMR specified microplastics quantification methods. This research focuses on the quantification with UV-VIS spectroscopy. 6 different microplastics concentrations same with previous experiments were applied.

PE was suspended in 99.5% Tolune-d8 from Cambridge Isotope Laboratories, Inc., United States(Peez et al., 2019). The residual proton of solvent worked as the internal standard. The temperature increase to 60° C. Peaks at 1.33, 0.93ppm range were integrated. PVC was suspended in dimethyl sulfoxide-d6 containing 1%(v/v) TMS from Sigma-Aldrich Korea Ltd., SIAL(Peez & Imhof, 2020). 1% TMS worked as the internal standard. The analysis was conducted at room temperature and peaks at 4.7~4.05ppm range were integrated.

Table 14. Q-(H)NMR experimental condition

	PE	PVC
Solvent	Toluene-d8	DMSO-d6
Internal standard	Residual proton of solvent	1% TMS
temperature	60° C	RT(room temperature)
range	1.33, 0.93 ppm	4.7-4.05 ppm

5.2.2. Results and discussions

Representative Q-(H)NMR spectra results are featured in Fig 24 and 25. PE and PVC had internal standard peak with 100 times scanning. The H atom concentration can be calculated with the solvent concentration and TMS concentration for each microplastics. Therefore, with these peaks as the standard, the sum of H atom peaks was compared with the standard. The concentration range of this research was narrowed down compared to the literature to simulate the actual environmental concentration. In this range, strong linearity was not discovered (see Fig 26 and 27). Stable detection range and reasonable limit of detection and quantification are not determined yet. Also, the real environmental condition should be considered for better application.

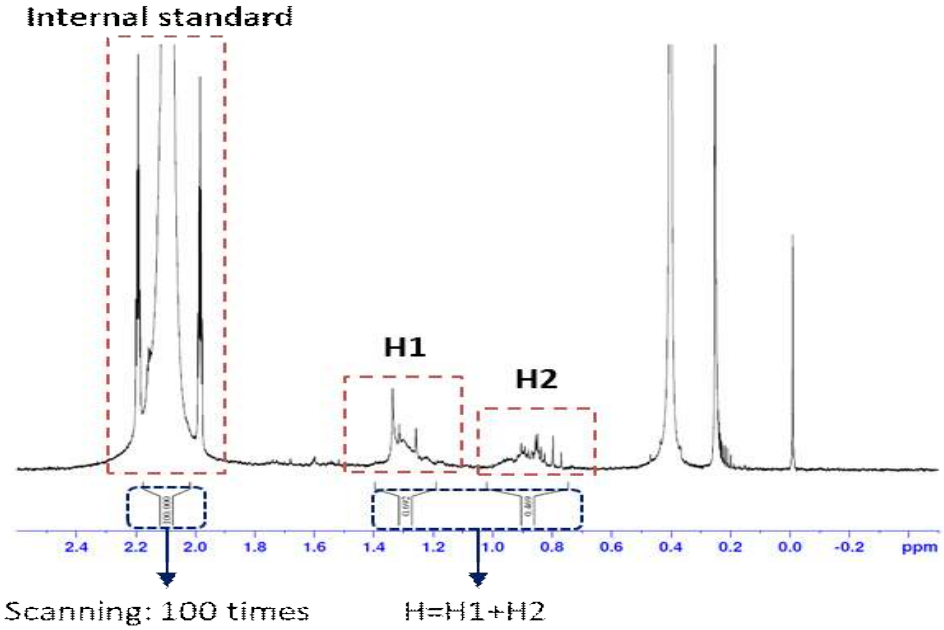


Fig 24. Q-(H)NMR spectra: PE

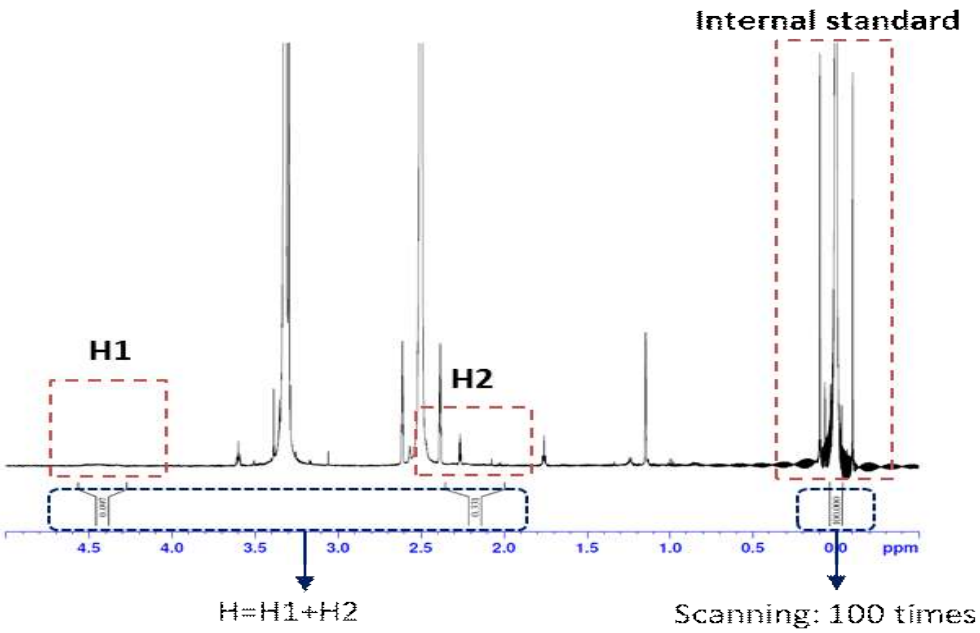


Fig 25. Q-(H)NMR spectra: PVC

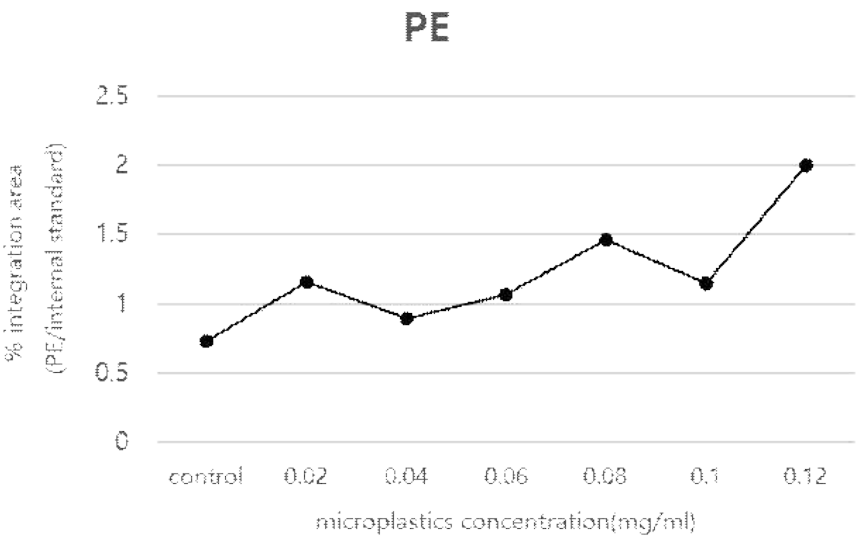


Fig 26. Microplastics quantification with Q-(H)NMR: PE

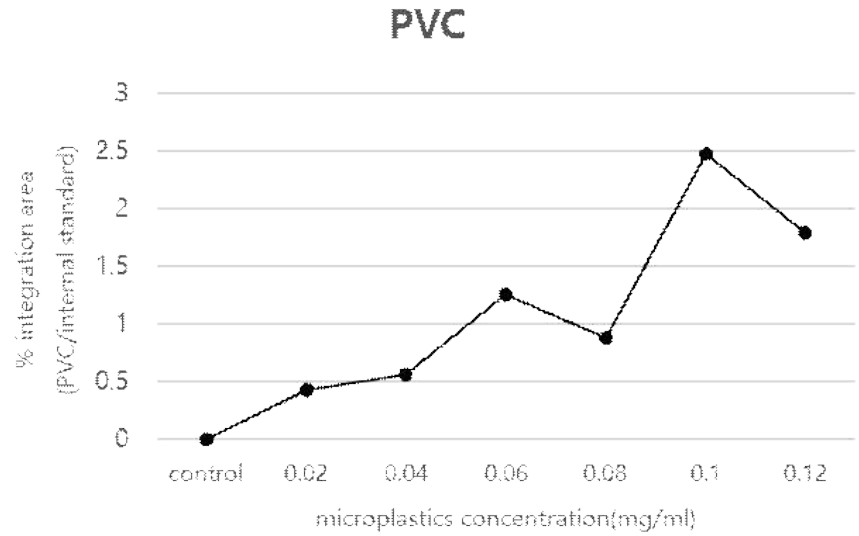


Fig 27. Microplastics quantification with Q-(H)NMR: PVC

5.3 TED-GC-MS

5.3.1. Principle and methods

Thermal Extraction and -desorption combined with Gas Chromatography-Mass Spectrometry(TED-GC-MS) is a potential microplastics quantification technique by pyrolysis, adsorption, desorption, and mass check by GC-MS depending on the microplastics type(Steinmetz et al., 2020)(Duemichen et al., 2019). The applicable mass range is 20-50mg. It takes two hours and 20 minutes to measure one sample.

5.3.2. Limitation of TED-GC-MS

This method is not mainly used for bulk samples. This is derived to figure out the number of microplastics in small-sized products like beverages and tea bags. There is a gap between actual environmental conditions and equipment specifications about the precision and quantification range. Therefore, it is had to apply the objective of this research to apply the actual environmental condition. Therefore, the comparison with this method was not conducted.

5.4 Gravimetric analysis

5.4.1. Principle and methods

Gravimetric analysis is a potential quantification method of the analyte based on its mass value using the scale. The microplastics particles are filtered with 0.45 μm, Ø 47mm GF/C membrane filter. For samples, it should be rinsed with deionized water sufficiently for the hydrolysis of the remaining matters on the filtered sample. To calculate the remaining microplastics mass, the latter mass of the filter and microplastics is subtracted by the original filter mass.

$$\frac{M_2 - M_1}{C} * 100$$

M_1 : $Filter\ paper\ weight(g)$
M_2 : $Filter\ paper\ with\ microplastics\ weight\ (g)$
C: $input\ microplastics\ concentration$

5.4.2. Results and discussions

The recovery rate of microplastics was on average 87.2% for PE, 83% for PMMA, and 82.8% for PVC. The overall recovery rate is as Fig. 28~30. The possibility of sample loss is higher than other quantification methods. Also, because of low sensitivity, the more delicate method might be required to apply for the microplastics quantification of real environmental conditions.

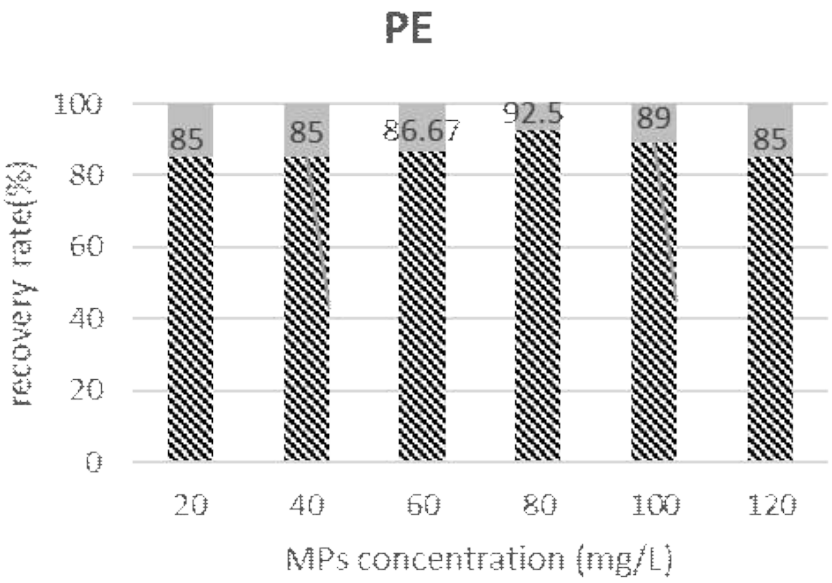


Fig 28. Microplastics quantification with gravimetric analysis: PE

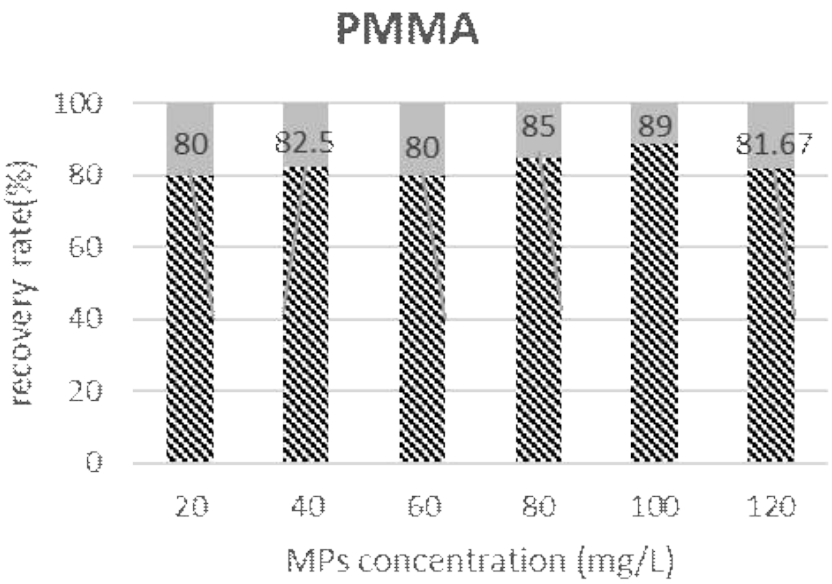


Fig 29. Microplastics quantification with gravimetric analysis: PMMA

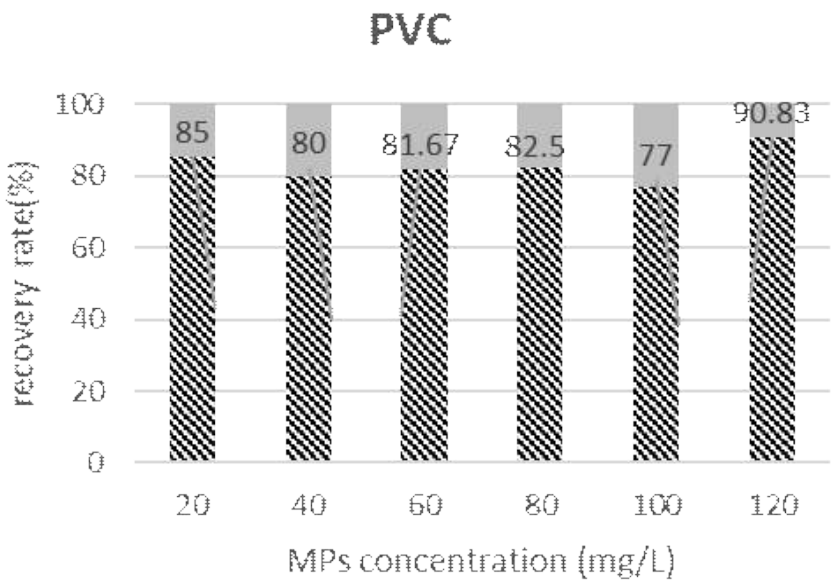


Fig 30. Microplastics quantification with gravimetric analysis: PVC

5.5 Overall comparison with UV-VIS Spectroscopy

Results of all potential microplastics quantification methods were compared with the UV-VIS spectroscopy results from the previous part. Because of the low linearity of Q-(H)NMR, it showed a weak correlation with UV-VIS spectroscopy. On the other hand, the correlation between visual identification, gravimetric analysis, and UV-VIS spectroscopy was strong (see Table 15).

Also, with consideration of quantitative analysis parameters of simultaneous qualification ability, time consumption for the analysis, operation difficulty, analytical sensitivity, and linearity, four methods were compared (see Table 16). 4 is the highest score, and 1 is the lowest score. UV-VIS spectroscopy and visual identification got the same best score.

Therefore, it is possible to propose UV-VIS spectroscopy for concentration-based quantification to compensate for the limitation of visual identification of which the quantification principle is counting.

Table 15. Overall comparison of potential microplastics quantification methods with UV-VIS spectroscopy

(x-axis: UV-VIS spectroscopy)

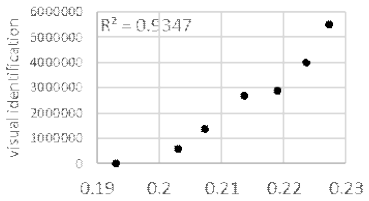
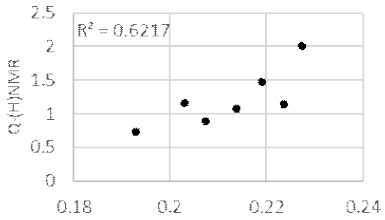
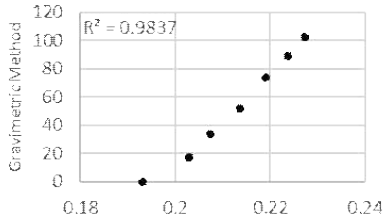
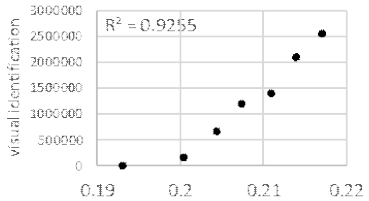
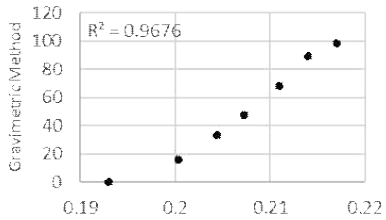
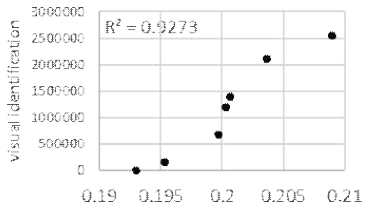
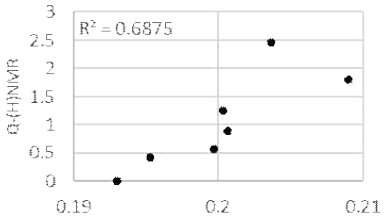
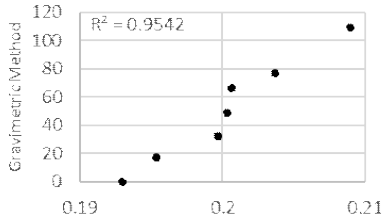
	(1) Visual identification	(2) Q-(H)NMR	(3) Gravimetric analysis
PE			
PMMA		-	
PVC			
Correlation	strong	weak	strong

Table 16. Overall comparision of potential microplastics quantification methods on analytical ability parameters

	UV-VIS spectroscopy	Visual Identification	Q-(H)NMR	Gravimetric analysis
simultaneous qualification	1	1	1	1
time consumption	4	1	2	3
operation	4	4	1	3
sensitivity	4	2	3	1
linearity	3	4	1	2
Sum	12	12	8	10

6. Conclusions

This research aimed to propose a proper analytical method for detection and quantification to deal with microplastics issues, which are sorted as emerging contaminants that can affect human health and the water environment. The objective of this research was to propose UV-VIS spectroscopy, which is a quick, simple, and accurate quantification method, as a new and potential microplastics quantification method.

First, TWEEN 80 worked not only for microplastics suspension but also for the role of analysis aid. TWEEN 80, a non-ionic surfactant could suspend microplastics in the water. Also, the absorbance change by microplastics themselves was not detectable alone. TWEEN 80 worked to detect microplastics as a base substance for UV-VIS analysis.

Second, microplastics quantification using UV-VIS spectroscopy was validated with five validation parameters. For density same or less than 1g/cm^3, this method was applicable. Then, by comparison with other potential microplastics quantification methods, its application possibility was investigated.

In conclusion, UV-VIS spectroscopy has its limitation at the linearity for the quantification and the consideration of the effect of the particle size. If another proper pre-treatment method for more accurate analysis derives and a compact analysis kit is developed, UV-VIS spectroscopy can be a promising alternative microplastics

quantification technology with its simplicity and convenience. With its strength in speed and accuracy, it can be utilized for real-time microplastics concentration monitoring by further research.

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국문초록

마이크로플라스틱은 인체 독성 발현의 가능성이 있는 신종오염물질로 분류되고 있다. 마이크로플라스틱이 가지고 있는 넓은 표면적 때문에 수중의 다른 오염물질이 쉽게 부착될 수 있다. 이 특징에 의해 수중 오염물질의 이동의 매개체가 될 수 있다. 이러한 문제를 해결하기 위한 적합한 해결책을 제시하기 위해서는 마이크로플라스틱의 양에 대한 정확하고 충분한 데이터베이스가 필요하다. 따라서, 세계적으로 자연 수계, 수처리장, 그리고 액체를 담고 있는 제품들에서 검출이 되는 마이크로플라스틱의 정량 분석 데이터를 수집하고 있다. 이러한 연구의 흐름에 따라 다양한 정량 분석 방법들이 연구되고 있으나, 간단하고 간편한 방법이 요구된다. 따라서, 본 연구에서 빠르고 정확한 분석능력을 가지고 있으며 조작이 간단한 UV-VIS 분광법을 선정하였다. 본 연구의 목표는 UV-VIS 분광법을 새롭고 잠재적인 마이크로플라스틱 정량 분석 방법으로 제안하는 것이다.

먼저, 마이크로플라스틱 분석을 위해 적합한 분산 방법을 찾고 계면활성제를 선정했다. 연구에 사용한 계면활성제들 중 비이온성 계면활성제인 TWEEN 80은 마이크로플라스틱 분산을 시킬 수 있을 뿐만 아니라 분석 보조제로의 역할도 가능했다. 마이크로플라스틱은 그 자체만으로는 검출이 되지 않기 때문에 TWEEN 80이 마이크로플라스틱에의 흡착을 통해 검출이 가능하도록 했다.

다음으로, UV-VIS 분광법의 마이크로플라스틱 정량 분석 방법으로서의 유효성을 평가하기 위해 검량선법이 적용되었다. 이 분석법은 정량한계 및 검출한계, 직선성, 정확성, 정밀성, 그리고 완건성의 총 5개 유효성 검사 항목을 통해서 검증이 되었다. 또한 이 분석법은 밀도가 1g/cm^3 이하이거나 비슷한 플라스틱 종들에 적용가능할만한 방법이라는 것이 확인

되었다. 이후, 현미경법, Q-(H)NMR, 무게측정법의 다른 잠재적인 마이크로플라스틱 정량 분석 방법들과의 비교를 통해, UV-VIS 분광법의 적용 가능성을 파악했다.

결론적으로, UV-VIS 분광법은 그 간단하고 편리한 조작 및 분석의 특성을 살려 대체할만한 마이크로플라스틱 정량 분석법이 될 수 있다. 이러한 분석법은 분석 기간을 단축시킬 수 있으며, 이는 마이크로플라스틱 문제의 해결책을 보다 빠르게 제시할 수 있도록 해줄 것이다. 현재 입자 사이즈의 영향 고려 불충분 및 직선성 확보의 한계점을 가지고 있다. 이 문제를 해결하기 위한 향후 연구를 진행한다면 UV-VIS 분광법은 보다 완성도있는 분석법으로 자리매김할 것이다.

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주요어 : 마이크로플라스틱, 정량분석, UV-VIS 분광법,
신종오염물질, 환경분석
학 번 : 2019-20281