

ANALYSIS OF MICROPLASTICS IN BOTTLED WATER

Manjusha M. P.*, Adithya P. S., Glisten P. O., Nancy Sara Jacob, Nandhana A. P. Sreelakshmi V. S., Bindu R., Shyni Bernard

College of Pharmaceutical Sciences, Govt. Medical College, Kottayam, Kerala, Affiliated to Kerala University of Health Sciences, Thrissur.

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Corresponding Author*Dr. Manjusha M. P.**

College of Pharmaceutical Sciences, Govt. Medical College, Kottayam, Kerala, Affiliated to Kerala University of Health Sciences, Thrissur.

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ABSTRACT

Microplastics are pieces of plastic, any kind, smaller than five millimetres. These are synthetic, high molecular weight chemicals with a slow rate of biodegradation and as a result the majority of them stay in the environment and negatively impact human health. The primary method of exposure can be breathing in indoor air and drinking water from plastic bottles. Human consumption of microplastic is estimated to be between 0.1 and 5g per week worldwide. The study looks into the possibility of microplastics in three samples of commercially available drinking bottled water. Chemical evaluations of the sample pH, dissolved solid content, calcium content, and chloride level were performed in addition to physical evaluations like colour, odour, taste, and turbidity. Using a stereomicroscope, the microplastics were visually identified and characterised. Using confocal Raman spectroscopy with AFM, microplastics were analysed spectroscopically. The analysis verifies that all of the samples include microplastics, and fall into the 5mm size range. All the standardisations were done according to BIS guidelines. According to our findings, there is a clear need for focused study to learn more about the uptake, destiny, and health impacts of microplastics in appropriate exposure circumstances.

KEYWORDS: Microplastics, Stereomicroscope, Raman spectroscopy, Analysis.**INTRODUCTION**

Plastics are a wide range of synthetic or semisynthetic materials that use polymers as the main ingredient. According to the type of plastic which is best suited for the processing of a given product, there are two main types of plastics need to be considered. Thermoplastics and thermosetting plastics. Common examples of thermoplastics includes, Polyethylene PE, Polypropylene PP, Polystyrene PS, Polyvinylchloride PVC, Polyethylene terephthalate PET, low and high density polyethylene and that of thermosetting plastics include, epoxy resin, phenolic resins, silicone, cyanate ester, polyurethane, and vulcanized rubber. PET is frequently used to produce bottles for bottled water, whereas PP and PE are used to make bottle caps. These materials are frequently subject to regulations to make sure that they don't leach contaminants such as monomers, plasticizers or other additives into drinking water at most amounts that are dangerous. Based on the size the plastic fragments are classified as macroplastics (≥ 25 mm), mesoplastics (5mm -25mm), minimicroplastics (<1mm - 1 μ m) and nanoparticles (<1 μ m). Plastics of all sizes and shapes degrade into tiny fragments when they reach water ways due to exposure to sunlight, oxygen reaction,

and physical impacts from sand and waves. These microscopic pieces of plastics are called microplastics. Microplastics are fragments of any type of plastic less than 5mm (0.201 inch) in length according to the U.S National Oceanic and Atmospheric Administration (NOAA) and the European Chemical Agency. They are synthetic, high molecular weight compounds and have low biodegradable rate, thus mostly remain in the environment and adversely affect the human body.^[1-5] Microplastics are classified into two categories: primary and secondary. Primary microplastics are those which are intentionally synthesised and introduced into consumer and commercial products like pharmaceuticals, cosmetics, insecticides, skin care products. Micro beads enter water systems and eventually natural waterways because they are minuscule and wash down through sinks. Secondary micro plastics are those which are unintentionally formed by the breakdown of larger plastics by chemical, physical or biological processes such as plastic bottles, plastic bags, plastic food containers and many more. Microplastics have been found in fresh water by runoff from land-based sources, wastewater effluent, and improperly managed plastic garbage. Migration of microplastic in bottled water occur

due to a number of factors including physical stress during transportation, bottle shaking, injection of water into the bottle with high pressure and so on. Also, the fragmentation process is further exacerbated by

temperature impact during storage. Compared to single use PET bottles, reusable PET water bottles contain more microplastics particles because of the friction created due to frequent opening and closing of the bottles.^[6-8]



Figure 1: Microplastics.

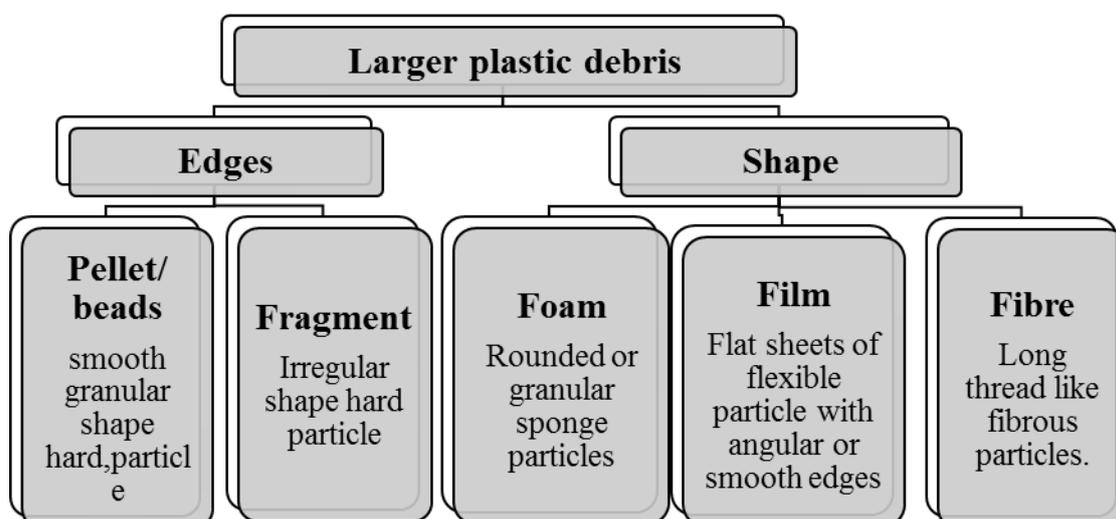


Figure 2: Classification of microplastic based on size and shape.^[9]

Pharmaceutical interactions of microplastics

Microplastics can transform, adsorb, and aggregate with pharmaceuticals, becoming vectors for contaminants like heavy metals, PFAS, PAHs, and PPCPs. Their solid surfaces and hydrophobic nature necessitate studies on sorption to control environmental impact. Pharmaceuticals such as antibiotics, psychiatric drugs, NSAIDs, and cardiovascular drugs can sorb onto microplastics. Ciprofloxacin and tetracycline are well-studied, with interactions including electrostatic,

hydrophobic, van der Waals, π - π , and pore filling. Polymer properties and antibiotic structures influence sorption, potentially forming antibiotic-resistant genes (ARG) and bacteria (ARB). Lower salinity increases microplastic-pharmaceutical complex formation, risking freshwater bodies and low-salinity seas. Aquatic organisms ingest these complexes, passing them up the food chain, affecting gut microbes, and damaging organs. Interactions may cause pharmaceutical degradation into hazardous byproducts. Internal and

external factors influence sorption, bioaccumulation, and toxicity. Microplastics might also decrease pharmaceutical bioaccumulation by promoting degradation and reducing bioavailability, potentially interfering with metabolism in aquatic organisms.^[10-14]

Physiological effects of microplastics in human

Bioaccumulation of microplastics in the human body can potentially lead to wide range of health issues, including respiratory disorders like lung cancer, asthma and hypersensitivity pneumonitis, inflammatory bowel disease. They can induce apoptosis in cells and have genotoxic and cytotoxic effects. Microplastics may cause changes in the intestinal microbiome, resulting in an imbalance between beneficial and harmful bacteria which can lead to various gastrointestinal symptoms. Patients with IBD have a higher concentration of microplastics in their stool (41.8 pcs/g) compared to healthy individuals (28.0 pcs/g). The skin could potentially be permeable to microplastics. These particles might infiltrate the body via sweat glands, hair follicles or skin injuries.^[16-19] Microplastics usually adsorb other pollutants and carry toxic chemicals such as heavy metals and organic pollutants which can adversely affect the human body.^[20-21]

BUREAU OF INDIAN STANDARDS (BIS) GUIDELINES FOR DRINKING WATER

BIS Certification is a symbol of conformity with the requirements for bottled drinking water, which are intended to protect public health and encourage safe usage.

In this regard, BIS has established a number of standards for distinct goods, including IS 14543, a standard intended to guarantee the safety and quality of packaged drinking water. The exact Indian standard for bottled drinking water that the BIS developed is IS 14543. It establishes standards for each step of the making, packing, and distributing of drinking water.

STEREOMICROSCOPY

An optical microscope that shows a specimen in three dimensions is called a stereomicroscope. It is also known as dissecting or stereoscopic microscopes and is a low magnification (5x-250x) digital optical microscope that collects light reflected from the specimen's surface rather than light reflected from the actual object.^[22]

A stereomicroscope operates using two distinct light paths employed by the microscope objective and eyepiece. Providing unique viewing angles through its binocular eyepieces. It has two lights: one for bottom viewing and another for top illumination during dissection. The binocular eyepieces offer a comfortable viewing area and display light pathways. As a digital microscope, it shows 3D images in real time on a computer monitor, enabling close examination of small specimens like insects, with images larger than the sample size (macro-photography).^[23-24] The

magnification can be adjusted by changing the eyepiece lenses. The Galilean optical system, positioned between fixed and zoom magnification, uses fixed-focus lenses for varying sets of magnifications (e.g., two sets for four magnifications, three sets for six magnifications).^[25]



Figure 3: Stereomicroscope.

RAMAN SPECTROSCOPY

Raman spectroscopy relies on inelastic scattering of light by molecules, with elastic scattering (Rayleigh scattering) involving no energy loss and inelastic scattering (Raman scattering) involving energy exchange. When a sample is exposed to laser light, most photons undergo Rayleigh scattering, while a small fraction (1 in 10^6 - 10^8) undergo Raman scattering, causing frequency shifts (Raman shifts). Raman spectroscopy analyses these shifts to identify molecular bond vibrations. Energy can be transferred from molecules to photons (anti-Stokes Raman scattering) or from photons to molecules (Stokes Raman scattering). Each Raman peak in the spectrum corresponds to a chemical bond, creating a unique vibrational fingerprint for molecular identification.^[26-28] Raman spectroscopy instrumentation comprises several key components: a laser source for monochromatic light, a sample holder, optical components like lenses and mirrors to direct and collect scattered light, a monochromator or filter to separate Raman from Rayleigh scattering, a detector (typically a CCD) to capture and convert scattered light into an electrical signal, and a spectrometer to analyse the light signal and produce a Raman spectrum. These components work together to measure and analyse inelastic light scattering, providing a molecular fingerprint of the sample.^[29-30]



Figure 4: Confocal Raman Microscope with AFM.

MATERIALS AND METHODS

Chemicals required

- Ammonium oxalate
- Dilute acetic acid
- Calcium standard solution
- Dilute nitric acid
- Silver nitrate solution
- Chloride standard solution
- Triple distilled water (Grade 3)

Apparatus required

- Petri Dish : 3 petri dishes of 60mm diameter
- Beaker : 1L volume capacity beaker
- Nessler's cylinder
- Glass rod
- Measuring cylinder

- Filtration unit: Glass microfiber filters of diameters 55mm with pore size of 1.5 μm was used.

Instruments required

- Microprocessor turbidity meter : LT-35, Labtronics
- DIGITAL pH METER DP 505
- Stereomicroscope : Carl – Zeiss Stemi 508, Germany
- Raman spectroscopy : WITEC ALPHA300 – Confocal Raman Microscope with AFM

METHODS^[37-43]

Sample collection

Three local brands of 1L water bottles were collected from the market for the analysis.

Table 1: Sample Collection.

Sample Number	Sample Brands	Mfg.Date	Exp.Date	Batch Number	Volume
1.	X	29/12/23	6 months from the date of packing	770	1L
2.	Y	25/03/24	24/09/24	PL482	1L
3.	Z	17/04/24	6 months from the date of packing	6FP57	1L

Physical evaluation

The physical characteristics were determined by analysing the colour, odour, taste and turbidity. The colour of the 3 samples were determined by using spectrophotometer. The samples were opened and

smelled and the odour was recorded. Later taste was recorded by sipping the samples. The turbidity was measured using a turbidity meter for the 3 samples. And the results were measured in NTU units.

Table 2: Evaluation of Physical Characteristics.

CHARACTERISTIC	ACCEPTABLE LIMIT	PERMISSIBLE LIMIT
Colour	Colourless	Colourless
Odour	Agreeable	Agreeable
Taste	Agreeable	Agreeable
Turbidity (NTU)	1	5

Chemical evaluation

The pH of the 3 samples were measured using the pH meter. Total dissolved solids were measured using TDS meter. The limit test was conducted as follows;

Calcium: To 0.2ml of alcoholic calcium standard solution, 1ml of ammonium oxalate solution was added. After 1 minute, a mixture of 1ml dilute acetic acid and 15ml of the sample solution were added and shaken. Prepared the standard in the same manner using a mixture of 10ml aqueous calcium standard solution, 1ml dilute acetic acid and 5ml of distilled water. After 15

minutes, the opalescence between the test and standard were compared.

Chloride: To 15ml of the prescribed solution 1ml of dilute nitric acid was added and poured the mixture as a single addition into a test tube containing 1ml of silver nitrate solution. Prepared the standard in the same manner using 10ml of chloride standard solution and 5ml of water. The test tubes were examined laterally against black background. After standing for 5 minutes protected from light, opalescence between the test and standard were compared.

Table 3: Evaluation of Chemical Characteristics.

CHARACTERISTIC	ACCEPTABLE LIMIT	PERMISSIBLE LIMIT
pH value	6.5 – 8.5	No relaxation
Total dissolved solids	500	2000
Calcium (mg/L, Max)	75	200
Chloride (mg/L, Max)	250	1000

Filtration of microplastic in bottled water

The filtration unit is set up. The bottle containing 1L of water to be analysed is emptied into the apparatus. The empty sample bottles during each filtration step were rinsed thrice with distilled water to ensure all the contents were filtered. The glass fibre filter membrane is transferred to a sterilized petri dish, which was covered with another petri dish to avoid external contamination. This is dried in a hot air oven at 40°C before proceeding to identification and counting. Similarly carry out the same procedure for the remaining two samples.

Visual identification of microplastic using Stereomicroscopy

Ensure that the machine and the surrounding platforms were dust free before particle identification. The dried filter paper is mounted on the stage plate. Adjust the focus knob, lighting controls and magnification to obtain visual identification of the microplastics present. Using the ZEN Microscopy Software the abundance (No. of

microplastics), shape, size and colour of the microplastics present in the sample is obtained. The stereomicrograph (images) was captured. Three samples of each brand were analysed similarly.

Determination of microplastics in bottled water available in market using Raman spectroscopy

Before conducting the analysis, ensure that the instruments and the lasers are switched on. In order to maintain the working temperature of the instrument, the detector and the laser should be switched on 30 minutes prior to the analysis. Transfer the sample on the scanning stage using a tweezer, set the objective lens to 20x magnification and focus the sample. This produces an image on the screen. The objective lens was manually moved to locate and focus the microplastics in order to optimise its detection. This ensures precise positioning and illumination of the sample. Laser of 532 nm was applied for 10 seconds and spectrum will be obtained which is then identified using a spectral library.

RESULTS AND DISCUSSIONS

OBSERVATION

Steps of filtration



Figure 5: Step 1

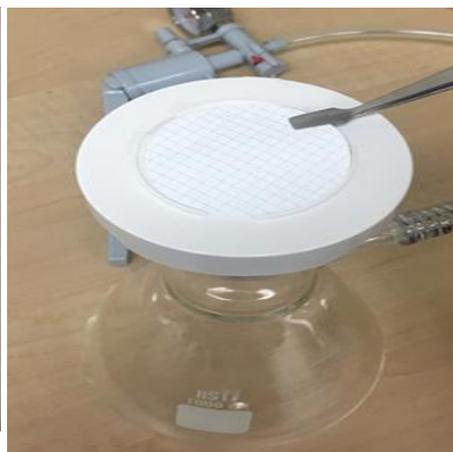


Figure 6: Step 2



Figure 7: Step 3.



Figure 8: Filtration unit.



Figure 9: Glass fibre filter membrane.

Samples after filtration

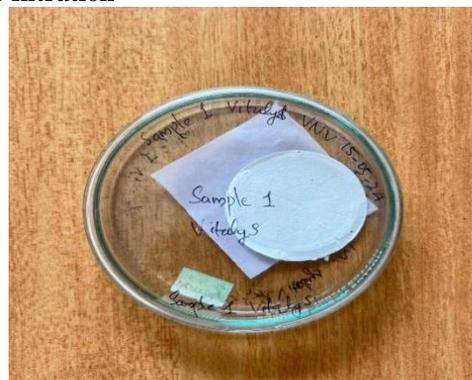


Figure 10: Sample X.



Figure 11: Sample Y.



Figure 12: Sample Z.

RESULTS OF PHYSICAL EVALUATION

Table 4: Report of physical evaluation.

CHARACTERISTIC	SAMPLE X	SAMPLE Y	SAMPLE Z
Colour	Colourless	Colourless	Colourless
Odour	Agreeable	Agreeable	Agreeable
Taste	Agreeable	Agreeable	Agreeable
Turbidity (NTU)	4.6	4.8	4.9

RESULTS OF CHEMICAL EVALUATION**Table 5: Report of chemical evaluation.**

CHARACTERISTIC	SAMPLE X	SAMPLE Y	SAMPLE Z
pH value	7.3	6.9	7.5
Total dissolved solids (ppm)	230	40	220
Calcium	Opalescence less than standard	Opalescence less than standard	Opalescence less than standard
Chloride	Opalescence less than standard	Opalescence less than standard	Opalescence less than standard

RESULT OF STEREOMICROSCOPIC ANALYSIS**Table 6: Visual identification and characterization.**

Sl. No.	Sample Id	Abundance (No. of Microplastic)	Shape of Microplastics	Size of Microplastics (Size range in μm)	Colour of Microplastics
1	Sample X	18	Fiber and fragment	23.93 to 2418.41	Blue Red Transparent Green Black
2	Sample Y	12	Fiber	178.08 to 2279.63	Blue Transparent Black
3	Sample Z	12	Fiber	101.33 to 2237.01	Blue Red Transparent Yellow

**Figure 13: Fibre blue X****Figure 14 : Fibre blue Y****Figure 15 : Fibre red Z****Figure 16 : Fibre transparent X****Figure 17: Fibre transparent Y****Figure 18: Fibre transparent Z**



Figure 19: Fragment red X



Figure 20: Fragment transparent X

RESULT OF RAMAN SPECTROSCOPIC ANALYSIS

Sample X

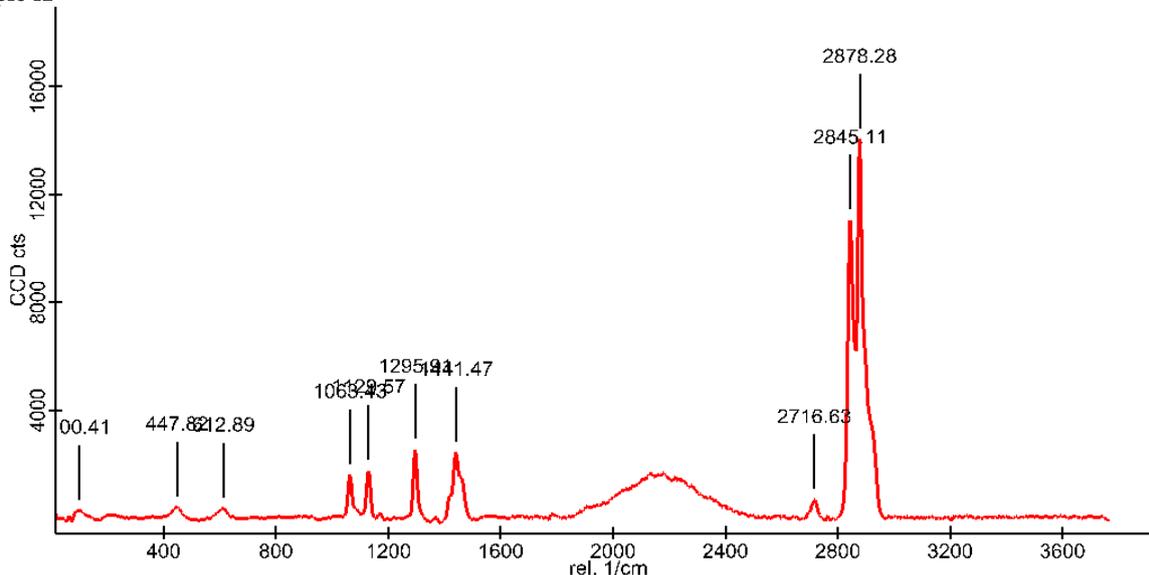


Figure 21: Sample peak (X).

Sample Y

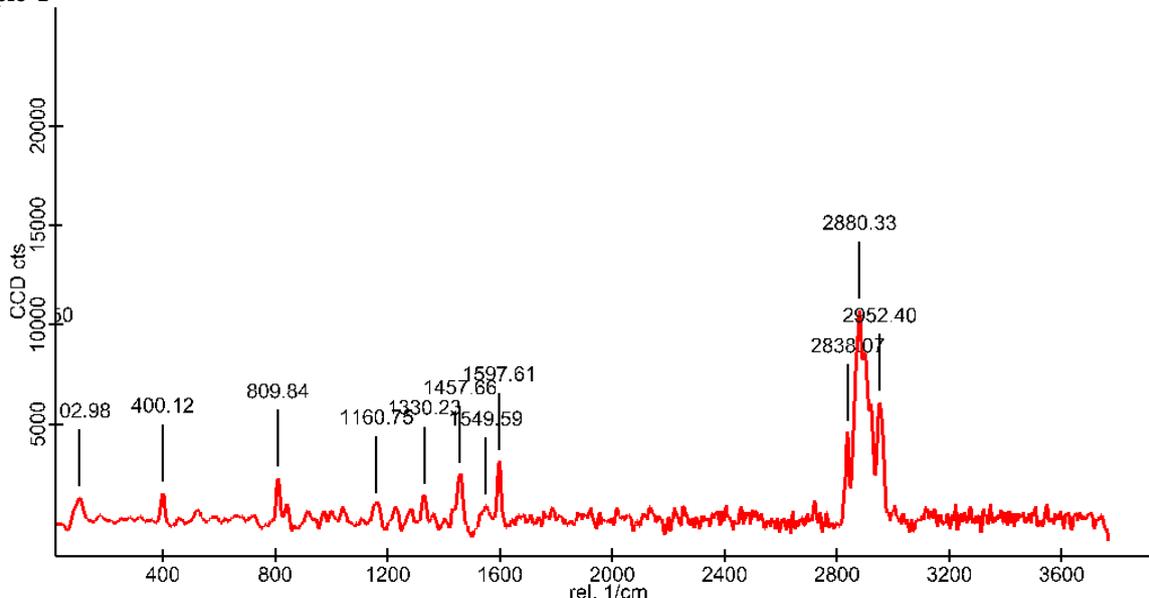


Figure 22: Sample peak (Y).

Sample -Z

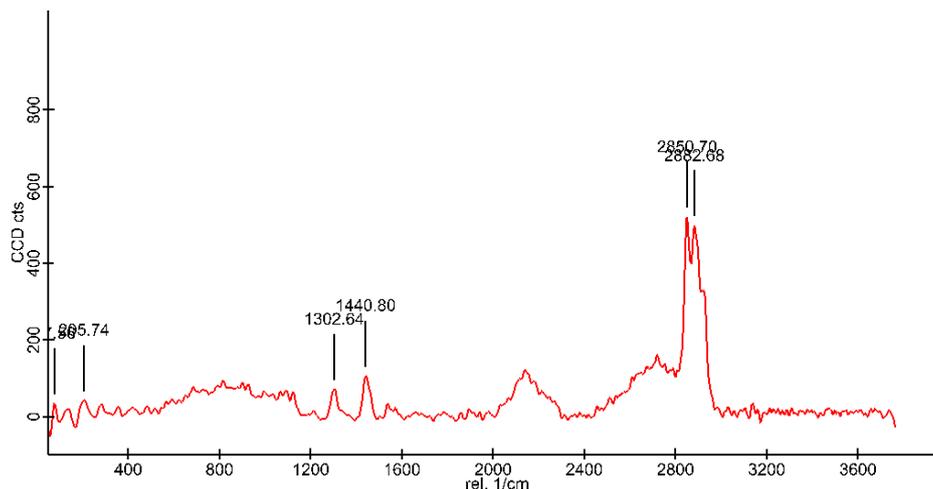


Figure 23: Sample peak (Z).

DISCUSSION

The analysis of microplastics in drinking bottled water was conducted using three local brands namely Vitalys, Basic and Mount mist. Visual identification and characterisation of the microplastics was carried out using a stereomicroscope. The stereo micrograph (images) of the selected fibers and fragments was presented in Fig. 13 to 19.

The size range of microplastics in sample x is 23.93 to 2418.41 μm sample Y is 178.08 to 2279.63 μm and sample Z is 101.33 to 2237.01 μm . All the observation were found to be within 5mm size range thus confirming them to be microplastics. Abundance of microplastics in sample 1, 2 and 3 was found to be 18, 12 and 12 respectively. Sample X and sample Z had microplastics less than 150 μm size range thus proving to be most toxic. The spectroscopic analysis of microplastics was done using confocal Raman spectroscopy with AFM. The spectrum plotted for the three samples are given in the Fig.21-23. From the spectrum the microplastics in. Sample X was identified to be polyethylene. Sample Y was identified to be polypropylene and. Sample Z was identified to be polyethylene.

SUMMARY AND CONCLUSION

Under this study we analysed the presence of microplastics in bottled water collected from Kottayam, Gandhinagar locality. FT-IR spectroscopy was commonly used in the analysis of microplastics, this study incorporates a novel approach using Confocal Raman Spectroscopy with AFM under which limited studies have been conducted. It is an analytical technique based on the inelastic scattering on microplastics which provides a vibrational fingerprint spectra. The water bottle samples taken were filtered using a glass filter membrane and examined with a stereomicroscope. Image captured from this examination were further analysed with Raman spectroscopy. The results showed that all three bottles contained microplastics in fragment and fibre forms of various sizes ranges. Bottled water being a

necessary source for human water consumption, especially in location with a high concentration of hospitals and also in urban areas thus it is essential to reduce the amount of microplastics in bottled water in order to safeguard human health. It is also important to regulate and lessen the amount of microplastic contamination in water bottles in order to enhance the quality of drinking water and thereby reduce the chance of toxic effects it may impose.

FUTURE PROSPECTS

The study provides a scope for future research on microplastics in drinking water sources, beverages, pharmaceutical products etc. Further research on physiological effect on human health can be conducted. Overcoming the restraints of factors like cost, time, availability of instruments and facilities we can confirm the source of microplastics.

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