ORIGINAL ARTICLE

Evaluating the Suitability of ATR-FTIR Spectroscopy for Screening Suspected Microplastics (MPs) in Human Breast Milk (HBM)

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ABSTRACT

Background: Microplastics (MPs) are increasingly detected in human biological samples, including human breast milk (HBM), raising concerns regarding infant exposure during early development. However, analysing MPs in HBM remains challenging due to its complex composition and limited standardised detection methods. This study aimed to evaluate the suitability of Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectroscopy for identifying MPs in HBM following enzymatic digestion and oxidative treatment. **Methods:** Processed samples were filtered, visually screened under a stereomicroscope, and analysed using ATR-FTIR. Spectral matches were compared with a polymer reference library. **Results:** Several polymer signatures were detected, including polypropylene (PP), polyvinylidene fluoride (PVDF), and chlorosulfonated polyethylene (CSPE), with match scores ranging from low to moderate confidence (0.43–0.55). These findings show that ATR-FTIR can support preliminary MP screening in HBM, but accuracy is limited by spectral resolution, library constraints and contamination risks. Improved contamination control upgraded software libraries, and integration of complementary techniques such as Raman spectroscopy and pyrolysis-GC-MS are recommended to strengthen reliability. **Conclusion:** Overall, this work provides early analytical insight into MP detection in HBM and highlights key considerations for method optimisation in future studies.

INTRODUCTION

Microplastics (MPs) are now recognised as a growing global health concern. They have been detected not only in the environment but also in complex biological samples, including human breast milk (HBM) (Zuri et al., 2023; Roslan et al., 2024). MPs are tiny plastic fragments that are formed through the breakdown of larger plastic items by processes such as photodegradation, biological degradation, and chemical reactions (Bajt, 2021). Since plastics are widely used in everyday life, their presence in the human body is becoming increasingly common. Their small size allows them to move through different media and potentially interact with human tissues, raising questions about possible health effects (Roslan et al., 2024). Infants are one of the most vulnerable groups, and they rely heavily on breast milk for healthy development. HBM contains essential nutrients and bioactive compounds that support growth, immune function, and overall well-being (Kim & Yi, 2020). Therefore, the discovery of MPs in HBM is concerning, as it may expose infants to plastic particles during a critical stage of life. Although the long-term effects of MP exposure in infants are still unclear, this issue deserves attention, given the continuing rise in plastic use and environmental contamination.

Studying MPs in HBM is challenging because breast milk is a complex biological fluid of fats, proteins, and other organic components that can interfere with analysis (Arshad et al., 2024; Saraluck et al., 2024). At present, there is no widely established standard method specifically designed for detecting MPs in HBM, and most existing protocols are adapted from environmental research. Advanced techniques like Raman spectroscopy, micro-FTIR, and ATR-FTIR have been used in previous studies, however, each method presents distinct advantages and limitations when applied to milk samples.

In this study, ATR-FTIR spectroscopy was used to detect and identify MPs in HBM after sample extraction and

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digestion. This study aimed to evaluate the reliability of this technique in identifying MPs within a complex matrix and contribute to ongoing efforts to understand and monitor MP exposure in infants.

MATERIALS AND METHODS

Ethical Approval and Sample

This study was ethically approved on 22nd March 2025 by the IIUM Research Ethical Committee (IREC), Malaysia, in accordance with the principles outlined in the Declaration of Helsinki, the International Conference on Harmonisation - Good Clinical Practice (ICH-GCP), the Malaysian Guidelines for Good Clinical Practice (Malaysia GCP), and the Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines (Approval ID: IREC 2025-058). Donated human breast milk (HBM) samples were obtained from Halimatussaadia Mother's Milk Centre (HMMC), Malaysia, with all samples anonymised to maintain donor confidentiality.

Chemicals

The key chemicals and reagents used in this study included hexane (HMBG Chemicals, Germany), 30% H_2O_2 solution (HMBG Chemicals, Germany), 70% ethanol (HMBG Chemicals, Germany), lipase Type VII from *Candida rugosa* with an activity of 700 units/mg solid (Sigma-Aldrich, United States), pH 7.2 buffer tablets (Sigma-Aldrich, United States), KOH pellets (Sigma-Aldrich, United States), and ultra-pure distilled water (milli-Q). All chemical and reagents used are analytical grade. The *Candida rugosa* lipase was stored at 2–4 °C prior to use, as recommended by the manufacturer, while other chemicals such as hexane, H_2O_2, and KOH were stored at room temperature in designated chemical safety cabinets.

Sample Processing

Donated HBM samples were processed following the standardized protocols of the operating milk bank and subsequently stored at −20°C to ensure preservation of compositional integrity. Upon thawing, noticeable physical variations were observed among samples, allowing classification into two primary types: Type A - high-fat milk exhibiting a thick, creamy texture with discernible lipid aggregates, and type B - low-fat milk displaying a more diluted appearance. This classification facilitated comparative analysis of digestive performance across samples differing in lipid content. A systematic sample nomenclature was adopted to streamline tracking and maintain procedural consistency throughout digestion and oxidation experiments. This consistent labelling framework minimized handling errors, improved traceability, and ensured reproducibility in subsequent data analysis.

Controls

Negative controls consisted of three samples prepared using only ultra-pure water (Milli-Q) without any HBM. These were subjected to the full digestion protocol using lipase, KOH, and H_2O_2 to detect any background contamination introduced through reagents, glassware, or environmental exposure. Positive controls were prepared by spiking HBM with known MPs: three nylon 66 (NY66) fragments (blue), three polyethylene terephthalate (PET) fragments (green), and three polypropylene (PP) fragments (pink). These polymers were selected based on their common occurrence in consumer packaging and their reported detection in breast milk and food-contact plastics (Ragusa et al., 2022; Liu et al., 2023). The positive controls were used to validate the integrity of the digestion method and to assess the compatibility of the protocol with polymer preservation under oxidative and enzymatic conditions. Both positive and negative controls were processed using the same reagent volumes, incubation durations, and temperature conditions as the experimental samples.

Digestion.

HBM samples (10 mL each) underwent a multi-step digestion protocol to remove lipids and organic matter while preserving MPs. Lipids were first reduced by hexane extraction, using 1:1 and 2:1 hexane: HBM ratios were prepared and vortexed, then left to stand for ≥2 hours until clear phase separation occurred. The lower aqueous phase (liquid matter, LM) containing proteins, MPs, and other soluble was collected for further treatment (Shang et al., 2021). Residual lipids were then enzymatically digested using a 5% lipase solution prepared in phosphate buffer (pH 7.2). Pre-warmed HBM (37 °C) was incubated with lipase at milk-to-enzyme ratios ranging from 1:1 to 20:1 at approximately 37 - 37.4 °C for 2 - 7 days, with pH monitoring, and successful digestion indicated by a final pH of approximately 4. Subsequently, protein digestion was performed with 10% (w/v) KOH, added at stepwise HBM:KOH ratios (1:1 to 1:7) and incubated at 40 - 45 °C for 2 - 4 hours. Successful protein degradation was evidenced by a dark brown colour change and decreased viscosity. Where necessary, 70% ethanol (1:1) was added to disrupt emulsions and aid filtration. Remaining organic matter was oxidised using 10% H_2O_2 at milk-to-H_2O_2 ratios of 1:1 - 1:3, typically at room temperature for approximately 12 hours, with an optional secondary oxidation at 40 - 50 °C for approximately 20 minutes until solutions became clear or

lightly coloured. After optimisation, the final protocol was applied identically to all experimental samples and to controls: negative controls to assess background contamination, and positive controls to verify MP recovery and polymer stability under the combined enzymatic, alkaline, and oxidative digestion conditions (Enders et al., 2016; Shang et al., 2021; Ragusa et al., 2022; Liu et al., 2023).

Filtration and ATR–FTIR Analysis

Following digestion, each HBM sample and all positive and negative controls were vacuum filtered using 47 mm diameter Whatman GF/C glass fibre filters (pore size 1.2 µm) (Shang et al., 2021), then dried at 60 °C for 24 hours (Prata et al., 2019). Putative MP particles retained on the filters were first screened under a stereomicroscope. Colour, shape and surface texture were used to distinguish plastic-like particles from residual organic matter, with fibrous, film-like and irregular fragment morphologies selected in line with recommended visual pre-screening criteria (Giri et al., 2024; Mariano et al., 2021). Visually suspected MPs were carefully transferred from the filters to the ATR crystal using a clean, fine-tipped metal tweezer, and each particle was placed directly onto the diamond crystal for spectral acquisition. All observable suspected MPs on each filter were subjected to ATR-FTIR analysis. Spectra were collected using a PerkinElmer Frontier (2013) FTIR spectrometer equipped with a diamond ATR accessory, operating from 4000 to 600 cm^{-1} at 4 cm^{-1} resolution with 32 co-added scans per particle. Spectra were processed in Spectrum software and matched against the commercial polymer libraries supplied by the manufacturer (e.g. Hummel Polymer and Additives Library, PerkinElmer ATR-Polymer Library). Library match scores in this study ranged from 0.42 to 0.55, below the widely accepted thresholds of ≥0.60 (reliable) and ≥0.70 (high-confidence) used for MP identification (Rathore et al., 2023). Given the reduced spectral clarity expected in lipid- and protein-rich matrices such as HBM, where residual organic matter can interfere with ATR-FTIR analysis (Ivleva, 2021; Rani et al., 2023), a pragmatic match-score threshold of 0.50 was adopted. This approach reflects the lack of universal standardisation in MP FTIR identification and the variability in spectral quality across complex matrices (Rathore et al., 2023). Therefore, spectra with scores ≥0.50 were considered tentative identifications, while lower scores were interpreted with caution and supported by morphological characteristics (Soliz et al., 2024; Giri et al., 2024). For each confirmed or suspected MP, we recorded polymer type, software match score, stereomicroscopic morphology and qualitative notes on possible contamination or artefacts, consistent with current recommendations to couple morphological and chemical descriptors when reporting MPs in complex biological samples (Dzierżyński et al., 2024; Soliz et al., 2024).

Quality Assurance

To mitigate atmospheric MP contamination, all surfaces were subjected to ethanol wiping prior to analysis. Glassware was used for all apparatus, and plastic materials were avoided except for the micropipette. Apart from that, glassware and equipment were meticulously cleansed with filtered distilled water and covered with aluminium foil (Davidson & Dudas, 2016; Li et al., 2015; Qu et al., 2017; Su et al., 2018). Every procedural step included both negative and positive controls to monitor contamination during the processes (Santana et al., 2017).

RESULTS

The ATR-FTIR analysis revealed several polymer signatures in the filtered and dried samples, as summarized in Table 1. Detected materials included polyvinylidene fluoride (PVDF), alginic acid sodium salt, polypropylene (PP), and chlorosulfonated polyethylene (CSPE). Spectral match scores obtained from the reference library ranged 0.42 - 0.55. For this study, spectra with scores ≥0.50 were considered reliable identifications, whereas values below 0.50 were interpreted as low confidence matches.

Table 1: ATR-FTIR polymer identification and match scores for HBM samples. All entries represent analysed samples (L2–L10). Procedural blank controls were conducted in parallel to assess potential contamination but are not included in this table.

Sample	Suspected Polymer Identified (Best Match)	Match Score
L2	(AP0078) Polyvinylidene fluoride (PVDF)	0.440426
L3	(AP0002) Alginic acid sodium salt	0.55236
L4	(AP0065) Polypropylene isotactic (PP)	0.420418
	AP0078 Polyvinylidene fluoride (PVDF)	0.432505
L6	AP0078 Polyvinylidene fluoride (PVDF)	0.439063

Sample	Suspected Polymer Identified (Best Match)	Match Score
L9	AP0078 Polyvinylidene fluoride (PVDF)	0.427609
L10	AP0054 Polyethylene chlorosulfonated (CSPE)	0.459408
S1	*(AP0054)* Polyethylene chlorosulfonated (CSPE)	0.429755
S2	(AP0054) Polyethylene chlorosulfonated (CSPE)	0.428505
S3	(AP0054) Polyethylene chlorosulfonated (CSPE)	0.452859

DISCUSSION

ATR-FTIR was employed in this study due to its accessibility, non-destructive nature, and proven reliability for polymer identification in complex biological and environmental matrices (Miskon et al., 2024; Ng et al., 2018). The technique operates by detecting characteristic vibrational modes of chemical bonds, which are then compared against reference spectra in polymer libraries (Ng et al., 2018).

The relatively low match scores (0.42 - 0.55) observed in this study can be attributed to several analytical and matrix-related factors. Human breast milk is a lipid- and protein-rich matrix, and residual organic matter may persist despite digestion, leading to spectral interference and reduced signal clarity (Ivleva, 2021; Rani et al., 2023). Such residual materials may hinder effective contact between particles and the ATR crystal and contribute to less distinct spectral features. In addition, the small size and irregular morphology of the particles can result in weak absorbance signals and reduced signal-to-noise ratios as well. Environmental MpPs may also undergo weathering or surface modification, further altering their spectral features compared to reference libraries (Ivleva, 2021).

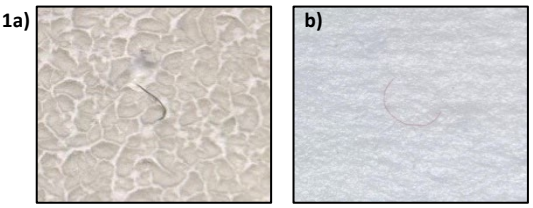

Figure 1: a) PVDF and b) PP under stereomicroscope

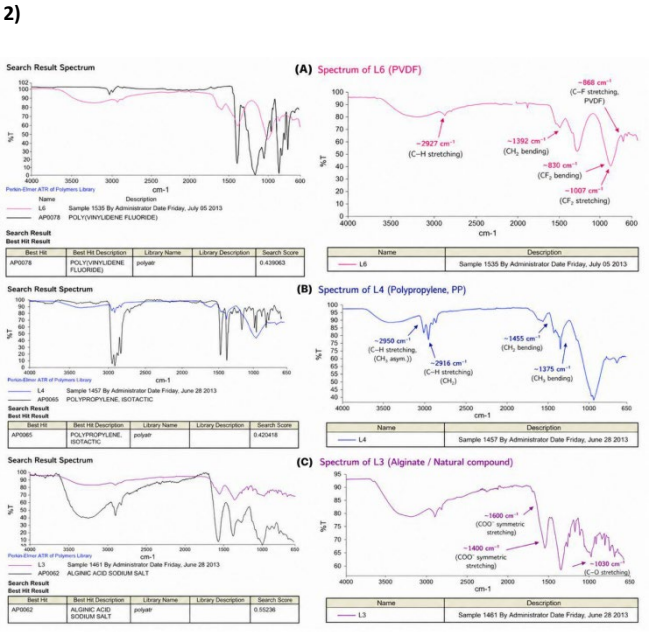

Figure 2: Composite ATR-FTIR spectra of representative samples showing polymer identification and diagnostic peak assignment. The left panels present the comparison between sample spectra and reference library spectra, where the black line represents the reference polymer and the coloured line represents the analysed sample. The right panels display the corresponding sample spectra with labelled diagnostic peaks indicated by arrows and their respective wavenumbers (cm^{-1}). (A) Sample L6 exhibiting PVDF-like features, with characteristic peaks at ~868 cm^{-1} (C–F stretching), ~830 cm^{-1} (CF_2 bending), and ~1007 cm^{-1} (CF_2 stretching), indicative of fluorinated polymers. (B) Sample L4 identified as polypropylene (PP), showing typical peaks at ~2950 and ~2916 cm^{-1} (C–H stretching), ~1455 cm^{-1} (CH_2 bending), and ~1375 cm^{-1} (CH_3 bending), consistent with an aliphatic hydrocarbon backbone. (C) Sample L3 showing alginate-like characteristics, with peaks at ~1600 cm^{-1} (COO^- asymmetric stretching), ~1400 cm^{-1} (COO^- symmetric stretching), and ~1030 cm^{-1} (C–O stretching), suggesting the presence of a naturally derived polysaccharide. Only representative and well-resolved diagnostic peaks are annotated to ensure clarity and to avoid overinterpretation of weak or non-specific signals. The significance of the labelled peaks is interpreted based on characteristic functional group vibrations to support tentative material identification.

Among the materials tentatively identified, PP is of particular interest, as shown in sample L4, as shown in Figure 1(b) and summarised in Table 1. The identification was supported by ATR-FTIR spectral matching and the presence of characteristic diagnostic peaks corresponding to C–H stretching and bending vibrations. In aquatic

environments, polypropylene (PP) has been reported to account for approximately 14% of MPs, followed by polystyrene (8.5%), polyvinyl alcohol (6%) and polyvinyl chloride (2%) (Ashrafy et al., 2023), underscoring its ubiquity in environmental matrices. Beyond marine ecosystems, PP is extensively used in food-contact materials, including microwaveable and thin-walled containers, as well as in medical and healthcare devices (Hossain et al., 2024; Kadac-Czapska et al., 2023). These applications suggest multiple potential pathways for PP to enter HBM, for instance through contact with food packaging, breast pumps, or storage containers. PP fibres and non-woven textiles are also common in consumer products due to their durability and low moisture absorption (Hossain et al., 2024). These broad applications suggest several plausible pathways for PP to reach HBM, including contact with PP-containing food packaging, breast pumps, storage containers, and clothing or nursing textiles during milk expression. Nonetheless, given the modest match scores observed, PP-like spectra in this study should be regarded as indicative of possible, rather than confirmed, PP contamination.

PVDF-like signatures were also detected in several samples such as L2, L4, L6 and L9. PVDF is a highly durable fluorinated polymer with a high melting point, strong mechanical strength, and resistance to chemicals, UV radiation, and hydrolysis (Dallaev et al., 2022). It is widely used in coatings, membranes, and components for pipelines and building materials (Dallaev et al., 2022). The biological plausibility of PVDF transfer into HBM is less straightforward than that of PP, and the detection of PVDF and CSPE in control samples strongly suggests that at least some of these signals may be attributable to airborne contamination or contact with laboratory materials rather than genuine *in vivo* exposure. It is acknowledged that polymers identified in this study, such as PVDF and CSPE, may potentially originate from laboratory-related sources (e.g., filtration membranes, tubing, or instrument components) rather than environmental contamination. Therefore, their presence should be interpreted with caution. While procedural controls were implemented, the possibility of laboratory-derived contamination cannot be fully excluded, particularly when working with complex matrices. This observation highlights the susceptibility of MP analysis to contamination and reinforces the importance of rigorous contamination-control measures, including the systematic use of field and procedural blanks.

Sample handling also influenced data quality. The formation of irregular, flaky residues on the filter surface likely impaired crystal-particle contact during ATR-FTIR measurements, contributing to weak absorbance signals and low match scores for several particles. Such manual handling artefacts are increasingly recognised as a critical source of variability in MP analysis, particularly when working close to the detection limits. Despite these limitations, the workflow applied here, combining enzymatic and chemical digestion with stereomicroscopic pre-screening and ATR-FTIR analysis, demonstrated that it is technically feasible to recover and chemically characterise particles from HBM. These findings reinforce the need for stringent contamination control measures, especially when working with trace-level MPs.

Particles identified as alginic acid sodium salt highlight the complexity of interpreting ATR-FTIR spectra in biological matrices. Unlike conventional synthetic polymers, alginate is a naturally derived polysaccharide extracted from brown algae and widely used in food and pharmaceutical applications (Sanchez-Ballester et al., 2021). It is commonly employed as a stabiliser and thickening agent in food products and as an excipient in pharmaceutical formulations (Sanchez-Ballester et al., 2021). Therefore, its detection in HBM does not necessarily indicate MP contamination but may reflect the presence of naturally derived or semi-synthetic compounds potentially originating from dietary or environmental sources.

Given that most match scores fell below commonly applied confidence thresholds (e.g. 0.6), these identifications should be regarded as tentative. In this study, such particles were not classified as confirmed MPs but were retained as ‘suspected’ materials to avoid over-interpretation of the data. Manual sample handling, particularly during drying and crystal contact, likely contributed to variability and lower reproducibility.

While ATR-FTIR remains an affordable and practical tool of MPs in biological samples, the present findings highlight several limitations of relying on this technique alone. Complementary methods such as Raman spectroscopy may provide improved sensitivity for smaller particles, whereas pyrolysis-GC-MS can offer more definitive chemical characterisation (Zhou et al., 2022; Sheriff et al., 2024; Rani et al., 2023).

Enhanced software capabilities, broader and better-curated spectral libraries, and rigorous contamination-control protocols (including field blanks and multiple procedural controls) are essential to generate robust, comparable data on MPs in HBM in the future. Such methodological improvements are a prerequisite for accurately characterising infant exposure and for informing risk assessment efforts related to early life MP ingestion. Overall, this study highlights that precise sample handling, and appropriate analytical tools are essential to confidently detect and classify MPs in biological samples.

CONCLUSION

This study evaluated the feasibility of applying ATR-FTIR spectroscopy to screen for suspected MPs in human breast milk, a complex biological matrix. By combining enzymatic and chemical digestion with microscopic pre-screening and spectral analysis, several materials, including PVDF- and PP-like polymers and the natural compound alginic acid sodium salt, were tentatively detected in HBM extracts. However, match scores were generally low, likely reflecting software limitations, restricted spectral library coverage, spectral interference from residual organic matter, and challenges in particle handling. Moreover, the occurrence of PVDF and CSPE in control samples highlights the susceptibility of the workflow to laboratory contamination and necessitates cautious interpretation of polymer assignments in HBM.

Despite these constraints, positive control experiments confirmed that the digestion protocol preserved MPs, supporting its suitability as a pre-treatment step for HBM. The findings therefore illustrate both the potential and the current limitations of ATR-FTIR as a preliminary screening tool for MPs in biological samples, particularly when working close to the detection limits. Future work should prioritise improved access to comprehensive spectral libraries, software upgrades, and rigorous contamination-control strategies, alongside the integration of complementary techniques such as Raman spectroscopy and pyrolysis-GC-MS to achieve more definitive polymer identification and characterisation. By outlining these practical considerations, this study contributes to ongoing efforts to refine analytical approaches for assessing early-life exposure to MPs and to support the development of robust biomonitoring frameworks.

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