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Review of Detection and Identification Techniques for Nanoplastics and Microplastics

Introduction

Microplastics and nanoplastics are plastic fragments that can be created via heating, friction and the recycling of a plastic surface [1]. Microplastics range from a size of 5 millimeters to 1 micron, and nanoplastics range from 1 to 1000 nanometers. Both nanoplastic and microplastic particles have been found to be potential toxicity hazards to the digestive, nervous, and reproductive system [2]. As a result, detection techniques are vital for determining if a food or water source was contaminated by potentially hazardous particles. However, there are flaws in mainstream techniques like standard Raman spectroscopy and Fourier-transform infrared (FTIR) spectroscopy, thus newer techniques are being turned to for migration particle analysis. Out of these newer techniques, Raman mapping, Raman spectroscopy with the addition of the peak extraction and retention (PEER) algorithm, Stimulated Raman Scattering (SRS) imaging, and Stimulated Emission Depletion (STED) imaging are the most promising. Raman mapping and Raman spectroscopy paired with the PEER algorithm provide more accurate analysis of microplastic particles compared to standard Raman spectroscopy. In addition to this, SRS imaging and STED imaging are able to analyze particle migration at the nano level. Analysis of migrated particles on the nano level allows scientists to gather information about migrated particle size, chemical composition and toxicity. This research would reduce gaps in knowledge about nanoplastic migration and its effects on the body, and allow the FDA to create better guidelines surrounding the topic of nanoplastic migration. Thus, the purpose of this literature review is to discuss the effectiveness of techniques used for the detection and identification of plastic particles between 50 nanometers and 100 microns.

Toxicity of Plastic Particles

Detection of plastic particles is important in the food packaging industry, as the plastic fragments have been linked with toxicity to the nervous, digestive, and reproductive system [2]. All plastic food packaging has the potential to migrate microplastics and nanoplastics. Migration of microplastics and nanoplastics are promoted in plastic packages that either experience mechanical stress or heat, or are stored for extended periods of time [8]. After conducting a study on microplastic and nanoplastic toxicity, Yin et. al concluded that generally, the smaller a microplastic particle, the more toxic it is [2]. The finding in a study done by Jeong et al. supported this, as it was conlcued that the increased toxicity of smaller particles was attributed to them having higher surface/volume ratios, which increase their odds of being engulfed by a cell or interacting with other parts of the body [3][4]. However, there have been contradictory findings to this general conclusion, as in the liver specifically Yin et al. found that larger microplastic particles were more toxic than smaller microplastic particles and nanoplastic particles [2]. Additionally, the chemical composition and structure of plastics can vary widely and result in a difference in toxicity. There are also significant gaps in knowledge about how different types of plastics interact with the systems in the body and their levels of toxicity to biological tissues. By filling in these gaps in knowledge, the FDA will be able to provide better guidelines about what size and type of plastic particles will be more harmful to the body.

Fourier-transform infrared (FTIR) spectroscopy

Fourier-transform infrared (FTIR) spectroscopy is a vibrational spectroscopy technique that measures molecular vibrations based on periodic changes of dipole moment. Infrared (IR) spectroscopy, which includes FTIR spectroscopy, measures light absorption by electric dipolar interactions. The corresponding IR absorption spectra generated by this vibrational spectroscopy technique provides information about the chemical composition or structure of the sample analyzed. FTIR has a limited spatial resolution, which affects its ability to detect microplastics below 10 microns [1]. This prevents FTIR from being an applicable technique for detection for all microplastics as some microplastics will be smaller than 10 microns. Moreover,

it also prevents FTIR from being used for nanoparticle analysis. However, FTIR is still useful for microplastic detection as most microplastics are within the field of mid infrared (MIR), which is between 2.5–30 μm wavelength, and is within the scope of FTIR [5]. While FTIR is able to detect larger microplastic particles and is an accessible detection technique, other alternatives should be investigated to analyze smaller microplastic particles below 10 microns and nanoparticles.

Standard Raman Spectroscopy

Standard Raman spectroscopy is the most commonly used technique for microplastic detection, and is able to identify the compounds by probing the vibrations of molecules. Standard Raman spectroscopy is based on the inelastic scattering of light [6]. Inelastic scattering of light is when a scattering light transfers energy to a scattering material, in this case microplastic particles, producing a change in direction and wavenumber of the scattered light, and causing the frequency to disperse [7]. Standard Raman spectroscopy has the capability to identify particles down to the size of 1 micron due to its high spatial resolution, meaning it has the potential to identify any size microplastic particle, but cannot identify nanoparticles. However, while Standard Raman spectroscopy has the theoretical capability to identify particles down to 1 micron, it is unreliable in terms of identifying smaller microplastic particles in actual practice. A study conducted by Guo et al. found that 12-63% of microplastics identified during the experiment failed to be characterized as a particular type of plastic [8]. As standard Raman spectroscopy can only consistently detect microplastic particles between 1-100 microns, but not provide information about what type of plastic is detected, this technique is not the best way to detect and analyze microplastic particles. Furthermore, as standard Raman spectroscopy cannot identify nanoparticles it is not a sufficient technique to detect and identify plastic particles between 50 nm and 100 microns. Supplementary analysis techniques that have been developed to pair with Raman spectroscopy are useful ways to reliably gather information about particle chemical composition for identification.

Raman Mapping

Raman mapping is a technique that involves developing a chemical image based on a sample's Raman spectra. The bright spots in the pseudo-color map or chemical image generated by Raman mapping aid the deduction of a sample's type, shape, and size, significantly improving both the efficiency and accuracy of particle detection [9]. Raman mapping is still limited like standard Raman spectroscopy, as it can only detect particles down to 1 micron, thus it cannot detect or identify nanoplastics. However, because Raman mapping can provide additional information for analysis via its chemical image it is much more reliable for particle identification than either FTIR or standard Raman spectroscopy. As a result, Raman mapping is commonly used for analysis of a molecular structure and chemical composition of particles, even in complex samples. This is key as other techniques used to identify types of plastic particles, such as the combination of thermogravimetric Fourier-transform infrared spectroscopy coupled with gas chromatography/mass spectrometry (TGA-FTIR-GC or MS), struggle to differentiate between types of plastic particles [9]. This quality makes Raman mapping extremely useful, as it is more accurately able to determine the types of particles within a more complex sample, which is more common in real world testing. In 2023 Lui et al. conducted a study in relation to this, and found that the Raman imaging and mapping mode was able to accurately distinguish microplastic particles (polypropylene (PP) and polyethylene (PE)) from environmental impurities [9]. In addition to Raman mapping, Raman spectroscopy with the utilization of the PEER algorithm was also able to reliably identify and determine all sizes of microplastic particles.

Raman Spectroscopy + PEER Algorithm

The peak extraction and retention (PEER) algorithm was developed to pair with Raman spectroscopy and help extract information from weak Raman signals. This data processing algorithm was created with the purpose of bolstering the success of identification of microplastics as standard Raman spectroscopy by itself does not have a 100% identification

rate of particles. To try to identify unknown plastic particles in samples, random forest models were employed. Random forests are an ensemble learning model that uses bagging and random feature selection to make multiple independent decision tree structures. They then integrate the voting results of each decision tree in order to make a prediction [10]. These forests are able to handle noisy and high dimensional data while providing interpretable results with high accuracy. Random forests are extremely useful for microplastic detection as they are able to discriminate between the five most common microplastic contaminants in complex environments with an accuracy of 98.8%, average sensitivity of 98.5%, and average specificity of 100%. Tests utilizing the PEER algorithm on samples with water mixed with plastic particles had an identification rate of over 97% [10]. This is significantly higher than standard Raman spectroscopy, as it was only able to identify 37-88% of the microplastics detected [8]. The high identification rate of the PEER algorithm paired with Raman spectroscopy is a strength, but studies have not elaborated if they also provide significant data on microplastic particle sizes. Additionally, like Raman mapping, Raman spectroscopy with the addition of the PEER algorithm lacks the capability to detect and identify nanoparticles. Thus, it is only able to detect a fraction of the migrated plastic particle contamination in a sample. SRS imaging is a new variation of Raman spectroscopy that was developed with the purpose of providing information on detected plastic particles on a micro and nano scale in order to try to fill gaps in knowledge about both types of particles.

SRS Imaging

Hyperspectral stimulated Raman scattering (SRS) imaging is a spectroscopy technique that was developed to improve the analysis of both microplastics and nanoplastics. SRS imaging uses SRS signals, generated by two laser beams, to conduct selective imaging of chemical bonds that allow for visualization and analysis of both nano-sized and micro-sized particles [11]. The goal of the technique is to analyze particles using single-particle imaging and collecting data on chemical specificity with sufficient throughput [12]. This is something

unachievable by FTIR and standard Raman spectroscopy as they have poorer instrumental resolution and detection on sensitivity for smaller microplastics and nanoplastics. FTIR and standard Raman imaging also are only able to determine composition at a microplastic level and are only reliable when the microplastics are larger, whereas SRS is able to determine composition reliably on a smaller scale [13]. Moreover, while Raman mapping and standard Raman spectroscopy paired with the PEER algorithm have better sensitivity for smaller microplastics, they still cannot detect nanoplastics making them inferior identification and detection techniques compared to SRS imaging. Additionally, electron microscopy and atomic force microscopy, which have nanometer resolution, do not have the chemical specificity to distinguish between different compounds, and thus different types of plastics. Using SRS imaging, Qian et al. conducted a particle detection study, and found that 90% of all the particles detected were nanoplastics, and only 10% were microplastics. Additionally of the 10% of particles that were determined to be microplastics, most were under 2 microns [12]. Thus, traditional particle identification techniques would have struggled to identify the majority of the microplastics detected via SRS imaging and would not have been able to detect any of the nanoplastics. The ability to detect both microplastics and nanoplastics makes SRS a more reliable technique of detecting plastic particles between 50 nm and 100 microns in comparison to previously discussed techniques.

STED Imaging

Stimulated Emission Depletion (STED) microscopy is a super resolution, fluorescence microscopy technique that is able to circumvent the optical diffraction limit. Super resolution is achieved by using two laser pulses to selectively deplete fluorescence at the edges of the excitation spot, which “turns off" fluorescence outside of the focal point, resulting in a sharper image of the target structure [14]. This sharper image is the main reason it is used for nanoplastic detection in addition to microplastic detection. Ngyen et al. found that STED microscopy when paired with labeling techniques can be used to resolve nanoplastics of

different shape and composition as small as 50 nm [15]. This can be useful when studying toxicity of nanoparticles as it is able to detect and identify particles in the nanoparticle range in not just water or other chemical solutions but also in biological tissues. STED imaging would allow for more accurate localization and quantification of these particles in humans and animals in exposure studies, and thus offer more information about the interactions they have within the body.

Conclusion

In this literature review, the effectiveness of current techniques used for detection and identification of plastic particles between 50 nm and 100 microns was evaluated. Of the techniques discussed, Raman mapping and SRS imaging are the most accessible techniques in terms of detection and identification as they provide information about microplastic particles' chemical composition and size. An additional benefit to SRS imaging is it can be used at the nanoparticle level rather than just at the microplastic level. The ability to study nanoplastics in addition to microplastics opens up additional opportunities to expand toxicity studies and understand how varying sizes of plastic fragments affect the body.

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Peer Review Suggestions and Edits:

During peer review it was highlighted that I had not included any discordant findings. To do this I wanted to broaden my paper to a larger range of plastic particles (50 nm to 100 microns), so I could include nanoplastics. Once I had partially rewritten the paper to include nanoplastics, I remedied the lack of discordant findings in the toxicity paragraph by highlighting that a general rule that was concluded relating smaller particle size to higher toxicity was contradicted when looking specifically at the liver. I also tried to make it clear as to the why of my paper by including a toxicity paragraph to make it clear as to why being able to detect all types of plastic particles is important. I tried to also highlight the gaps in knowledge about microplastic and nanoplastic toxicity as a whole, as well as how developing better methods of detection would lead to better FDA guidelines in relation to microplastic and nanoplastics. I tried to also improve transitions between paragraphs.