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INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI:10.21474/IJAR01/24369
DOI URL: <http://dx.doi.org/10.21474/IJAR01/24369>



RESEARCH ARTICLE

INFLUENCE OF FILTER MORPHOLOGY ON PYROLYSIS MASS SPECTRAL RESPONSE

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Manuscript Info

Manuscript History

Received: 12 August 2026

Final Accepted: 14 September 2026

Published: October 2026

Key words:

pyrolysis mass spectrometry; biological sample collection filter; filter morphology; spatial distribution; sucrose; peak-height ratio; biological detection

Abstract

Pyrolysis mass-spectral response in filter-based detection systems may be influenced not only by instrumental conditions but also by the characteristics of the sample-collection medium. This study investigated the influence of biological sample collection filter morphology on simulant distribution and pyrolysis mass-spectral response using sucrose as a nonpathogenic biological simulant. Type A, Type B, and modified filters were evaluated using a military biological detector, with ten independent filters tested in each group. Optical observations showed clear structural differences between Type A and Type B filters. Despite identical simulant loading, less sucrose remained visible on the Type A surface, and cross-sectional observations indicated deeper penetration into the filter interior, where as sucrose remained predominantly near the surface of Type B filters. The primary comparison variable was the m/z 123-to-95 peak-height ratio, a spectral indicator relevant to the detector classification algorithm. Median ratios were 2.31%, 9.73%, and 12.85% for Type A, Type B, and modified filters, respectively. The three-group difference was significant (Kruskal–Wallis $H(2) = 23.907$, $p < 0.001$), and all pairwise comparisons remained significant after Holm adjustment. Type A filters were associated with repeated misidentification, whereas all observed tests using Type B and modified filters resulted in correct classification. These findings support an association among filter morphology, simulant distribution, and relative pyrolysis mass-spectral response, and indicate that the sampling medium should be considered an engineering factor when evaluating and troubleshooting pyrolysis mass spectrometric detection systems.

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Introduction:-

Rapid and accurate detection and classification of biological agents are important for protecting personnel and supporting military operations. Pyrolysis mass spectrometry is one analytical approach to characterizing collected

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samples: the sample is thermally decomposed, and the resulting mass spectrum is used to assess its characteristics [1].

Pyrolysis mass spectra can depend on sample composition, sample amount, and pyrolysis conditions [2, 3, 4]. In systems that concentrate samples on a collection medium and then pyrolyze them directly, the morphology and internal structure of that medium may influence where the sample is retained. Differences in spatial distribution may, in turn, alter exposure to the heat source and the relative spectral response. This interpretation is consistent with the importance of sample configuration and heat transfer in analytical pyrolysis [4, 5, 6]. Related studies of sampling wipes further indicate that collection-medium characteristics and sampling conditions should be considered when interpreting subsequent analytical responses [7, 8, 9].

The military biological detector used in this study collects and concentrates samples on a biological sample collection filter and uses an infrared (IR) laser for pyrolysis. During testing with the same biological simulant, both correct classification and misidentification were observed. Checks of the IR laser, heater, sensors, and sample-transfer functions did not reveal abnormalities. Subsequent observations showed differences between filter types in the surface retention and internal penetration of the simulant. These findings motivated an investigation of the collection medium as a potential contributor to spectral variation.

This study compared the morphology of Type A and Type B filters and the surface and internal distributions of the biological simulant. The primary comparison variable was the peak-height ratio of m/z 123 to m/z 95; the individual peak heights were secondary variables. Modified filters incorporating the Type B morphology were subsequently evaluated to examine the relationship between filter morphology, relative mass-spectral response, and ancillary detection characteristics. The results showed distinct differences in relative mass-spectral response among the three filter groups and supported an association between filter morphology, simulant distribution, and analytical response.

Materials and Methods:-

Biological detector and analytical workflow:-

Figure 1 summarizes the analytical workflow. Material entering the inlet is concentrated and collected on a biological sample collection filter. IR-laser irradiation pyrolyzes the collected material. As the transfer capillary is heated, the volatilized products reach the mass spectrometer at different times. Signal amplification and mass detection yield a mass spectrum, and the instrument compares peak positions and intensity patterns with its internal reference library to classify the sample.

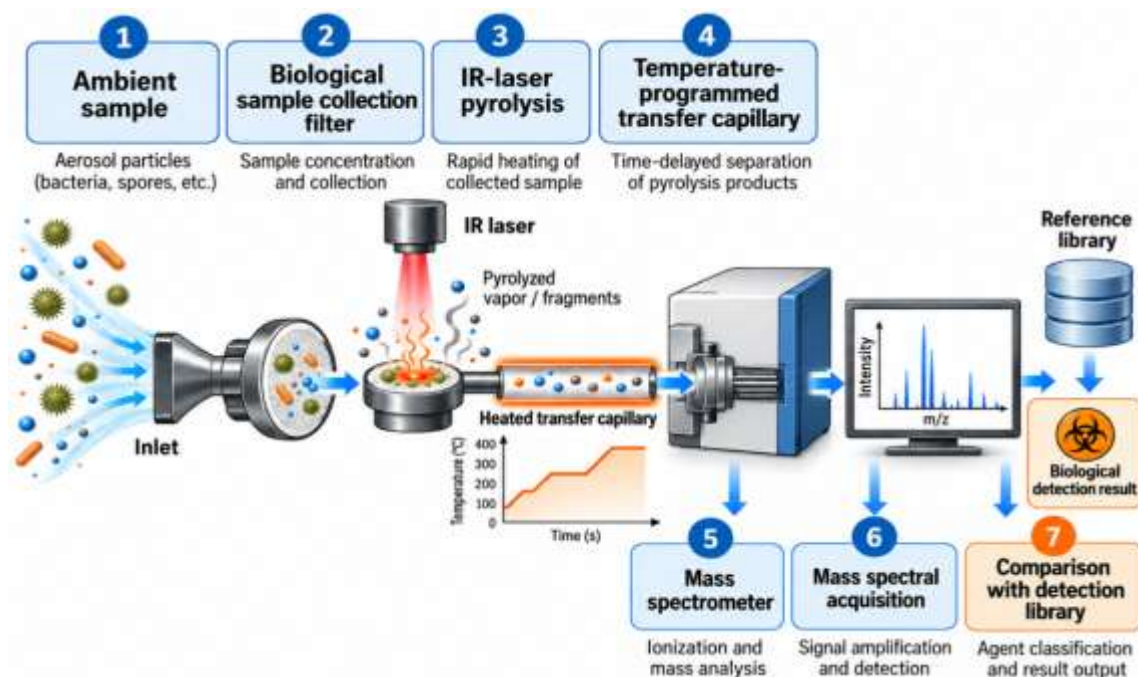


Figure 1. Schematic of biological sample collection, infrared (IR)-laser pyrolysis, and mass spectrometric analysis.

Biological simulant and spectral response variable:-

Sucrose was used as a nonpathogenic biological simulant for instrument testing and was not selected ad hoc for this study. According to the equipment specification, nonpathogenic or low-pathogenic simulants are used for instrument testing in place of actual biological agents because of the associated biosafety risks, and albumin or sucrose is specified for this purpose. Accordingly, sucrose was used in the present experiments as the specified test simulant. Sucrose is a disaccharide composed of glucose and fructose units, and its thermal degradation produces furan-containing compounds, including furfural and related derivatives [10, 11, 12, 13]. 2,5-Furandicarboxaldehyde is also among the pyrolysis products of sucrose [10,13]. The electron ionization reference spectrum of furfural contains prominent peaks at m/z 95 and m/z 96, whereas that of 2,5-furandicarboxaldehyde contains prominent peaks at m/z 95, m/z 123, and m/z 124 [14]. Among these signals, m/z 95 and m/z 123 are used by the detector evaluated in this study, and the peak-height ratio of m/z 123 to m/z 95 is one of the variables incorporated into its classification algorithm. For this detector, higher values of this ratio favor correct classification, whereas lower values are unfavorable to classification and are associated with misidentification. Accordingly, the m/z 123-to-95 peak-height ratio was selected as the primary comparison variable in this study and was calculated using Equation (1).

$$\text{Peak-height ratio (\%)} = \frac{\text{Peak height at } m/z \text{ 123}}{\text{Peak height at } m/z \text{ 95}} \times 100 \quad (1)$$

Figure 2 illustrates spectra with contrasting ratios: approximately 1% in panel (a) for a misidentified sample and approximately 8% in panel (b) for a correctly classified sample.

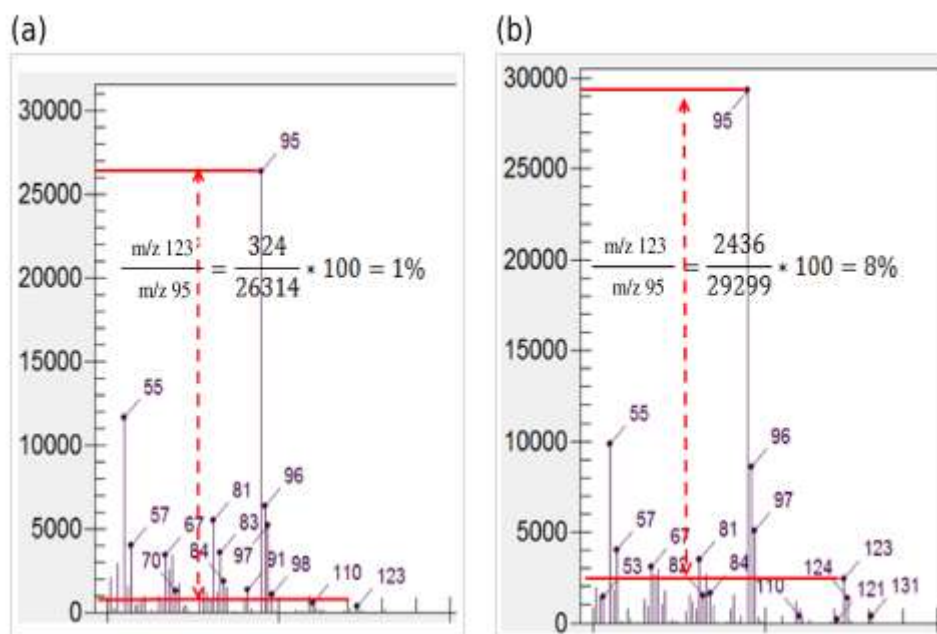


Figure 2. Representative mass spectra with (a) a low peak-height ratio (approximately 1%) for a misidentified sample and (b) a high peak-height ratio (approximately 8%) for a correctly classified sample.

Filter classification and microscopy:-

The biological sample collection filters were fabricated from sintered stainless-steel fiber media. The specified material requirements included a filter rating of 11 μm and a porosity of 85%. The filters were classified according to the presence or absence of a visible ejector hole at the center of the filter: Type A had a central ejector hole, whereas Type B did not (Figure 3). The two filter types were examined visually and by optical microscopy before and after sucrose application using a micropipette. Surface views and filter cross-sections were examined qualitatively to compare simulant distribution.

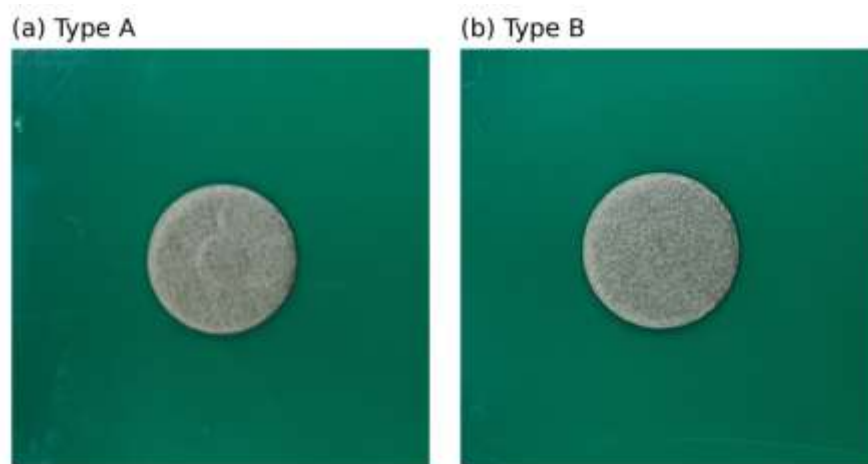


Figure 3. Morphological classification of biological sample collection filters: (a) Type A and (b) Type B.

Comparison and validation experiments:-

Ten Type A and ten Type B filters were analyzed using the biological detector. For each test, 3 μg of the biological simulant was deposited onto the filter and allowed to dry for 5 min before analysis. Ambient conditions were maintained at 23 °C and 50% relative humidity. Prior to each test, the detector was cleaned, the absence of carryover from the preceding analysis was confirmed, and the calibration status was verified. The normal operation of the IR laser, heater, sensors, biological multi-position valve (Bio MPV), and related input/output functions was also checked to minimize the influence of equipment-related factors. Mass spectra were obtained from the detector data using dedicated proprietary software developed for the detector, and the peak heights at m/z 95 and m/z 123 were extracted from the spectra. Their ratio was calculated using Equation (1). Type A filters were analyzed sequentially from A1 to A10, followed by Type B filters from B1 to B10.

For validation, the filter mold and manufacturing process were modified to reproduce the Type B morphology selected on the basis of the comparison experiment. Ten newly manufactured modified filters were tested later as a separate validation series using the same analytical approach, including the 3 μg simulant loading and 5 min drying procedure.

Statistical analysis:-

The peak-height ratio of m/z 123 to m/z 95 was the primary comparison variable. Individual peak heights at m/z 95 and m/z 123 were secondary variables. Ratios were calculated from the individual peak heights without intermediate rounding; table and figure labels were rounded for display. Descriptive statistics included the mean, median, and interquartile range (IQR; 25th–75th percentiles calculated by linear interpolation). Differences among Type A, Type B, and modified filters were assessed using the Kruskal–Wallis test with the chi-squared approximation and two degrees of freedom [15]. When the overall test was significant, two-sided Mann–Whitney U tests with exact permutation p-values were used for post hoc comparisons [16]. All 184,756 allocations of 20 observations to two groups of ten were enumerated for each comparison. Two-sided p-values were twice the smaller inclusive tail probability, capped at one. U refers to the first group in each named comparison. The Holm method was applied separately to the three pairwise comparisons for each variable [17]. Adjusted p-values below 0.05 were considered significant. The analyses were performed in Python 3.12.14 with SciPy 1.17.0 and statsmodels 0.14.6. Exact p-values were checked by a separate exhaustive rank-sum enumeration, and Holm adjustments were checked against statsmodels. Cliff's delta (δ) was additionally calculated as a descriptive nonparametric effect-size measure for the pairwise comparisons [18]. Negative values indicate lower observations in the first group, and $\delta = \pm 1$ indicates complete separation of the observed samples.

Results:-

Filter morphology and simulant distribution:-

Optical examination revealed clear morphological differences between the two filter types (Figure 4). The Type A filter showed a visible ejector hole at its center (Figure 4a), whereas no central ejector hole was observed in the Type

B filter (Figure 4b). Optical micrographs further showed a relatively open and irregular surface network in Type A (Figure 4c), while Type B exhibited a denser and more uniform network structure (Figure 4d).

After the same amount of sucrose was deposited onto each filter, less simulant was visible on the surface of Type A than on that of Type B (Figure 5). Despite the identical simulant loading, the lower amount of visible surface deposit on Type A suggested that a portion of the simulant may have penetrated into the filter interior, whereas a greater proportion appeared to remain on the surface of Type B.

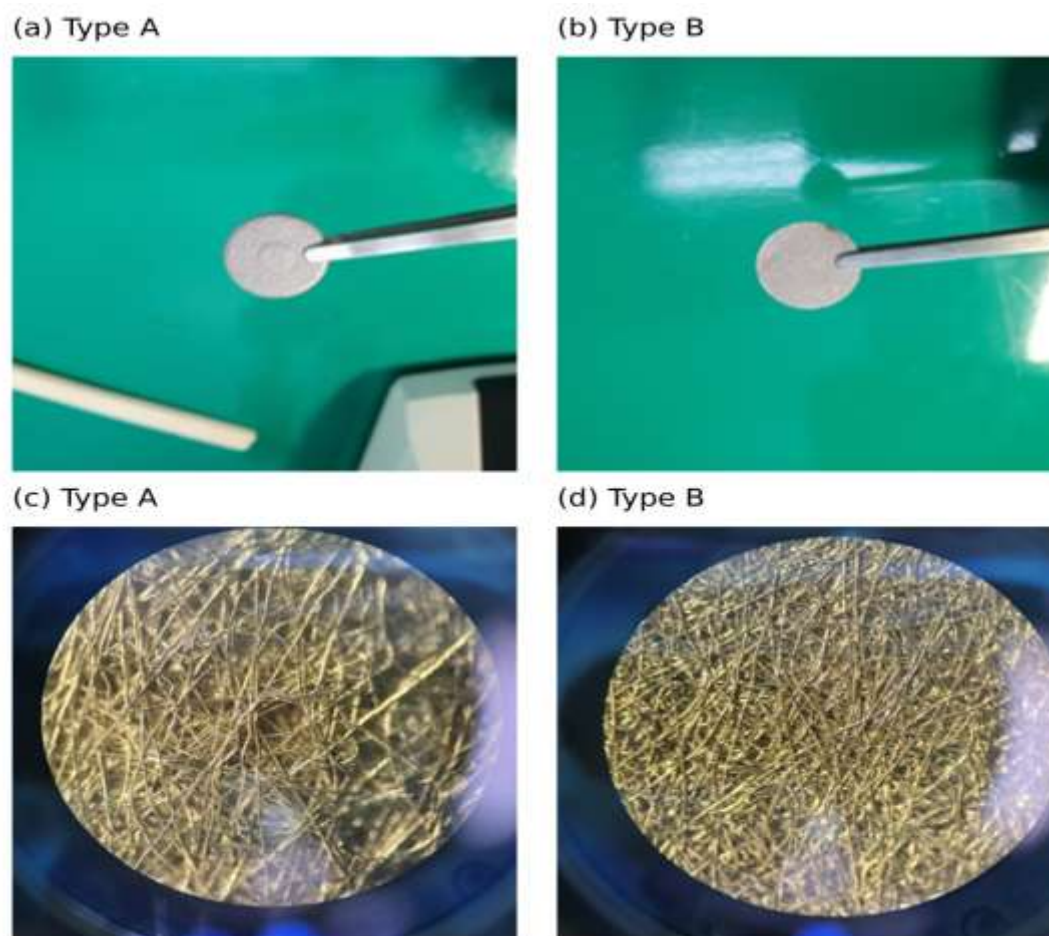


Figure 4. Comparison of Type A (left column) and Type B (right column) filters: (a,b) filters during handling; (c,d) optical micrographs of their surface structures. The panels show separate views for qualitative comparison.

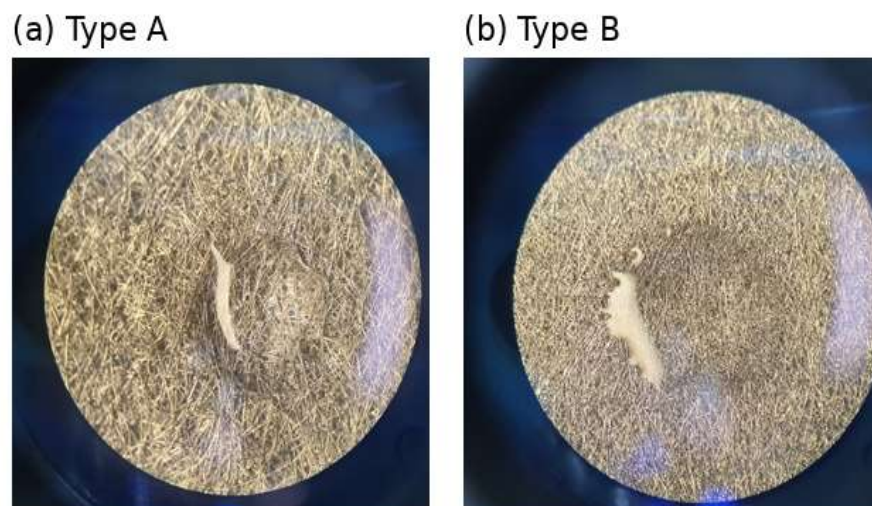


Figure 5. Surface distribution of the biological simulant on (a) Type A and (b) Type B filters. Panels are separate views; a common calibrated magnification is not implied.

Cross-sectional observations showed simulant crystals within Type A filters, whereas crystals were observed primarily on the surface of Type B filters, without visible internal penetration in the examined sections (Figure 6). Together, the surface and cross-sectional images indicate differences in the spatial distribution of the simulant between the filter types.

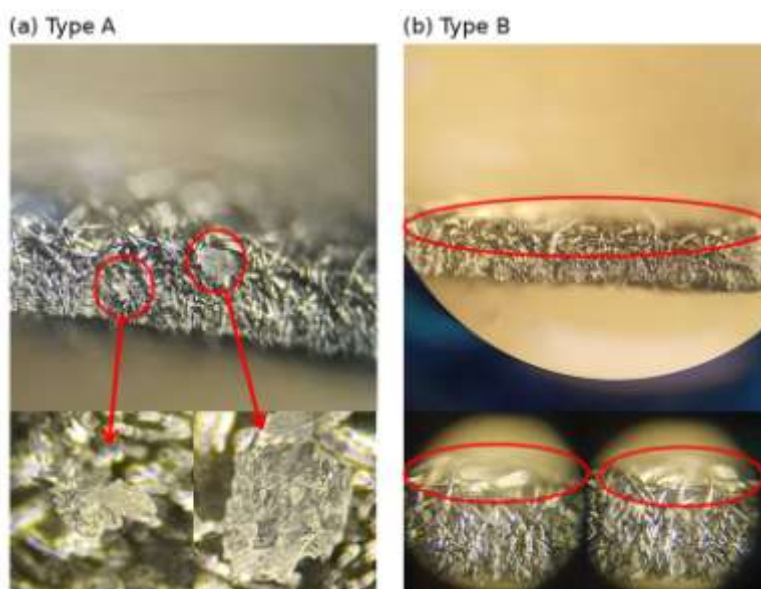


Figure 6. Cross-sectional distribution of the biological simulant in (a) Type A and (b) Type B filters. Red circles and ellipses mark regions highlighted in the source images; arrows link the marked regions to the enlarged views. Panels are separate views; a common calibrated magnification is not implied.

Spectral comparison of Type A and Type B filters:-

Table 1 lists the individual peak heights and peak-height ratios for Type A and Type B filters. Figure 7 shows representative spectra for sample 1 from each group.

Table 1. Mass-spectral peak responses of Type A and Type B filters.

Type	No.	Peak height at m/z 95	Peak height at m/z 123	Peak-height ratio (%)
A	1	106,454	2,758	2.59
A	2	98,337	1,994	2.03
A	3	121,044	5,452	4.50
A	4	117,265	1,783	1.52
A	5	86,903	688	0.79
A	6	209,981	1,797	0.86
A	7	92,202	4,534	4.92
A	8	74,125	1,377	1.86
A	9	391,116	11,103	2.84
A	10	36,060	935	2.59
B	1	152,315	14,914	9.79
B	2	345,722	30,049	8.69
B	3	126,596	9,649	7.62
B	4	156,158	15,090	9.66
B	5	112,340	6,333	5.64
B	6	518,672	56,571	10.91
B	7	499,525	63,377	12.69
B	8	478,778	60,483	12.63
B	9	392,828	39,178	9.97
B	10	214,418	18,112	8.45

Peak-height ratio (%) is defined in Equation (1).

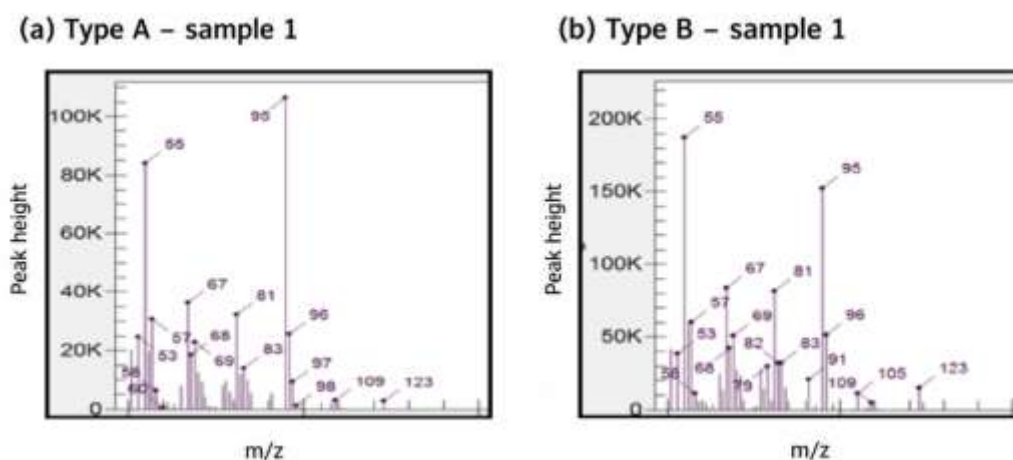


Figure 7. Representative pyrolysis mass spectra for sample 1 obtained with (a) Type A and (b) Type B filters.

The median peak-height ratio of m/z 123 to m/z 95 was 2.31% for Type A and 9.73% for Type B, with mean values of 2.45% and 9.61%, respectively. The distributions differed significantly (Mann–Whitney $U = 0$, Holm-adjusted $p < 0.001$), indicating that Type B filters produced markedly higher m/z 123-to-95 peak-height ratios than Type A filters. For this detector, higher values of this ratio favor correct classification; therefore, the shift observed with Type B was in the favorable direction. Consistent with this pattern, misidentification occurred repeatedly with Type A filters, whereas Type B filters were consistently classified correctly.

Modified filters and three-group comparisons:-

Table 2 presents the results for the ten modified filters. Their median peak-height ratio was 12.85%, and their mean was 12.95%. Correct classification was consistently observed as an ancillary outcome.

Table 2. Mass-spectral peak responses of modified biological sample collection filters.

No.	Peak height at m/z 95	Peak height at m/z 123	Peak-height ratio (%)
1	160,640	20,496	12.76
2	146,252	19,244	13.16
3	127,868	20,956	16.39
4	80,195	11,370	14.18
5	156,030	17,016	10.91
6	151,304	19,576	12.94
7	139,853	17,844	12.76
8	135,239	14,401	10.65
9	124,883	18,297	14.65
10	111,195	12,369	11.12

Peak-height ratio (%) is defined in Equation (1).

The median peak-height ratios (IQRs) were 2.31% (1.60–2.78%) for Type A, 9.73% (8.51–10.67%) for Type B, and 12.85% (11.53–13.92%) for the modified filters (Figure 8).

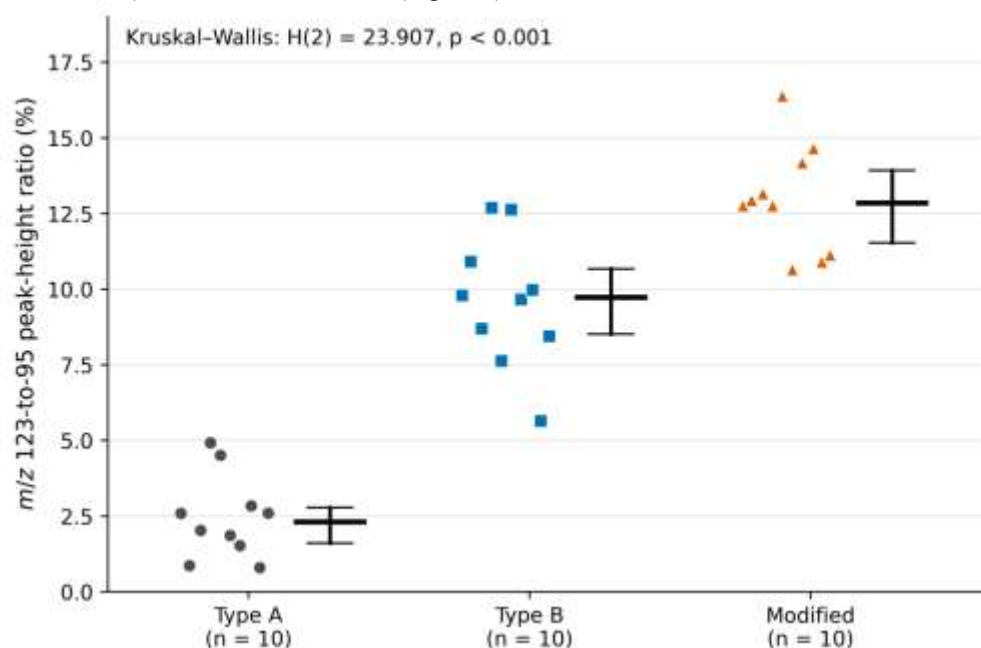


Figure 8. Individual peak-height ratios of m/z 123 to m/z 95 for Type A, Type B, and modified filters (n = 10 per group). Each symbol represents one physical filter. Thick horizontal marks indicate medians, and capped vertical lines indicate IQRs. Horizontal jitter is used only for visibility and does not indicate test order or pairing.

The three groups differed significantly (Kruskal–Wallis $H(2) = 23.907, p < 0.001$). Post hoc Mann–Whitney U tests identified significant differences between Type A and Type B ($U = 0$, Holm-adjusted $p < 0.001$), Type A and modified filters ($U = 0$, Holm-adjusted $p < 0.001$), and Type B and modified filters ($U = 8$, Holm-adjusted $p = 0.000725$; Table 3). The ratio increased from Type A to Type B and was highest in the modified filters. Cliff's δ values of -1.00 for Type A versus Type B and Type A versus modified filters indicate complete separation of the observed ratio values between these groups, whereas $\delta = -0.84$ for Type B versus modified filters indicates substantial separation. Thus, both Type B and modified filters showed higher m/z 123-to-95 ratios, in the direction associated with correct classification for this detector.

Individual peak heights also differed among groups at m/z 95 ($H(2) = 11.097, p = 0.00389$) and m/z 123 ($H(2) = 18.846, p < 0.001$; Figure 9). At m/z 95, Type A differed from Type B ($U = 12$, adjusted $p = 0.00864$), and Type B differed from modified filters ($U = 82$, adjusted $p = 0.0294$), whereas Type A and modified filters did not differ significantly ($U = 28$, adjusted $p = 0.105$). At m/z 123, Type A differed from Type B ($U = 2$, adjusted $p < 0.001$) and from modified filters ($U = 0$, adjusted $p < 0.001$), whereas Type B and modified filters did not differ significantly (U

= 61, adjusted $p = 0.436$). All pairwise p -values were Holm-adjusted within each variable (Table 3). The different groupwise patterns of the two peaks indicate that the change in the m/z 123-to-95 ratio was not simply attributable to a uniform change in overall signal magnitude. The joint distribution of the two selected peak heights is shown in Figure 10.

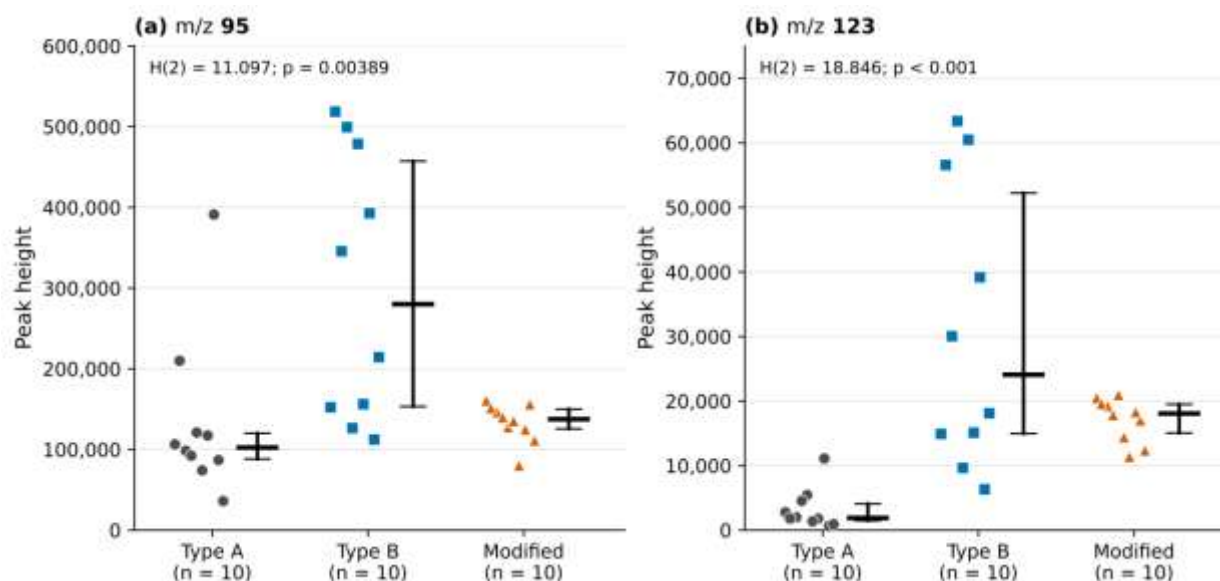


Figure 9. Individual peak heights at (a) m/z 95 and (b) m/z 123 for Type A, Type B, and modified filters ($n = 10$ per group). Thick horizontal marks indicate medians, and capped vertical lines indicate IQRs. The panels use different vertical-axis ranges.

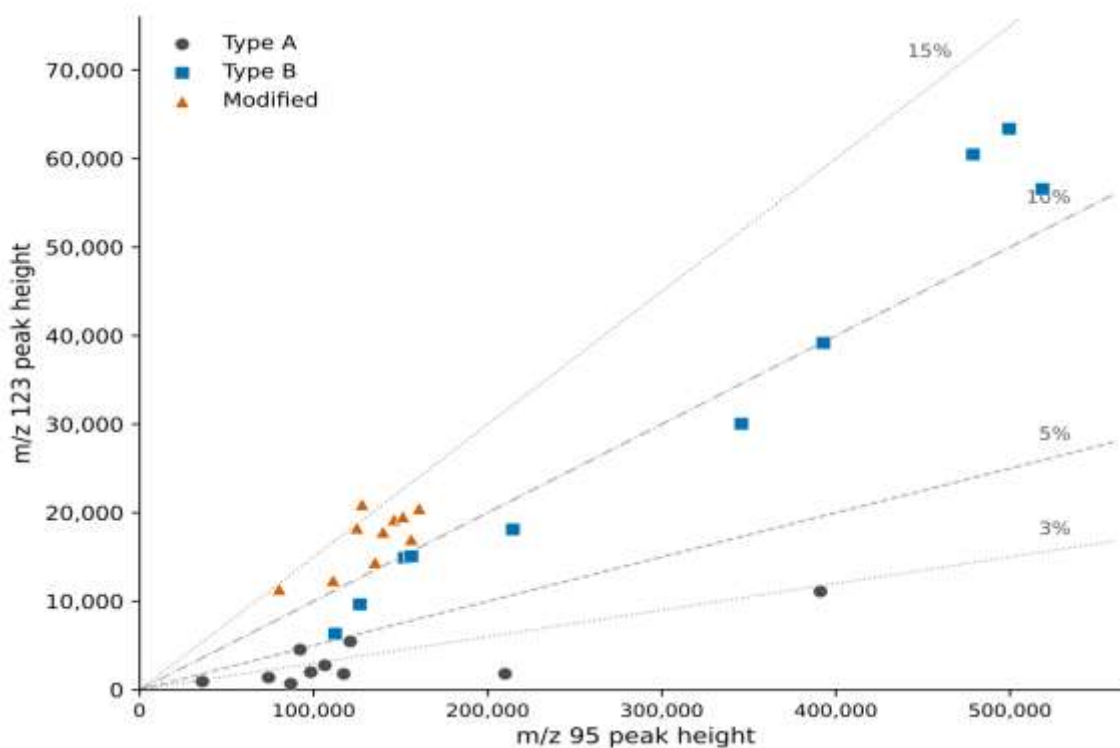


Figure 10. Joint distribution of m/z 95 and m/z 123 peak heights for the same 30 filters. Reference lines indicate constant m/z 123-to-95 peak-height ratios of 3%, 5%, 10%, and 15%, according to peak height at m/z 123 = (ratio/100) $\times$ peak height at m/z 95. These lines are descriptive visual guides, not classification thresholds or fitted regression lines.

Table 3. Exact pairwise comparisons and descriptive effect sizes.

Variable	Comparison	Mann–Whitney U	Exact p-value	Holm-adjusted p-value	Cliff's δ
Peak-height ratio (%)	Type A vs. Type B	0	1.08251e-05	3.24753e-05	-1.00
Peak-height ratio (%)	Type A vs. Modified	0	1.08251e-05	3.24753e-05	-1.00
Peak-height ratio (%)	Type B vs. Modified	8	0.000725281	0.000725281	-0.84
m/z 95	Type A vs. Type B	12	0.00287947	0.00863842	-0.76
m/z 95	Type A vs. Modified	28	0.105122	0.105122	-0.44
m/z 95	Type B vs. Modified	82	0.0146896	0.0293793	0.64
m/z 123	Type A vs. Type B	2	4.33004e-05	8.66007e-05	-0.96
m/z 123	Type A vs. Modified	0	1.08251e-05	3.24753e-05	-1.00
m/z 123	Type B vs. Modified	61	0.435872	0.435872	0.22

n = 10 filters per group. Exact two-sided p-values enumerate all 184,756 allocations. Holm adjustment applies separately to the three comparisons for each variable. U and Cliff's δ refer to the first group in each comparison; negative δ indicates lower observations in that group.

Discussion:-

The observed differences in surface morphology were accompanied by differences in simulant distribution and relative mass-spectral response. The observed group differences were not only statistically significant but also consistent with the expected direction of the detector response. Type A filters exhibited the lowest m/z 123-to-95 ratios and were associated with repeated misidentification, whereas Type B and modified filters showed markedly higher ratios together with correct classification. Cliff's δ further showed complete separation between Type A and the other two groups and substantial separation between Type B and modified filters, indicating that the observed differences were large within the tested samples rather than merely statistically detectable.

The deeper internal distribution observed in Type A filters suggests that the amount and location of material directly exposed to the IR laser may differ from those in Type B filters. Sample amount and configuration can affect heat transfer and the time–temperature history during pyrolysis [4]. Because thermal conditions can influence the distribution of sucrose-derived products [10], differences in simulant distribution may contribute to the observed changes in the peak-height ratio of m/z 123 to m/z 95. Experiments with droplets on porous substrates likewise show substrate-dependent spreading and imbibition, providing a qualitative analogy for differences in deposited-material distribution [19].

Previous studies collectively indicate that pyrolysis responses depend on thermal history, sample configuration, and transport conditions. Temperature and residence time can alter carbohydrate pyrolysis product distributions [10,20], while sample form, transport conditions, heating profiles, and structural characteristics of the pyrolyzed material can influence the resulting product distribution or mass-spectrometric response [21–23]. Together with previous analyses of heat-transfer effects during pyrolysis [4], these findings provide a plausible basis for interpreting the filter-dependent spectral responses observed here in terms of differences in simulant distribution and thermal exposure. This interpretation is also consistent with the observed differences in simulant distribution, peak-height ratio, and the response of the modified filters.

The modified filters, which incorporated the Type B morphology, exhibited higher median and mean peak-height ratios than either original group, and all three pairwise comparisons of the peak-height ratio were significant. These observations support an association between filter morphology and spectral response. The accompanying correct-classification observations were consistent with this association, although classification performance was not formally quantified.

Several limitations should be considered when interpreting these results. The study included ten filters per group and used sucrose as a single nonpathogenic simulant; therefore, the findings should not be directly generalized to other production lots, biological agents, or complex sample matrices. The microscopy observations were qualitative and were not used to quantify penetration depth or pore-scale structure. In addition, local temperature distributions and

individual pyrolysis products were not measured, so the specific thermal and chemical mechanism underlying the spectral-response differences remains unresolved. Potential manufacturing-batch or test-sequence effects were also not independently evaluated and cannot be completely excluded, although routine cleaning, carryover checks, calibration verification, and instrument-function checks were performed throughout the experiments.

More broadly, material–sensor interaction has also been shown to affect stress measurements in granular media, illustrating the engineering relevance of the measurement interface [24]. Overall, the findings indicate that the morphology and collection characteristics of the sampling medium should be considered alongside instrument performance in pyrolysis mass spectrometry.

Conclusion:-

This study demonstrated differences in pyrolysis mass-spectral response associated with the morphology of biological sample collection filters. The different surface network structures of Type A and Type B filters were accompanied by differences in the spatial distribution of the simulant. Sucrose penetrated more deeply into Type A filters, whereas it remained predominantly on the surface of Type B filters.

The median m/z 123-to-95 peak-height ratio, a spectral indicator relevant to the detector classification algorithm, was 2.31%, 9.73%, and 12.85% for Type A, Type B, and modified filters, respectively, with significant differences among all three groups. The higher ratios observed for Type B and modified filters were in the direction favorable to correct classification for this detector; correspondingly, repeated misidentification was observed with Type A filters, whereas all observed tests using Type B and modified filters resulted in correct classification.

Taken together, these findings support an association among sampling-medium morphology, simulant distribution, and relative pyrolysis mass-spectral response. They further indicate that the sampling medium itself should be considered as an engineering factor when evaluating and troubleshooting pyrolysis mass-spectrometric detection systems.

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