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Refining thyroid function evaluation: a comparative study of preprocessing methods in diffuse reflectance spectroscopy

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ABSTRACT

Thyroid dysfunction, comprising conditions such as hyperthyroidism and hypothyroidism, represents a substantial global health challenge, necessitating timely and precise diagnosis for effective therapeutic intervention and patient welfare. Conventional diagnostic modalities often involve invasive procedures, that could cause discomfort and inconvenience for individuals. The non-invasive techniques like diffuse reflectance spectroscopy (DRS) can offer a promising alternative. This study underscores the critical role of preprocessing methods in enhancing the accuracy of thyroid hormone functionality through a non-invasive approach. In the proposed study the spectral data acquired from the DRS setup are subjected to different preprocessing techniques to improve the efficacy of the prediction model. Thirty individuals with thyroid dysfunction were included in the study, and preprocessing methods such as baseline correction, multiplicative scatter correction (MSC), and standard normal variate (SNV), were systematically evaluated. The study highlights that SNV preprocessing outperformed other methods with a root mean square error (RMSE) of 0.005 and an R^2 of 0.99. In contrast, MSC resulted in an RMSE of 0.87 and an R^2 of 0.86, while baseline correction showed a RMSE of 0.84 and an unusual R^2 of 1.09, indicating potential issues. SNV proved to be the most effective technique.

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1. INTRODUCTION

Pre-processing of spectral data is crucial for achieving reliable outcomes. Preprocessing methods are crucial for model performance, as spectra can be affected by various disturbances that impact measurement accuracy [1]–[4]. Major influences include measuring geometry-such as sample thickness, detector distance, contact pressure, and light source angle [5], [6]. Eliminating scattering effects from differently sized particles is also essential in preprocessing. This discussion will concentrate on the pre-processing of data obtained from diffuse reflectance spectroscopy for non-invasive thyroid hormone functioning assessment.

Different spectroscopic methods encounter specific challenges. Near-infrared spectroscopy typically contends with consistent or linear shifts in the baseline due to scattered light, Raman spectroscopy often exhibits polynomial backgrounds from fluorescence, and mid-infrared spectra are affected by variations in sample thickness [7], [8]. The purpose of preprocessing is to remove these interferences while retaining the critical information within the spectrum. Diffuse reflectance spectroscopy (DRS) has proven to be a valuable

asset in medical diagnostics. The accurate interpretation of spectral data from DRS is highly dependent on efficient preprocessing techniques, such as multiplicative scatter correction (MSC), standard normal variate (SNV), and baseline correction. These methods are crucial for addressing baseline shifts and variations in diffuse reflectance spectra, ensuring that the data is properly normalized and ready for in-depth analysis.

Multiplicative signal correction (MSC) addresses major effects by defining a reference spectrum usually the mean of the calibration data and the correcting spectra for baseline and multiplicative scattering effects, aligning with the Kubelka–Munk theory [9], [10]. SNV removes constant offset terms by subtracting the spectrum's mean and scaling by its standard deviation, making it a popular method for its simplicity [11]. SNV and MSC often produce similar, interchangeable results [12], [13]. The importance of preprocessing in DRS: preprocessing techniques like MSC, SNV, and baseline correction play a fundamental role in enhancing the quality of spectral data. MSC is used to correct for scattering effects that can distort the spectra. It adjusts the spectra by aligning them to a reference spectrum, which minimizes variations caused by particle size, shape, and other physical properties of the sample. SNV is another technique that normalizes each spectrum by removing scatter effects and centering the data around zero. It is particularly useful for dealing with multiplicative interferences. Baseline correction addresses any shifts or drifts in the baseline of the spectra, which can result from instrument variations or sample inconsistencies. By correcting these baseline issues, the spectra become more comparable and reliable for further analysis [14].

The SNV technique was meticulously implemented to significantly diminish the multiplicative interference resulting from scatter. This approach involved subtracting the mean value of the entire spectrum, effectively removing constant offset terms. Additionally, it normalized the scale of all spectra by dividing each spectrum by the standard deviation of the complete spectrum [15]. MSC and SNV are frequently used interchangeably, producing results that are typically similar [16]. SNV is distinguished as a preferred preprocessing method, known for its straightforwardness and efficacy [17]. MSC, and SNV enhance the predictive capabilities of spectroscopic analyses. These preprocessing methods ensure that the spectral data used in predictive models are accurate and reliable, leading to better clinical outcomes [18]. DRS has proven useful in other medical areas. For instance, it has been employed in the diagnosis of breast lesions and the assessment of tumor margins during surgeries highlighting its ability to provide real-time feedback during surgical procedures [19]. In summary, diffuse reflectance spectroscopy, when coupled with appropriate preprocessing techniques like MSC, SNV, and baseline correction, holds significant promise in medical diagnostics, including thyroid assessment.

2. METHOD

2.1. Dataset

A randomized study was conducted during this examination to collect real-time spectrum signals. With folio number 8462/IEC/2022 serving as proof, the SRM Medical College Hospital and Research Center's Ethical Committee granted the necessary ethical clearance for this study. Thirty volunteers (N=30) both male and female, aged eighteen and up, who have regular clinical visits to maintain thyroid hormone imbalance were included in the study.

2.2. Experimental setup of diffuse reflectance spectroscopy

The experimental configuration for DRS is shown in Figure 1. It encompasses a Tungsten Halogen light source (LS-1) specifically tailored for the visible near-infrared (NIR) wavelength range, spanning from 360 to 2,500 nm. Additionally, the setup features a spectrometer (USB 4000) equipped with interface capabilities and high-speed electronics. The USB 4000 showcases responsiveness within the wavelength range of 360 to 1,100 nm.

Figure 2 shows the real-time DRS setup. This section outlines the detailed setup, components, and procedures involved in the DRS measurements. The primary light source used in the DRS setup is a Tungsten halogen lamp (LS-1). This light source is specifically chosen for its ability to emit a broad spectrum of light, covering both the visible and NIR wavelength ranges. The emission spectrum of the LS-1 spans from 360 to 2,500 nm, making it ideal for capturing a wide range of optical properties from the tissue. A USB 4000 spectrometer, equipped with high-speed electronics and a computer interface, is used to capture the diffusely reflected light from the tissue. The USB 4000 is responsive across a wavelength range of 360 to 1,100 nm. This spectrometer is selected for its ability to provide high-resolution spectral data quickly and accurately. The spectral data collected by the USB 4000 are essential for analyzing how light interacts with the tissue, including absorption, scattering, and reflection properties, which are indicative of tissue composition and health. The light emitted from the Tungsten Halogen source is transmitted to the tissue through a specialized fiber optic reflectance probe (R400). The R400 probe is equipped with bifurcated optical fibers arranged in a specific configuration to optimize light delivery and collection. The probe

contains 7 optical fibers, each with an inner diameter of 400 micrometers. One fiber is positioned at the center, surrounded by six other fibers. The six surrounding fibers are responsible for delivering light to the tissue, while the central fiber collects the diffusely reflected light.

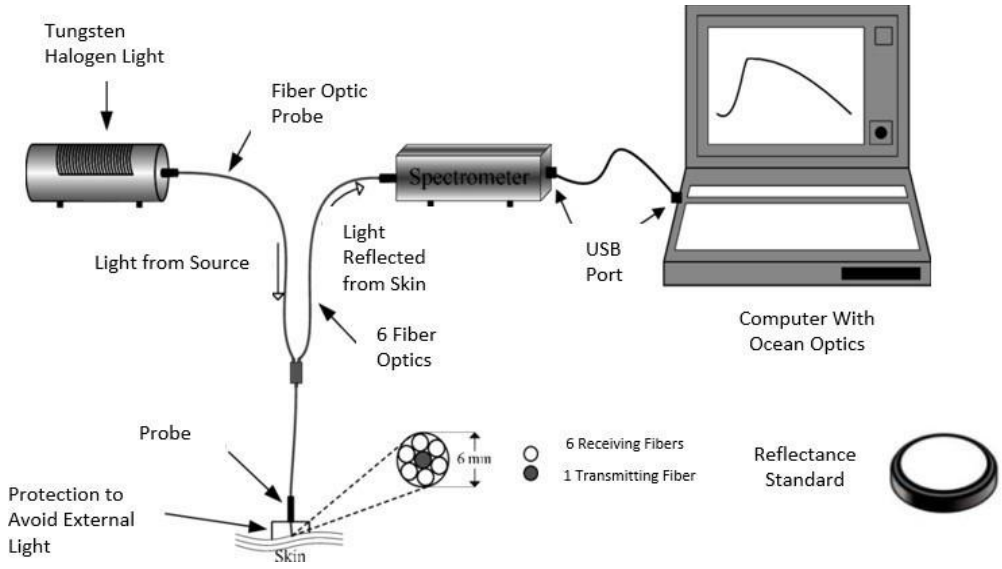


Figure 1. DRS configuration

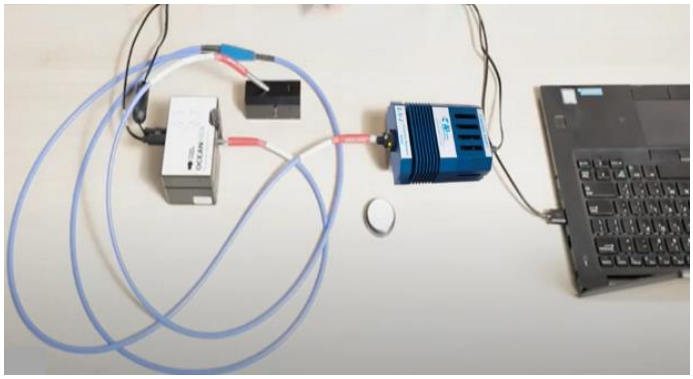


Figure 2. Real-time DRS setup

Accurate DRS measurements require careful calibration of the system to ensure that the spectral data accurately reflect the tissue's properties. *Barium Sulphate* ($BaSO_4$) is used as a reflectance standard. $BaSO_4$ is chosen for its high reflectance of approximately 99%, making it an ideal reference material. The light from the Tungsten halogen source is directed onto the $BaSO_4$ surface. The reflected light from the $BaSO_4$ is captured by the spectrometer to generate a reference spectrum. This reference spectrum serves as a benchmark against which tissue spectra are compared.

To account for ambient light and electronic noise, a dark spectrum is acquired by blocking the light source. This step ensures that any non-signal-related components are removed from the spectral data, enhancing the accuracy of the subsequent tissue measurements. After acquiring the reference and dark spectra, the system is set to reflectance mode using the Spectra Suite software.

In this study, participants were initially briefed on the non-invasive approach and safe utilization of near-infrared light (NIR) on the neck region. The measurement site and probe tip were cleansed with an alcohol-based solution to ensure accurate spectral readings and participants were safe from infections. The fiber optic probe is positioned on the neck. The light from the Tungsten halogen source is transmitted through the probe and onto the tissue. The reflected light, carrying information about the tissue's optical properties, is captured by the central fiber and transmitted back to the spectrometer. The Spectra Suite software is used to capture and

analyze spectral data in real time. The collected data are immediately transferred to a computer connected to the spectrometer, ensuring the integrity and accuracy of the measurements.

2.3. Spectral preprocessing

Spectral preprocessing techniques are utilized mathematically to enhance spectral data. The objective is to rectify unwanted influences like unpredictable noise, variations in light path length, and light scattering due to diverse physical properties of samples or instrument-related factors. This stage is typically executed before employing multivariate modeling, aiming to mitigate, remove, or standardize these influences on the spectra, thereby significantly improving the reliability of the calibration model [20]. In this study, three spectral preprocessing approaches are comparatively explored: SNV, MSC, and baseline correction.

2.3.1. Multiplicative scatter correction

Multiplicative scatter correction (MSC) is a robust technique used to address scatter effects in spectral data, which arise due to variations in particle size, surface texture, and other physical properties of the sample. These variations can distort the light path and intensity, leading to inaccuracies in the data. MSC regresses each spectrum against a reference spectrum and corrects using the slope and intercept of the linear fit. This minimizes baseline offsets and multiplicative effects [21]. The process of MSC begins with calculating the mean spectrum X_m from the entire calibration set. This mean spectrum acts as the reference spectrum. For each spectrum (X_i), a linear regression is performed against the mean spectrum to determine the slope (B_i) and intercept (A_i). The regression model is expressed as (1),

$$X_i = A_i + B_i X_m + E_i\, X_i, \tag{1}$$

where E_i represents the error term that includes the actual information. Once the intercept and slope are determined, the corrected spectrum ($X_{msc,i}$) is obtained using (2).

$$(X_{msc,i}) = \frac{X_i - A_i}{B_i}, \tag{2}$$

This correction process removes both multiplicative and additive scatter effects, normalizing the spectra to the mean spectrum and effectively reducing baseline shifts and multiplicative variations.

2.3.2. Standard normal variate

Standard normal variate (SNV) is an additional preprocessing technique that normalizes each spectrum independently to eliminate multiplicative scatter effects and adjusts for baseline variations. SNV individually centers and scales each spectrum by subtracting the mean and dividing by the standard deviation. This corrects additive and multiplicative effects [22]. The SNV process involves calculating the mean ($\overline{L_i}$) and standard deviation (σ_i) for each spectrum (L_i). The mean is calculated as (3),

$$\bar{L}_i = \frac{1}{n}\sum_{j=1}^{n} L_{ij,} \tag{3}$$

and the standard deviation is calculated as (4),

$$\sigma_i = \sqrt{\frac{1}{n-1}\sum_{j=1}^{n}\left(L_{ij} - \bar{L}_i\right)^2}\,, \tag{4}$$

where *n* is the number of data points in the spectrum. Each data point in the spectrum is then standardized using (5).

$$S_i = \frac{L_{ij} - \bar{L}_i}{\sigma_i}, \tag{5}$$

This transformation results in spectra that have zero mean and unit variance, which reduces the influence of scatter and enhances the spectral features. By making the spectra independent of the original scale and sample set characteristics, SNV ensures that the data is more consistent and easier to interpret.

2.3.3. Baseline correction

Baseline correction is a vital preprocessing method in spectroscopy that significantly enhances the quality and precision of data analysis. It is necessary to eliminate spectral artifacts that may result from

factors such as electronic interference, insufficient digital filtering, or incomplete digital sampling [23]. Baseline correction is a preprocessing technique used to remove baseline drifts and background noise from spectral data. These baseline drifts can be caused by instrument instability, environmental changes, or sample inconsistencies, and can obscure the spectral features of interest. The process of baseline correction starts with identifying the baseline of the spectrum using an appropriate method, such as polynomial fitting, moving average, or other baseline fitting algorithms. The determined baseline is subsequently subtracted from the original spectrum to derive the corrected spectrum, represented as (6),

$$L_{Corrected} = L_{Original} - L_{Baseline}(x). \quad (6)$$

here, $L_{Original}$ is the original spectrum, and $L_{Corrected}$is the spectrum after baseline correction. This correction process ensures that the spectral features related to the chemical composition are not obscured by baseline variations, leading to more accurate spectral analysis.

2.4. Performance-metrics

R-squared (R^2) and root mean square error (RMSE) are the most used performance metrics for evaluating the accuracy of predictive models in spectroscopic analysis. R^2 indicates the proportion of variability in the dependent variable that can be explained by the independent variables, whereas RMSE measures the average size of the prediction errors in the same units as the dependent variable [24]. R^2 is determined by squaring the correlation between the predicted and observed values, with a value of 1 representing an ideal fit. Table 1 shows the evaluation metrics with description. The choice of spectral preprocessing technique can significantly impact these metrics showing superior performance in certain applications [25].

Table 1. Evaluation metric table

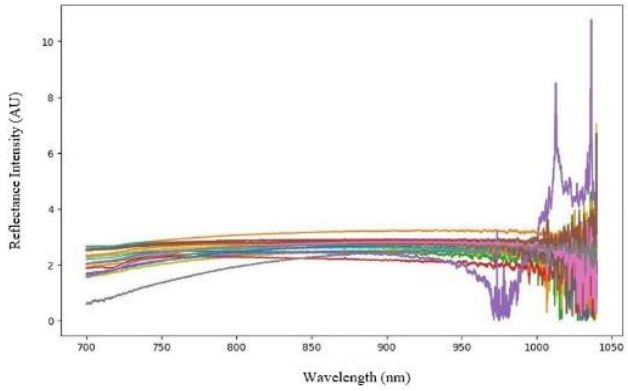
S.NO	Metric	Formula	Description
1	R^2	$R^2 = 1 - \frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - \bar{y})^2}$	where y_i is the actual value, $\hat{y}_i$ is the predicted value, and $\bar{y}$ is the mean of the actual value.
2	RMSE	$RMSE = \sqrt{\frac{\sum(y_i - \hat{y})^2}{n}}$	where y_i is the actual value, $\hat{y}_i$ is the predicted value and n is the number of samples

3. RESULT AND DISCUSSION

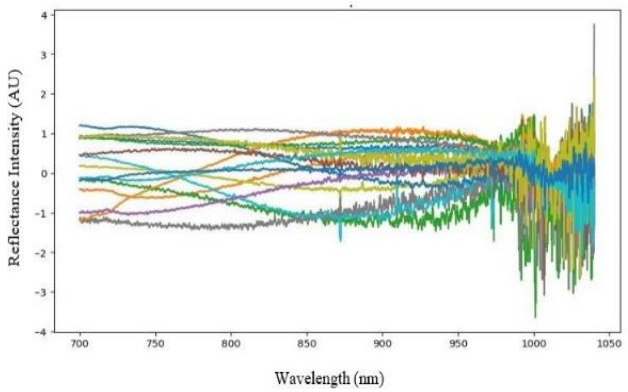
The spectral data acquired through diffuse reflectance spectroscopy contains nonlinearities that can impact the accuracy of predictive models. To address this issue, three preprocessing techniques were applied to the data: SNV, MSC, and Baseline Correction. The raw spectral data plot for 30 participants with thyroid dysfunction is presented in Figure 3(a). From the figure it is inferred that, around 700 to 950 nm, the reflectance intensity remains relatively stable and low. Beyond 950 nm, there is an increase in noise and variability in the reflectance intensity among participants. Some spikes and abrupt changes in intensity are observed around 1,000 nm, which may indicate increased sensitivity or variability in that region. The relatively stable reflectance intensity between 700 and 950 nm suggests that the participants' spectral responses are consistent in this region. This stability is often desirable in spectral analysis as it can indicate a uniform response to the light across participants.

Figure 3(b) shows the spectral plot of SNV preprocessing, which normalizes the distribution across the wavelength range. In the SNV process, the data are mean-centered and scaled by their standard deviation, resulting in spectra centered around zero with uniform variance. This normalization helps to reduce multiplicative effects, enhancing the comparability and interpretability of the spectral data. The SNV preprocessed plot demonstrates a standardized representation, minimizing baseline shifts and scaling inconsistencies.

Figure 3(c) displays the spectral plot after baseline correction using Savitzky-Golay filtering. This technique successfully eliminates baseline irregularities, smoothing the spectral baseline for a more consistent and stabilized data representation. The corrected plot reveals enhanced clarity of spectral features, as unwanted fluctuations and distortions are mitigated. This plot highlights the effectiveness of the baseline correction in improving the quality and interpretability of spectral data. Figure 3(d) presents the spectral plot after MSC preprocessing. MSC effectively addresses scattering effects, resulting in a more uniform spectrum across the entire wavelength range. By reducing the impact of scattering, this normalization process enhances the comparability and interpretability of the spectral information.



(a)



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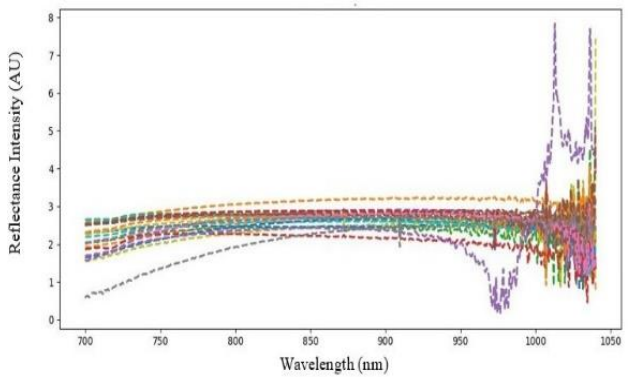
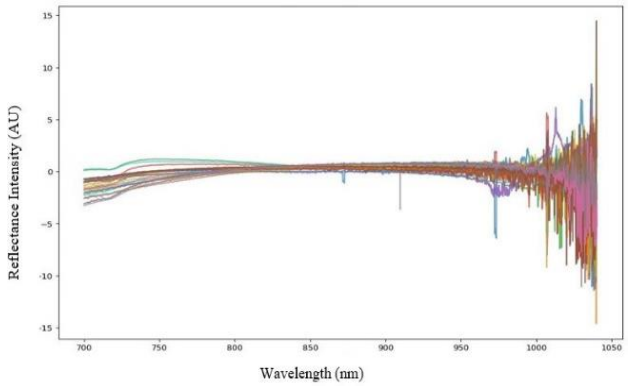


(c)



(d)

Figure 3. Preprocessed spectral plots (a) raw spectral data plot of 30 participants, (b) SNV, (c) baseline correction, and (d) MSC

The proposed preprocessing techniques were statistically analyzed based on the performance metrics as shown in Table 2, it is clear that SNV preprocessing approach significantly outperforms the other methods in terms of model accuracy and reliability. SNV resulted in an RMSE of 0.005 and an R^2 of 0.99. This highlights SNV's superior ability to normalize the spectral data, effectively mitigating multiplicative effects. In contrast, MSC shows an RMSE of 0.87 and an R^2 of 0.86. While MSC reduces scatter effects and improves the uniformity of the spectrum, it still exhibits a relatively high error margin and explains only 86 percent of the variance, making it less reliable compared to SNV. The higher RMSE indicates more significant errors in the predictions, suggesting that while MSC is beneficial, it does not achieve the same level of precision as SNV.

Baseline correction using Savitzky-Golay filtering presents an RMSE of 0.84 and an R^2 of 1.09. Although the RMSE is slightly lower than MSC, the R^2 value exceeding 1 is unusual and points to potential overfitting or anomalies in the model's evaluation process. This could imply that while baseline correction effectively smooths the spectral data, it might introduce artifacts or inconsistencies, thus affecting the overall model reliability and interpretability.

Table 2. Evaluation-metric of preprocessing technique

Preprocessing techniques	R^2	RMSE
MSC	0.86	0.87
SNV	0.99	0.005
Baseline correction	1.09	0.84

4. CONCLUSION

The study signifies the importance of preprocessing in spectroscopic analysis. In the proposed study, the selection of efficient preprocessing techniques to obtain improved model accuracy has been evaluated. The study involved various preprocessing techniques such as SNV, MSC and baseline correction out of which SNV outperformed as the most robust technique, significantly improving the clarity, comparability, and interpretability of spectral data, thereby enhancing diagnostic accuracy for thyroid dysfunction. While MSC and baseline correction have their merits, they do not match the performance of SNV in this context. The findings emphasize the necessity for meticulous selection and application of preprocessing techniques to ensure high-quality spectral data, ultimately leading to more accurate non-invasive diagnostics and better patient outcomes.

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