Rapid detection of gelatin in dental materials using attenuated total reflection fourier transform infrared spectroscopy (ATR-FTIR)

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Abstract. The presence of gelatin is not limited to food products but has also been found in pharmaceuticals. Most dental materials available in Malaysia are imported from other countries and might contain gelatin which is a protein derived either from porcine, bovine or other animal sources. Authentication of gelatin is crucial due to religious and health concerns. Therefore, this study aimed to detect gelatin in dental materials using ATR-FTIR. Forty two samples of dental material were purchased from dental suppliers and detection was done using ATR-FTIR. The spectrum from each sample was compared against standard bovine and porcine gelatin. Experimental dental paste containing bovine and porcine gelatin at concentrations of 5, 10, 15 and 20% were also prepared for quantification analysis. The results showed that gelatin was present in nine out of forty two samples of dental materials but the species of origin was not confirmed. Meanwhile, in the experimental bovine and porcine dental paste, it was seen that as the concentration increased, the intensity of the absorption of Amide group also increased. Thus, ATR-FTIR can be utilized as a reliable tool to detect gelatin in dental materials and other pharmaceuticals.

1. Introduction

In the recent years, Muslims are seeking halal integrity not only with meat-based products but also with a wide range of products. This has also spread to a growing awareness about cosmetics, personal care products and pharmaceuticals including dental products where a research has cited that more than 20% of Muslim consumers are concerned about the Halal status of products they are using [1]. The basic principle in Islam is that all things created by God (Allah) are permitted, with few exceptions that are specifically prohibited (non halal or haram). The basis for the prohibition of anything in Islam is purely and strictly Quranic guidance (The divine book) from Allah (The Creator) to the Prophet Mohammed S.A.W. for all mankind. This is clear in Chapter II, verse 172-173, Chapter V, verse 3-5 and Chapter VI, verse 145 of the Quran. Muslims of all ethnic and geographic origins strictly observe these laws. Besides that, the Jewish community also
has their own dietary laws called Kosher law which prohibits them from consuming porcine-derived products while Hindus abstain from the use of bovine or cow-based products [2].

Dental materials are materials used in both laboratory and practical dentistry and can be categorised as preventive materials, restorative materials or auxiliary materials. Preventive dental materials are materials which contain antibacterial, fluoride or other therapeutic agents against dental caries. Restorative dental materials can be applied in repairing or replacing tooth structure and mainly consists of synthetic components. Meanwhile, materials which are used in fabricating dental prostheses are known as auxiliary dental materials. One example of these materials includes impression material [3].

Most dental materials available in Malaysia are imported from other countries and might contain gelatin. The labelling of these products is not clear since the label does not mention the excipients used, which could include gelatin. Gelatin is a protein derived from hydrolysed collagen of bones, hides and skins from various animals such as cattle and pig. It was reported that gelatin derived from porcine (pig) skin is more abundant compared to gelatin derived from other animals [4]. Authentication of gelatin is crucial not only due to religious reasons but also includes health concerns such as the outbreak of Bovine Spongiform Encephalopathy (BSE) or mad cow disease which is transmitted through infected cattle [5].

Analytical methods to detect the presence of gelatin have been carried out by researchers. These methods include amino acid analysis [6-7], spectroscopic analysis [8-10], and DNA analysis [2, 11-14].

The use of Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) spectroscopy has been reported to be useful since it produces reliable, accurate and rapid results as it requires less than 2 minutes per sample analysis. This method is also known to be non-destructive and requires simple sample preparation to obtain information on the chemical and molecular properties of various substances [15]. Most of the studies reported focus on detection of raw gelatin, or gelatin in processed foods or capsules and not in dental materials. Hence, the present study was aimed to detect gelatin in dental materials using ATR-FTIR spectroscopy.

2. Materials and Methods
2.1 Samples
Porcine skin gelatin and bovine skin gelatin were purchased from Sigma-Aldrich (St. Louis, MO, USA). 42 samples of dental materials used in Polyclinic, Kulliyah of Dentistry were purchased from various legal dental suppliers in Malaysia.

2.2 Sample Preparation
Samples of dental products were classified into 4 types such as powder, paste, gel and liquid. The powder sample was homogenized and dissolved in deionized water. The other samples were directly placed into the ATR surface area. Control samples of pure porcine and bovine gelatin powder were dissolved in deionized water and incubated at 50 °C for 10 minutes until a clear solution was obtained.

2.3 Experimental (home-made) dental paste preparation
Dental paste containing bovine and porcine gelatin at concentrations of 5%, 10%, 15% and 20% were prepared. Bovine gelatin dental paste was made by mixing bovine skin powder, sodium bicarbonate and sodium chloride. Meanwhile, porcine dental paste was prepared by mixing porcine skin powder, sodium bicarbonate and sodium chloride. The mixture was mixed until it achieved a paste-like texture.

2.4 Infrared spectroscopy measurements
Perkin Elmer spectrometer (PerkinElmer, Inc., USA) with an ATR detector was used in the measurements. All spectra were recorded within a range of 4000-400 cm⁻¹ with a 2 cm⁻¹ resolution and
16 scans. All measurements were performed at room temperature (25 ± 0.5 °C) in a dry atmosphere. A background of air spectrum was scanned before measurement of samples. The air background spectrum was subtracted from the spectra obtained from samples and the results were presented in absorbance units.

3. Results and Discussion

3.1 Results

3.1.1 Classification of dental materials

List of dental products used are shown in Table 1. Forty two samples of dental materials were classified based on type of material and batch number. Each sample was assigned product number BDM 01 until BDM 42. The dental materials were classified into haemostatic agent, restoration material, dental prophylaxis, oral surgery, impression material, dental anaesthetic agent, oral rinse, prosthetic material and preventive material.

| Product number | Brief description on material | Type of material |
|----------------|------------------------------|------------------|
| BDM01          | Haemostatic agent            | Sponge           |
| BDM02          | Haemostatic agent            | Sponge           |
| BDM03          | Haemostatic agent            | Sponge           |
| BDM04          | Haemostatic agent            | Liquid           |
| BDM05          | Haemostatic agent            | Paste            |
| BDM06          | Restoration material         | Liquid           |
| BDM07          | Restoration material         | Liquid           |
| BDM08          | Restoration material         | Powder           |
| BDM09          | Restoration material         | Paste            |
| BDM10          | Restoration material         | Powder           |
| BDM11          | Dental prophylaxis           | Gel              |
| BDM12          | Restoration material         | Paste            |
| BDM13          | Restoration material         | Liquid           |
| BDM14          | Restoration material         | Paste            |
| BDM15          | Dental anaesthetic agent     | Liquid           |
| BDM16          | Dental prophylaxis           | Paste            |
| BDM17          | Restoration material         | Paste            |
| BDM18          | Oral surgery                 | Paste            |
| BDM19          | Restoration material         | Paste            |
| BDM20          | Impression material          | Paste            |
| BDM21          | Impression material          | Gel              |
| BDM22          | Restoration material         | Liquid           |
| BDM23          | Restoration material         | Paste            |
| BDM24          | Restoration material         | Paste            |
| BDM25          | Impression material          | Paste            |
| BDM26          | Restoration material         | Paste            |
| BDM27          | Dental anaesthetic agent     | Gel              |
| BDM28          | Preventive material          | Gel              |
| BDM29          | Dental anaesthetic agent     | Gel              |
| BDM30          | Preventive material          | Liquid           |
Table 1. Continued

| Product number | Brief description on material | Type of material |
|----------------|-------------------------------|------------------|
| BDM 31         | Oral rinse                    | Liquid           |
| BDM 32         | Restoration material          | Liquid           |
| BDM 33         | Prosthetic material           | Powder           |
| BDM 34         | Periodontal dressing          | Paste            |
| BDM 35         | Restoration material          | Paste            |
| BDM 36         | Restoration material          | Gel              |
| BDM 37         | Dental prophylaxis            | Paste            |
| BDM 38         | Dental prophylaxis            | Paste            |
| BDM 39         | Dental prophylaxis            | Paste            |
| BDM 40         | Dental prophylaxis            | Paste            |
| BDM 41         | Dental prophylaxis            | Powder           |
| BDM 42         | Dental prophylaxis            | Paste            |

3.1.2 Infrared spectrum of control samples
Figure 1 shows the infrared spectra of bovine and porcine gelatin acquired by means of ATR-FTIR. Based on Figure 1, both spectra of porcine and bovine gelatin showed similar patterns and four regions were observed; 3600-2300 cm⁻¹ (Amide A), 1656-1644 cm⁻¹ (Amide I), 1560-1335 cm⁻¹ (Amide II) and 1240-670 cm⁻¹ (Amide III).

![Figure 1. FTIR spectra of bovine and porcine gelatin](image)

3.1.3 Detection of gelatin in dental material using FTIR
Detection of gelatin in various dental materials is shown in Table 2. Nine out of forty two samples were found to contain gelatin from either porcine or bovine sources. Four of them were categorized under haemostatic agents, two samples under restorative material and one sample each under dental prophylaxis, impression material and oral rinse.
Table 2. Detection of gelatin in dental materials using ATR-FTIR

| Sample    | Category                         | Presence of Gelatin |
|-----------|----------------------------------|---------------------|
| BDM 01    | Haemostatic agent                | +                   |
| BDM 02    | Haemostatic agent                | +                   |
| BDM 03    | Haemostatic agent                | +                   |
| BDM 04    | Haemostatic agent                | -                   |
| BDM 05    | Haemostatic agent                | +                   |
| BDM 06    | Restoration material             | -                   |
| BDM 07    | Restoration material             | -                   |
| BDM 08    | Restoration material             | -                   |
| BDM 09    | Restoration material             | -                   |
| BDM 10    | Restoration material             | -                   |
| BDM 11    | Dental prophylaxis               | -                   |
| BDM 12    | Restoration material             | -                   |
| BDM 13    | Restoration material             | -                   |
| BDM 14    | Restoration material             | +                   |
| BDM 15    | Dental anaesthetic agent         | -                   |
| BDM 16    | Dental prophylaxis               | +                   |
| BDM 17    | Restoration material             | -                   |
| BDM 18    | Oral surgery                     | -                   |
| BDM 19    | Restoration material             | -                   |
| BDM 20    | Impression material              | -                   |
| BDM 21    | Impression material              | -                   |
| BDM 22    | Restoration material             | -                   |
| BDM 23    | Restoration material             | -                   |
| BDM 24    | Restoration material             | -                   |
| BDM 25    | Impression material              | -                   |
| BDM 26    | Restoration material             | -                   |
| BDM 27    | Dental anaesthetic agent         | -                   |
| BDM 28    | Preventive material              | +                   |
| BDM 29    | Dental anaesthetic agent         | -                   |
| BDM 30    | Preventive material              | -                   |
| BDM 31    | Oral rinse                       | +                   |
| BDM 32    | Restoration material             | -                   |
| BDM 33    | Prosthetic material              | -                   |
| BDM 34    | Periodontal dressing             | -                   |
| BDM 35    | Restoration material             | -                   |
| BDM 36    | Restoration material             | +                   |
| BDM 37    | Dental prophylaxis               | -                   |
| BDM 38    | Dental prophylaxis               | -                   |
| BDM 39    | Dental prophylaxis               | -                   |
| BDM 40    | Dental prophylaxis               | -                   |
| BDM 41    | Dental prophylaxis               | -                   |
| BDM 42    | Dental prophylaxis               | -                   |
3.1.4 Spectra of dental materials

Figure 2 shows the spectra for haemostatic agents (BDM 01, BDM 02, BDM 03 BDM 04 and BDM 05) compared against standard bovine and porcine gelatin. In the range of 3700-3100 cm\(^{-1}\) and 1700-1600 cm\(^{-1}\), all samples showed similar patterns to both bovine and porcine gelatin. Therefore, all samples (BDM 01, BDM 02, BDM 03 BDM 04 and BDM 05) contained gelatin.

The ATR-FTIR spectra for other samples compared to standard bovine and porcine gelatin are illustrated in Figure 3. BDM 14, BDM 16, BDM 28, BDM 31 and BDM 36 showed a similar peak at the area between 1700 and 1600 cm\(^{-1}\) when compared to standard bovine and porcine gelatin.

![Figure 2](image2.png)

**Figure 2.** Spectra of haemostatic agents (BDM 01, BDM 02, BDM 03, and BDM 05) compared with standard bovine and porcine gelatine

![Figure 3](image3.png)

**Figure 3.** Spectra of other dental materials (BDM 14, BDM 16, BDM 28, BDM 31 and BDM 36) compared with standard bovine and porcine gelatin
3.1.5 FTIR spectra for dental paste containing gelatin

Figure 4 (a) and 4 (b) show FTIR spectra of dental paste containing bovine gelatin and porcine gelatin. It is seen that as the concentration increases, the intensity of the absorption of Amide I and Amide II also increased. Thus, FTIR-ATR can be utilized to detect gelatin at different concentrations.

![Figure 4 (a)](image)

**Figure 4 (a).** Concentration-dependent FTIR spectra of dental bovine paste (5%, 10%, 15% and 10%)  

![Figure 4 (b)](image)

**Figure 4 (b).** Concentration-dependent FTIR spectra of dental porcine paste (5%, 10%, 15% and 10%)

3.2 Discussion

Based on the results, it is seen that bovine and porcine gelatin exhibit similar patterns of spectra. Both bovine and porcine gelatin produced four regions which are 3600-2300 cm\(^{-1}\) (Amide A), 1660-1620 cm\(^{-1}\) (Amide I), 1560-1300 cm\(^{-1}\) (Amide II) and 1240-670 cm\(^{-1}\) (Amide III). These findings were in concordance with a previous study reported by Hashim *et al.* [9]. Amide I absorption
represents the stretching of carbonyl C=O (peptide bond) with less involvement of the stretching C-N bond. Meanwhile, Amide II absorption is due to N-H bond-bending mode and stretching vibration of C-N bond [16]. It is reported that secondary structure of protein could be determined based on Amide I absorption instead of Amide II. This is owing to the contribution of only one of the amide functional groups [17]. However, Amide III absorption could also be beneficial in determining the secondary structure of protein [17-18]. According to Bandekar [19], stretching vibrations of C-N combined with in plane bending vibrations of N-H with weak contributions from stretching of C-C bond and C=O in plane bending gave rise to Amide III absorption. For that reason, Amide III showed a less defined vibrational mode with varying protein vibration [16].

The present study showed that FTIR can be utilized as an analysis tool to screen the presence of gelatin. The detection is possible since Infrared (IR) spectrum was able to identify the functional groups present in each sample. However, we could not differentiate gelatin from different species because both gelatins produced indistinguishable spectra. Several studies reported that discrimination of gelatin from different species was successful by means of FTIR combined with chemometric analysis [8-10]. The analysis was continued further with the detection of gelatin without discriminating either bovine or porcine gelatin. Detection of gelatin in dental material was done by comparing the spectra of each sample with the standard bovine and porcine gelatin.

The present study indicates that nine out of forty two samples were identified to contain gelatin with most of samples being categorised as haemostatic agents. Haemostatic agents are applied to control bleeding due to tooth extraction, oral surgery and implants [20]. Some of the haemostatic agents used in dental treatment are derived from bovine and porcine gelatin [21]. These type of haemostatic agents are called mechanical haemostats which act as a first line agent and are beneficial in the control of minor bleeding [22]. It was reported that collagen derived from bovine is effective to control haemorrhage [23].

For the detection of gelatin in dental materials, it was found that gelatin was successfully detected using ATR-FTIR. However, we could not differentiate gelatin from different species. On the contrary, this technique was found to be effective in detecting adulteration of food products [24-27] as well as discrimination analysis of gelatin from different species as reported by Hashim et al. [9] and Cebi et al. [8].

4. Conclusion
In conclusion, ATR-FTIR technique can be utilized as a screening tool to detect gelatin in dental material. However, further studies need to be done in order to distinguish gelatin from different animal species.

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