CYTOTOXICITY AND CHARACTERISATION OF POLYMETHYL METHACRYLATE DENTURE BASE ENHANCED WITH LINSEED OIL (PMMA-L)

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Abstract

Objectives: Polymethyl methacrylate (PMMA) is a widely used polymer for a denture base material. Dibutyl phthalate was previously utilised as a synthetic plasticiser in PMMA, but concerns about ester leaching have raised awareness of using organic plasticisers, such as linseed oil. The objective of this research was to assess the cytotoxic effects of linseed oil and to characterise the experimental polymethyl methacrylate enhanced with linseed oil (PMMA-L).

Method: The cytotoxicity of linseed oil (LO) was evaluated by exposing Human Gingival Fibroblast (HGF) cells to different linseed oil concentrations (1%, 3%, 5%, 7%, and 10%) for 24, 48, and 72 hours. Afterwards, cell viability was measured with the WST-1 assay, and a spectrophotometer was used for cell quantification. Five groups of PMMA-L were prepared, each containing different percentages of linseed oil (1%, 3%, 5%, 7%, and 10% by weight). These samples underwent an ageing process involving 5,000 cycles of thermocycling between 5°C and 55°C with a 30-second dwell time. Compositional and leaching analyses of PMMA-L were performed using attenuated total reflection fourier-transform infrared spectroscopy (ATR-FTIR).

Results: The results from the WST-1 assay consistently showed cell viability exceeding 95% across all doses and incubation periods, indicating the non-cytotoxicity of LO. Composition analysis revealed that the ATR-FTIR spectra of PMMA-L and linseed oil were identical within the C=C stretching vibration range (1536-1652 cm^{-1}), originating from unsaturated triglyceride compounds found in linseed oil but not in PMMA. Additionally, the leaching analysis did not detect any leaching of linseed oil from PMMA-L.

Conclusions: The research offers proof of the non-cytotoxic nature of linseed oil. Moreover, the successful creation of PMMA-L is evident, as indicated by the incorporation of the linseed oil component in PMMA-L and the absence of linseed oil in the leaching analysis.

Keywords: Polymethyl Methacrylate, Linseed Oil, Dentures, Plasticiser, Bio-based

Introduction

Polymethylmethacrylate (PMMA) has remained the most popular denture base material since its introduction in 1937 due to its desirable properties, such as low density, affordability, aesthetic appeal, and optimal mechanical and physical properties. PMMA polymer is created through the free radical addition and polymerisation of methyl methacrylate (1).

However, a significant drawback of PMMA is its poor strength, driven by polymerisation shrinkage (2), which leads to frequent denture repairs. Consequently, numerous studies have explored the addition of fillers to enhance the properties of PMMA (3-5). However, enhancing one set of characteristics without compromising others presents a significant challenge.

Plasticisers like dibutyl phthalate were used to improve PMMA's properties, such as water sorption properties (6).

However, in vitro (7) and in vivo (8) studies have shown the leaching of dibutyl phthalate ester from PMMA, causing allergic asthma, disruption of the endocrine system, estrogenic and carcinogenic properties, and environmental hazards (9, 10). Consequently, current PMMA formulations in the market exclude chemical plasticisers from their monomer.

Since plasticisers are crucial for the PMMA properties, there is a need to explore possible types of biocompatible plasticisers that can improve the properties of PMMA as a denture base material. One such natural-based plasticiser is linseed oil, a triglyceride vegetable oil extracted from the flax plant (*Linum usitatissimum*) that contains Oleic Acid (18.88%), Linoleic Acid (16.10%), and Linolenic Acid (53.73%). Linseed oil and fibres offer promising health advantages, demonstrating the potential to reduce the risk of various conditions such as heart problems, autoimmune disease and diabetes. Additionally, flax protein plays a role in preventing and treating heart disease while boosting the immune system. Furthermore, linseed oil has found its way into various food items, including dairy products, baked goods and dry pasta (11). Multiple studies have incorporated linseed oil in biomedical material research. Nanocapsules derived from linseed oil incorporating CdSe/ ZnS quantum dots exhibit promise as a biocompatible carrier for hydrophobic quantum dots, demonstrating low cytotoxicity in mouse embryonic fibroblasts (12). Moreover, an in vitro study by Lewinska *et al*. also showed that linseed oil exhibited strong wound-healing activity by promoting cell proliferation (13).

Linseed oil has shown excellent compatibility and a homogenous blend with PMMA (14). In addition, linseed oil has also been widely used in various industries as a plasticiser for polymers. Multiple studies have shown that linseed oil has improved the physical properties of polymers, such as plasticity, light and heat stability (9, 15).

So far, there have been no efforts to utilise natural-based linseed oil as a plasticiser to PMMA used in denture base materials. Therefore, this study aimed to investigate the linseed Oil cytotoxicity effect and characterise the chemical composition and molecular structure of the linseed oil-enhanced polymethyl methacrylate (PMMA-L) via attenuated total reflectance fourier-transform infrared spectroscopy (ATR- FTIR).

Materials and Methods

In vitro cytotoxicity of linseed oil

Cell culture media

Human Gingival Fibroblast (HGF) (ATC.CRL-2014, ATTC, US) were grown as cultures under 37 ^{O}C and 5% CO_2 in a 75 cm^2 vented cap tissue culture flask in the complete growth media of the Dulbecco's Modified Eagle Medium-Nutrient Mixture F-12 (DMEM/ F12) (Gibco, UK) supplemented with 10% (v/v) Fetal Bovine Serum (FBS) (Gibco, UK) and 1% (v/v) Penicillin-streptomycin (Pen/ Strep) (Gibco, UK). The study had three biological replicates from each HGF Passage 8, 9 and 10.

WST-1 cytotoxicity assay

Once the cell confluency achieved 80% of the 75 cm^2 flask, the number of cells was then acquired by a haemocytometer under the microscope. The cells were seeded in 96-well plates at 5×10^4 cells/ well in 100 µL complete growth media (as the manufacturer suggested) and incubated for 24 hours at 37°C and 5% CO_2. Five different concentrations of linseed oil (1%, 3%, 5%, 7% and 10%) (Sigma Aldrich, CAS no: 8001-26) were then used to treat the seeded cells. Dimethyl Sulfoxide (DMSO) (Sigma Aldrich, US) of the same concentration were used to treat the seeded cells as the positive control. The cells were incubated in pure growth media (DMEM/F12) without any linseed oil concentration as the negative control. They were then incubated in the incubator for 24, 48 and 72 hours at 37^{O}C and 5% CO_2. After the incubation period, the WST-1 cytotoxicity assay was performed.

The viability of the HGF cells was analysed by WST-1 assay (Sigma Aldrich, US), and the colourimetry assay relies on the breakdown of WST-1 tetrazolium salt by mitochondrial dehydrogenases (16, 17). Cell viability was assessed by measuring the optical density of formazan byproducts. Subsequently, the WST-1 assay was employed following the manufacturer's guidelines, involving the addition of 10 µl WST-1 in each well and incubated for 2 hours at 37°C and 5% CO_2. The absorption of cells was read with a spectrophotometer plate reader (Tecan Infinite M200 Plate Reader, Switzerland) at 420-480 nm.

For the viability of cells, the formula as shown below was used:

% Viability of cells = [(mean absorbance of treated cells) – (mean absorbance of blank cells)/ (mean absorbance of control cells) – (mean absorbance of blank cells)]×100

Data analysis

The percentage of cell viability was statistically analysed with an unpaired t-test using Prism Software (GraphPad Software, Inc) 9.0.0 for MacOS. Cytotoxicity levels were categorised based on cell viability compared to controls: non-cytotoxic (>90% cell viability), slightly cytotoxic (60-90% cell viability), moderately cytotoxic (30-59% cell viability), and strongly cytotoxic (<30% cell viability) (17).

Fabrication of the experimental PMMA-L samples

A commercially available Vertex BasiQ 20 rapid heat-curing powder, PMMA (polymethyl methacrylate), and Vertex BasiQ 20 liquid (MMA, methyl methacrylate) (Vertex-Dental B.V, The Netherlands) were used as the denture base material. In addition, linseed oil (Sigma Aldrich, CAS no: 8001-26) was used as a natural-based plasticiser.

The ratio of 24 gram:10 mL acrylic resin powder-to-monomer ratio was used acrylic resin powder-to-monomer

for the PMMA-L samples with the addition of linseed oil in weight percentage at 1%, 3%, 5%, 7%, and 10%. A mould of size 20 mm (length) X 30 mm (width) x 2 mm (thick) was prepared by embedding a wax sheet in dental stone in the dental flask. After de-waxing, sodium alginate (Metrodent Limited, United Kingdom) was applied to the mould as a separating medium to remove the processed sample easily. The mould was then packed with a dough-like acrylic, and the denture flask was clamped before heat curing at 100°C for 20 minutes. Finally, the flask was removed to allow the air to cool to ambient temperature for 30 minutes. After the end of the curing process, the sample was removed from the denture flask, and a tungsten carbide bur was used to trim the excess. Samples were placed in the ultrasonic cleaning machine (Renfert easy-clean, Germany) to clean any foreign bodies from processing. Later, pumice was used to polish the samples in a polishing machine (Mestra, Spain). All samples subsequently underwent an ageing process, subjecting them to 5,000 thermocycling cycles between 5°C and 55°C, with a dwell time of 30 seconds each (Zecttron, Germany).

Characterisation and in vitro leaching analysis of PMMA-L.

Each PMMA-L group sample was subjected to ATR- FTIR spectroscopy (PerkinElmer series 600) to obtain an infrared spectrum over a wavelength range from 400 cm^{-1} to 4000 cm^{-1}. The data was presented as the transmittance spectrum of the ATR-FTIR. The peak visible in the transmittance spectrum of the ATR-FTIR was compared to the functional group presented in previous studies of edible fats and oils (18). For leaching analysis, distilled water was used as the immersion media of the PMMA-L samples for seven days at room temperature. The distilled water was then characterised under ATR- FTIR to detect the presence of leached linseed oil.

Results and Discussion

In vitro cytotoxicity of the linseed oil

Figure 1 shows the percentage cell viability for different concentrations of linseed Oil (1%, 3%, 5%, 7%, and 10%) compared to different concentrations of DMSO at 24, 48 and 72. The negative control group, where the cells were only incubated in pure DMEM/F12 growth media without any linseed oil (0%), exhibited 100% cell viability for all the sets. The cell viability percentage for the linseed oil group remained above 94% at all concentrations and incubation periods up to 72 hours, indicating that exposure to linseed oil did not have a cytotoxicity effect on the HGF cells at any concentration (Figure 1). However, with HGF cells that were treated in DMSO, there is a marked decrease in the viability percentage, especially at all concentrations at 72 hours of treatment compared to 24 hours. This was shown by the decline in the mean percentage of cell viability in the DMSO group from 102.5% at 1% concentration after 24 hours of incubation to 86.3% after 72 hours.

The unpaired t-test in Table 1 proves there is a statistically significant difference in cell viability percentage at 48 hours (p<0.05, p-value=0.0121) and 72 hours (p<0.05, p-value=0.0028) between the means of the linseed oil group as compared to DMSO group. However, no statistical significance was found between the groups within 24 hours (p>0.05, p-value=0.3124).

In this study, the WST-1 assay was used to evaluate the effect of linseed oil on the percentage of HGF cell viability. Known for its precision and sensitivity, the WST-1 assay assessed both cell cytotoxicity and proliferation while estimating the count of viable cells in cultures. Its convenience lies in the fact that adherent cells can be cultured in a microplate, and the optical density was observed with a spectrophotometer after the treatment with WST-1 assay, which assessed the transformation of the tetrazolium salt into formazan by cellular dehydrogenases. The resulting formazan, appearing as dark yellow, was measured at 450 nm and directly associated with the cell count (19).

From the above results, we observed an insignificant influence of the percentages of linseed oil towards the percentage of cell viability of the HGF cells. Therefore, the null hypothesis was accepted: linseed oil is not cytotoxic to HGF cells. Figure 1 shows that the incubation time from 24 to 72 hours had no significant impact on cell viability. Cell viability stayed above 94% of all the linseed oil concentrations with no significant changes even at 72 hours. This indicated that linseed oil is not cytotoxic with HGF, as Sjögren et al. (20) suggested. Their study found that if the cell viability exceeds 90%, the medium is categorised as non-cytotoxic. Table 1 showed a statistically significant difference between the cells incubated in linseed oil compared to DMSO at 48 hours (p<0.05, p-value=0.0121) and 72 hours (p<0.05, p-value= 0.0028). DMSO is a highly effective polar aprotic solvent suitable for water-insoluble substances like linseed oil. It is commonly employed in biological research and often used as a drug therapy carrier. Multiple studies have shown that the use of DMSO above 1% exhibited a cytotoxic effect by a noticeable decrease in cellular viability due to the reduction in Collagen I expression of the mesenchymal cell, which is crucial in biological processes of cell attachment, cell proliferation, and tissue remodelling (21–23).

The result of cell viability at all concentrations of linseed oil was also in compliance with the status Generally Recognised as Safe (GRAS) appointed by the United States Food and Drug Administration (FDA) for linseed oil, which means that it is safe for applications and consumptions at levels of 1.0 – 10.0%. Moreover, Lewinska et al. (13) supported this finding with reports of linseed oil as edible oil utilised as a functional dietary component with multiple health advantages, including management of cardiovascular diseases, diabetic therapy, and anti-inflammatory and autoimmune disorders (11, 24).

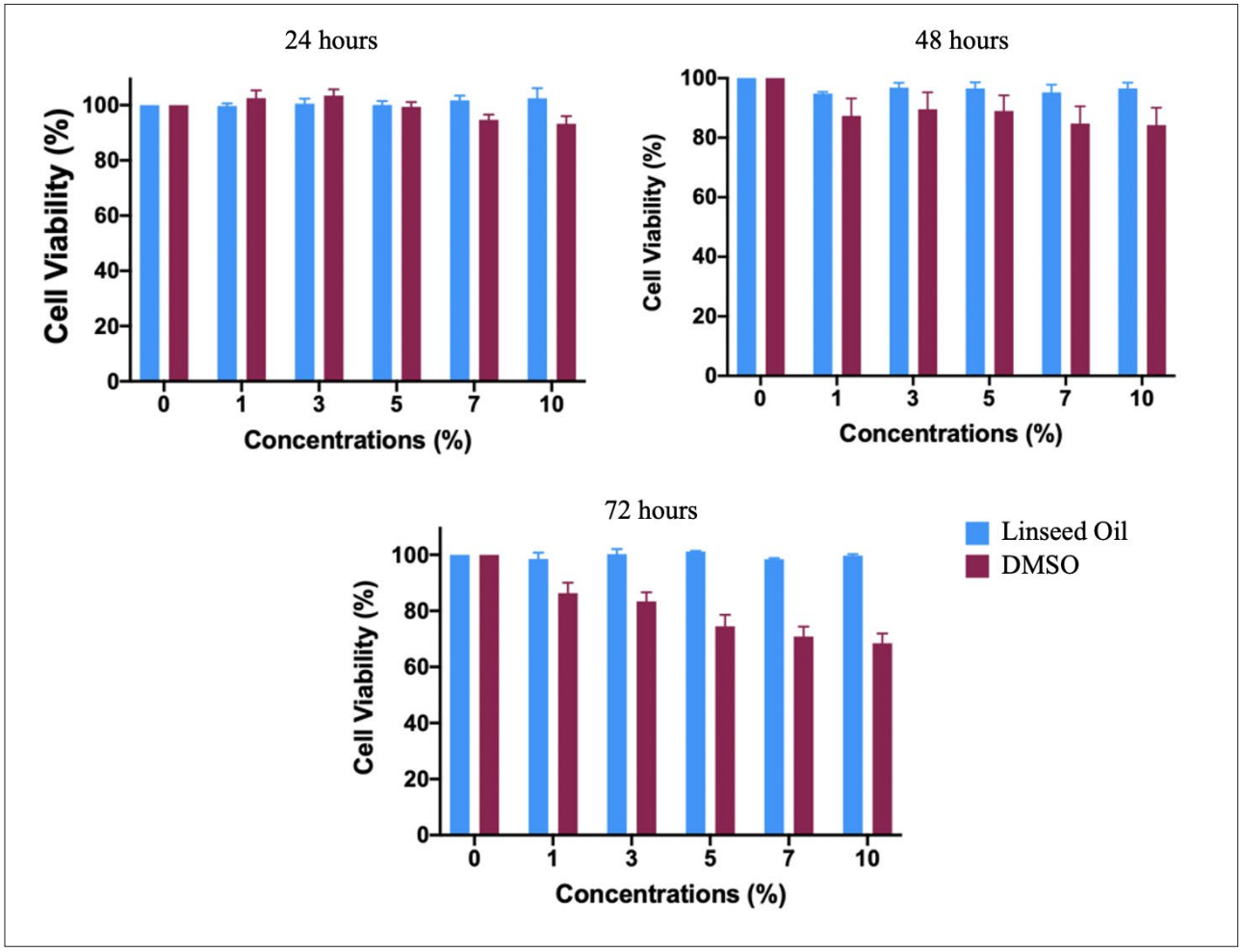

Figure 1: Cell viability of HGF cells treated with different concentrations of linseed oil and DMSO at 24, 48 and 72 hours incubation periods based on WST-1 assay

Table 1: The unpaired t-test between the six concentrations of linseed oil and DMSO within 24, 48 and 72 treatment hours

Treatment time (hours)	n	Standard Deviation	95% Confidence Intervals		p-value
			Lower Bound	Upper Bound	
24	18	1.537	-5.741	2.030	0.3124
48	18	0.991	-12.99	-2.033	0.0121*
72	18	3890	-29.85	-8.297	0.0028*

DMSO=dimethyl sulfoxide, Statistical significance of differences between linseed oil and DMSO within incubation time were determined using unpaired t-test, significant level was set at $p<0.05$ (*)

Characterisation of PMMA-L.

Figure 2 shows the infrared spectra analysis, which displayed several peaks of the pure linseed oil, pure PMMA, and PMMA-L. Only the spectrum for 10% of PMMA-L was shown here since no significant differences were observed in the spectrum peak and intensity for different percentages of linseed oil loadings. Table 2 listed the wavenumbers and functional groups corresponding to the linseed oil spectrum, pristine PMMA spectrum and PMMA-L spectrum. Few apparent peaks for linseed oil detected are C-H stretching vibrations in the aliphatic chains (3006 – 2856 cm^{-1}), C=O stretch of triglycerides (1742 cm^{-1}), C=C stretching vibrations of unsaturated fatty acids (1652 cm^{-1}), bending vibrations of -CH_2 groups (1454 cm^{-1}), C-O stretching vibrations of a secondary alcohol (1098 cm^{-1}) and rocking vibrations of =CH_2 groups (712 cm^{-1}). On the other hand, the apparent peaks observed for PMMA are C-H stretching vibrations in the methyl and methylene groups (2984-2856 cm^{-1}), C=O stretch of carbonyl groups in the ester units (1720 cm^{1-}), bending vibrations of -CH_3 and -CH_2 groups (1446 cm^{-1}), symmetric bending vibrations of the methyl groups (1380 cm^{-1}), C-O stretching vibrations of ester groups (1244 cm^{-1}) and C-O-C stretching vibrations in the ester units (1136 cm^{-1}).

Since the molecular compounds of linseed oil and PMMA were similar, as shown in Figure 2, peaks of C-H stretching vibrations, C=O stretching vibrations, CH_2 and CH_3 bending

vibrations, and C-O-C stretching vibrations were detected for both pure linseed oil spectrum and pristine PMMA spectrum. As for the PMMA-L spectrum, these peaks are believed to overlap due to similar molecular compounds (25). However, the peak corresponding to the C=C stretching vibration of unsaturated fatty acids appears only in the linseed oil spectrum and PMMA-L spectrum but not in the pure PMMA spectrum. This is because linseed oil contains unsaturated fatty acids, such as alpha-linolenic acid, which contribute to characteristic peaks related to C=C stretching vibrations. These findings proved that linseed oil is successfully integrated into PMMA-L.

The results in Table 2 and Figure 2 confirm the presence of common molecular compounds between linseed oil and PMMA. The overlapping transmission peaks observed in specific frequency ranges indicate the existence of similar molecular compounds shared by both substances, as shown in Figure 3. Furthermore, the C=C stretching vibration of unsaturated fatty acids confirms the successful physical incorporation of linseed oil into PMMA, creating PMMA-L samples.

This finding aligns with Cunnane et al. 1993 findings, which establish linseed as the richest plant source of α-linolenic acid (ALA), an omega-3 fatty acid. In addition, linseed oil exhibits a low proportion of saturated fatty acids (9%), moderate levels of monounsaturated fatty acids (18%), and a significant concentration of polyunsaturated fatty acids (73%) (26). The predominant fatty acid in linseed oil is α-linolenic, ranging from 39.00% to 60.42%, with other notable lipids including oleic, linoleic, palmitic, and stearic acids.

Table 2: Wavenumber and the functional group of Linseed oil, PMMA, and PMMA-L

Linseed Oil		PMMA	
Wavenumber (cm^{-1})	**Functional Group**	**Wavenumber (cm^{-1})**	**Functional Group**
3006 - 2856	C-H stretching vibrations in the aliphatic chains	**2984-2856**	C-H stretching vibrations in the methyl and methylene groups
1742	C=O stretch of triglycerides	**1720**	C=O stretch of carbonyl groups in the ester units
1652	C=C stretching vibrations of unsaturated fatty acids	**1446**	Bending vibrations of $-CH_3$ and $-CH_2$ groups.
1454	Bending vibrations of $-CH_2$ groups	**1380**	Symmetric bending vibrations of the methyl groups
1098	C-O stretching vibrations of secondary alcohol	**1244**	C-O stretching vibrations of ester groups
712	Rocking vibrations of $=CH_2$ groups	**1136**	C-O-C stretching vibrations in the ester units

PMMA-L	
Wavenumber (cm^{-1})	**Functional Group**
2990 - 2848	C-H stretching vibrations in the methyl and methylene groups
1720	C=O stretch of carbonyl groups in the ester units
1584 - 1536	C=C stretching vibrations of unsaturated fatty acids.
1442	bending vibrations of $-CH_3$ and $-CH_2$ groups.
1144	C-O-C stretching vibrations in the ester units

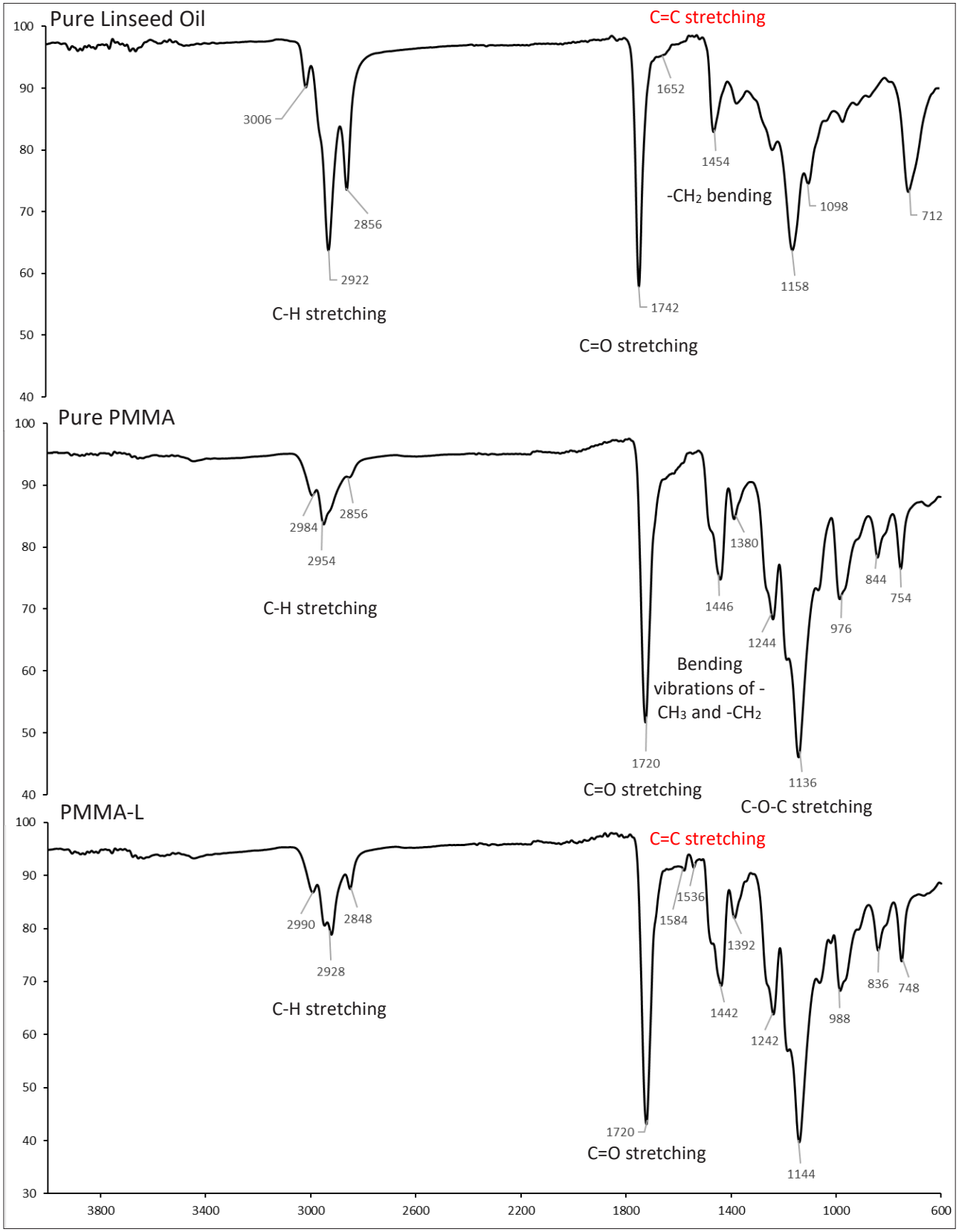

Figure 2: The spectrum of pure linseed oil, pure PMMA and PMMA-L

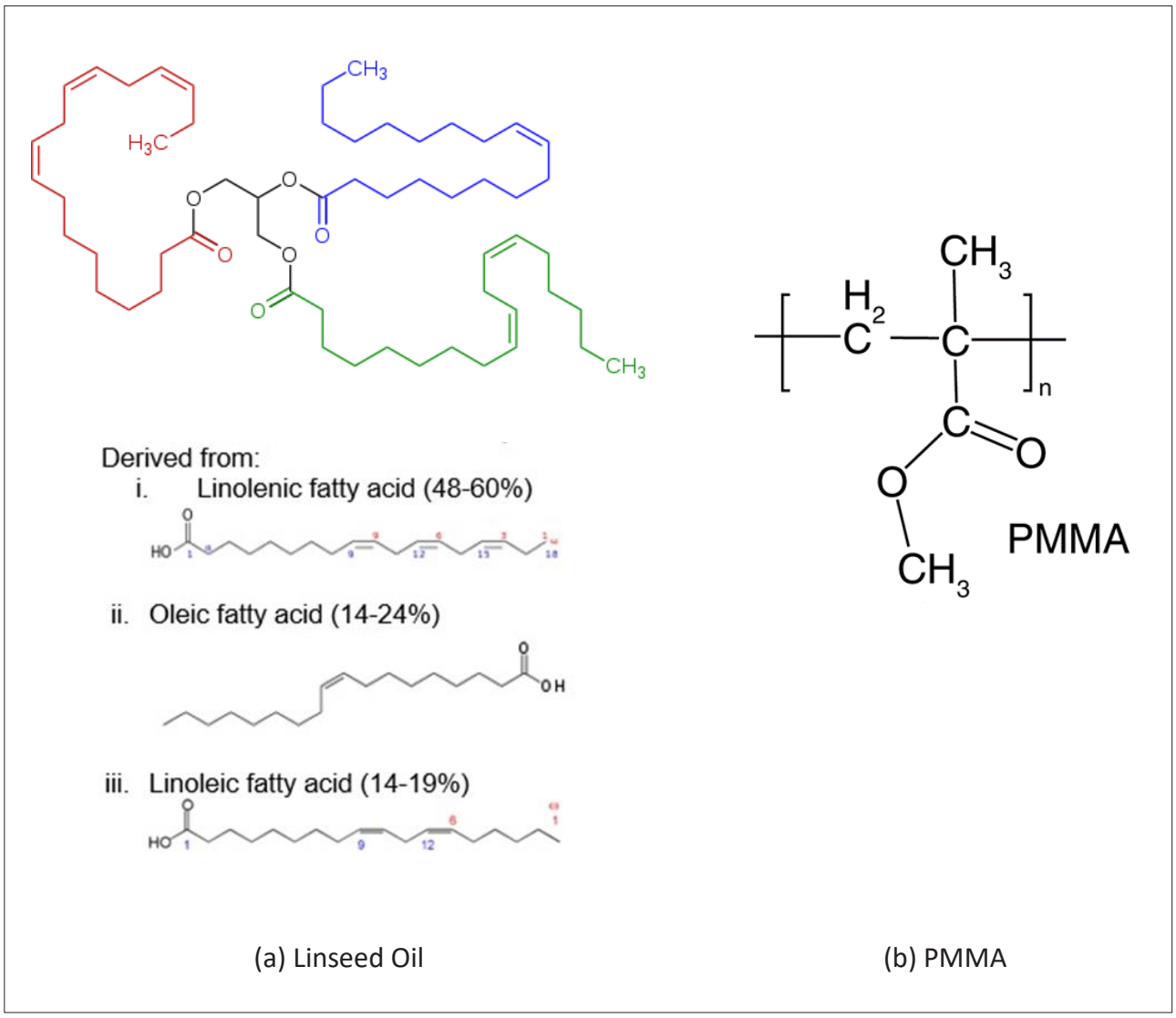

Figure 3: Chemical composition of linseed oil and pure PMMA

In vitro leaching analysis of PMMA-L

Figure 4 shows the percentage of the transmittance spectrum of the mixture of 1% linseed oil in distilled water. The visible central strong, broad peak would be from the O-H stretching vibration at the 3260-3270 cm^{-1} wavelength. The sharp and strong transmission peak at 1706 – 1818 cm^{-1} is characteristic of the C=O stretch of triglycerides and the C-H stretching vibrations in the aliphatic chains at 3006 – 2856 cm^{-1}, which are only present in the spectrum of the mixture of linseed oil in distilled water but absent in the leaching analysis of the other groups. Only broad O-H stretching (3273 cm^{-1}) and H-O-H bending (1634 cm^{-1}) of distilled water (27) at the same percentage of transmittance present in the ATR-FTIR spectrum, indicating no presence of linseed oil leaching into the distilled water after the immersion of all PMMA-L samples.

The spectrum in Figure 4 shows that linseed oil leaching from PMMA-L samples is absent. The only bending and stretching presence from O-H and H-O-H indicates no leaching out of linseed oil into the distilled water at room temperature. This study found no evidence of linseed oil leaching from the PMMA-L samples, indicating that the linseed oil was effectively integrated into the PMMA. In contrast to earlier research (7), which demonstrated the leaching of synthetic plasticisers like dibutyl phthalate from heat-cured acrylic resin after incorporation, the findings of this study indicate a positive outcome. The findings suggest that linseed oil is successfully integrated into PMMA-L, presenting it as a potentially safe and practical substitute for conventional PMMA materials.

However, this study's limitations are that it was performed in vitro and may not accurately reflect the behaviour of PMMA-L in vivo. Additionally, it is essential to note that while this study found no evidence of leaching, further studies are suggested to confirm the long-term stability of PMMA-L. On the other hand, this study has important implications for developing safe and effective denture base materials.

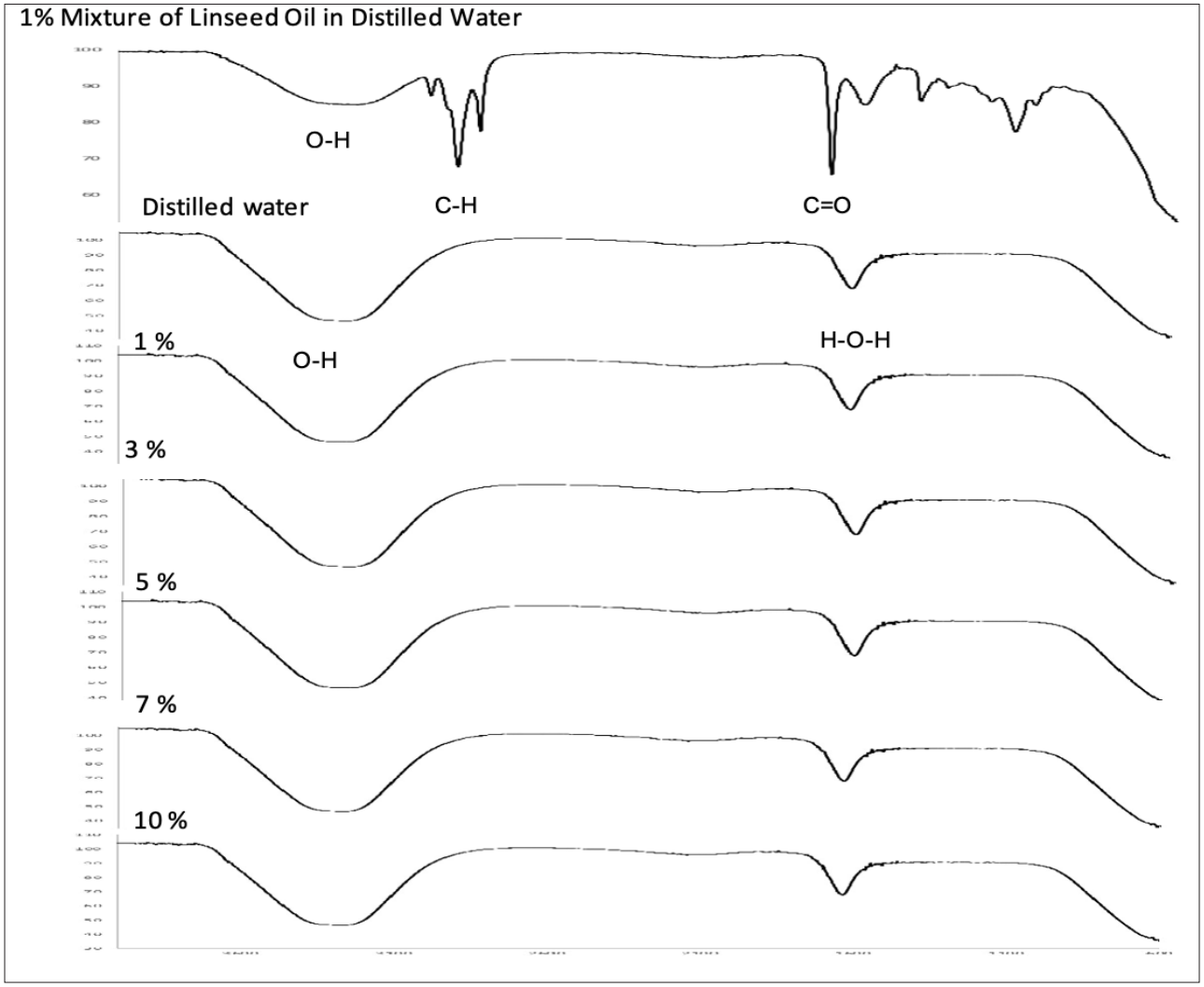

Figure 4: The spectrum of 1% mixture of linseed oil in distilled water, distilled water and leaching analysis of 1, 3, 5, 7 and 10% PMMA-L in distilled water

Conclusion

In conclusion, incorporating linseed oil as a bio-based plasticiser in the fabrication of PMMA-L presents a promising alternative to conventional PMMA for denture base material. This study reveals that linseed oil exhibits non-cytotoxicity against HGF cells, affirming its safety for integration into PMMA as a denture base material. Moreover, linseed oil demonstrates successful physical integration into PMMA-L, as evidenced by the presence of C=C stretching vibration observed in both the pure linseed oil group and the PMMA-L group through characterisation via ATR-FTIR analysis. Additionally, linseed oil shows no evidence of leaching after integration into PMMA-L, as indicated by the presence of only O-H and H-O-H molecular compounds in the leaching analysis. The successful fabrication of PMMA-L samples with varying linseed oil concentrations highlights the potential of linseed oil as a viable plasticiser in PMMA, suggesting its capability to replace synthetic alternatives in denture-based material production.

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Competing interests

The authors declare that they have no competing interests.

References

1. Zafar, MS. Prosthodontic applications of polymethyl methacrylate (PMMA): an update. Polymers (Basel). 2020; 12(10):2299.
2. Grumezescu V, Grumezescu A. Materials for Biomedical Engineering: Thermoset and Thermoplastic Polymers. 1st Ed. Amsterdam: Elsevier. 2019.
3. Gad MM, Abualsaud R, Rahoma A, Al-Thobity AM, Al-Abidi KS, Akhtar S. Effect of zirconium oxide nanoparticles addition on the optical and tensile

properties of polymethyl methacrylate denture base material. Int J Nanomedicine. 2018; 13:283-92.
4. Saeed F, Muhammad N, Khan AS. Prosthodontics dental materials: from conventional to unconventional. Mater Sci Eng C Mater Biol Appl. 2020; 106:110167.
5. Zidan S, Silikas N, Haider J, Alhotan A, Jahantigh J, Yates J. Evaluation of equivalent flexural strength for complete removable dentures made of zirconia-impregnated PMMA nanocomposites. Materials (Basel). 2020; 13(11):1-14.
6. Kalachandra S, Turner DT. Water sorption of plasticised denture acrylic lining materials. Dent Mater. 1989; 5:161-4.
7. Lygre H, Solheim E, Gjerdet NR. Leaching from denture base materials in vitro. Acta Odontol Scand. 1995; 53(2):75-80.
8. Lygre H, Solheim E, Gjerdet NR, Berg E. Leaching of organic additives from dentures in vivo. Acta Odontol Scand. 1993; 51(1):45-51.
9. Haryono A, Triwulandari E, Jiang P. Interaction between vegetable oil-based plasticiser molecules and polyvinyl chloride, and their plasticisation effect. AIP Conf Proc. 2017; 1803:1-10.
10. Lithner D, Larsson Å, Dave G. Environmental and health hazard ranking and assessment of plastic polymers based on chemical composition. Sci Total Environ. 2011; 409(18):3309-24.
11. Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. Flax and flaxseed oil: an ancient medicine & modern functional food. J Food Sci Technol. 2014; 51(9):1633-53.
12. Adamczak M, Krok M, E. Pamuła. Linseed oil based nanocapsules as delivery system for hydrophobic quantum dots. Colloids Surf B Biointerfaces. 2013; 110:1-7.
13. Lewinska A, Zebrowski J, Duda M, Gorka A, Wnuk M. Fatty acid profile and biological activities of linseed and rapeseed oils. Molecules. 2015; 20(12):22872-80.
14. Ahmad S, Ashraf SM, Abul Hasnat, Noor A. Studies on epoxidised oil and its blend with polystyrene and poly(methyl methacrylate). Indian J Chem Technol. 2001; 8(3):176-80.
15. Navarchian AH, Najafipoor N, Ahangaran F. Surface-modified poly(methyl methacrylate) microcapsules containing linseed oil for application in self-healing epoxy-based coatings. Prog Org Coat. 2019; 132:288-97.
16. Francoeur AM, Assalian A. MICROCAT: a novel cell proliferation and cytotoxicity assay based on WST-1. Biochemica. 1996; 3(1):6.
17. KonjhodzicPrcic A, Gorduysus O, Kucukkaya S, Atila B, Muftuoglu S, Zeybek D. In vitro comparison of cytotoxicity of four root canal sealers on human gingival fibroblasts. Med Arh. 2015; 69(1):24.
18. Guillén MD, Ruiz A, Cabo N, Chirinos R, Pascual G. Characterization of sacha inchi (Plukenetia volubilis L.) oil by FTIR spectroscopy and 1 HNMR. Comparison with linseed oil. J Am Oil Chem Soc. 2003; 80(8):755-62.
19. Sarı C. A comparative study of MTT and WST-1 assays in cytotoxicity analysis. Haydarpasa Numune Med J. 2021; 61(3):281-8.
20. Sjögren G, Sletten G, Dahl JE. Cytotoxicity of dental alloys, metals, and ceramics assessed by Millipore filter, agar overlay, and MTT tests. J Prosthet Dent. 2000; 84(2):229-36.
21. Santos NC, Figueira-Coelho J, Martins-Silva J, Saldanha C. Multidisciplinary utilisation of dimethyl sulfoxide:pharmacological, cellular, and molecular aspects. Biochem Pharmacol. 2003; 65(7):1035-41.
22. Timm M, Saaby L, Moesby L, Hansen EW. Considerations regarding use of solvents in in vitro cell based assays. Cytotechnology. 2013; 65:887-94.
23. Lee H, Park JB. Evaluation of the effects of dimethylsulphoxide on morphology, cellular viability, mRNA, and protein expression of stem cells culture in growth media. Biomed Rep. 2017; 7:291-6.
24. Rabail R, Shabbir MA, Sahar A, Miecznikowski A, Kieliszek M, Aadil RM. An intricate review on nutritional and analytical profiling of coconut, flaxseed, olive, and sunflower oil blends. Molecules. 2021; 26(23):1-24.
25. Nandiyanto ABD, Oktiani R, Ragadhita R. How to read and interpret FTIR spectroscope of organic material. Indones. j. sci. technol. 2019; 4(1):97-118.
26. Cunnane SC, Ganguli S, Menard C. High α-linolenic acid flaxseed (Linum usitatissimum):some nutritional properties in humans. Br J Nutr. 1993; 69(2):443-53.
27. Ping ZH, Nguyen QT, Chen SM, Zhou JQ, Ding YD. States of water in different hydrophilic polymers - DSC and FTIR studies. Polymers (Basel). 2001; 42(20):8461-7.