

Identification of functional groups present in bigel and estimating their concentration by FTIR spectroscopy; experimental study

Identificación de grupos funcionales presentes en bigel y estimación de su concentración por espectroscopia FTIR; estudio experimental

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ABSTRACT

Gel formulations of oleogel, hydrogel, and oleo-hydrogel (bigel) were evaluated as transdermal drug delivery systems, but recently various studies have proved that gel-based delivery systems are also able to improve the stability and bioavailability of many bioactive food ingredients. It has been proven that higher concentration of oleogel in bigel, precisely 7:3 OG:HG ratio, enhanced the viability of probiotic bacteria. And this study aims to identify all the organic compounds present in that bigel, determine their respective functional group and estimate their concentration. This work was an experimental research design conducted in Food Technology Laboratory at B.B. Ambedkar University with technical assistance provided by CytoGene Research and Development in Lucknow, India. The experimental work was conducted from January to April 2021. Spectroscopic technique was the primary analysing tool used which is based on the absorption or emission of IR radiation. Fourier Transform FT is an instrument that uses the mid-region of IR spectrum for qualitative and quantitative analysis of food. FTIR technique in food industry was basically established for quality control by evaluating industrially manufactured material, and can often serve as the first step in material analysis process (proximate analysis). The IR spectra of the sample in which %T was a function of wavenumber cm^{-1} had three main regions, from which the most important region ($1500\text{-}3000\text{ cm}^{-1}$) had three peaks, by using the frequency range reference table, it was found that Alkyl group (methyl) and Carbonyl group (ester) were the functional groups of the sample. Absorbance was calculated using Beer Lambert Law. Lastly, the concentration for which a standard curve of Fatty Acid Methyl Ester FAME was used 0.36 g mL^{-1} was the conc. of methyl and 0.14 g mL^{-1} was the conc. of ester. This study concluded that, FTIR had shown to provide higher accuracy and a significant reduction in total analysis time, and the ability to perform a variety of common fat and oil analyses such as iodine value, saponification number and peroxide value by FTIR is a realistic possibility.

Keywords: Bigel, FTIR, Functional groups, Transmittance, Absorbance.

RESUMEN

Las formulaciones en gel de oleogel, hidrogel y oleohidrogel (bigel) se evaluaron como sistemas de administración transdérmica de fármacos, pero recientemente diversos estudios han demostrado que los sistemas de administración basados en gel también son capaces de mejorar la estabilidad y biodisponibilidad de muchos ingredientes alimentarios bioactivos. Se ha demostrado que una mayor concentración de oleogel en bigel, precisamente la proporción 7:3 OG: HG, mejoraba la viabilidad de las bacterias probióticas. Este estudio pretende identificar todos los compuestos orgánicos presentes en ese bigel, determinar su respectivo grupo funcional y estimar su concentración. Este trabajo fue un diseño de investigación experimental llevado a cabo en el Laboratorio de Tecnología de Alimentos de la Universidad Babasaheb Bhimrao Ambedkar con la asistencia técnica proporcionada por *CytoGene Research and Development* en Lucknow, India. El trabajo experimental se llevó a cabo de enero a abril de 2021. La técnica espectroscópica fue la principal herramienta de análisis utilizada, que se basa en la absorción o emisión de radiación infrarroja (IR). La transformada de Fourier (FT) es un instrumento que utiliza la región media del espectro IR para el análisis cualitativo y cuantitativo de los alimentos. En la industria alimentaria, la técnica de Infrarrojo por Transformada de Fourier (FTIR) se estableció básicamente para el control de calidad mediante la evaluación del material fabricado industrialmente y, a menudo, puede servir como primer paso en el proceso de análisis del material (análisis proximal). El espectro IR de la muestra en el que %T era una función del número de onda cm^{-1} tenía tres regiones principales, de las cuales la región más importante ($1500\text{-}3000\text{ cm}^{-1}$) tenía tres puntos máximos; al utilizar la tabla de referencia de rango de frecuencia, se encontró que el grupo alquilo (metilo) y el grupo carbonilo (éster) eran los grupos funcionales de la muestra. La absorbancia se calculó mediante la ley de Beer Lambert. Por último, la concentración para la que se utilizó una curva estándar de éster metílico de ácido graso FAME $0,36\text{ g mL}^{-1}$ fue la concentración de metilo y $0,14\text{ g mL}^{-1}$ fue la concentración de éster. Este estudio concluyó que, FTIR había demostrado que proporciona una mayor precisión y una reducción significativa en el tiempo total de análisis, además de demostrar que es la posibilidad realista de la capacidad de realizar una variedad de análisis comunes de grasas y aceites, como el valor de yodo, el número de saponificación y el valor de peróxido por FTIR.

Palabras clave: Bigel, FTIR, Grupos funcionales, Transmitancia, Absorbancia.

INTRODUCTION

A hydrogel is a network of polymer chains that are hydrophilic, sometimes found as a colloidal gel in water as a medium, it is highly absorbent, possess a degree of flexibility and can encapsulate chemical systems. The first appearance of the term 'hydrogel' was in 1894 (Bemmelen. 1907). While an organogel/oleogel is a non-crystalline, non-glassy thermoreversible (thermoplastic) solid material composed of a liquid organic solvent, mineral oil, or vegetable oil. The solubility and particle dimensions of the structure are important characteristics for the elastic properties and firmness of the oleogel. Oleogels have potential for use in a number of applications, such as in pharmaceuticals (Kumar and Katare. 2005), cosmetics, art conservation (Carretti and et al. 2005) and food (Pernetti. 2007). The first semisolid formulation of bigel obtained by combining stable oleogel and hydrogel

was in 2008 by [I.F. Almedida](#). Formulations of bigel can be introduced in food to enhance the delivery of bioactive components and improve their survival ([Zhuang, 2020](#)). Combining these two structured gel systems at high shear produces a biphasic system that has several advantages compared to the pure oleogel or hydrogel alone. It has greater stability because both the continuous and the dispersed phases of the gel systems are semi-solid and therefore immobilized. Upon long-time storage, the capacity of internal phase leaching is decreased and hence, the separation process of phases is delayed. There is more control over bigel properties, not only can the concentration of the gelator be changed but also the proportion of gel phases can be manipulated to tailor the gel for a specific application and enhance its physical and mechanical properties. Each component brings unique properties to the bigel system and should be taken into account when designing the final product. The most important feature of this bigel is its ability to hold both lipophilic and hydrophilic substances since there is both the lipid and water phase present in the bigel. This gives the advantage of being compatible with a greater variety of bioactive compounds.

Infrared (IR) spectroscopy refers to measurement of the absorption of different frequencies of IR radiation by foods or other solids, liquids, or gases. Infrared radiation is electromagnetic energy with wavelengths (λ) longer than visible light but shorter than microwaves. The IR spectrum is divided into three wavenumber regions: far-IR spectrum ($<400\text{ cm}^{-1}$), mid-IR spectrum ($400\text{--}4000\text{ cm}^{-1}$) and near-IR spectrum ($4000\text{--}13000\text{ cm}^{-1}$). The mid-IR regions of the spectrum are most useful for quantitative and qualitative analysis of foods. Two types of spectrometers are used for mid-IR spectroscopy: dispersive instruments and Fourier transform (FT) instruments.

Food scientists and technologists determine the chemical composition and physical characteristics of foods routinely as part of their quality management, product development, or research activities. Proximate analysis of foods refers to determining the major components of moisture, ash (total minerals), lipids, protein, and carbohydrates.

Lipid, fat, butter, margarine, oil and all water-in-oil emulsion processing industry have a set of parameters that includes, iodine value IV, determines the degree of unsaturation of the fatty substance, saponification number SN, represents the number of milligrams of potassium hydroxide (KOH) required to saponify one gram of fat and peroxide value PV, detects oil degradation. Analytical processing of these parameters is time taking and effort consuming.

Proximate analysis of foods is one area which can benefit from FTIR, as food systems are mainly composed of fats, proteins, carbohydrates and moisture, all of which contribute to the gross spectrum obtained. Characteristic absorption bands are associated with these components, e.g., the carbonyl ester and CH signals associated with fat, the amide signals for protein, the CHO bands for carbohydrate and the HOH bending absorption of water ([Bellamy L.J. 1958](#)). In principle, simple standardized, quantitative preparation procedures can be developed to dissolve and disperse most food components in water, or another solvent if selective extraction is required, so that samples are suitable for FTIR analysis. Once in solution, the sample can be applied to the ATR crystal, the spectrum recorded and the solvent subtracted out, yielding a high-quality sample

spectrum from which quantitative data may be obtained (Fuller M. P. et al. 1988). Functional groups and their respective quantified frequencies data were adopted from reference (Coates, 2000).

MATERIALS AND METHODS

Sample preparation: The bigel was prepared from edible components containing an organogel (oleogel) and a hydrogel, the basic constituents of oleogel is a 10% oleogelator for which beeswax was used (10gm) and a 90% continuous phase which was made from soybean oil (90gm). Beeswax was mixed with soybean oil at temperature between 90-100°C. At the same time hydrogel was prepared with 10% hydrogelator for which gelatin was used (10gm) and a 90% continuous phase of water (90gm), gelatin and water was mixed at 40°C. At the time of homogenization, the temperature of gelatin was raised to 60°C and both gels were combined and homogenized at 24000 rpm for approximately two minutes. The hot mixture was immediately placed in the refrigerator to undergo dilation for 24 hours (Saffold and Acevedo. 2020). Different ratios were prepared of bigel, but only the best sample of bigel was selected i.e 7:3 OG:HG ratio which had shown to improve bacterial growth of *Clostridium butyricum* and *Lactic acid bacillus* LAB in probiotic supplement.

Interpreting IR spectra: IR is not generally used to determine the whole structure of an unknown molecule; it is a useful and quick tool for identifying the bonds present in a given molecule. And since the selected sample has a high concentration of lipophilic ingredients (oleogel), analysing the type of bonds present can help in determining the degree of unsaturation (iodine value). The peaks represent areas of the spectrum where specific bond vibrations occur (Ashenhurst J. 2020). The mid-IR region of the spectrum is the most useful for quantitative and qualitative analysis of foods, and it is divided into four regions:

- the single bond region (2500-4000 cm⁻¹)
- the triple bond region (2000-2500 cm⁻¹)
- the double bond region (1500-2000 cm⁻¹)
- the fingerprint region (600-1500 cm⁻¹) (Nandiyanto, 2019)

The IR active functional group is between 1500-3000 cm⁻¹, this range is the most important as it will determine the existence of double or triple bonds (Jackson. 2015)

Beer Lambert Law: The Beer Lambert law, which is also referred to as Beer's Law, describes the relationship among absorbance (A), the molar solute concentration in M (c) and the length of the path the light takes to get to the sample in centimetres (l). Absorbance is directly proportional to concentration and length:

$$A = \epsilon cl$$

ϵ is the wavelength-dependent molar absorptivity coefficient and it is constant for a particular substance. ϵ has units of L mol⁻¹ cm⁻¹. The Beer's law provides a linear relationship between concentration and absorbance that can be plotted to produce an easy-to-use graph.

Transmittance and Absorbance: Absorbance and transmittance are measurements used in spectrophotometry. Spectrophotometry measures how much radiant energy a substance absorbs at varying

wavelengths of light. The technique is useful for determining the identity of an unknown substance and, with the use of a set of standards, determining a substance's concentration in a sample.

Calculation of Absorbance from Transmittance:

Absorbance can be calculated from percent transmittance (%T) using this formula:

$$\text{Absorbance} = 2 - \log(\%T)$$

Transmittance (T) is the fraction of incident light which is transmitted. In other words, it's the amount of light that "successfully" passes through the substance and comes out the other side. It is defined as $T = I/I_0$, where I = transmitted light ("output") and I_0 = incident light ("input"). %T is merely $(I/I_0) \times 100$. For example, if $T = 0.25$, then %T = 25%. A %T of 25% would indicate that 25% of the light passed through the sample and emerged on the other side.

Absorbance (A) is the flip-side of transmittance and states how much of the light the sample absorbed. It is also referred to as "optical density." Absorbance is calculated as a logarithmic function of T:

$$A = \log_{10} (1/T) = \log_{10} (I_0/I)$$

Calculation of concentration from a standard curve:

Standard curves represent the relationship between two quantities. They are used to determine the value of an unknown quantity from one that is more easily measured (a standard). Due to pandemic, this standard curve was adopted from a previous research work ([Filipe L. 2018](#)).

RESULT AND DISCUSSION

The FTIR analysis of 7:3 OG:HG bigel was conducted to estimate the concentration of organic compound present the sample.

Figure 1, is a representation of the IR spectra where percent transmittance %T is the function of wavenumber cm^{-1} , each peak has a specific %T at specific cm^{-1} , this data is found more accurately in Table 1.

In Figure 2, the mid-region of IR spectra is divided into four regions: single bond, triple bond, double bond and finally finger-print region which traces substitution patterns of aromatic compounds that is not useful for a newly developed sample. The triple bond region was also neglected as there was no peak shown at that region, meaning the gel is free from triple bonds. The double bond region has a very sharp peak at 1742.64 cm^{-1} indicating the presence of carbonyl group, more precisely, ester (from the reference table of IR spectrum by frequency range). Lastly the single bond region showed two peaks at, 2919.00 cm^{-1} and 2851.24 cm^{-1} , both of these peaks are a C-H stretch of alkyl group, more precisely methyl.

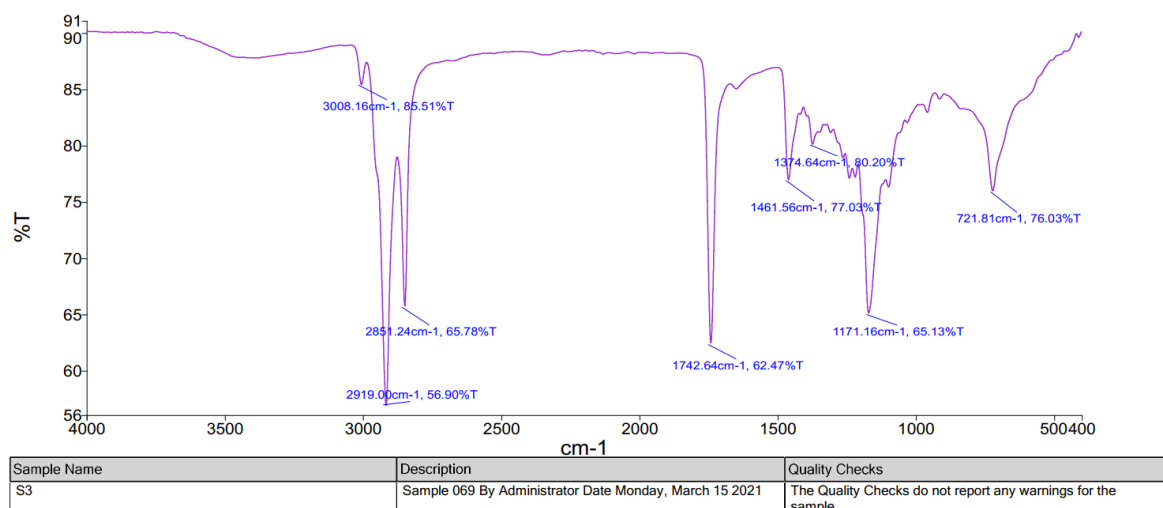


Figure 1: IR spectra where %T as a function of wavenumber cm-1

Table 1: cm-1 and %T of each single peak of IR spectra

Wavenumber cm-1	%T
3008.16	85.51
2919.00	56.90
2851.24	65.78
1742.64	62.47
1461.56	77.03
1374.64	80.20
1171.16	65.13
721.81	76.03

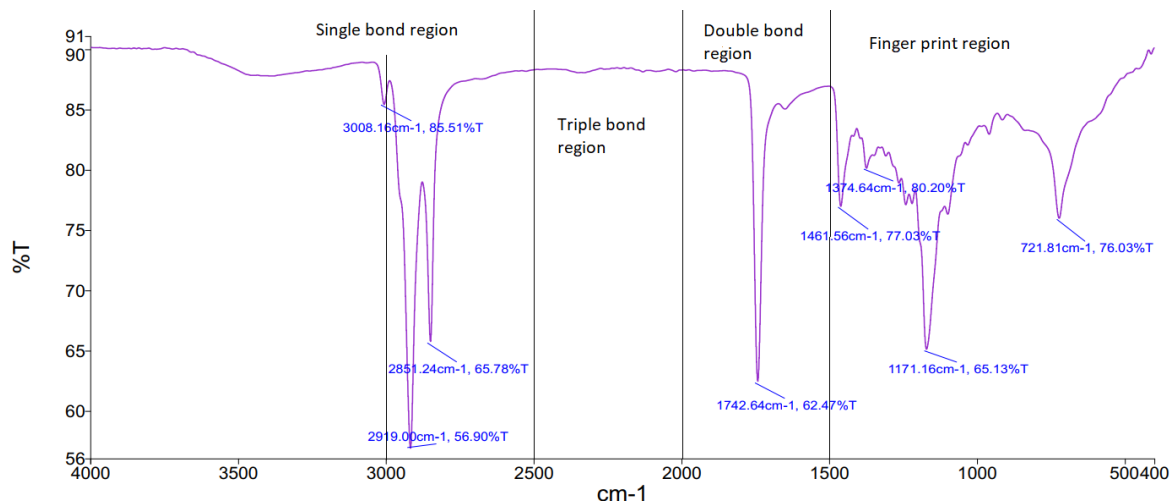


Figure 2: IR spectra divided to regions according to type of chemical bonds

Table 2: Important peaks within 1500-3000 cm-1 and their identified functional group (Ashenhurst J. 2020)

Wavenumber cm-1	Type of chemical bond	Functional group
2919.00	Single bond of C-H stretch	Alkane/alkyl group (methyl)
2851.24	Single bond of C-H stretch	Alkane/alkyl group (methyl)
1742.64	Double bond of C=O stretch	Carbonyl group (ester)

Since absorbance A is calculated as a logarithmic function of transmittance T, A of these methyl and ester are easily calculated using the formula: $A = 2 - \log(\%T)$. See Table 3.

In Figure 3, absorbance was plotted on a graph as a function of wavenumber to illustrate hoe transmittance curve can be converter to absorbance using Beer Lambert Law. For determining the concentration, a standard curve of Fatty Acid Methyl Ester FAME was adopted. This curve was a result of spectrophotometry technique used for analysis. See Figure 4. In Figure 5, the A values calculated from %T were plotted on the standard curve to determine the concentration. Each group was plotted in a different color to distinguish their concentration. Table 4 shows the final result i.e., the concentration of methyl and ester, 0.36 and 0.14 gmL-1. This concentration was found in gmL-1 unit and can be converted to other units.

Table 3: Absorbance of methyl and ester are calculated using the formula: $A = 2 - \log(\%T)$

Wavenumber cm-1	Functional group	%T	A
2919.00	Methyl	56.90	0.245
2851.24	Methyl	65.78	0.182
1742.64	Ester	62.47	0.203

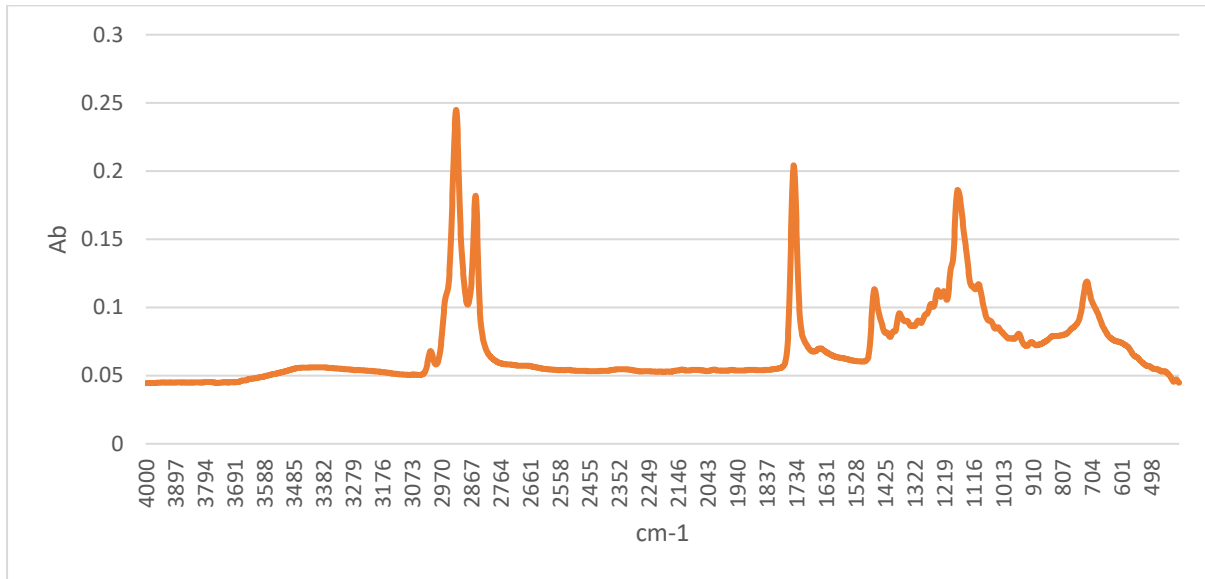


Figure 3: IR spectra where A as a function of wavenumber cm-1

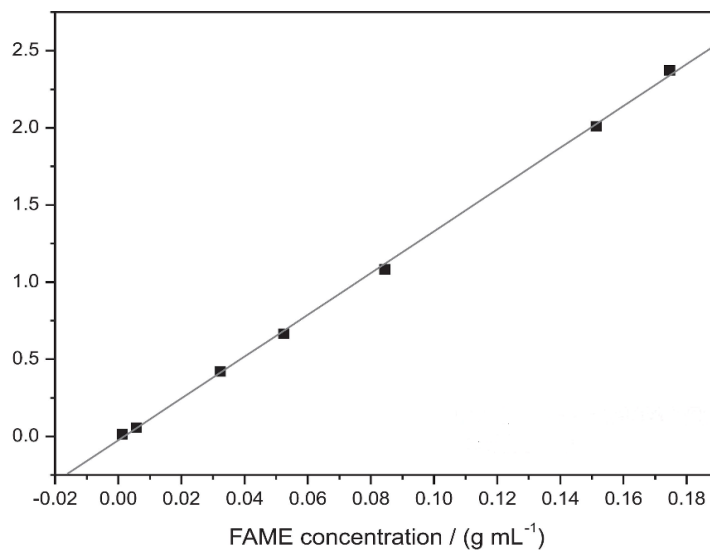


Figure 4: Fatty Acid Methyl Ester Standard A/conc. curve (Filipe L. et al. 2018)

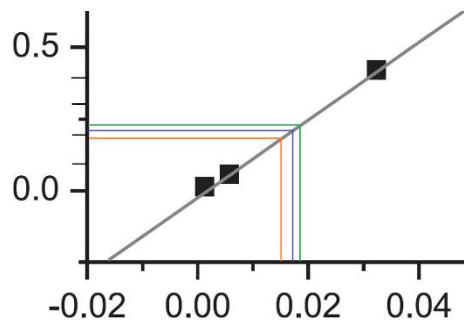


Figure 5: Determination of the alkyle-ester concentration in the sample using the std. curve

Table 4: Concentration values determined from the standard curve of alkyl ester in (gmL-1)

Functional group	A	Conc. (gmL-1)	Final conc. (gmL-1)
Methyl	0.245	0.19	0.36
Methyl	0.182	0.17	
Ester)	0.203	0.14	0.14

CONCLUSION

The FTIR method has been shown to provide higher accuracy and a significant reduction in total analysis time. Iodine value (IV) which determines the degree of unsaturation contained in fatty acids, the higher the iodine value, the more unsaturated fatty acid bonds are present in a fat. This can easily be analysed from FTIR since unsaturation is directly linked to the type of bond. Saponification number (SN) is a measure of the average molecular weight (or chain length) of all the fatty acids present in the sample as triglycerides. IV and SN are important parameters in the fats and oils processing industry, while the traditional methods for their determination are tedious. The IV and SN results obtained by FTIR can be helpful in reducing the analytical time and effort. Other application of FTIR spectroscopy is in the evaluation of oil degradation, as measured by the peroxide value. FTIR method can also be developed to measure fat and moisture. Because of the simplicity of preparing the calibrating standards, this method is not limited to certain type of lipid, infact, butter, margarine, and a variety of oil in water (e.g., mayonnaise, salad dressings) or water in oil emulsions (bigels) are all potential candidates for this approach. The ability to perform a variety of common fat and oil analyses simultaneously by FTIR is a realistic possibility.

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