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Prinsip, Metode, dan Aplikasi

Fourier-transformed Infrared Spectroscopy (Spektroskopi FTIR)

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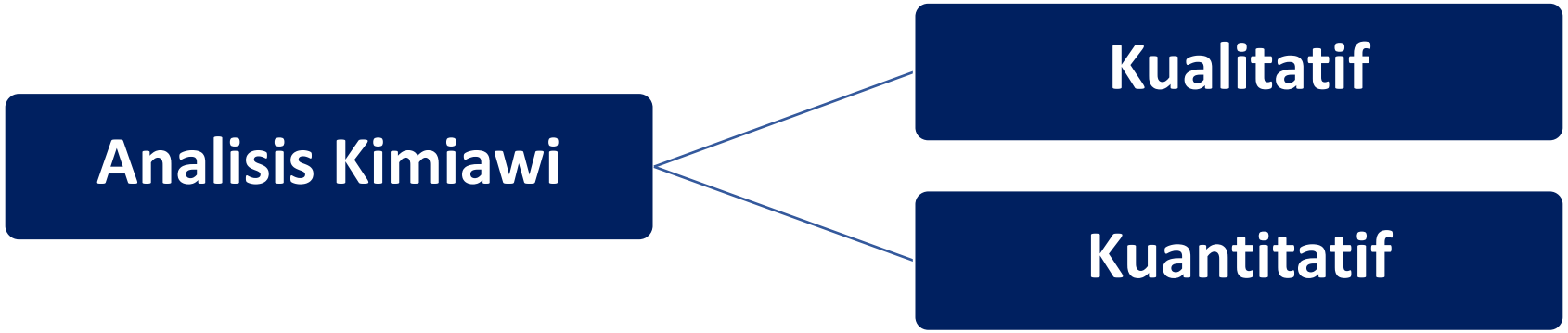
Prinsip Dasar dan Metode Analisis dengan Spektroskopi FTIR

Laboratorium Biomedika Kedokteran Gigi, Fakultas Kedokteran Gigi, UGM

Analisis Fisis dan Kimiawi:



1

Ada beragam cara untuk melakukan analisis kimiawi yang diperlukan untuk suatu studi, baik dalam bidang medis, agro, pengembangan berbagai jenis teknologi, industri, hingga arkeologi.

2

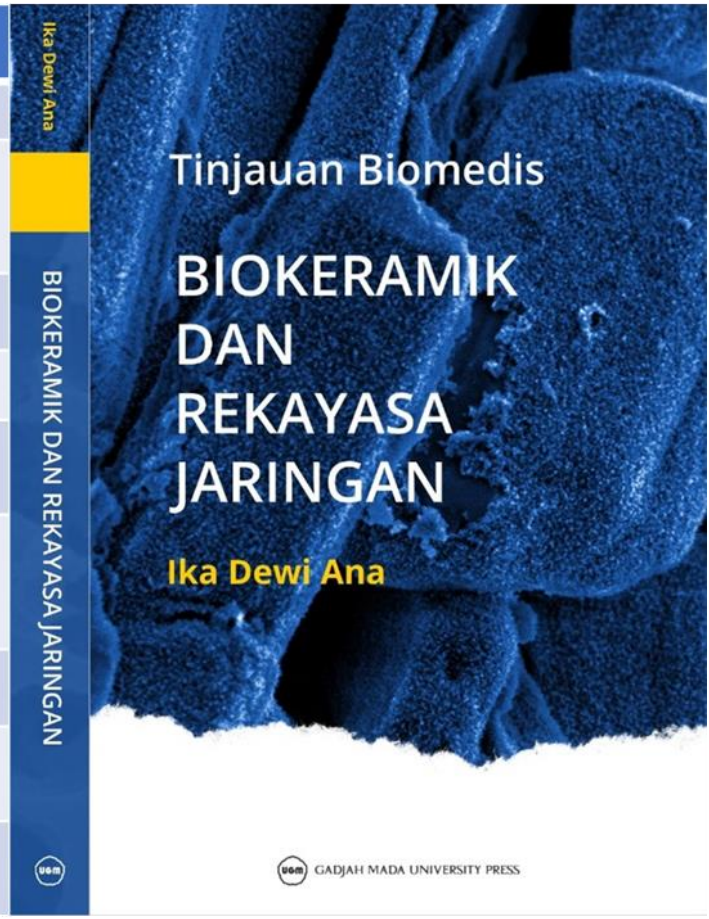
Dapat dilakukan menggunakan elektron, ion, atau fotoelektron dari suatu permukaan material.

3

Dapat juga dilakukan menggunakan cuplikan atau spesimen zat atau material yang akan diperiksa.

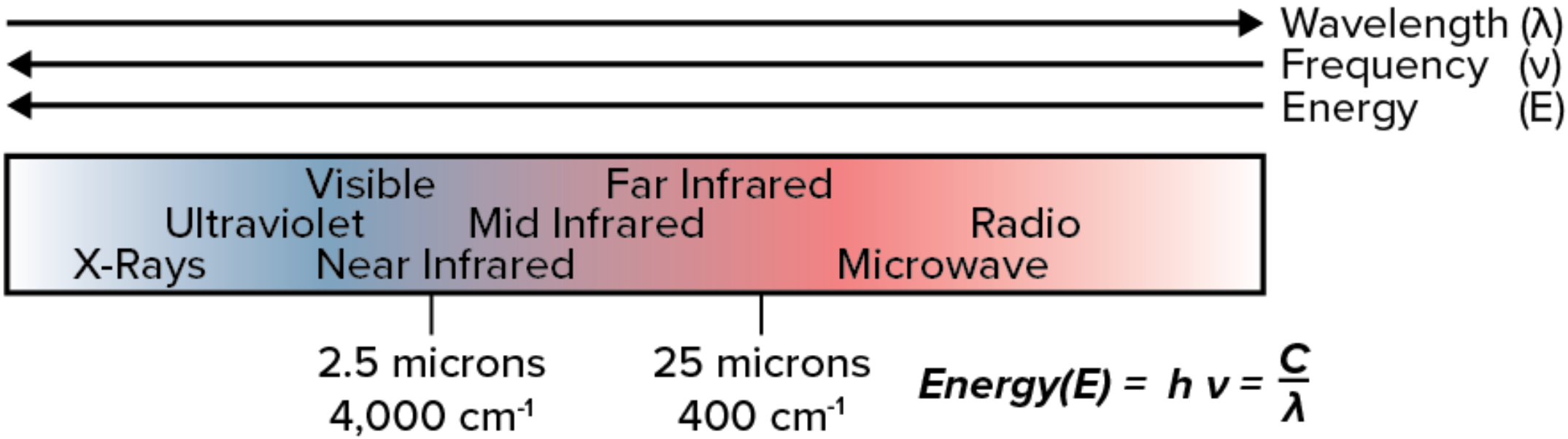
Beragam Instrumen untuk Analisis Fisis dan Kimiawi:

Nama Instrumen	
XRD (X-Ray Diffractometer)	Identifikasi pola difraksi suatu zat/ material.
XPS (X-Ray Photoelectron) Spectroscopy	Identifikasi unsur-unsur pasuatu permukaan suatu material, bilangan oksidasi, bilangan ikatan kimiawi, termasuk struktur elektronis dan unsur-unsur yang berikatan dengan elemen-elemen pada permukaan material.
XRF (X-Ray Fluorescent)	Identifikasi komposisi unsur suatu material.
EDX (Energy Dispersive X-Ray)	Analisis kimiawi secarasemi kuantitatif.
UV-Vis Spectroscopy	Deteksi suatu unsur berdasar absorbansi dan pantulan sehingga dapat diketahui komposisi unsur dalam suatu material secara kuantitatif.
AAS (Atomic Absorption Spectrophotometer)	Deteksi elemen (baik cair maupun padat) berdasar penyerapan radiasi optic atau cahaya oleh atom bebas dalam fase gas secara kuantitatif.
CHNS Analyzer	Analisis komposisi karbon, hydrogen, nitrogen, dan sulfur pada suatu material.
FTIR Spectroscopy	Identifikasi dan pengukuran unit struktural dalam suatu senyawa (Identifikasi gugus fungsional).
Raman Spectroscopy	Informasi kimia dan struktural, serta identifikasi zat melalui karakteristik Raman (cahaya yang tersebar digunakan untuk mengukur mode energi getaran suatu zat).

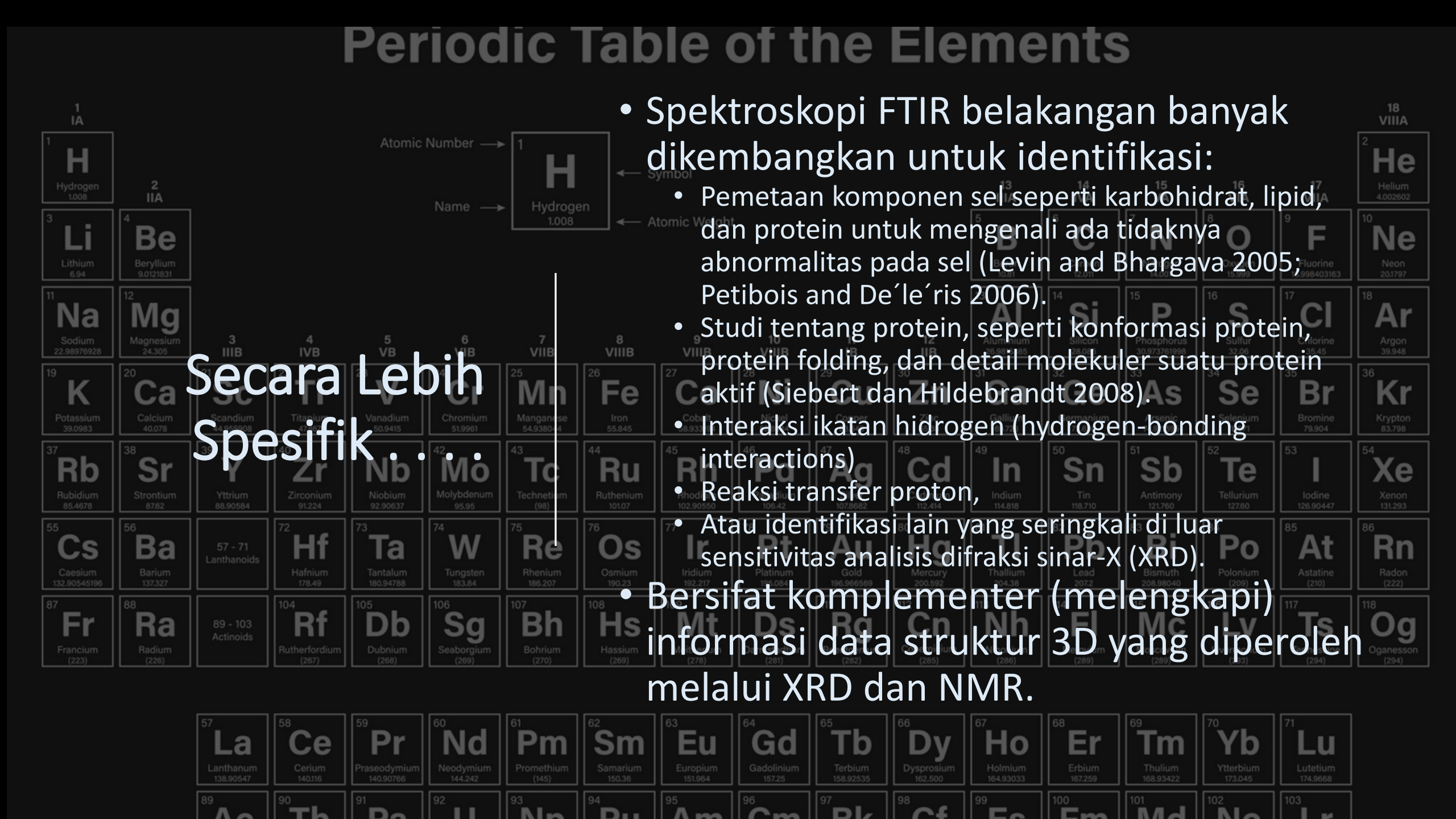


Spektroskopi FTIR (Fourie-transformed Infrared)

- Identifikasi gugus-gugus fungsional dalam suatu sampel, misal gugus hidroksil, fosfat, karbonat, amina, amida, asam amino, dsb.
- Identifikasi dilakukan dengan cara mengenali frekuensi fibrasinya.



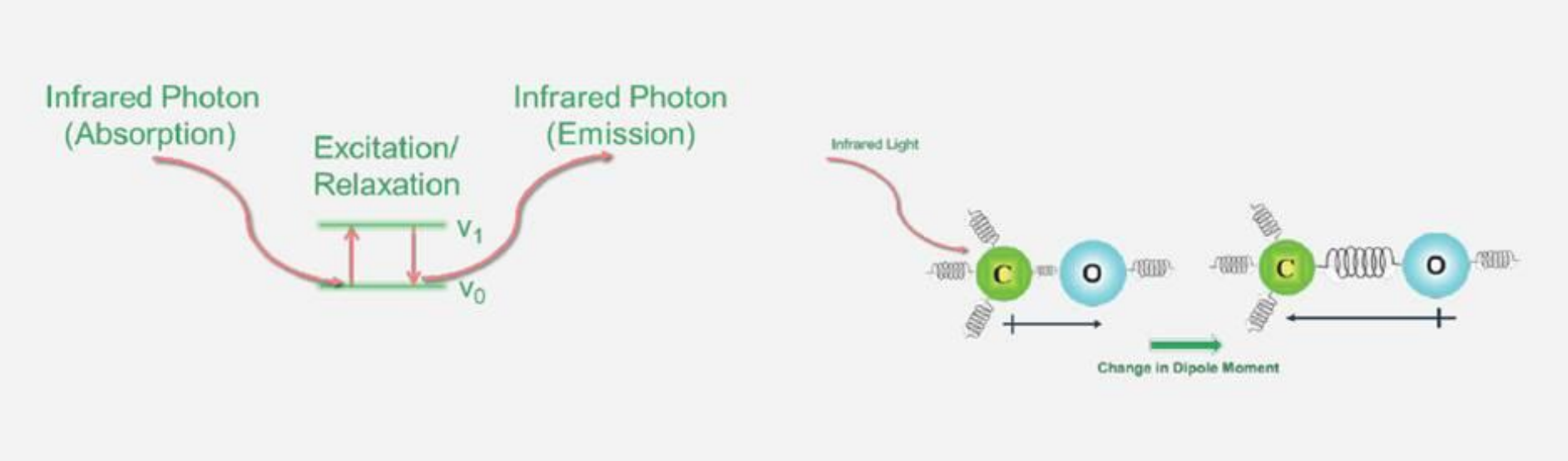
https://jascoinc.com/wp-content/uploads/2020/10/ftir-spectroscopy-figure-2-01.png



Secara Lebih Spesifik . . . .

- Spektroskopi FTIR belakangan banyak dikembangkan untuk identifikasi:
  - Pemetaan komponen sel seperti karbohidrat, lipid, dan protein untuk mengenali ada tidaknya abnormalitas pada sel (Levin and Bhargava 2005; Petibois and De´le´ris 2006).
  - Studi tentang protein, seperti konformasi protein, protein folding, dan detail molekuler suatu protein aktif (Siebert dan Hildebrandt 2008).
  - Interaksi ikatan hidrogen (hydrogen-bonding interactions)
  - Reaksi transfer proton,
  - Atau identifikasi lain yang seringkali di luar sensitivitas analisis difraksi sinar-X (XRD).
- Bersifat komplementer (melengkapi) informasi data struktur 3D yang diperoleh melalui XRD dan NMR.

Spektroskopi FTIR bekerja dengan prinsip cahaya IR yang datang mengubah momen dipol suatu molekul yang berkorespondensi dengan energi getaran spesifik (*specific vibrational energy*)



C - C	1,400 cm^{-1}
C = C	1,600 cm^{-1}
C ≡ C	2,200 cm^{-1}

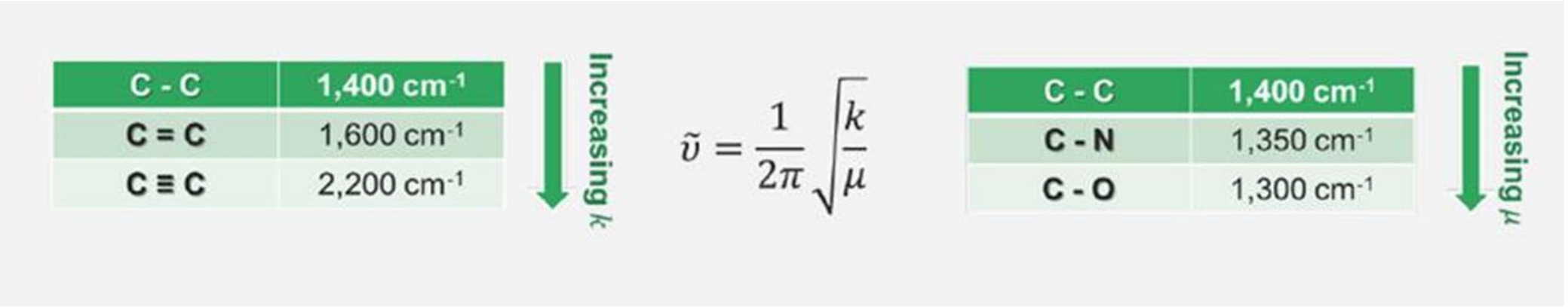
Increasing k

$$\tilde{\upsilon} = \frac{1}{2\pi}\sqrt{\frac{k}{\mu}}$$

C - C	1,400 cm^{-1}
C - N	1,350 cm^{-1}
C - O	1,300 cm^{-1}

Increasing μ

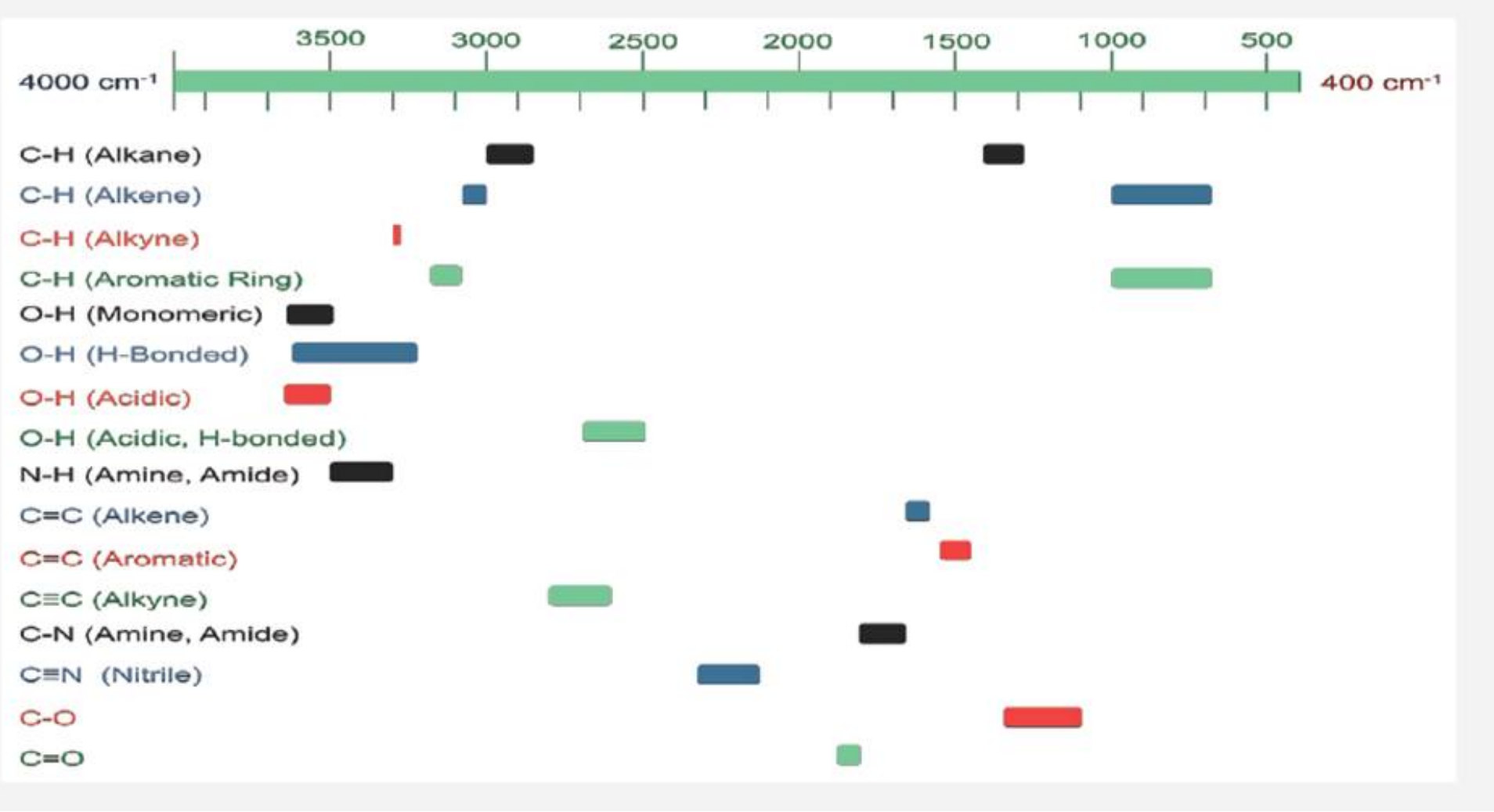
https://jascoinc.com/wp-content/uploads/2020/04/image002.jpg



https://jascoinc.com/wp-content/uploads/2020/04/image002.jpg

Sementara itu energi vibrasi berkorespondensi dengan 2 variabel:

- Berkurangnya massa atau *reduced mass* (μ)
- Konstanta pegas ikatan atau *bond spring constant* (k)
- Karena setiap gugus fungsi terdiri atas atom dan kekuatan ikatan yang berbeda, vibrasi yang dihasilkan unik untuk setiap gugus fungsi dan kelas gugus fungsi (misalnya regangan O-H dan C-H muncul masing-masing pada bilangan gelombang sekitar 3200 cm^{-1} dan 2900 cm^{-1}).



Beberapa Contoh Gugus Fungsional Spesifik pada Deteksi FTIR

Table 1. Characteristic IR Absorption Peaks of Functional Groups*

Vibration	Position (cm^{-1})	Intensity*	Notes
Alkanes			
C-H stretch	2990 – 2850	m to s	
Alkenes			
=C-H stretch	3100 – 3000	m	
C=C stretch	1680 – 1620 (sat.) 1650 – 1600 (conj.)	w to m	
=C-H bend	995 – 685	s	See Table 2 for detail
Alkynes			
≡C-H stretch	3310 – 3200	s	
C≡C stretch	2250 – 2100	m to w	
Aromatic Compounds			
C-H stretch	3100 – 3000	m to w	
C=C stretch	1625 – 1440	m to w	Hidden in fingerprint region
C-H bend	900 – 680	s	See Table 2 for detail
Alcohols**			
O-H stretch	3550 – 3200	br, s	Hydrogen bonded (typical)
Amines			
N-H stretch	3550 – 3250	br, m	Primary (two bands) Secondary (one band)
Nitriles			
C≡N stretch	2280 – 2200	s	
Aldehydes			
C-H stretch	2900 – 2800 & 2800 – 2700	s	H-C=O Fermi doublet
C=O stretch	1740 – 1720 (sat.) 1715 – 1680 (conj.)	s	
Ketones			
C=O stretch	1750 – 1705 (sat.) 1700 – 1665 (conj.)	s	
Esters**			
C=O stretch	1765 – 1735 (sat.) 1730 – 1715 (conj.)	s	
Carboxylic Acids**			
O-H stretch	3200 – 2500	br, m to w	
C=O stretch	1725 – 1700 (sat.) 1715 – 1680 (conj.)	s	
Amides			
N-H stretch	3500 – 3150	m	Primary (two bands) Secondary (one band)
C=O stretch	1700 – 1630	s	

https://www.sigmaaldrich.com/ID/en/technical-documents/technical-article/analytical-chemistry/photometry-and-reflectometry/ir-spectrum-table

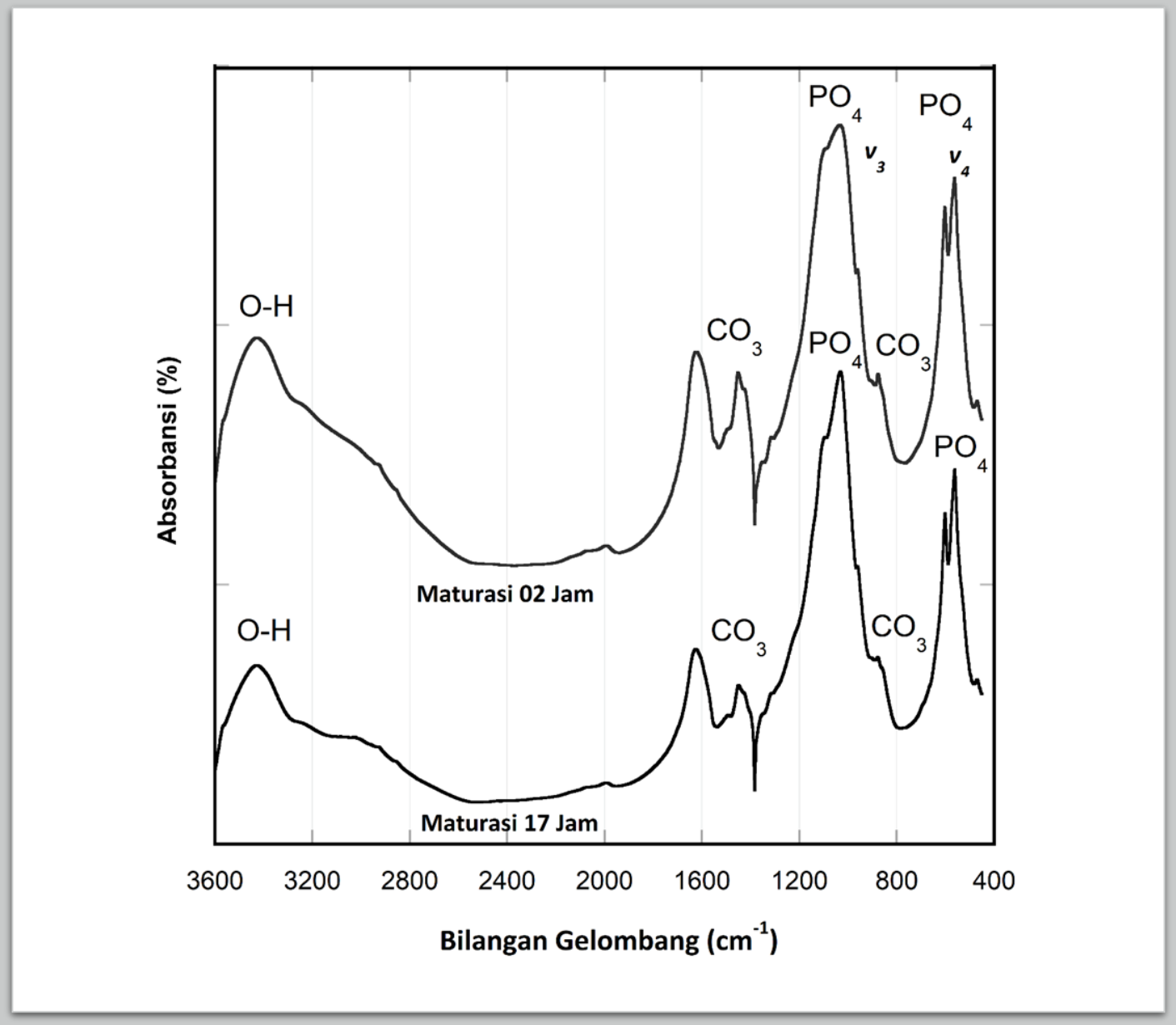
- Contoh Gugus Fungsional (Tabel FTIR)

Tipe Vibrasi Dasar FTIR:

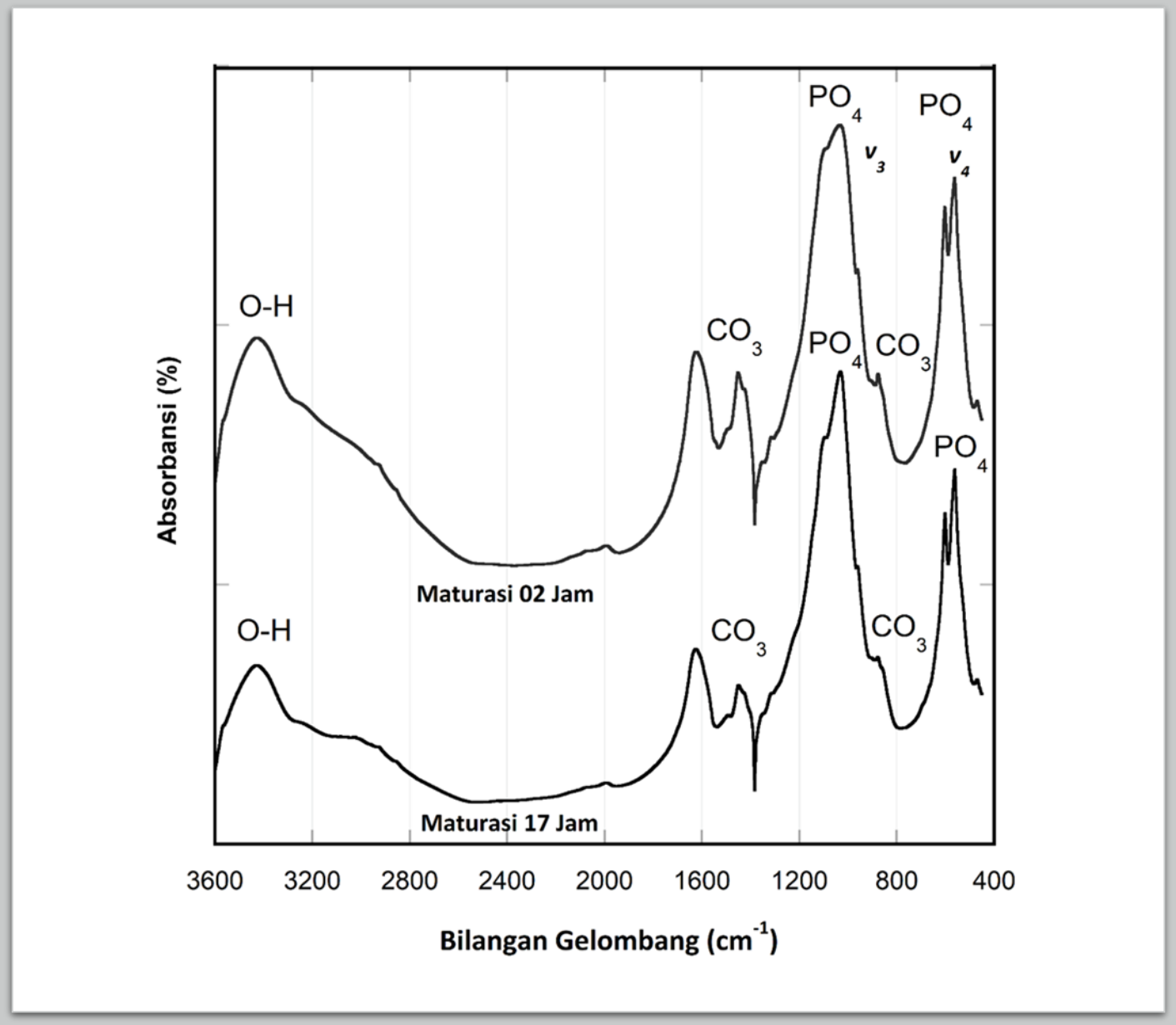
- Spektroskopi inframerah mengidentifikasi getaran (vibrasi) molekul (Colthup dkk., 1975; Griffith dan de Haseth 1986).
- Ada 4 tipe vibrasi dasar:
  - getaran peregangan atau *stretching vibrations* (satu atau lebih panjang ikatan berubah),
  - getaran lentur planar atau *planar bending vibrations* (satu atau lebih sudut ikatan berubah, sementara panjang ikatan tetap),
  - getaran tekukan di luar bidang atau *out-of-plane bending vibrations* (satu atom berosilasi melalui bidang yang ditentukan oleh setidaknya tiga molekul lain yang berdekatan), dan
  - getaran torsi atau *torsion vibrations* (sudut dihedral atau sudut antara dua bidang yang memiliki satu ikatan berubah).
- Di samping itu, dikenal pula adanya vibrasi simetris dan asimetris (Fadini dan Schnopel (1989), Rey dkk. (1989), dan Rey dkk. (1990).

Contoh Hasil Analisis FTIR:

- Spektra FT-IR apatit yang dihasilkan dengan metode presipitasi basah titrasi H_3PO_4 pada $Ca(OH)_2$ yang dilarutkan dalam air, dengan pengadukan magnetis dan maturasi 2-17 jam.



- Karena ion fosfat merupakan molekul tetrahedral (PO_4), maka fosfat memiliki 4 mode vibrasi atau getaran (v_1, v_2, v_3, dan v_4), tetapi hanya v_3, dan v_4 yang aktif terhadap inframerah dengan molekul yang simetris. Prosedur presipitasi kimiawi basah menghasilkan vibrasi fosfat (v_3, dan v_4) serta pita hidroksil (Le Geros, 2001) baik setelah maturasi 2 jam maupun 17 jam.
- Di samping itu, terlihat juga pita gugus karbonat pada rentang bilangan gelombang 1600-1400 cm^{-1} untuk v_3 (vibrasi regangan asimetris atau *asymmetric stretching vibration*), pada bilangan gelombang sekitar 880 cm^{-1} untuk v_2 (tekukan di luar bidang getaran atau *bending out-of-plane bending vibrations*), dan sekitar 755 cm^{-1} untuk v_4 (tekukan pada bidang getaran atau *bending-in-plane vivrations*), dengan merujuk pada penelitian-penelitian sebelumnya (Rey dkk., 1991).
- Intensitas pita pada bilangan gelombang 630 cm^{-1} yang berkorespondensi dengan gugus hidroksil tampak menghilang dalam reaksi tersebut, sebagai akibat substitusi gugus hidroksil oleh gugus karbonat. Gugus hidroksil masih terlihat dan belum atau tidak tersubstitusi oleh gugus karbonat pada kisaran bilangan gelombang 3572 cm^{-1}.



Pita HA Stoikiometris dan HA Nanokristal Hasil Pengujian dengan Spektroskopi Inframerah (FTIR) dan Raman (Eichert dkk., 2009).

Domain, Lokasi	HA Stoikiometris		HA Nanokristal	
	IR (cm^{-1})	Raman (cm^{-1})	IR (cm^{-1})	Raman (cm^{-1})
v_2 PO_4		433		432
	464	448	469	452
	474			
HPO_4 non apatit			533	
HPO_4 apatit			551	
v_4 PO_4	567	580	562	584
	572	591	575	590
	602	607	603	611
		614		
PO_4 non apatit			617	
v_L OH	633			
P-OH dalam HPO_4			870	873
Non apatit v_2 CO_3 tipe-B			866	
			871	
tipe-A			880	
v_1 PO_4	964	964	962	961
v_3 PO_4			1006	1005
			1020	
	1026	1029	1031	1032
	1034	1034		
	1044	1041	1044	1044
		1057	1059	
	1063	1064		
	1089	1077	1072	1071
			1091	
			1104	
HPO_4			1144	
v_1 CO_3 tipe-B				1071
v_1 CO_3 tipe-A				1103
B+ non-apatit			1420	
v_3 CO_3 A+B			1460-1470	
Non-apatit			1500	
A			1540	

Aplikasi FTIR untuk Riset Berbagai Bidang

Laboratorium Biomedika Kedokteran Gigi, Fakultas Kedokteran Gigi, UGM

Journal of *Functional Biomaterials*

MDPI

Article

Safe-by-Design Antibacterial Peroxide-Substituted Biomimetic Apatites: Proof of Concept in Tropical Dentistry

Ika Dewi Ana [1,*], Any Lestari [2], Prescillia Lagarrigue [3], Jérémy Soulie [3], Rahmi Anggraeni [2], Françoise Maube-Bosc [3,†], Carole Thouron [3], Benjamin Duployer [3], Christophe Tenailleau [3] and Christophe Drouet [3,*]

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Abstract: Bone infections are a key health challenge with dramatic consequences for affected patients. In dentistry, periodontitis is a medically compromised condition for efficient dental care and bone grafting, the success of which depends on whether the surgical site is infected or not. Present treatments involve antibiotics associated with massive bacterial resistance effects, urging for the development of alternative antibacterial strategies. In this work, we established a safe-by-design bone substitute approach by combining bone-like apatite to peroxide ions close to natural in vivo oxygenated species aimed at fighting pathogens. In parallel, bone-like apatites doped with Ag^+ or co-doped Ag^+/peroxide were also prepared for comparative purposes. The compounds were thoroughly characterized by chemical titrations, FTIR, XRD, SEM, and EDX analyses. All doped apatites demonstrated significant antibacterial properties toward four major pathogenic bacteria involved in periodontitis and bone infection, namely *Porphyromonas gingivalis* (*P. gingivalis*), *Aggregatibacter actinomycetemcomitans* (*A. actinomycetemcomitans*), *Fusobacterium nucleatum* (*F. nucleatum*), and *S. aureus*. By way of complementary tests to assess protein adsorption, osteoblast cell adhesion, viability and IC_{50} values, the samples were also shown to be highly biocompatible. In particular, peroxidated apatite was the safest material tested, with the lowest IC_{50} value toward osteoblast cells. We then demonstrated the possibility to associate such doped apatites with two biocompatible polymers, namely gelatin and poly(lactic-co-glycolic) acid PLGA, to prepare, respectively, composite 2D membranes and 3D scaffolds. The spatial distribution of the apatite particles and polymers was scrutinized by SEM and μCT analyses, and their relevance to the field of bone regeneration was underlined. Such bio-inspired antibacterial apatite compounds, whether pure or associated with (bio)polymers are thus promising candidates in dentistry and orthopedics while providing an alternative to antibiotherapy.

Keywords: peroxide; silver; biomimetic apatite; tropical dentistry; antibacterial properties; biocompatibility; porous scaffolds

check for updates

Citation: Ana, I.D.; Lestari, A.; Lagarrigue, P.; Soulie, J.; Anggraeni, R.; Maube-Bosc, F.; Thouron, C.; Duployer, B.; Tenailleau, C.; Drouet, C. Safe-by-Design Antibacterial Peroxide-Substituted Biomimetic Apatites: Proof of Concept in Tropical Dentistry. *J. Funct. Biomater.* **2022**, *13*, 144. https://doi.org/10.3390/jfb13030144

Academic Editors: Adriana Bigi and Elisa Boanini

Received: 21 July 2022
Accepted: 2 September 2022
Published: 7 September 2022

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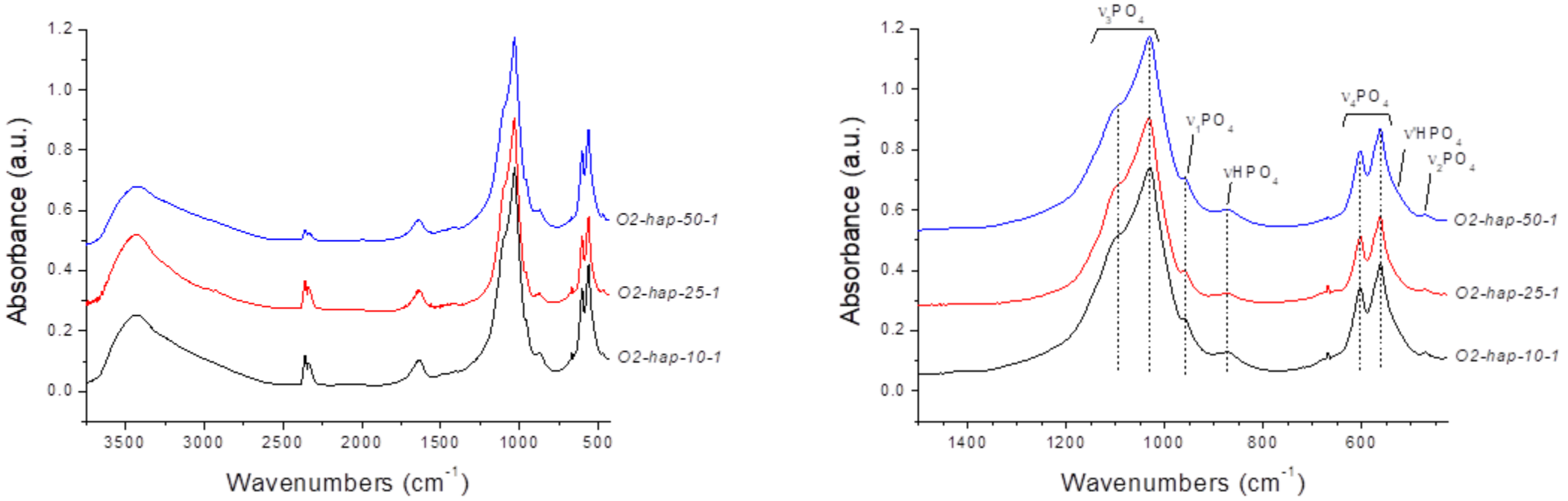
Pembuatan Apatit Berdaya Antibakteri dengan Penambahan Berbagai Ion

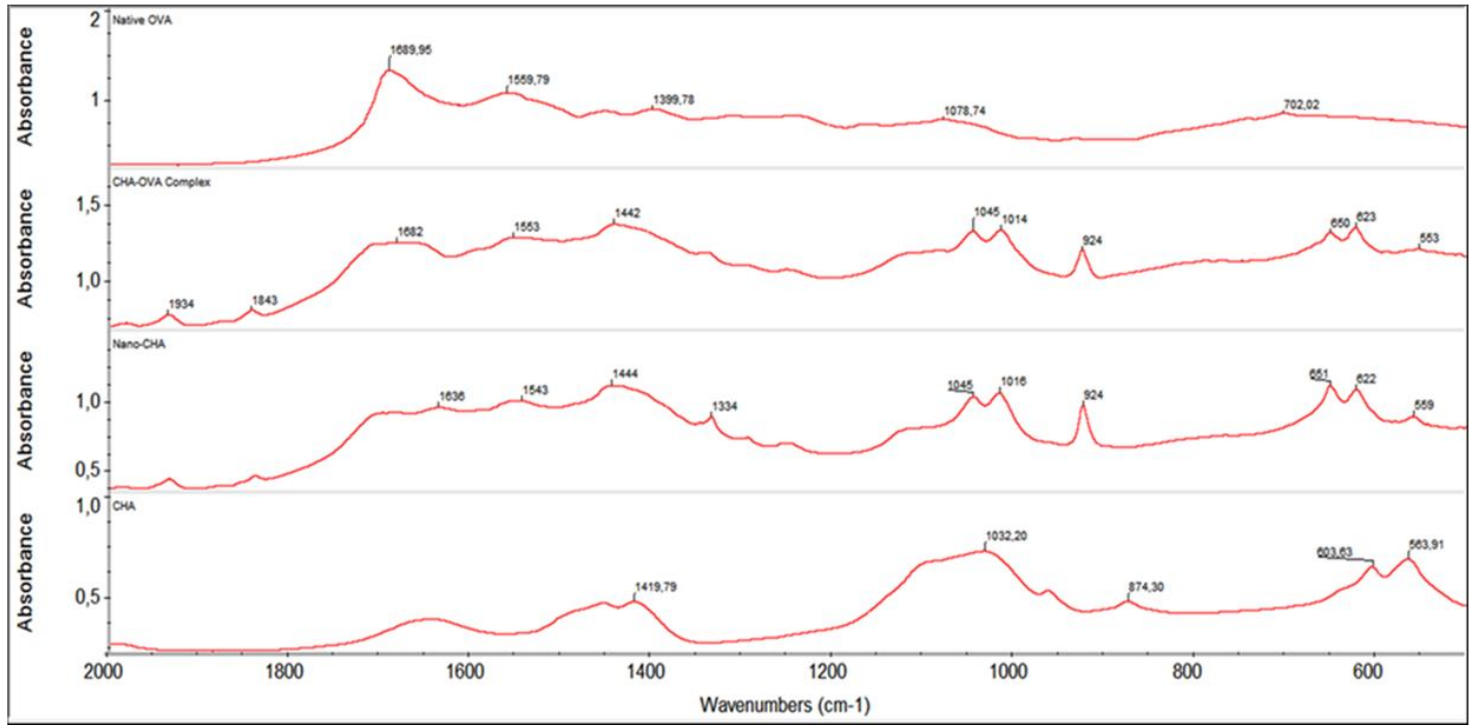
FTIR spectra for apatite compounds prepared under increasing initial amounts of H_2O_2 for an apatite maturation time of 1 day. The second graph is a zoomed view on the 425-1500 cm^{-1} domain. The main phosphate band attributions have been added, with reference to bone-like apatite.

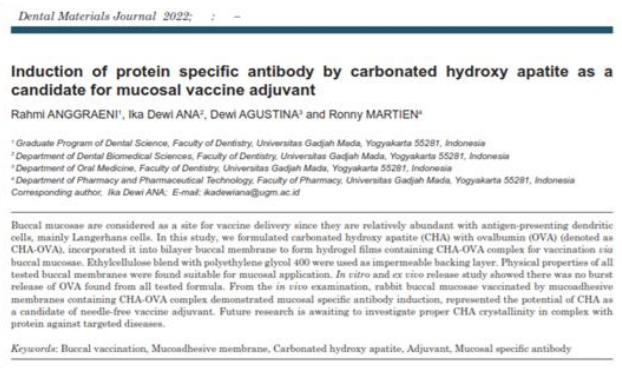


Ana et al., J. Funct. Biomater. 2022, 13(3), 144; https://doi.org/10.3390/jfb13030144

Riset ID Ana, R Anggraeni, dan H Wihadmadyatami

FT-IR spectroscopy was used to study the structure of the complex. Spectra of the CHA-OVA complex at band 1553 cm^{-1} suggested that COO^- groups of protein bound to calcium in the bidentate coordination mode. It describes that the Ca^{2+} binds equally with 2 oxygen atoms on the COO^- groups [10,11,12]. The band at 1442 cm^{-1} showed the carbonated side of hydroxy apatite. Carbonate band at 1442 cm^{-1} suggested that the synthesized CHA was type-B (range 1410 and 1455 cm^{-1}), because type-A CHA shows CO_3^{2-} band at 1455-1545 cm^{-1} [2]. The spectra at 1014 cm^{-1} showed the phosphate groups of CHA, while the band at 1682 cm^{-1} belonged to amide I (between 1600 and 1700 cm^{-1}). The amide I band is the most intense bands in the protein spectra when the protein is dissolved in water or buffer solution [13].





Dental Materials Journal 2022; : –

Induction of protein specific antibody by carbonated hydroxy apatite as a candidate for mucosal vaccine adjuvant

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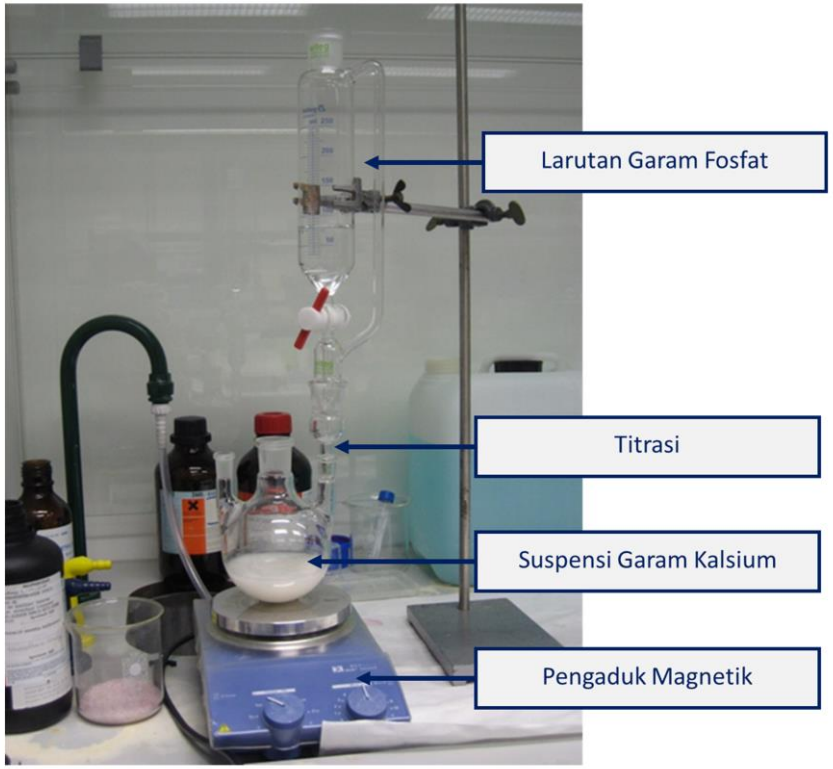
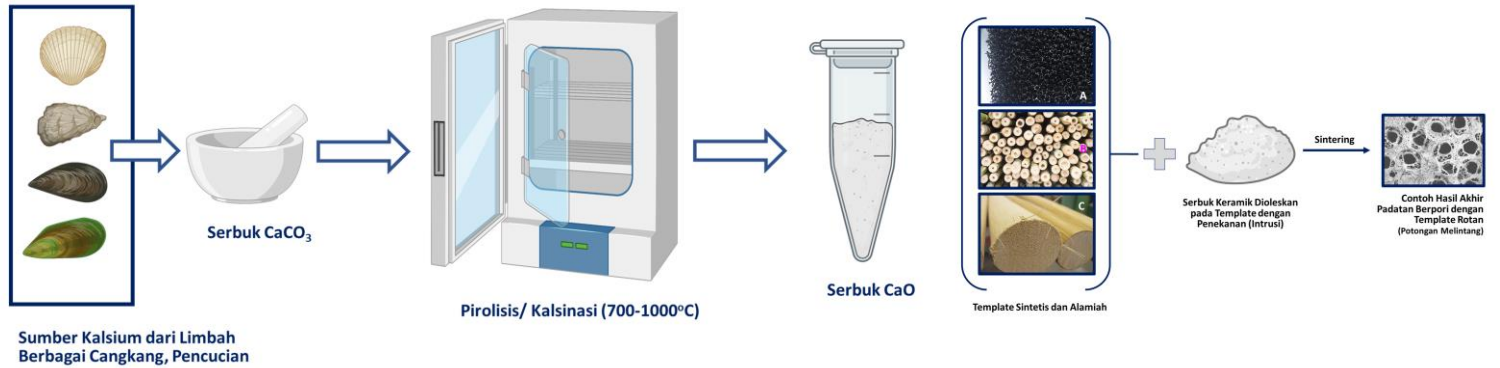
Buccal mucosae are considered as a site for vaccine delivery since they are relatively abundant with antigen-presenting dendritic cells, mainly Langerhans cells. In this study, we formulated carbonated hydroxy apatite (CHA) with ovalbumin (OVA) (denoted as CHA-OVA), incorporated it into bilayer buccal membrane to form hydrogel films containing CHA-OVA complex for vaccination *via* buccal mucosae. Ethylcellulose blend with polyethylene glycol 400 were used as impermeable backing layer. Physical properties of all tested buccal membranes were found suitable for mucosal application. *In vitro* and *ex vivo* release study showed there was no burst release of OVA found from all tested formula. From the *in vivo* examination, rabbit buccal mucosae vaccinated by mucoadhesive membranes containing CHA-OVA complex demonstrated mucosal specific antibody induction, represented the potential of CHA as a candidate of needle-free vaccine adjuvant. Future research is awaiting to investigate proper CHA crystallinity in complex with protein against targeted diseases.

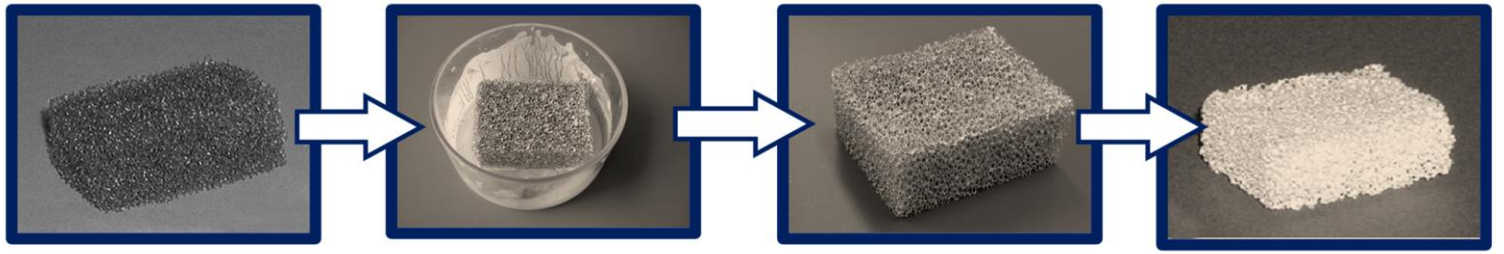
Keywords: Buccal vaccination, Mucoadhesive membrane, Carbonated hydroxy apatite, Adjuvant, Mucosal specific antibody

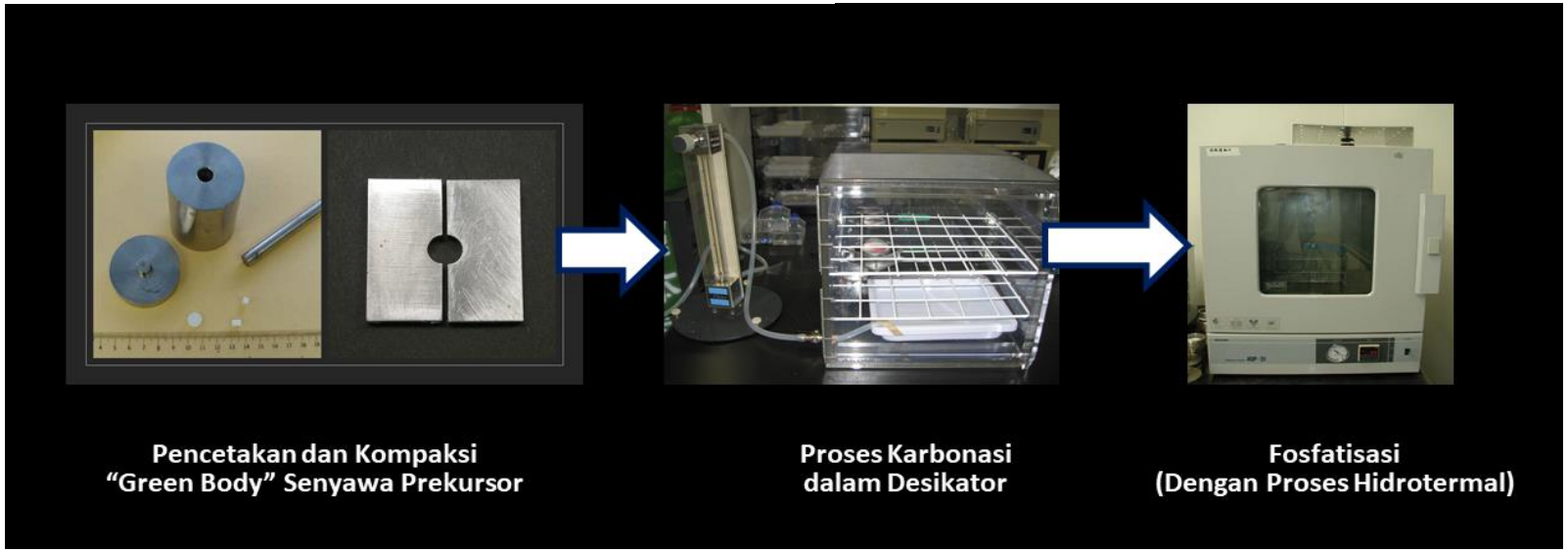
DMJ, 2022 (Article in Press)

Pengembangan Ajuvan Vaksin – FTIR digunakan untuk mengidentifikasi gugus fungsional kompleks protein-CHA


Larutan Garam Fosfat
Titrasi
Suspensi Garam Kalsium
Pengaduk Magnetik


Sumber Kalsium dari Limbah Berbagai Cangkang, Pencucian
Serbuk $CaCO_3$
Pirolisis/ Kalsinasi (700-1000°C)
Serbuk CaO
Template Sintetis dan Alamiah
Serbuk Keramik Dioleskan pada Template dengan Penekanan (Intrusi)
Sintering
Contoh Hasil Akhir Padatan Berpori dengan Template Rotan (Potongan Melintang)


Spons Poliuretan
Spons Poliuretan Dilapisi Bubur Serbuk Keramik
Spons Berlapis Keramik yang Telah Kering
Spons Biokeramik


Pencetakan dan Kompaksi "Green Body" Senyawa Prekursor
Proses Karbonasi dalam Desikator
Fosfatisasi (Dengan Proses Hidrotermal)

Sari *et al. Biomaterials Research* (2021) 25:2
https://doi.org/10.1186/s40824-021-00203-z

Biomaterials Research

RESEARCH ARTICLE Open Access

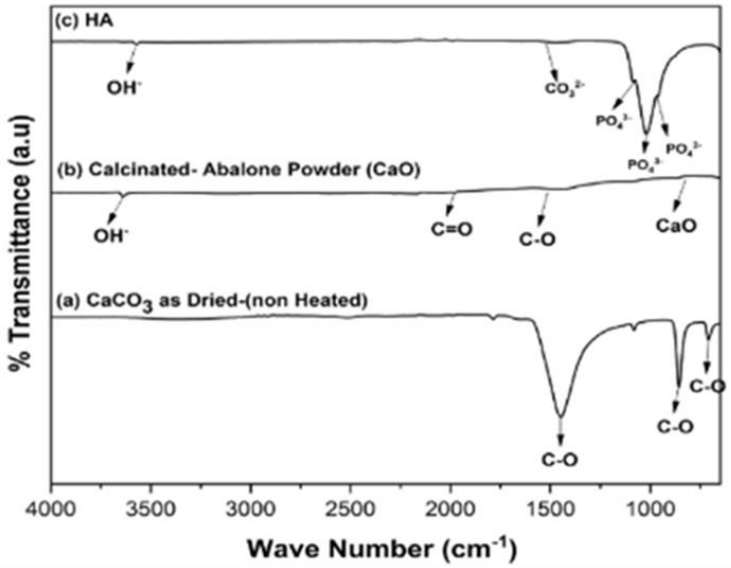
Bioceramic hydroxyapatite-based scaffold with a porous structure using honeycomb as a natural polymeric Porogen for bone tissue engineering

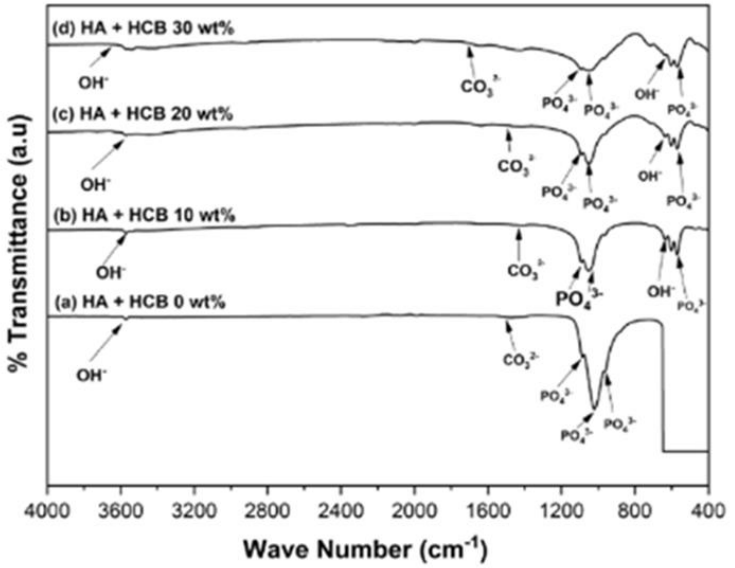
Mona Sari[1], Puspa Hening[2], Chotimah[1], Ika Dewi Ana[3] and Yusril Yusuf[1*]

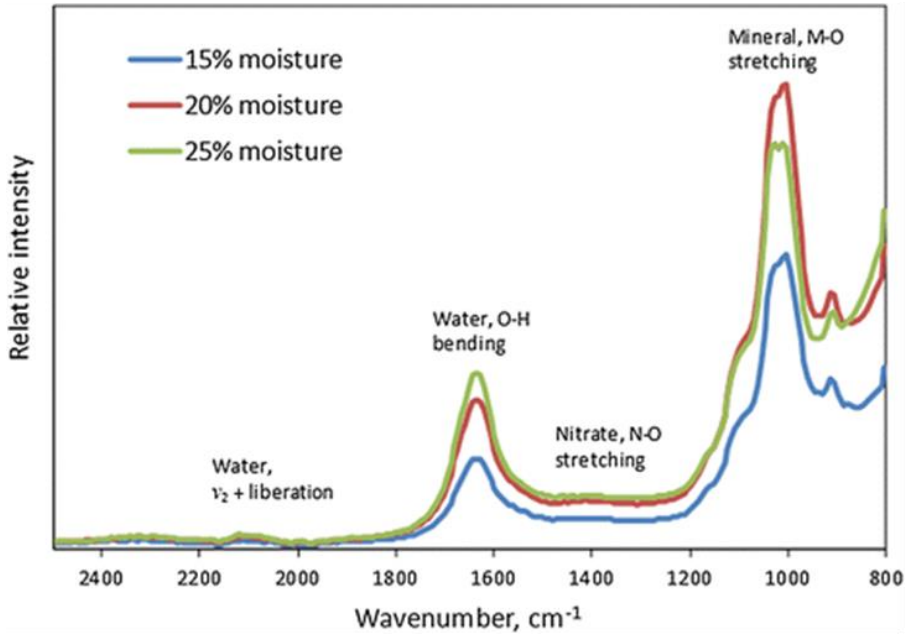
FTIR spectra analysis was performed to identify the functional groups of samples themselves. As shown in Fig. 4a-b, the non-calcined abalone mussel shells did not display the OH^- stretching mode, the bond functional group of C = O and CaO. The CaO bond functional groups, the C = O bond, and the OH^- stretching functional groups were present in the abalone mussel shells at 1000 °C. The functional groups of CaO, C –O, C = O, and OH^- were observed at 873.88 cm^{-1} and 1470.39 cm^{-1}, the C = O bond at 1792.50 cm^{-1} and 2043.34 cm^{-1},and the functional group of OH^- at 3640 cm^{-1}. As shown in Fig. 4c, the synthesized HA exhibited the functional group of HA. The HA exhibited the stretching mode of OH^- at 3571.66 cm^{-1} and the bending modes of stretching $_v$(P – O) mode of PO_4^{3-} at 963.28, 1020.31, and 1085.81 cm^{-1}. HA exhibited the functional group of CO_3^{2-} only at 1476.66 cm^{-1}.

HA-based honeycomb scaffold

The FTIR spectra data (Fig. 5) show that HA without HCB the functional groups of B-type CO_3^{2-} at 1476.66 cm^{-1}, PO_4^{3-} absorption at 963.28, 1020.31, and 1085.81 cm^{-1} and the absorption band attributed to hydroxyl at 3571.66 cm^{-1}. PO_4^{3-} absorption was observed at 602–570 cm^{-1} and 1091–963 cm^{-1} for all concentrations of HCB. For all variations in HA-HCB treatments, the absorption band attributed to hydroxyl was observed within the ranges of 636–635 cm^{-1} and 3570–3543 cm^{-1}.

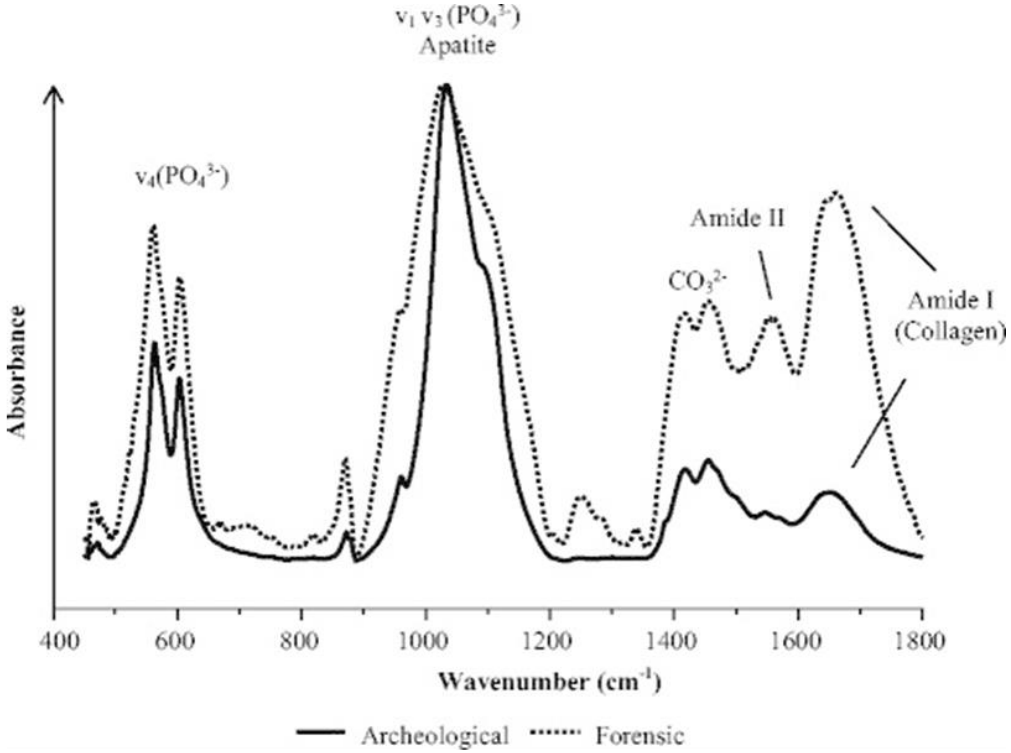






https://www.researchgate.net/publication/325129851/figure/fig1/AS:962150646431782@1606405998806/Differences-in-D-ATR-FTIR-spectra-of-the-same-soil-equilibrated-with-different-amounts-of.gif

Aplikasi untuk Agro

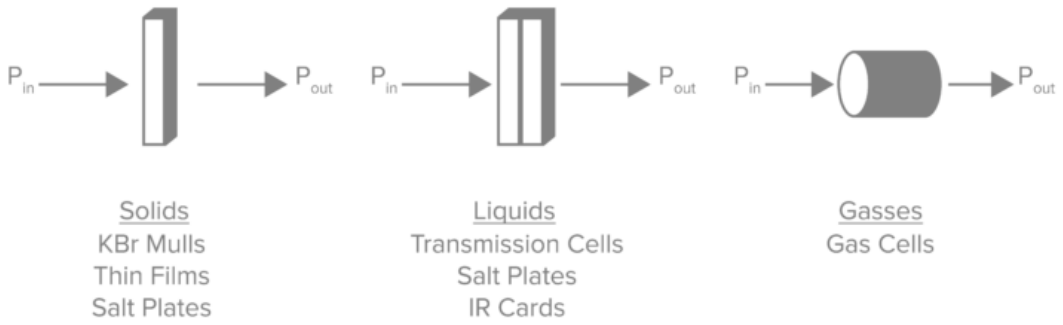


https://www.researchgate.net/publication/304361465/figure/fig3/AS:391199530733574@1470280644263/FTIR-spectra-of-the-archeological-bone-16-and-FTIR-spectra-of-the-humerus-bone-from-the.png

Aplikasi untuk Arkeologi dan Forensik

Bagaimana Cara Kerja Spektroskopi FTIR?

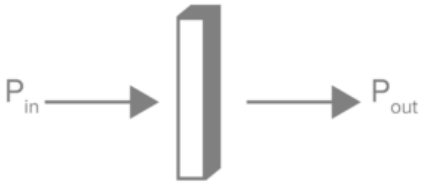
Laboratorium Biomedika Kedokteran Gigi, Fakultas Kedokteran Gigi, UGM



Solids
KBr Mulls
Thin Films
Salt Plates

Liquids
Transmission Cells
Salt Plates
IR Cards

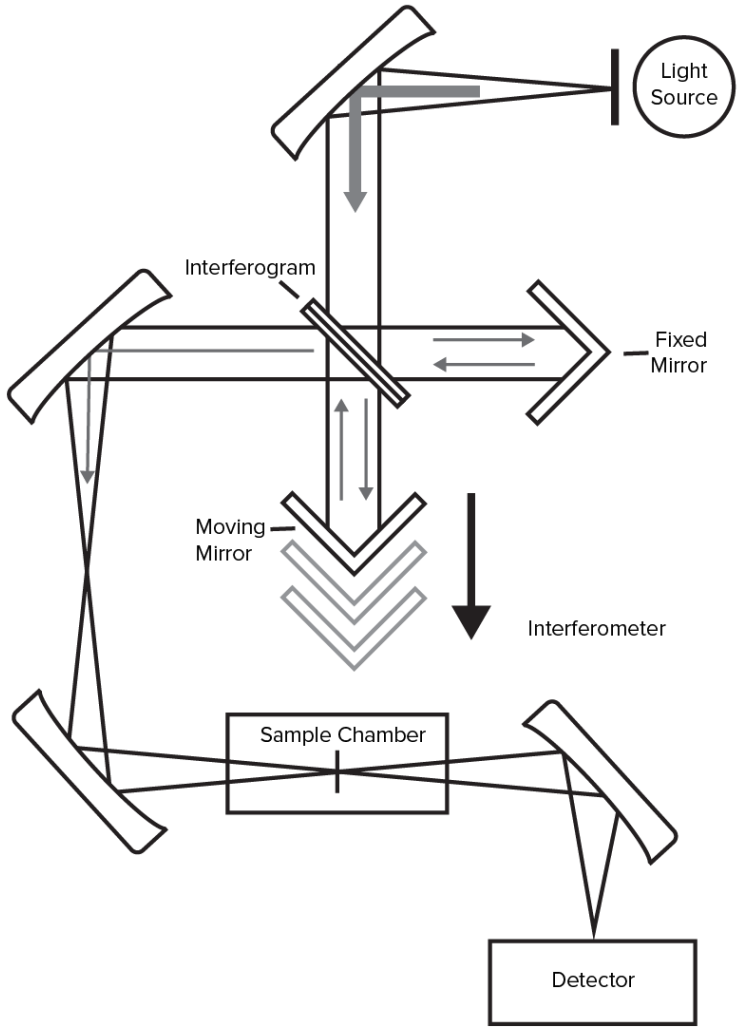
Gasses
Gas Cells



Sample

Y-Axis Units

P_{in} = Radiant Power from IR Source
P_{out} = Radiant power to detector
Absorbance(A) = $\log P_{in}/P_{out}$) = Light absorbed by sample
Transmittance(%T) = (P_{in}/P_{out}) x 100 = Light transmitted through sample



https://jascoinc.com/wp-content/uploads/2021/08/Y-Axis-Units-03-768x278.png

Future Perspectives

In Dentistry and Medicine, Biological System Need Engineering and Interdisciplinary Approaches: Modulating Extracellular Signaling and Intracellular Reprogramming

Terima Kasih