UNIVERSITATEA
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CLUJ-NAPOCA

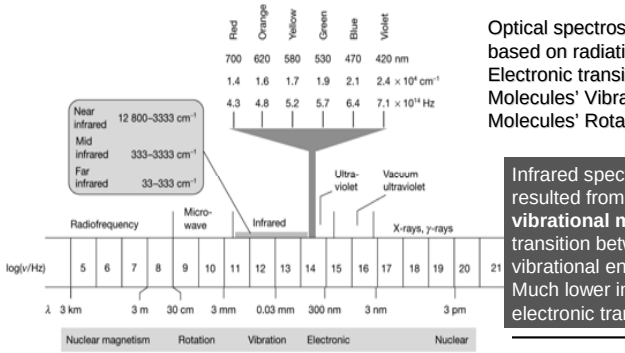
FTIR SPECTROMETRY- A VERSATILE METHOD TO INVESTIGATE MICROCAPSULES' COMPOSITION

Prof. Dr. Carmen Socaciu

OUTLINE

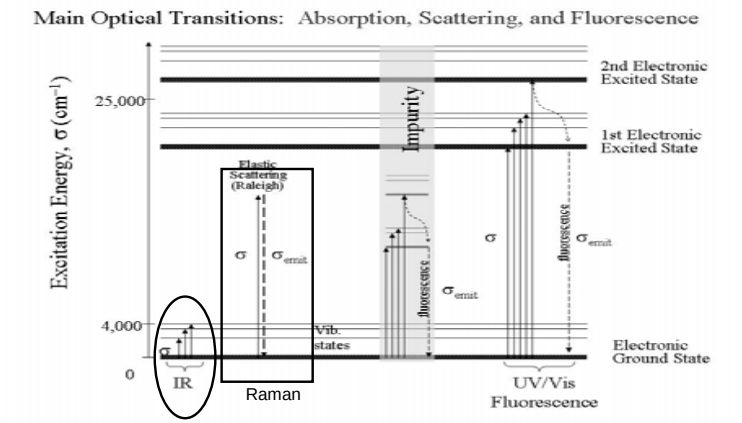
1. Basics on Optical transitions and IR spectroscopy
2. FTIR applications - versatile molecular fingerprint
3. Carbohydrate matrices: Recognition of IR markers
4. Case study: FTIR spectroscopy to identify specific oils in hydrocolloid /carbohydrate capsules
5. Needs for calibration/validation/standardization

Light & the Electromagnetic Spectrum



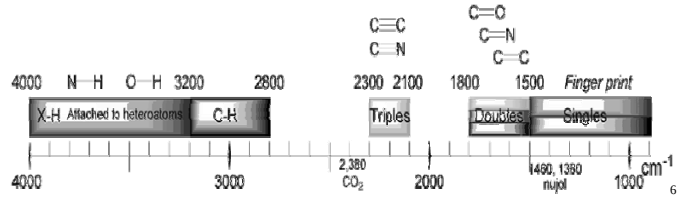
Optical spectroscopy-based on radiations'effects by:
Electronic transitions (UV-Vis)
Molecules' Vibration (IR-)
Molecules' Rotation (IR, MW)

Infrared spectra are resulted from **excitation of vibrational modes** --- transition between the vibrational energy levels. Much lower in energy than electronic transitions.

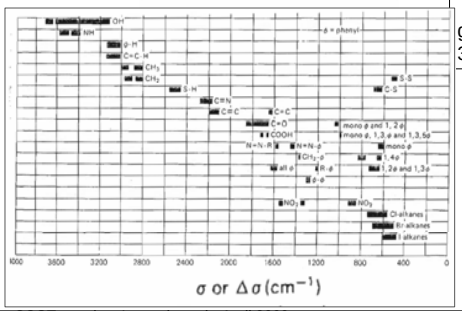


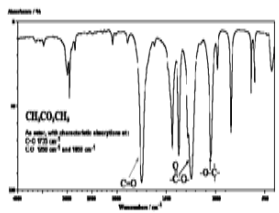
Which molecules can absorb IR radiation ?

1. Polar molecules with a dipol moment
2. IR absorption is active when the energy of radiation = the difference between the 2 vibrational and/or rotational levels.
3. Vibrational IR absorption is observed in organic molecules (fall in MIR region)
4. Rotational IR absorptions fall in far IR region.



Region	Wavelength (μm)	Wavenumber (cm⁻¹)
Near	0.8-2.5	13,000 - 4,000
Middle	2.5-50	4,000-200
Far	50-1000	200-10





Examples of IR induced vibrational modes

Energies associated with the vibrational found between 4000 - 650 cm-1

Rotational energies are generally much smaller (below 300 cm-1)

Harmonic oscillator

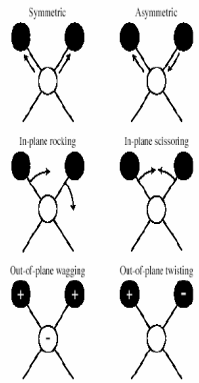
IR radiation interacts with molecules and induce a net change in dipole moment as a result of vibrational or rotational motion

Bonds between atoms vibrate when absorbing specific wavelength of their functional groups

Vibration Modes

Two basic vibration categories:

1. Stretching:
2. Bending:
   scissoring, rocking, wagging, twisting.



IR spectra (A, T) and BANDS

IR Bands

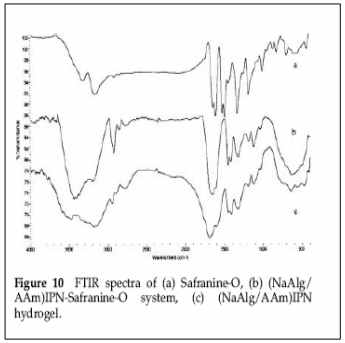


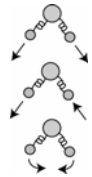
Figure 10 FTIR spectra of (a) Safranine-O, (b) (NaAlg/AAm)IPN-Safranine-O system, (c) (NaAlg/AAm)IPN hydrogel.

POSITION	REDUCED MASS	LIGHT ATOMS HIGH FREQUENCY
	BOND STRENGTH (STIFFNESS)	STRONG BONDS HIGH FREQUENCY
STRENGTH	CHANGE IN 'POLARITY'	STRONGLY POLAR BONDS GIVE INTENSE BANDS
WIDTH	HYDROGEN BONDING	STRONG HYDROGEN BONDING GIVES BROAD BANDS

http://www.ftir-libraries.com/ftir_databases.htm

4000-3000 cm^{-1}	3000-2000 cm^{-1}	2000-1500 cm^{-1}	1500-1000 cm^{-1}
O-H N-H C-H	C≡C C≡N	C=C C=O	C-O C-F C-Cl deformations

Increasing energy



H_2O : 3657 cm^{-1}
3756 cm^{-1}
1594 cm^{-1}

The most useful regions are as follows:
1680-1750 cm-1: C=O stretches feature very strongly in IR spectra and carbonyl group
2700-3100 cm-1: C-H stretching vibrations.
3200-3700 cm-1: O-H and N-H stretching vibrations.

➢**Only vibrations that cause a change in 'polarity' give rise to bands in IR spectra**

Typical spectral regions for IR spectroscopy:

1. near-IR: excites overtones or harmonics of fundamental vibrations (multiple level transition). Instrumentation - similar to UV-VIS absorption.

2. mid-IR: excites fundamental vibrations (single level transition). The most widely used for IR spectroscopy, generates spectral fingerprints of IR active organic molecules.

3. far-IR: excites low-energy vibrations and higher energy rotations. Few analytical uses, used in industry for quality control.

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3. Microcapsules characterization
4. Carbohydrate matrices: Recognition of IR markers
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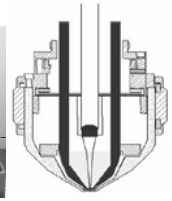
6. Needs for calibration/validation/standardization

FTIR –sensitive analytical technique

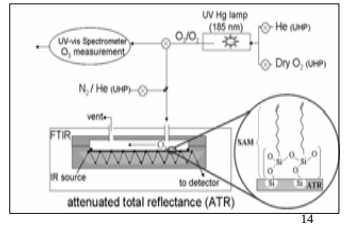
- **Fast data acquisition** -spectra are collected based on measurements using time-domain of IR radiation by temporal coherence of a radiative source.
- **Simple to operate, fast**
- **Non-destructive**
- **Useful for fingerprint different samples (powders, extracts, emulsions, gels)**
- **Better qualitative than quantitative**
- **Need validation by accurate methods (GC, LC)**

FTIR coupled with Attenuated Total Reflectance (ATR)

Most versatile instrumentation
Micro diamond ATR objective to handle micro-areas
– The red area indicates the IR beam path
– Penetration depth can be controlled



To perform the analysis, the sample is placed in contact with the surface of an IR transmitting crystal. The IR light is reflected from the inside surface of the crystal, but also penetrates a small distance into the sample and therefore is partially absorbed.



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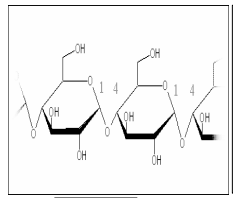
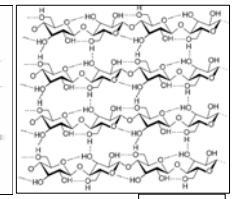
Homopolysaccharides
Cellulose
Starch: amylose & amilopectin

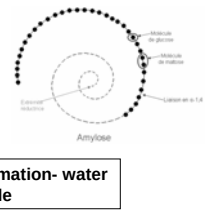
Heteropolysaccharides
Alginate
Carragenans
Chitosan
Gums: xanthan, guar



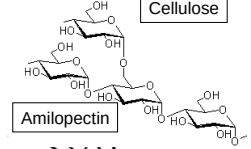
Amylose



Cellulose



Amylose

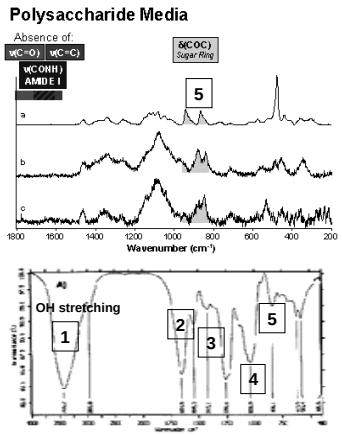


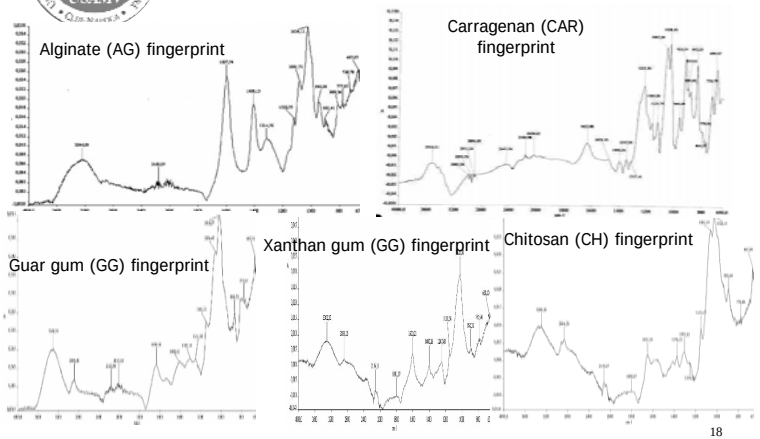
Amilopectin

Specific hydrocolloid formation- water link plays an essential role

FTIR spectra reveals:

1. OH stretching vibrations (> 3000 cm^{-1})
2. Sugar ring stretching (1630 cm^{-1})
3. C-OH bending (alcoholic) (1250-1310 cm^{-1})
4. CH_2-OH stretch (1050-1150 cm^{-1})
5. Glycosidic link (700-900 cm^{-1})





Functional group and vibration	AG	CAR	GG	XG	CH
O–H stretching vibration	3244	3514 PolyOH groups	3299	3302	3289 O-H + N-H strech
C–H stretching of CH2 group	2926	2953, 2911, 2894	2884	-	2935
C-O stretching (COOH)	1597	-	1636	-	1651
Deformations of CH2 group (bending)	1408	1474, 1400	1408	1400	1428
O-H bending		1223 (S=O strech sulphate ester	1350	1247	
C-O and C-C ring stretching	1200-1000		1145	1150	1151
–CH2OH stretching mode	1054	1063	1054		1061
C–OH alcoholic (C-O stretching saccharide)	1024	1024		1025	1024
–CH2 twisting vibration	948, 902, Gululonic & mannuronic	924, 910 Polyhydroxy groups	1016		
Glycosidic links	809	842 Galactose sulphate, glycosidic link	866,777 (1,4; 1,6) link galactose and mannose	785 C-H rocking, bending C-C stretching	892, 776

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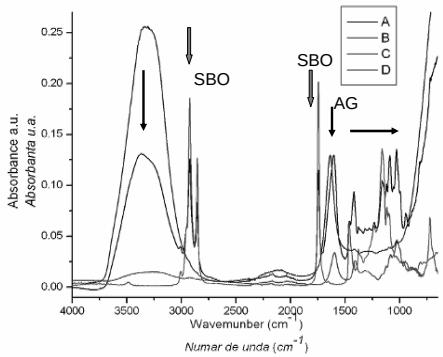
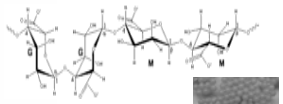
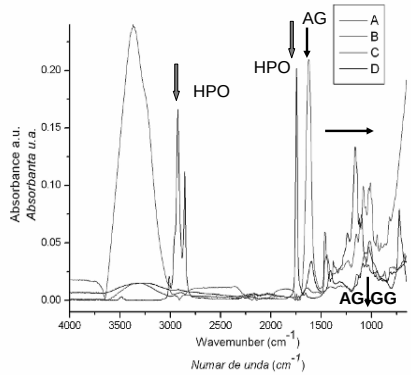


FTIR-ATR spectra
A. AG 2% SBO
B. AG powder;
C. SBO;
D. AG 2% empty

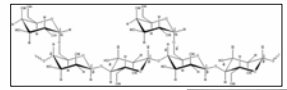


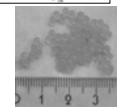
Encapsulation induce:

✓Decrease abs at 3400 (proportional with increase of oil %)
✓LW Shifts in reg. 1000-1500 against SBO



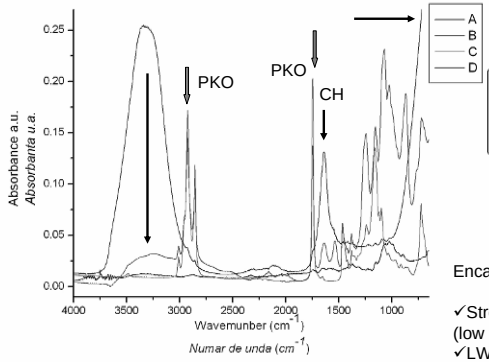
FTIR-ATR spectra
A. AG powder;
B. GG powder;
C. AG-GG-HPO (ratio 0.5:0.5)
D. HPO



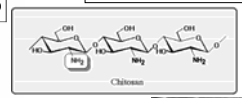


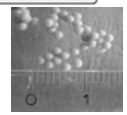
Encapsulation induces:

✓Increase abs at 3400 (high water absorption, less oil %)
✓LW Shifts in reg. 1000-1500 against HPO



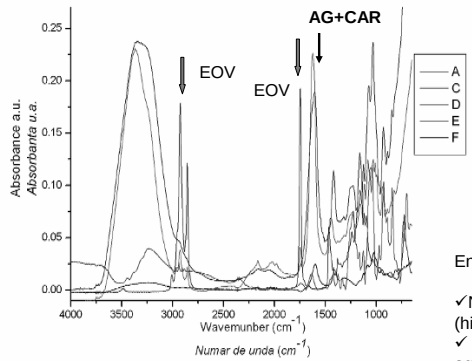
FTIR-ATR spectra
A. CH powder;
B. CH 2% PKO;
C. PKO;
D. CH 2% empty



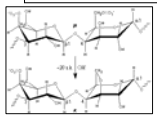


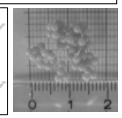
Encapsulation induces:

✓Strong decrease abs at 3400 (low water content)
✓LW Shifts in reg. 1000-1500 against PKO



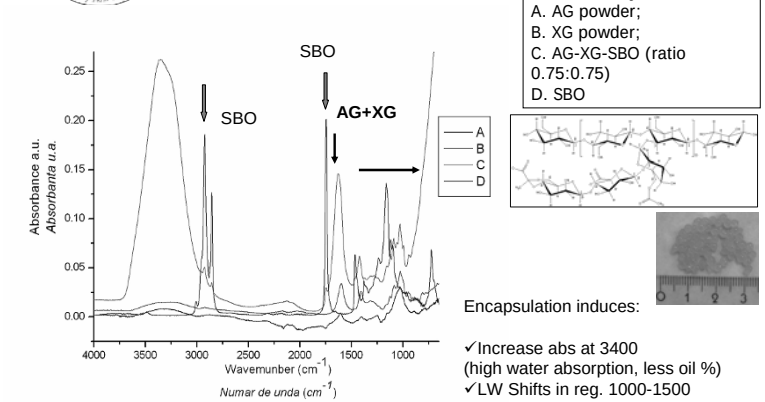
FTIR-ATR spectra
A. AG powder;
C. CAR powder;
D. AG-CAR-EOV(0.75:0.75)
E. EOV
F. AG-CAR beads (0.75:0.75)





Encapsulation induces:

✓No modifications at 3400 (high water content)
✓ NO Shifts in reg. 1000-1500 against PKO

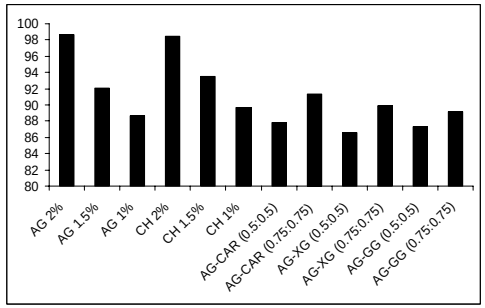


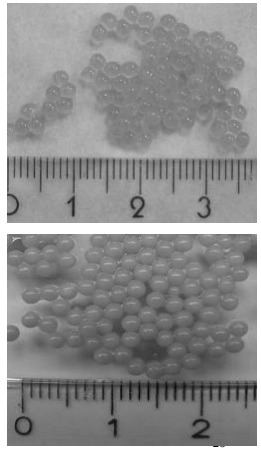
FTIR-ATR spectra
A. AG powder;
B. XG powder;
C. AG-XG-SBO (ratio 0.75:0.75)
D. SBO

Encapsulation induces:

✓Increase abs at 3400 (high water absorption, less oil %)
✓LW Shifts in reg. 1000-1500 against HPO

Different encapsulation efficiencies





Less water, more oil (matt) at increased AG, CH conc.

More water, less oil (translucide) At decreased concentrations.

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5-points-conditions for standardization

To make adequate and accurate interpretation of FTIR spectra of encapsulated target molecules in specific (polysaccharide) matrices, some *sine qua non* conditions have to be respected:

1. Correct and adequate calibrations (FTIR-ATR spectra) with free matrices
2. Adequate calibrations with "empty microcapsules" containing the matrix hydrogels without target molecules
3. Evaluation of the FTIR-ATR) fingerprint of the target molecule in its free form (oil, oleoresin, oleosomes)
4. Complementary quantitative analysis of target molecules by GC-FID or HPLC-PDA using standardized methods
5. Elaboration of standardized protocols for microcapsule composition via FTIR(ATR) – HPLC (GC) complementary methods

Such protocols can cover qualitative and quantitative evaluations of microcapsule composition and stability

TRADITION and MODERNITY