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Evaluation of Graft Copolymerization of Acrylic Monomers Onto Natural Polymers by Means Infrared Spectroscopy

José Luis Rivera-Armenta¹, Cynthia Graciela Flores-Hernández¹,
Ruth Zurisadai Del Angel-Aldana¹, Ana María Mendoza-Martínez¹,
Carlos Velasco-Santos² and Ana Laura Martínez-Hernández²

¹*Instituto Tecnológico de Ciudad Madero,
División de Estudios de Posgrado e Investigación*

²*Instituto Tecnológico de Querétaro,
Departamento de Metal-Mecánica
México*

1. Introduction

Infrared spectroscopy (IR spectroscopy) is a technique based on the vibrations of atoms of a molecule. An IR spectrum is commonly obtained by passing IR radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The energy at which any peak in an absorption spectrum appears corresponds to the frequency of a vibration of a part of a sample molecule (Bickford, 2008).

For a molecule to show IR absorptions, it must possess specific feature: electric dipole moment of molecule must change during the movement that means changes in molecular dipoles which are associated with vibrations and rotations. The atoms in molecules can move relative to one another. This is a description of stretching and bending movements that are collectively referred to as vibrations. Vibrations can involve either change in bond length (stretching) or bond angle (bending). Some bonds can stretch in-phase (symmetrical stretching) or out-of-plane (asymmetric stretching) (Bickford, 2008).

The IR is divided into three regions; the near-, mid- and far- IR. The mid-IR is the most common region to identification and study of organic compounds based on fundamental vibrations and associated rotational-vibrational structure.

IR spectroscopy is a popular method for characterizing polymers. This spectroscopy may used to identify the composition of polymers, to monitor polymerization processes, to characterize polymer structure, to examine polymer surface, and to investigate polymer degradation processes. There are several reports of use of IR spectroscopy to evaluate grafting of acrylic monomers onto natural materials as carboxymethyl cellulose (CMC) and chicken feathers (CF) (Martínez et al, 2003, 2005, Vasile et al, 2004 , Zohuriann-Mehr et al, 2005, Joshi and Sinha, 2006).

Now a days have been an increasing interest in obtation and use of polymeric materials using naturals sources from a diversity of systems, getting attention such biopolymers in research areas as medicine, electronics, textil, corrosion and nanotechnology among others. This rising interest is due variety of properties that offers on depending of chemical structure and source (Martínez et al, 2008).

Use of natural fibers is an research area that allows obtain materials for cotidiane applications, using more resistance materials and with outstanding properties and specially materials that are environmental friendly. Keratin is a natural protein which can be found in wool, hair, claws, horns or nails, and is the main component in birds’ feathers, represents from 5% to 7% of the body weight of chickens. Keratin is durable and resistant to organic solvents and chemically unreactive, which give benefits when is exposure to environmental conditions, thinking in industrial applications.

Chicken feathers (CF) are more abundant material of keratin in nature. Birds’ feathers characterize by a complex branched structure formed by keratinic filaments that grow in a unique mechanism in cylindric feather follicles. This branched structure is a distinctive characteristic in feathers morphology and its origin is biologic evolution (Xu et al., 2001, 2003).

CF are considered residues of a byproduct of poultry, corresponding to more than 5 million tons around the world (Barone et al 2005). Aminoacids content of CF on depend of breed, feeding and environment of study animals. The aminoacids present in CF are mainly aspartic acid, glutamic acid, arginine, proline, glycine, phenylalanine, alanine, cystine, isoleucine, among others (Schmidt 1998) (see table 1).

Functional group	Aminoacid	Content (as % mole)
Negatively charged	Aspartic acid	5
	Glutamic acid	7
Positively charged	Arginine	5
Conformationally special	Proline	12
	Glycine	11
Hydrophobic	Phenylalanine	4
	Alanine	4
	Cystine	7
	Valine	9
	Isoleucine	5
	Leucine	6
	Tyrosine	1
Hydrophilic	Threonine	4
	Serine	16

Table 1. Aminoacid content in keratin fiber from chicken feather (Martínez et al 2005).

Carboxymethyl cellulose (CMC) is a very important cellulose derivative and it is known by its superabsorbent properties. It is anionic polymer water soluble. It is produced by reaction of alkali cellulose and monochloroacetic sodium salt under strict reaction conditions (Klemm et al, 1998). Byproducts as sodium chloride and sodium glycolate are obtained in reaction whose are removed obtaining CMC sodium salt highly purified. Higher swelling capacity of CMC can be reached and controlled by addition of crosslinking agent, thermal treatment or ionic state conversion. The conversion of some hydroxyl groups from cellulose in hydrophobic substituents diminishes hydrogen bonding decreasing crystallinity and increasing water solubility. Multiple applications of Na-CMC in several areas of food industry made it and almost indispensable material. Other applications are: laundry detergent for instance, mainly as a viscosity modifier or thickener, as a soil suspension to deposit onto cotton and other cellulosic fabrics (Klemm et al, 1998). Other use of CMC is as a lubricant in non-volatile eye drops. These properties on depend of preparation method, degree of polymerization and degree of substitution (DS), that means how much of hydroxylic groups are displaced for carboxymethyl groups in anhydroglucose (AGU) structure. The more useful DS value is from 0.7 to 1.5. Due these CMC is an interesting material and properties can be modify by means graft reactions.

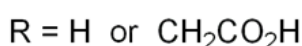
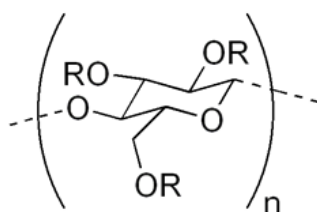


Fig. 1. Carboxymethyl cellulose structure.

Modification of natural protein through grafting have become in a widely used path and some works have carry out studies with acrylic monomers as ethylacrylate, acrylic acid, methacrylic acid and methyl methacrylate (Mostafa, 1995; Athawale and Rathi, 1997; Jia and Yong, 2006; Zampano, et al 2009), which carried out graft of natural fibers and proteins. However, just few of works report grafting of hydroxyethyl methacrylate onto naturals fibers (Joshi and Sinha, 2006) (Martínez et al., 2003, 2005).

Graft copolymerization of acrylic monomers onto natural polymers is one of the most useful paths to modify physicochemical properties on order to add new properties to final copolymer with minimal. Acrylic monomers are the most grafted monomers in this kind of research works; being acrylonitrile, acrylic acid, methyl methacrylate more studied species. Redox initiation is an efficient method frequently used to obtain graft copolymers. Polymers with OH groups can react with ceric ion or an oxidant agent to form polymer radical capable to initiate copolymerization. In a redox initiation frequently there is a minimum degree of homopolymerization, due only polymer radicals can be formed. However, limitation of this method is only useful with polymers with functional groups; there are reports of polymers with amides, urethane and nitrile groups by means redox initiation.

Several natural polymers as chitin, cellulose, functionalized cellulose and natural fibers are some of most studied natural polymers in graft copolymerization using redox system as initiator, being cerium ion one with more reports.

Infrared spectroscopy is a useful tool to identify functional groups through vibrational frequencies in polymers to evaluate changes in structure. This research was focused in graft copolymerization of Hydroxyethyl methacrylate (HEMA) onto chicken feathers fibers (CFF) and carboxymethyl cellulose (CMC), evaluating effect of reaction conditions (time reaction, monomer concentration, initiator concentrations) on grafting yield and probe presence of HEMA in copolymers by means Infrared Spectroscopy (IR).

2. Methodology

2.1 Raw materials

Chicken Feathers (CF) were obtained from a local slaughterhouse. A clean up procedure was carried out before use them for reaction, CF were washed several times with ethanol and dried at room temperature to have them clean white, sanitized and odor-free. Manual procedure by cutting to separate fibers from barbs and barbules was carried out. Chicken feather fiber (CFF) was used in graft copolymerization reaction.

CMC supplied by Sigma-Aldrich with 0.7 degree of substitution was used to graft reactions, without further purification process, same as all other reactivities: Hydroxyethyl methacrylate (HEMA) (98%) and malic acid, potassium permanganate (KMnO_4), hydrochloric acid (HCl), methanol, ethanol, sulfuric acid (H_2SO_4), Cerium ammonium nitrate were from Sigma-Aldrich. Distilled water was used as reaction medium and finally to wash the homopolymer residues.

2.2 CFF graft copolymers

To carry out the grafting reaction procedure proposed by Martínez-Hernández et al. (2003) was used. In this process the following reagents were used: 0.5 g CFF, distilled water, malic acid (0.005M), sulfuric acid (0.01M), KMnO_4 (0.003M) and HEMA monomer in 3 different levels (0.025, 0.05 and 0.075 M).

The substances were mixed at a temperature of 60 °C, under constant magnetic stirring. The reactions were carried out at three different times: 2, 3 and 4 h. Once the reaction time passed, proceeded to filter and wash the reaction product with hot water and methanol in order to remove residual monomer and homopolymer. Grafting yield was evaluated after a hydrolysis to reaction product, to determinate HEMA amount grafted in CFF. Hydrolysis procedure was: 1g of CFF grafted was swamped in a 6M HCl solution at 130°C for 24 hours in a soxhlet extractor. Once the time was over, hydrolysis residue was dried and weight was determinate, this weight correspond to grafted HEMA, due CFF decompose in hydrolysis process.

Grafted polymer percentage was determinate according with next formulae (Martínez-Hernández et al, 2003; Gupta and Sahoo, 2001):

$$\% \text{Grafted PHEMA} = (X_4 / X_1) * 100 \quad (1)$$

Where:

X_1 = CFF without modification

X_2 = container

X_3 = container + hydrolyzed sample

X_4 = residual polymer after hydrolysis ($X_3 - X_2$)

2.3 CMC graft copolymers

2.3.1 Initiator preparation

It was necessary preparation of Ce (IV) initiator solution. The required amount of cerium ammonium nitrate salt (0.1, 0.25 and 0.5 M), was dissolved in 100 mL of 1M HNO₃ solution. This solution was stored under refrigeration in an amber bottle.

2.3.2 Grafting reaction

For graft reaction 5 g of CMC were placed in the reactor with distilled water and HEMA was added in selected amount (3 levels: 0.2, 0.3 or 0.4 M), under constant stirring at 70 ° C, the initiator was added and reaction was carried out for 3 hours. Once the reaction ends, it was necessary to neutralize the mixture with a NaOH 10% w/w solution, copolymer was precipitated with acetone, and then the material was milled and washed with 90% methanol solution, then dried. After, the material was subjected to a Soxhlet extraction with methanol, in order to extracting the material that has not been in the copolymer. Grafting yield was determinated by weight differences according with next formulae:

$$\text{Grafting yield} = ((W_1 - W_0)/W_0) * 100 \quad (2)$$

Where:

W_0 = CMC weight

W_1 = graft copolymer weight

2.4 IR Spectroscopy characterization of graft copolymers

IR analysis was carried out to evaluate structural changes of CMC and CFF and its grafted copolymers, by means main functional groups signals. IR spectra were recorded with a Perkin-Elmer Spectrum One Fourier Transform IR spectrophotometer, using an Attenuated Total Reflectance (ATR) accessory, with ZnSe plate, using 12 scans and resolution of 4 cm⁻¹, ranging from 4000 to 600 cm⁻¹.

3. Results and discussion

Results will be presented in sections following next order. First, the effect of HEMA concentration and time reaction over grafting yield onto CFF and HEMA and CAN concentration over grafting yield onto CMC. Next, IR spectra are presented for ungrafted and grafted CFF, as well as a comparison of IR spectra for different grafting yield of CFF. Also for CMC grafted and ungrafted are presented the IR spectra and comparison to different grafting yield of HEMA onto CMC.

3.1 CFF-g-HEMA copolymer

Effect of reaction conditions over graft yield were studied. Table 2 shows effect of time reaction and HEMA concentration on grafting yield of CFF, initiators concentration was constant. It can observe that an HEMA concentration increase causes an increase of grafting yield value at 3 reaction times studied. That increase is due the amount of free radicals formed in reaction system, and CFF main component is keratin which posses several pendant functional groups, for like -NH₂, -COOH, -SH and -OH, which can form active sites where HEMA can be grafted. This behavior was also reported by Martínez et al (2003) that observed a maximum graft yield value. A higher HEMA concentration value was studied (1 M), but a fiber saturation was observed and also homopolymerization predominated over graft reaction which is not convenient for this study. Scanning Electron Microscopy shows the HEMA cover the CFF structure growing poly HEMA chains (figure 2) on fiber surface of CFF, but CFF reached a saturation of active sites and then homopolymerization happens.

Time/hr	HEMA conc./ M	Grafting yield
2	0.075	85%
2	0.05	70%
2	0.025	26%
3	0.075	92%
3	0.05	83%
3	0.025	57%
4	0.075	76%
4	0.05	38%
4	0.025	35%

Table 2. Effect of time reaction and HEMA concentration on grafting yield over CFF

The increase on graft yield can be also attributed that keratin forms a charge transfer complex with HEMA molecules, so it is possible increase monomer activity at higher concentrations of HEMA which leads homopolymerization.

Other reports (Joshi and Sinha, 2006) indicate an increase of monomer concentration increases graft yield, until a maximum and then decreases the obtained percentage. The difference is attributed to graft yield diminish due the saturation of available active sites in CFF. Also on depend of initiator system.

In the other hand, it can observe that when reaction time increase the graft yield has a maximum value at 3 h and then decrease at 4 h. this behavior is attributed that at higher reaction time the keratin pendant groups open and diffusion of HEMA molecules into the structure occurs, allowing the grafting on keratin structure not only on surface of CFF. However, when reaction time is high, a denaturalization of keratin can occurs, due the acidic medium where reaction is carried out, leading to HEMA homopolymerization. Other reason of this decrease in grafting yield is a reduction in number of free radicals available for grafting as the reaction proceeds, creating a saturation of active sites.

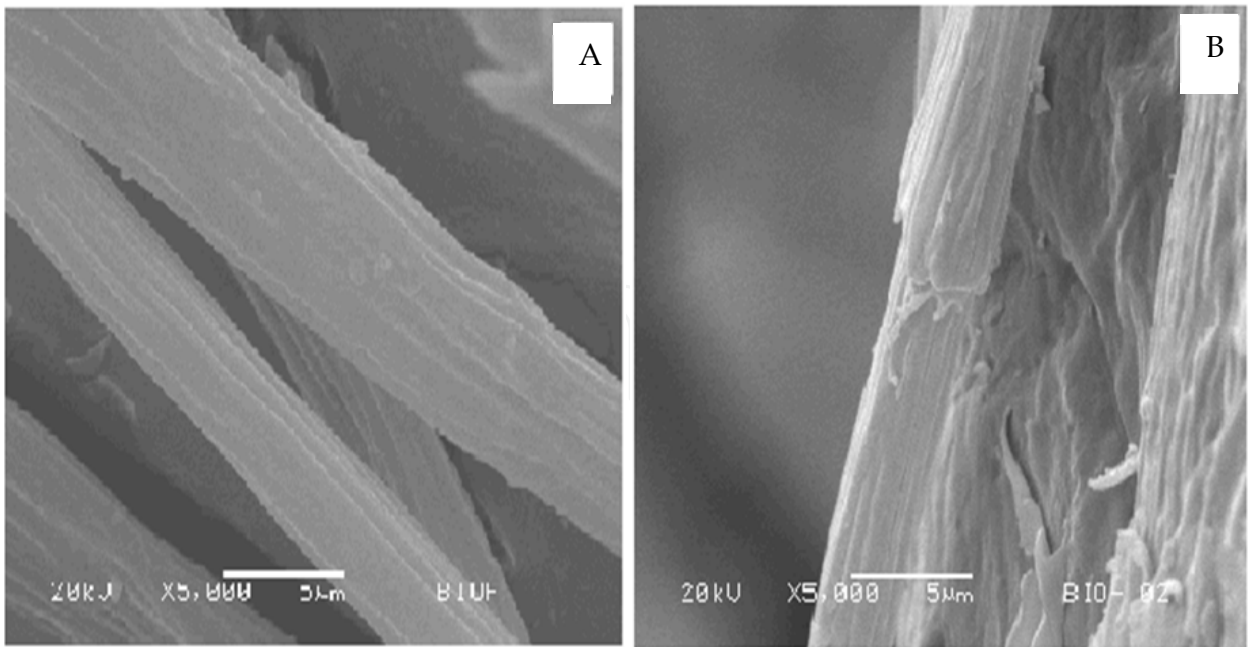


Fig. 2. SEM micrograph of CFF unmodified and CFF-g-HEMA

3.2 CMC-g-HEMA copolymer

The graft copolymerization of HEMA onto CMC had differences compared with HEMA-g-CFF: Redox initiator system used was CAN and was conducted at 3 different concentrations; HEMA concentrations values were higher 0.05, 0.1 and 0.15M and reaction time was constant in 3 hours. Table 3 resumes the effect of HEMA and CAN concentrations over grafting yields values of CMC.

CAN [M]	HEMA [M]	Grafting yield
0.1	0.05	69 %
0.1	0.1	207 %
0.1	0.15	249 %
0.25	0.05	88 %
0.25	0.1	214 %
0.25	0.15	275 %
0.5	0.05	82 %
0.5	0.1	179 %
0.5	0.15	397 %

Table 3. Effect of CAN and HEMA concentration on grafting yield over CMC

Graft copolymerization of HEMA onto CMC present a similar behavior as grating of HEMA onto CFF, grafting yield increased continuously with increase in concentration of HEMA when CAN concentration was constant, and reaches a maximum value with 0.15M at 3 CAN concentrations. This behavior could be explained by the fact of that an increase of HEMA

concentration lead to the accumulation of monomer molecules in close proximity to CMC backbone. There are reports of modified polysaccharides grafted with HEMA that found with CAN concentration of 0.2 M around 200% attributed the primary radicals attack the monomer instead of reacting with backbone polymer (Joshi and Sinha 2006). In this research higher CAN concentration studied was 0.5 M and higher grafting yield was reached with this concentration (397%). Also lower CAN concentrations (0.05 M) were tried in this research work, but not good results in grafting yield was obtained

In the other hand, about effect of CAN concentration over grafting yield, results show the initial increase in grafting yield with increase in initiator concentration levels off and decrease with further increase in CAN concentration. It would seem that termination of graft copolymerization would proceed by the reaction of the growing graft polymer chain with ceric ions, the reaction point is OH groups of anhydroglucose, which form a complex with ceric ion. This complex may dissociate, and giving rise to free radical sites onto the polysaccharides backbone and these radicals initiate the graft copolymerization (Zohuriaan-Mehr et al 2005).

Figure 3 shows SEM micrographs of CMC unmodified and CMC-g-HEMA. It is clearly seen that CMC morphology was totally modified by grafting of HEMA. CMC shows a porous surface which is covered by grafting of HEMA resulting in a homogeneous surface, which suggests that HEMA chains formed are long.

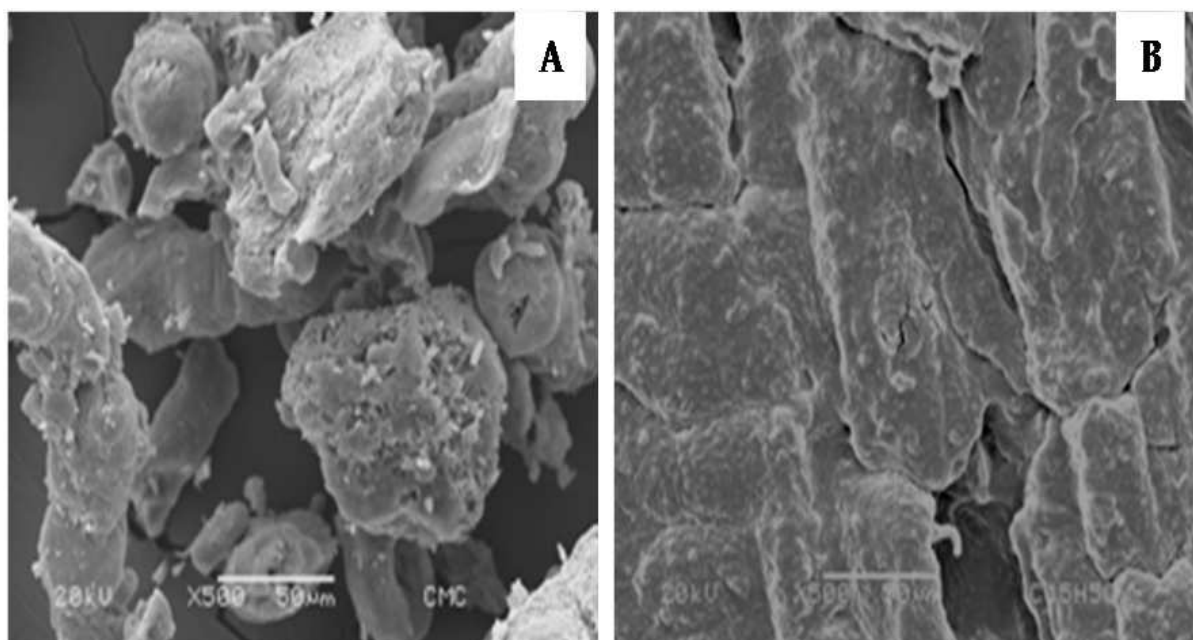


Fig. 3. SEM micrograph of CMC unmodified (A) and CMC-g-HEMA (B).

3.3 IR of CFF-g-HEMA copolymer

IR spectroscopy is a useful technique to evaluate if a graft reaction is carried out and also to evaluate grafting yields on graft copolymers. Several research works used IR with this purpose for keratin (Martínez et al 2003; Martínez et al 2008; Kavitha et al 2005), starch (Meshram et al 2009), modified starch (Cao et al 2002), chitosan (Mun et al 2008), carboxymethyl chitosan (Joshi and Sinha, 2006), cellulose (Zampano et al 2009),

carboxymethyl cellulose (Okieimen and Ogbeifun, 1996; Vasile et al , 2004), ethyl cellulose (Kang et al 2006), which make it a powerfull tool in graft reactions.

Figure 4 shows IR spectrum of CFF, which main component is keratin, a mixture of aminoacids as serine, proline, glycine, valine, cysteine, and others. The main vibrations attributed to CFF structure were identified according to wavenumber. The region around 3300 cm^{-1} corresponds to range of amide bands, figure shows a peak in 3297 cm^{-1} associated with ordered regions of NH group of amide A α -helix conformation, peak in 2945 cm^{-1} is assigned to the assimetric vibration of CH group od metyl, the strong band at 1715 cm^{-1} is matched with vibration of amide I of β -sheet conformation, band at 1650 cm^{-1} assigned to C=O group of amide I α -helix conformation, peaks at 1520 , 1449 and 1243 cm^{-1} attributed to in plane bending of NH group corresponding to β -sheet conformation, bending of CH_3 group and CN group of amide III respectively; vibrations on 1136 , 1074 and 1023 cm^{-1} corresponds to assigned to vibrations of C-C group; and finally a peak around 700 cm^{-1} attributed to vibration of C-S group.

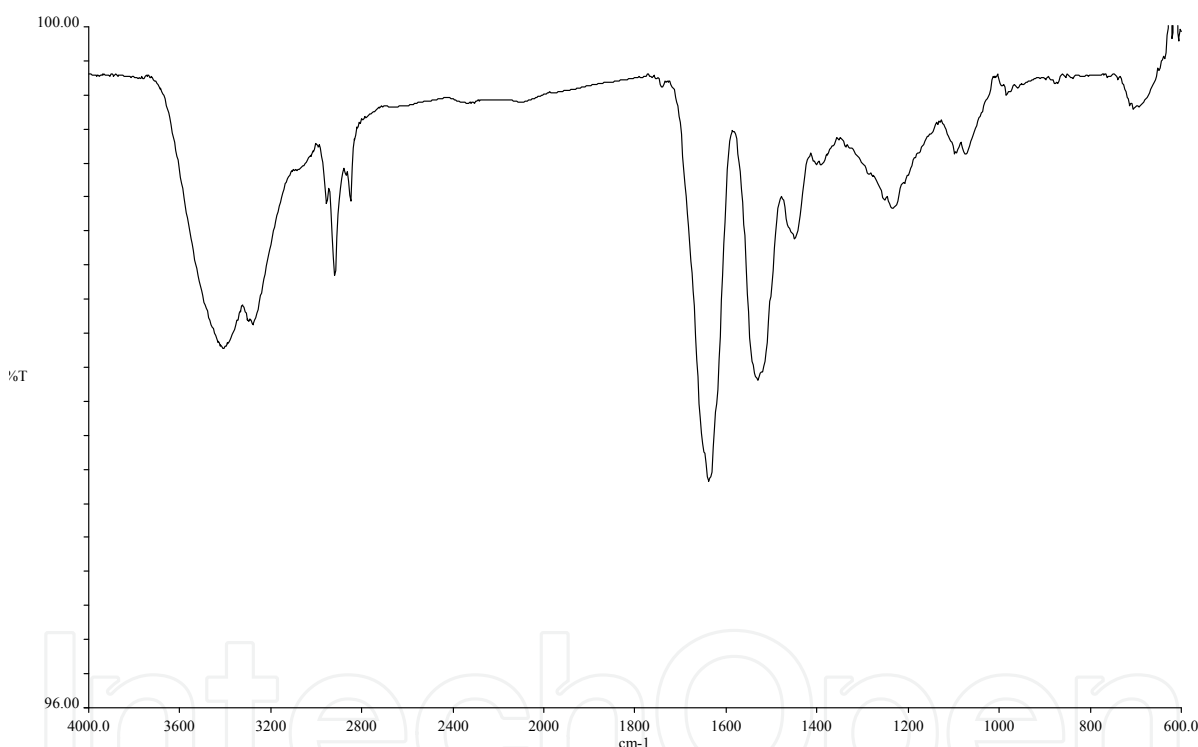


Fig. 4. IR spectrum of CFF ungrafted.

In figure 5 presents IR spectrum of PHEMA which presents signals at 3394 cm^{-1} attributed to vibration of OH group, 2956 cm^{-1} from antisymmetric vibration of CH_2 and CH_3 , 2925 cm^{-1} symetric vibration of CH_2 , CH_3 , 2855 cm^{-1} symetric vibration of CH_2 , 1720 of stretching C=O, a small shoulder around 1652 from stretching C=C, 1369 cm^{-1} deformation of CH_2 , CH_3 , a shoulder at 1260 cm^{-1} from C=O stretiching, 1164 vibration of C-O-C, 1048 stretching of CO(H), and 771 cm^{-1} CH_2 coupled with skeletal stretching. This is according for reports of IR spectrum of PHEMA (Prachayawarakorn and Boonsawat, 2007).

Figure 6 shows IR spectra of CFF and CFF grafted. It can observe that main differences are IR spectrum of CFF grafted show peaks at 1720 , 1160 and a shoulder around 1260 cm^{-1} ,

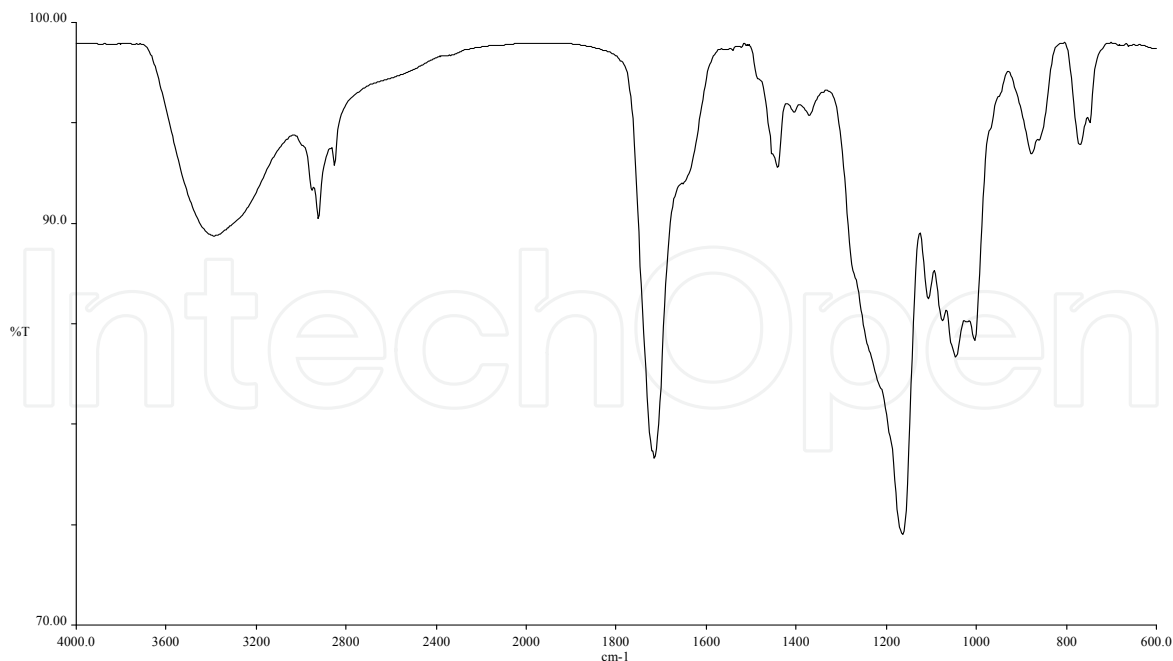


Fig. 5. IR spectrum of PHEMA

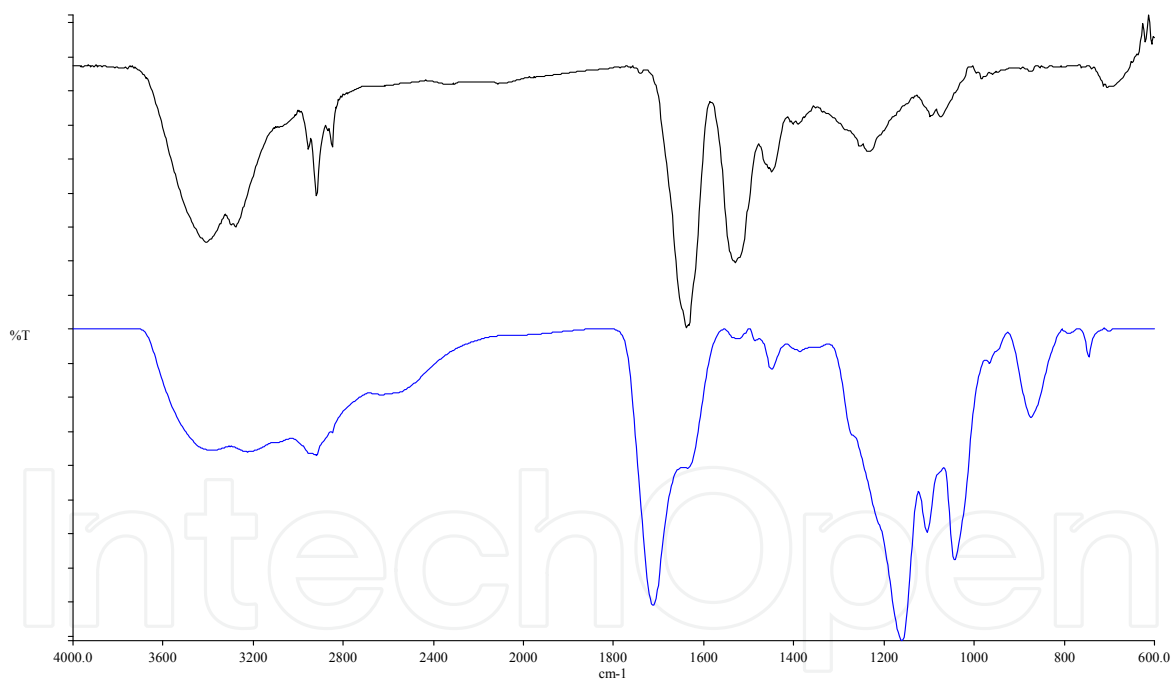


Fig. 6. IR spectra of ungrafted CFF (black) and CFF grafted (blue).

attributed to groups C=O and OH respectively, from HEMA structure, and which does not appear in CFF spectrum, which can indicate the graft reaction is carried out. CFF posses functional groups as -NH₂, -SH nd -OH where graft reaction can carry out, signals from NH (3290, 3080 and 1525 cm⁻¹) changed which make it suppose that graft reaction is taking place between NH groups and free radical of HEMA.

A comparison of main assignments from functional groups of ungrafted CFF and PHEMA are sumarized in table 4.

PHEMA		CFF (Martínez 2005)	
Approximate assignments, cm ⁻¹	Functional groups	Approximate assignments, cm ⁻¹	Functional groups
3390	v (OH)	3290	v (NH) Amide A, α-helix
2950	v _a (CH ₃ , CH ₂)	3080	v _a (NH) Amide B
2920	v _s (CH ₃ , CH ₂)	2930	v _a (CH ₃)
2850	v(CH ₂)	1700	v(C=O) Amide I β-sheet
1720	v(C=O) ester group	1650	v(C=O) Amide I α-helix
1650	(C=C) stretching	1525	δ(N-H) Amide II β-sheet
1370	δ (CH ₃ , CH ₂)	1450	δ _α (C-H ₃)
1260	C=O stretching	1230	v(CN) Amide III
1160	v (C-O-C)	1170	v(C-C)
1050	(CO-H) stretching	1075	v(C-C)
770	(CH ₂) stretching	685	v(C-S)

Table 4. IR assignments of main functional groups of ungrafted CFF and PHEMA.

In figure 7 present IR spectra of CFF grafted with 26 and 92% grafting yield, to evaluate effect of grafting on main assignments. It is evident when graft yield increase assignments attributed to PHEMA are more evident in spectrum (3390 and 1720, 2850 and 1150 cm⁻¹), and signals attributed to NH and OH groups disappear because there are sites where graft reaction is carrying out. There are several reports about use of IR spectroscopy for evaluate graft reactions on keratin from chicken feathers (Martínez et al 2003, Martínez et al 2008 and Kavitha et al 2005). They use IR spectra for identify main functional gruops of keratin and of acrylic monomers in ungrafted and grafted copolymers, in addition to evaluate changes in peaks according with grafting yield.

3.4 IR of CMC-g-HEMA copolymer

IR spectra of the CMC is presented in figure 8. We can notice the characteristic broad band attributed to OH stretching vibration at 3360 cm⁻¹ due the CMC has a degree of substitution of 0.7, in average 2.3 OH groups of anhydroglucose ring are present in structure, peak at 2920 cm⁻¹ due stretching of C-H, peak at 1620 cm⁻¹ a strong absorption band that confirm the presence of carboxy group (COO-), 1420 and 1320 cm⁻¹ are assigned to -CH₂ scissoring and hydroxyl group bending vibration respectively, signal at 1060 cm⁻¹ is due to >CH-O-CH₂ stretching vibration and vibrations of the ether groups at 1060, 1110 cm⁻¹. It is worth to remark that CMC used in this work was as sodium salt. The assignments are according with reports of IR spectroscopy studies of CMC (Bono et al 2009, Heydarzadeh et al 2009, Vasile et al 2004).

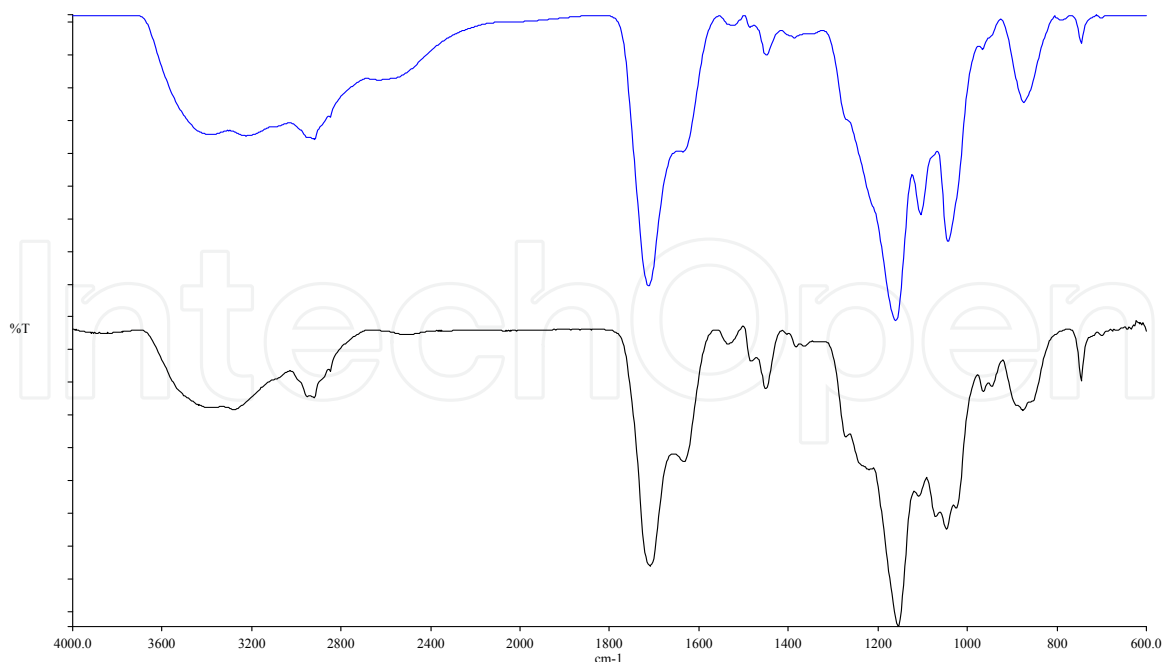


Fig. 7. IR spectra of CFF-g-HEMA with 26 (blue) and 92% (black) graft yield

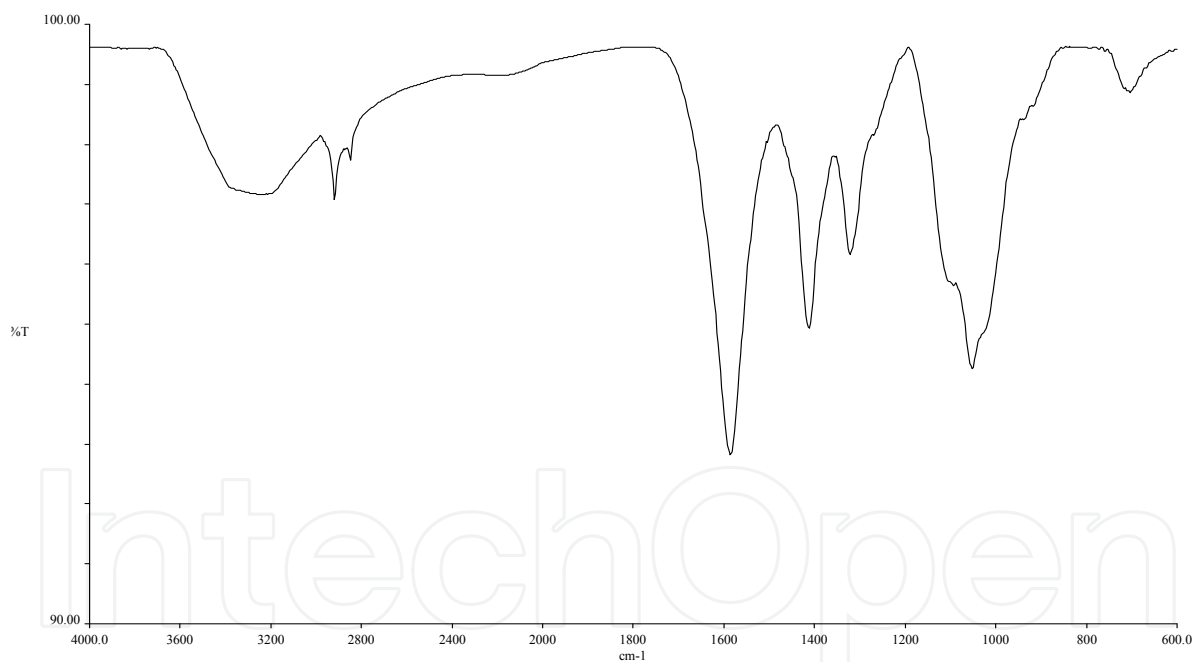


Fig. 8. IR spectrum of CMC

Figure 9 presents a comparative of IR spectra of CMC ungrafted and grafted with HEMA. Before, the main assignments for PHEMA were discussed. The CMC grafted present appearance of peak at 2930 cm^{-1} due antisymmetric stretching CH_3 , 1715 cm^{-1} from stretching vibration of $\text{C}=\text{O}$ and peaks at 1410 , 1250 , 1150 and 902 cm^{-1} assigned to bending vibration of CH_3 , CH_2 , stretching of $\text{C}=\text{O}$, stretching of CO-H and $=\text{CH}_2$ groups respectively, all those signals are from PHEMA structure, and also peaks attributed to CMC structure as 3340 , 1650 and broad peak at 1050 cm^{-1} , which are from OH , COO^- and C-O-C from CMC, which is indicative the graft reaction was carried out.

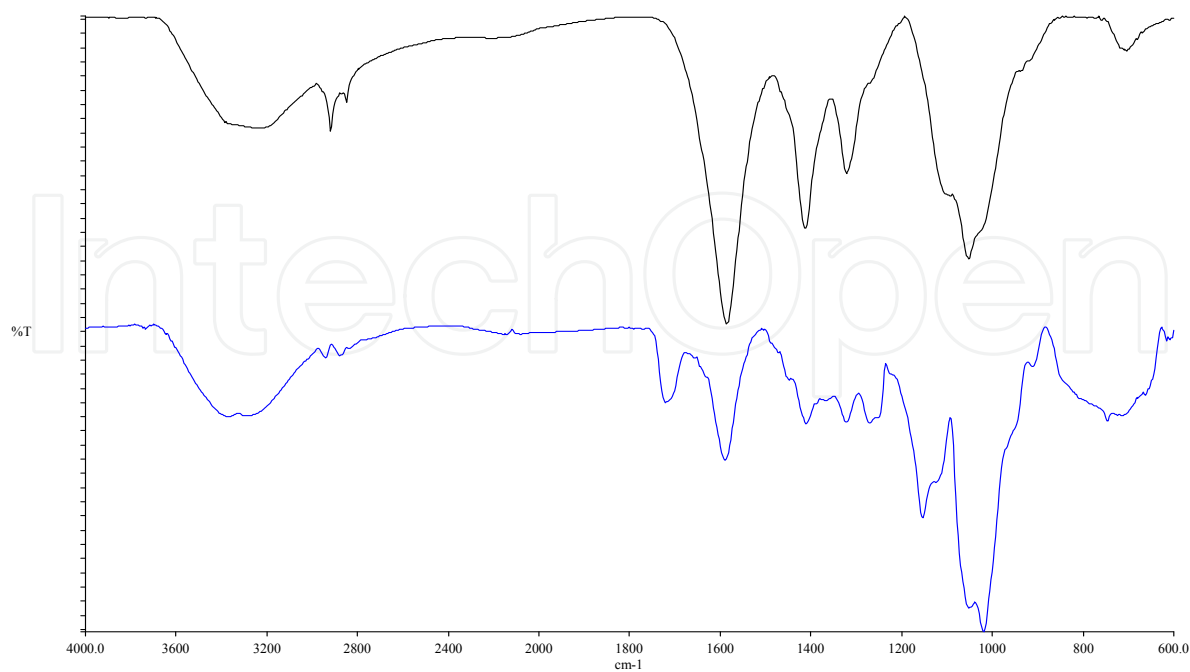


Fig. 9. IR spectra of CMC ungrafted (black) and with 69% grafting yield (blue)

Effect of grafting yield on IR assignments was evaluated in figure 10. It can observe that main peaks attributed to PHEMA (2940, 1720, 1270 and 1060 cm^{-1}) increase according with graft yield, which makes sense due the PHEMA is an higher proportion compared with CMC.

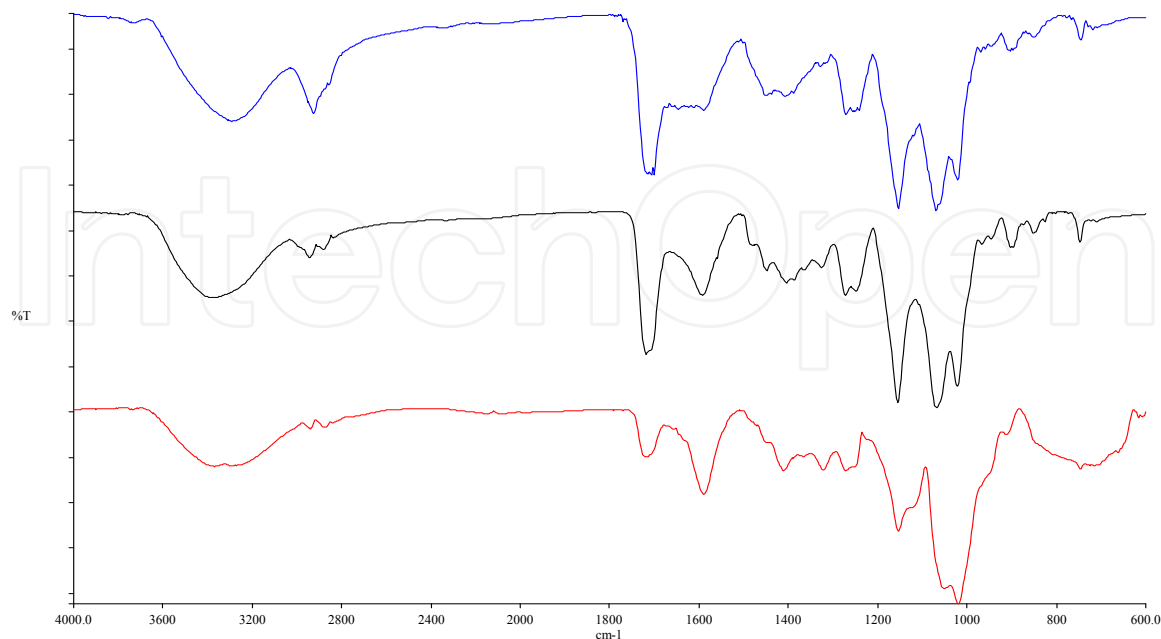


Fig. 10. IR spectra of CMC grafted with 69% (red), 249 % (black) and 397% (blue) grafting yield.

4. Concluding remarks

From results presented in this research work it can be conclude that is possible graft HEMA onto CFF and CMC using different initiator system, and that grafting yield obtain on depends of kind of initiator system, being higher with CAN initiator system than KMnO₄ malic acid. SEM microgrpah give evidence that the grafting reaction takes place on CFF and CMC surface until a saturation of active sites, and then homopolymerization happens.

One of the most common applications of IR spectroscopy is to the identification of organic compounds. In polymers, may be used to identify the composition, to monitor polymerization process, to characterize polymer structure. In present research, IR spectroscopy showed to be a usefull toll to evaluate if the graft reaction takes place. It was possible identify main functional groups of CFF and CMC ungrafted, and PHEMA. The IR spectra of grafted copolymers presented assignments due to ungrafted materials and PHEMA giving evidence that the graft reaction carried out. Futhermore, it was possible evaluate the changes in peaks of the grafted materials according with grafting yield, increasing signals attributed to PHEMA.

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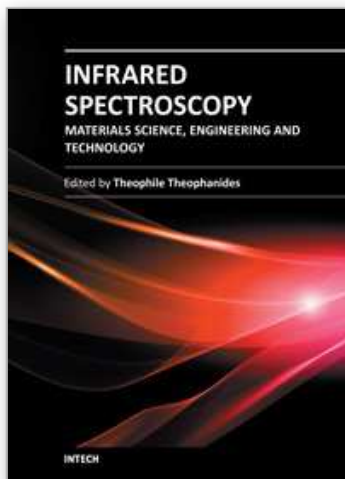
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Phone: +86-21-62489820
Fax: +86-21-62489821

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