CHAPTER 3
RESEARCH METHODOLOGY
CHAPTER – 3

RESEARCH METHODOLOGY

3.1 Introduction

This chapter deals with the descriptions of relevant materials, equipments and their experimental techniques used in the present research work.

- Materials and Methods

- Synthesis
  - Synthesis of Cross-linked Hydrogels in Air
  - Microwave Induced Synthesis of Cross-linked Hydrogels
  - Synthesis of Cross-linked Hydrogels under Vacuum

- Characterization Techniques
  - UV-Visible Spectroscopy
  - Fourier Transforms Infrared (FTIR) Spectroscopy
  - Scanning Electron Microscopy (SEM)

- Properties
  - Swelling Behavior
  - Current-Voltage Characteristics
  - Thermal Studies

- Applications
  - Dye Removal Studies
  - Soil Water Retention Studies

- Biodegradation Studies
  - Soil Burial Method
  - Composting Method
3.2 Materials and Methods

3.2.1 Chemical Used

The various chemicals used during present experimental work are listed in the table 3.1. Their sources of procurement are mentioned against their names.

**Table 3.1: Chemical used During Studies along with Source of Procurement**

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Acronym</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Guar Gum</em> [Backbone]</td>
<td>Ggum/GG</td>
<td>LOBA-CHEMIE</td>
</tr>
<tr>
<td>Ammonium persulfate</td>
<td>APS</td>
<td>LOBA-CHEMIE</td>
</tr>
<tr>
<td>Hexamine [Cross-linker]</td>
<td>-</td>
<td>LOBA-CHEMIE</td>
</tr>
<tr>
<td>Acrylic Acid [Monomer]</td>
<td>AA</td>
<td>MERCK</td>
</tr>
<tr>
<td>Itaconic Acid [Monomer]</td>
<td>IA</td>
<td>MERCK</td>
</tr>
<tr>
<td>Hydrochloric Acid</td>
<td>HCl</td>
<td>MERCK</td>
</tr>
<tr>
<td>Sodium Hydroxide</td>
<td>NaOH</td>
<td>SIGMA-ALDRICH</td>
</tr>
<tr>
<td>Aniline [Monomer]</td>
<td>ANI</td>
<td>LOBA-CHEMIE</td>
</tr>
<tr>
<td>N-methylpyrrolidone</td>
<td>NMP</td>
<td>MERCK</td>
</tr>
<tr>
<td>Methylene Blue</td>
<td>MB</td>
<td>SIGMA-ALDRICH</td>
</tr>
<tr>
<td>Acetone</td>
<td>-</td>
<td>LOBA-CHEMIE</td>
</tr>
</tbody>
</table>

Different soil samples (dry and crushed) were collected from the Solan (Himachal Pradesh) India for the study of moisture retention. Compost was collected from the site of domestic waste effluent discharge at NIT-Jalandhar (Punjab). Soil for the biodegradation studies under soil burial method was collected from garden, NIT-Jalandhar (Punjab). Electronic balance (LIBROR AEG-220 Shimadzu, Japan) was used for weighing of samples. All the chemicals used are AR grade and their purity ensured is more than 99%. The distilled water was used for all the reactions.
3.2.2 Equipment Used

3.2.2.1 Hot Air Oven
The hot air oven (Navyug, India, Q-5247) was used to carry out reaction in air. The hot air oven is used to maintain the temperature during reactions.

3.2.2.2 Microwave
The reactions were carried out in microwave equipment (IFB, 30SC2) at 2450 MHz and 850 W microwave power. The reaction mixture was irradiated for different time duration at different microwave power ranging from 20-100%.

3.2.2.3 Vacuum Oven
The reaction was carried out in vacuum oven (Lobco, India, ANISO9001) by maintaining its vacuum from 350-550 mm/Hg. The reactions were carried out under vacuum while maintaining temperature conditions.

3.2.3 Technical Program /Details

The following technical program of work was conducted during course of investigation:

3.2.3.1 IPN Preparation with Different Vinyl Monomers under Different Reaction Conditions
Grafting of different vinyl monomers such as acrylic acid (AA) and itaconic acid (IA) onto Guar gum with interpenetration of polyaniline in the matrix was carried out under different reaction conditions like in air, under the influence of microwave radiations and under vacuum.

3.2.3.1.1 In-Air Synthesis

3.2.3.1.1.a Synthesis of Semi-Interpenetrating Networks (Semi-IPNs)
A polymer matrix composed of Guar gum and vinyl monomers i.e. Ggum-cl-poly(AA) and Ggum-cl-poly(IA) were prepared by using hexamine as crosslinker and
APS as initiator in hot air oven. To get the graft copolymer Guar gum (1.0 g) was mixed in 20 ml of distilled water in a 100 ml beaker. To the reaction mixture a calculated amount of APS as an initiator and hexamine as cross-linker were added followed by drop wise addition of vinyl monomers with continuous stirring. The reaction mixture was continuously stirred to get homogeneity. The reaction mixture was kept in hot air oven at 60 °C for fixed time period to get the product. The resulting product was washed with distilled water in order to remove unreacted homopolymer. Finally, the homopolymer free product was dried in the hot air oven at 50 °C. Various reaction parameters such as monomer concentration, initiator concentration, cross linker concentration, polymerization time, reaction temperature, amount of solvent and pH of reaction mixture were optimized so as to get the candidate polymer with maximum water absorption capacity or percentage swelling ($P_S$) and $P_S$ was calculated as per Eq. 3.1 and results are depicted in Section 4.2.1.1.b, Figs. 4.1a-g and 4.2a-g under Chapter 4.

3.2.3.1.1.b Synthesis of Conducting Interpenetrating Networks (IPNs)

Semi-IPNS prepared under Section 3.2.3.1.1.a were converted into conducting IPNs using PANI. For each experiment calculated amount of semi-IPN (1.0 g) was added to the aqueous medium containing known volume of aniline monomer in a 150 ml beaker. The resulting reaction mixture was kept for 16 h at room temperature, which resulted in the absorption of aniline monomer in the semi-IPN and the formation of swollen sample. Pre-optimized amount of APS and hexamine was added to this reaction mixture with continuous stirring.

The light brown colour of the reaction solution changed to greenish on addition of pre-optimized amount of APS as thermal initiator slowly to the reaction mixture during the polymerization. As the polymerization progresses, protonation of the PANI chains takes place and a change in colour was observed. The resulting reaction mixture was again kept in a hot air oven at 60 °C for a pre-optimized time to get the IPN. The resulting product was washed with 1-methyl-2-pyrrolidone (C$_5$H$_9$NO) in order to remove homopolymer. Finally, the homopolymer free product was dried in vacuum oven at 50 °C. Optimization was done with respect to aniline concentration so as to get product with maximum swelling capacity (Chapter 4, Section 4.2.1.1.c, Figs. 4.3a and c). The synthesis of the conducting IPN was carried out in similar
fashion containing 0.5N aqueous acidic solution of HCl in the reaction flask instead of the neutral medium (Chapter 4, Section 4.2.1.1.c, Figs. 4.3b and d). Optimization of HCl concentration was also done so as to get the IPN with better conductivity (Chapter 4, Section 4.2.1.1.d, Figs. 4.4a-e).

3.2.3.1.2 Synthesis under the Influence of Microwave Radiations

3.2.3.1.2.a Synthesis of Semi-Interpenetrating Networks (Semi-IPNs)

Semi-IPNs of Guar gum such as Ggum-cl-poly(AA) and Ggum-cl-poly(IA) were prepared by using hexamine as crosslinker and APS as initiator under the influence of microwave radiations. Guar gum (1.0 g) was mixed in 20 ml of distilled water in a reaction flask. To this reaction mixture, a calculated amount of APS as an initiator and hexamine as cross-linker were added followed by drop wise addition of vinyl monomers with continuous stirring. The reaction mixture was continuously stirred to get homogeneity.

The reaction mixture was kept in microwave equipment at 100% microwave power (850 W) for fixed time period to get the product. The resulting product was washed with distilled water in order to remove unreacted homopolymer. Finally, the homopolymer free product was dried in the hot air oven at 50 °C until a constant weight was obtained. Various reaction parameters such as monomer concentration, initiator concentration, cross linker concentration, polymerization time, microwave power, amount of solvent and pH of reaction mixture were optimized so as to get the candidate polymer with maximum water absorption capacity or percentage swelling (Pₛ) (Chapter 4, Section 4.2.1.2.b, Figs. 4.11a-g and 4.12a-g).

3.2.3.1.2.b Synthesis of Conducting Interpenetrating Networks (IPNs)

Ggum-cl-poly(vinyl monomers-ipn-ANI) conducting hydrogels were synthesized by using Ggum-cl-poly(vinyl monomers) as a flexible matrix. Semi-IPN (1.0 g) was added to an aqueous medium with a known amount of aniline. The resulting mixture was kept for 16 h at room temperature so as to allow the swelling of semi-IPN and penetration of aniline monomer into the polymer matrix. Reaction was carried out under preoptimized reaction conditions using hexamine as cross-linker and APS as an
initiator, under microwave radiations for a pre-optimized time to get the IPN. The
colour of the reaction mixture changed from slight brown to greenish with the
progress of the reaction.

The resulting product was washed with 1-methyl-2-pyrrolidone (C₅H₉NO) in order to
remove homopolymer. Finally, the homopolymer free product was dried in vacuum
oven at 50 °C. Optimization was done with respect to aniline concentration so as to
get product with maximum swelling capacity (Chapter 4, Section 4.2.1.2c, Figs.
4.13a and c). The synthesis of the conducting IPN was carried out in similar fashion
containing 0.5N aqueous acidic solution of HCl in the reaction flask instead of the
neutral medium (Chapter 4, Section 4.2.1.2c, Figs. 4.13b and d). Optimization of
HCl concentration was also done so as to get the IPN with better conductivity
(Chapter 4, Section 4.2.1.2.d, Figs. 4.14a-e).

3.2.3.1.3 Under Vacuum Synthesis

3.2.3.1.3.a Synthesis of Semi-Interpenetrating Networks (Semi-IPNs)

Preparation of Ggum-cl-poly(vinyl monomers-ipn-ANI) conducting hydrogel is
synthesized by two-step polymerization. Firstly the superabsorbent based on Guar
gum and vinyl monomers was prepared using hexamine as cross-linker and APS as an
initiator under vacuum at 60 °C for different time intervals. In a typical experiment,
1.0 g Guar gum was dissolved in 20 ml distilled water in a reaction flask. To the
reaction mixture a calculated amount of APS as an initiator and hexamine as cross-
linker were added followed by drop wise addition of vinyl monomers with continuous
stirring. The reaction mixture was continuously stirred to get homogeneity. The
reaction mixture was kept in vacuum oven at 60 °C for fixed time period to get the
product. The resulting product was washed with distilled water in order to remove
unreacted homopolymer. Finally, the homopolymer free product was dried in the hot
air oven at 50 °C. Various reaction parameters such as monomer concentration,
ininitiator concentration, cross linker concentration, polymerization time, reaction
temperature, amount of solvent, vacuum and pH of reaction mixture were optimized
so as to get the candidate polymer with maximum water absorption capacity or
percentage swelling ($P_S$) (Chapter 4, Section 4.2.1.3.b, Figs. 4.21a-h and 4.22a-h).
3.2.3.1.3.b Synthesis of Conducting Interpenetrating Networks (IPNs)

In case of each experiment a calculated amount of semi-IPN prepared under section 3.2.3.1.3.a was added to the aqueous medium with a known amount of aniline monomer. The resulting mixture was kept for 16 h at room temperature, for the absorption of aniline monomer in the swollen semi-IPN matrix. Pre-optimized amount of APS and hexamine was added to this reaction mixture with continuous stirring. The colour of the reaction mixture changed from slight brown to greenish with the progress of the reaction. The reaction was carried out under vacuum at 60 °C to complete the polymerization process.

The resulting product was washed with 1-methyl-2-pyrrolidone (C$_5$H$_9$NO) in order to remove homopolymer. Finally, the homopolymer free product was dried in vacuum oven at 50 °C. Optimization was done with respect to aniline concentration so as to get product with maximum swelling capacity (Chapter 4, Section 4.2.1.3c, Figs. 4.23a-d). The synthesis of the conducting IPN was carried out in similar fashion containing 0.5N aqueous acidic solution of HCl in the reaction flask instead of the neutral medium. Optimization of HCl concentration was also done so as to get the IPN with better conductivity (Chapter 4, Section 4.2.1.3.d, Figs. 4.24a-e).

3.2.4 Swelling Studies of Hydrogel

Various reaction parameters were optimized with respect to percentage swelling ($P_s$). The swelling ratio was defined as the ratio (wet weight - dry weight)/(dry weight). Swelling behavior of the hydrogels was investigated in distilled water. After definite time intervals, samples were wiped and weighed. The process was repeated until equilibrium was achieved. After weighing the swollen hydrogel on an analytical balance (accuracy ± 0.00001 g), percentage swelling ($P_s$) was calculated by using the following equation (Pourjavadi and Mahdavinia, 2006; Singh et al., 2007)

$$P_s = \frac{W_s - W_d}{W_d} \times 100 \quad (3.1)$$

Where, $W_s$ and $W_d$ are the weight of swollen and dry hydrogel, respectively.
The percentage grafting was calculated by using the following equation (Kalia and Kaith, 2008)

\[
P_g = \frac{W_f - W_b}{W_b} \times 100
\]

(3.2)

Where, \( W_f \) and \( W_b \) are the weight of the functionalized polymer and backbone polymer, respectively.

### 3.2.5 Preparation of Solutions of different pH

The solutions with different pH values were prepared by using HCl (0.1 N) and NaOH (0.1 N) solutions. Alkaline solutions were prepared with NaOH pellets and acidic solutions were prepared by the addition of HCl. The pH value of the solutions was determined by a pH meter (Cyberscan 1100, EUTECH INSTRUMENTS).

### 3.3 Characterization Techniques

#### 3.3.1 Fourier Transform Infra-red Spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectra of backbone and cross-linked samples were recorded by Perkin Elmer FTIR spectrophotometer using KBr pellets in Punjab University, Chandigarh. FTIR spectra of the samples were analyzed in the wave number range of 450-4000 cm\(^{-1}\) with a resolution of 2 cm\(^{-1}\). Fourier transform infrared spectrophotometer (FTIR) can be used to identify the structure of unknown composition or its chemical group and the intensity of the absorption spectra associated with molecular composition of the chemical group (Surewicz et al., 1993).

#### 3.3.2 Scanning Electron Microscopy (SEM)

The surface morphologies of the backbone and cross-linked samples were studied with the help of Scanning electron microscopy (SEM). SEM studies were made on
LEO scanning electron spectroscopy machine 435 VF in the Indian Institute of Technology Ropar (Pb). The image resolution was set at 1000X and at 1500X. Before focusing electron beam on the samples, the samples were gold-sputtered in order to make them conducting. The SEM electron beam is focused probe of electrons accelerated to moderately high energy and positioned onto the sample by electromagnetic fields. These electron beams interact with atoms in the specimen by a variety of mechanisms when they impinge on a point on the surface of the specimen. The electron beams produce image on the screen of the SEM. The electromagnets can be used to control the magnification. The most common form of sample preparation for the SEM is the deposition of a metal thin film onto the specimen surface. Vacuum evaporation and ion sputtering of metals are common methods of depositing these thin films. The metal thin film provides electrical conductivity, enhances the signal and may add strength to the specimen (Joshi et al., 2008).

### 3.3.3 UV-Visible Spectroscopy

Perkin Elmer Lambda 950 UV-Visible spectrophotometer was used to study the adsorption tests of methylene blue dye on the crosslinked hydrogels.

### 3.4 Properties

#### 3.4.1 Electrical Conductivity

The surface DC electrical conductivity of the samples pressed pellets (mass~0.3 g, diameter = 9 mm, thickness = 1±0.07 mm, pressure 8 tons/cm$^2$) was measured by a two probe method using a Keithley electrometer (Model 6514). The resistivity of the sample was calculated using the following equation (Tiwari and Singh, 2008).

$$\rho = R(A/l)$$  \hspace{1cm} (3.3) \nonumber

Where, $\rho$, $R$, $A$, and $l$ are resistivity (Ω cm), resistance (Ω), area of the pellet (cm$^2$) and thickness of the pellet (cm), respectively.
Conductivity can be computed using the relationship, $\sigma = 1/\rho$ where $\sigma$ is the conductivity and $\rho$ is the resistivity of the sample.

3.4.2 Thermogravimetric Analysis (TGA)

Thermogravimetric studies of the synthesized samples were done on TGA/DTA 6300, SII EXSTAR 6000 in air. All the samples were subjected to a constant heating rate of 10 °C/min. TGA is an experimental technique in which the weight of a sample is measured as a function of sample temperature or time. The sample is typically heated at a constant heating rate. Thermal studies were performed over a temperature range of 0 °C to 700 °C in the air with flow rate of 200 ml/min. The results of a TGA measurement are usually displayed as a TGA curve in which percent weight is plotted against temperature. Different effects can cause a sample to lose, or even gain, mass, these include the following:

1. Evaporation of volatile constituents; such as desorption of gases and moisture
2. Thermal decomposition in an inert atmosphere with the formation of gaseous products
3. Oxidation of constituents (Gabbott, 2008).

3.5 Applications

3.5.1 Dye Removal Studies

Adsorption tests of methylene blue dye on the cross-linked hydrogels were carried out using UV-Visible spectroscopic technique. Firstly a solution of methylene blue dye with concentration $2 \times 10^{-5} \text{ mol L}^{-1}$ was prepared. Distilled water was used as the reference. A volume of 100 ml of methylene blue solutions, with concentration $2 \times 10^{-5} \text{ mol L}^{-1}$ was taken in 200 ml beakers. To determine the degree of dye adsorption, 0.250 g of each test sample prepared under different reaction conditions such as in air, under the influence of microwave radiations and under vacuum was immersed in 100 ml aqueous solution of methylene blue dye ($2 \times 10^{-5} \text{ mol L}^{-1}$) for 4 hours at room temperature. After shaking the beakers, the supernatant was analyzed.
spectrophotometrically using a UV-VIS spectrometer at wavelength ($\lambda$) of 662 nm at 40 minutes intervals to determine the remaining dye concentration in the solution. The supernatant was analyzed continuously after every 40 minutes for the remaining dye concentration for four hours. In order to get the cross-linked samples with maximum percentage removal capacity, the effect of different reaction parameters such as adsorption time, amount of sample, pH and temperature were studied. The percentage removal of MB dye was calculated using the following expression (Zendehdel et al., 2011):

$$\text{Dye adsorption (\%)} = \frac{(C_o - C_t)}{C_o} \times 100 \quad (3.4)$$

Where, $C_o$ is the initial absorbance of MB dye and $C_t$ represents the absorbance after every 40 minutes.

### 3.5.1.a Adsorption Kinetics

To study the adsorption kinetics, at specified time intervals, the amount of adsorbed MB dye was evaluated using a UV-VIS spectrometer at $\lambda_{\text{max}} = 662$ nm. The content of adsorbed dye ($q_t$, mg/g) was calculated using following Eq. 3.5 where, $C_o$ is the initial concentration of dye (mg/L), $C_t$ is the remaining dye concentrations in the solution at time $t$, $V$ is the volume of dye solution used (L) and $m$ is the weight of the sample (g). The equilibrium adsorption capacity of cross-linked samples, $q_e$ (mg/g), was determined using Eq. 3.5 (Bajpai et al., 2012). At this Eq. the $C_t$ and the $q_t$ will be replaced with equilibrium concentration of dye in the solution ($C_e$) and equilibrium adsorption capacity ($q_e$), respectively.

$$\text{Adsorption Capacity (q)} = \frac{(C_o - C_t)}{m} \times V \quad (3.5)$$
The kinetics of MB adsorption was analyzed using pseudo first order and pseudo second order models. The model expressions and their linearized forms are given in Table 3.2. In order to obtain model calculations, we can plot log(qe-qt) against t for pseudo-first-order and t/qt against t for pseudo-second-order. Model calculations for semi-IPNs and IPNs synthesized under different reaction conditions are given under Chapter 5.

Table 3.2: Expressions for Kinetics Models and their Linearized Form

<table>
<thead>
<tr>
<th>Kinetics Model</th>
<th>Expression</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudo first order</td>
<td>[ \log(q_e - q_t) = \log q_e - \frac{k_1 t}{2.303} ]</td>
<td>(k_1, q_e)</td>
</tr>
<tr>
<td>Pseudo second order</td>
<td>[ \frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} ]</td>
<td>(k_2, q_e)</td>
</tr>
</tbody>
</table>

3.5.2 Moisture Retention Studies of Semi-IPNs and IPNs in different type of Soil

Soil texture and organic matter are the key components that determine soil water holding capacity. In terms of soil texture, soil such as silt and clay consisting of smaller particle size and larger surface area has greater water holding capacity. Sand in contrast has large particle size which results in smaller surface area and low water holding capacity. The water retention studies were performed in different type of dry soil samples. In present study, different semi-interpenetrating and interpenetrating networks (1.0 g) were well mixed with known weight (40 g) of different type of crushed and dry soils and were kept in pre-weighed ventilated paper cups. Known volume (20 ml) of distilled water was slowly added to each test sample and initial weight of each test sample was recorded \(W_1\). The test samples were maintained at ambient temperature and were weighed at a definite interval of time \(W_i\) i.e. after every 12 hours till no detectable weight loss was observed (Wang and Wang, 2010). The water evaporation ratio (W%) of different soil samples was calculated using the following equation (Wu et al., 2008):
\[ W\% = \frac{(W_1 - W_i)}{20} \times 100 \]  

Where, \( W_1 \) is the Initial weight and \( W_i \) is the final weight of each cup.

### 3.6 Biodegradation Studies of the Guar gum-based Superabsorbents

#### 3.6.1 Soil Burial Method

Synthesized cross-linked samples were subjected to biodegradation studies using soil burial method as per the standard method mentioned elsewhere (Fig. 3.1) (Maiti et al., 2010). The soil for these studies was collected from one of the garden of NIT Jalandhar and the samples under investigation, in the form of plates were buried in the soil. The soil was collected in the cleaned pots with an opening to the atmosphere. The water level of the pots was maintained such that it was just reached to the surface of the soil. Water was supplemented at every day to replenish the drying by evaporation and samples under test were kept in a room maintained at ambient temperature. The distance between test samples was 3 cm apart when placed in the soil. Weights of test samples were taken at a regular interval of 7 days. Biodegradation of the test samples was monitored at different stages of breaking down using FT-IR and SEM techniques.
Fig. 3.1: Biodegradation Studies of Semi-IPNs and IPNs Synthesized under Different Reaction Conditions using Soil Burial Method
3.6.2 Composting Method

Synthesized cross-linked samples were subjected to biodegradation studies using composting method (Fig. 3.2) (Unmar and Mohee, 2008). The compost was collected from the site of domestic waste water effluent discharge at NIT Jalandhar. Samples were prepared in the form of plates and buried into the compost for the biodegradation. The compost contained the varieties of actively growing and well balanced population of mixed microbial species for the degradation of the wide varieties of compounds. The microbes were well acclimatized to the variation of discharge loads and maintained a good population balance due to the regular discharge of the effluents in the site over a period of two decades. The compost was collected in cleaned pots with an opening to the atmosphere. The microbial species in the compost were regularly fed with the aqueous solution from the effluent discharge as supplement to maintain the population balance and to keep the viability of the culture. The aqueous level of the pots was maintained such that it was just reached to the surface of the compost. Aqueous solution was supplemented at every alternate day to replenish the drying by evaporation and samples under test were kept in a room maintained at ambient temperature. The distance between the test samples was 3 cm apart when placed in the compost. Weights of test samples were taken at a regular interval of 7 days. To monitor the progress of the biodegradation, FT-IR and SEM of the samples were taken at different stages of biodegradation. The percentage weight loss after particular time interval was determined as follows (Phetwarotai et al., 2013):

\[
B_s = \frac{W_i - W_f}{W_i} \times 100
\]  

(3.7)

Where, \(W_i\) is the initial weight of the cross-linked hydrogels and \(W_f\) represents the weight after 7 day’s intervals.
Fig. 3.2: Biodegradation Studies of Semi-IPNs and IPNs Synthesized under Different Reaction Conditions using Composting Method
3.6.3 Characterization of Biodegradable Samples

3.6.3.1 Fourier Transform Infra-red (FTIR) Spectroscopy

IR spectra of the samples at different stages of biodegradation were recorded with Perkin Elmer RXI spectrophotometer using KBr pellets (Sigma Aldrich).

3.6.3.2 Scanning Electron Microscopy (SEM)

Scanning electron micrographs of the samples under different biodegradation stages taken on LEO, 435VF, LEO Electron Microscopy Ltd. In order to have the conducting impact, the samples were gold plated and the scanning was synchronized with microscopic beam so as to maintain the small size over a large distance relative to the specimen. The resulting image had a great depth of the field. A remarkable three dimensional appearance with high resolution was obtained for each sample.