The synthesis and characterization of new materials with tailor-made properties is one of the most important areas in science today. Physicochemical characterization requires the selection of analytical techniques that are quantitative, specific and sensitive. Insight in the physico-chemical processes that play a role during the preparation of catalyst extricates its essential to design catalysts in a more controlled way. The main biomaterial related research carried out is the synthesis, functionalization and immobilization of enzymes and detailed characterization of inorganic or hybrid siliceous silica and clay supports and their structural characterization by various physicochemical techniques. The overall aim is to establish a rational link between the physicochemical and structural properties of the materials and their biological activity and response to external parameters such as temperature and pH. Immobilized biocatalysts have been developed for a variety of applications in the laboratory, for analytical purposes and in enzyme technology. In order to allow for their reproducible use, and to select an optimum biocatalyst for specific application, they must be well characterized- that is, their essential physicochemical (including mechanical), kinetic properties, and their stability under process conditions must be determined in standard procedures. The characterization of the immobilized biocatalysts-and especially comparative studies with the same enzyme immobilized in different supports used for the same enzyme process-can provide useful information on the properties of the supports that are important to improve the process.
3.1 Physico-Chemical Characterization

A number of techniques are used to characterize mesoporous molecular sieves. Among these, X-ray powder diffraction, transmission electron microscopy (TEM) and adsorption measurements are the essential ones to identify the structure of the mesoporous molecular sieves. Infrared spectroscopy, ultra violet spectroscopy, nuclear magnetic resonance spectroscopy and electron spin resonance spectroscopy have also been applied to obtain additional structural information about molecular sieves. A complete characterization of molecular sieves requires information from a number of physical, chemical and spectroscopic techniques. A combination of X-ray diffraction, sorption studies and TEM of mesoporous molecular sieves provides a reasonable idea about the structure of these materials. The applications of these techniques have been discussed in Chapter I. This chapter summarizes the results of the physicochemical characterization of montmorillonite K-10 clay and mesoporous silica foams. The discussion is focused on XRD, thermal analysis (TG/DTG), N2-adsorption-desorption, transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier Transform infrared spectroscopy (FTIR), CHN measurements, CPMAS Nuclear magnetic resonance spectroscopy (NMR) and Contact angle measurements of these materials. The present results are the full characterization research and structural comparison between the functionalized/immobilized samples with the pure supports.

3.2 Fourier Transform Infrared Spectroscopy

The most common application of infrared spectroscopy in catalysis is to identify adsorbed species and to study the way in which these species are chemisorbed on the surface of the catalyst. FTIR is a sensitive technique to probe into the secondary structure of proteins. The interaction between the supports and lipase molecules can be studied by comparing the IR spectra of the pure silica and clay, pure lipase, and lipase immobilized on silica and clay.
The infrared spectra of the calcined MCF samples are present in Fig. 3.1. The spectra of the pure siliceous MCF showed narrow vibration band at 3476 cm\(^{-1}\) belonging to isolated terminal silanol groups. The corresponding \(\text{–OH}\) bending mode around 1634 cm\(^{-1}\) correlate very well with the water adsorption property (hydrophilic property) of the catalysts. The peaks between 500 and 1200 cm\(^{-1}\) are assigned to framework vibrations. The intense peaks at 1038 cm\(^{-1}\) with a shoulder at 1220 cm\(^{-1}\) are due to internal and external asymmetric Si–O stretching modes. The bands around at 800 and 471 cm\(^{-1}\) are assigned to symmetric Si–O stretching and tetrahedral Si–O bending modes. The MCF 35 silica samples (Fig 3.2A) display bands due to the abundant free OH groups at 3704 cm\(^{-1}\). The bands at 806 cm\(^{-1}\) is due to the symmetric stretching vibrations of Si-O-Si bonds belonging to ring structures. The peak at 968 cm\(^{-1}\) is due to the stretching vibration of Si-O (H) bonds.

Figure 3.1 are spectra obtained for unmodified MCF silica, immobilized lipase, silane binded (MTS) and Glutaraldehyde binded (MTSG). Free lipase showed a typical spectrum of proteins, with the absorption band associated with their characteristic amide groups (CONH). Between the wave number range from 1600 to 1700 cm\(^{-1}\), there is the amide I band, due to the double bond CO stretching, the CN stretching and NH bending [1]. The amide II band (1600–1500 cm\(^{-1}\)) results from a combination of N–H in-plane bending and C–N stretching of the peptide groups.

The band position of lipase MCF is slightly shifted in comparison with that of lipase which is attributed to the weak interactions of MCF with lipase molecule. After loading on the MCF160, the two absorption flexible peaks shift to 1634 and 1550 cm\(^{-1}\), which may be due to the strong electrostatic interactions between the charged amino acid residues on the surfaces of the protein molecules and the silanol groups on the mesoporous structure; these shifts may also arise from hydrophobic interactions between protein molecules and the supports. This was further evidenced from an intense OH stretching band in the range of 3451 cm\(^{-1}\) after enzyme binding depicting the presence of
enzyme on the surface of the particles. The \(-CH\) stretching band due to the enzyme groups occurs at 2942 cm\(^{-1}\). These results suggest that lipase immobilized in these matrices retains its native structure and biological activity. The same observation is observed with MCF 35 and KI-10.

The presence of APTES applied in the functionalization of MCFs was identified by methylene stretching bands of the propyl chain in the region of 2929 cm\(^{-1}\) (Fig. 3.1B and 3.2B), which cannot be observed in the FT-IR spectra of bare MCFs. The corresponding simple bending vibrations occur at 1407 cm\(^{-1}\) for the silane modified supports. At 693 cm\(^{-1}\), a weak peak was observed, which was due to the bending of N\(-H\) bonds. A strong band around 1550 cm\(^{-1}\) associated with the \(-NH\) bending vibrations indicate the presence of aminopropyl functional group in all the silane binded samples [2, 3].

As expected, C-H stretching vibration frequency is seen at 2936 cm\(^{-1}\) for all spectra except the pure silica and clay supports with contributions from the organosilane, glutaraldehyde, and the enzyme [4]. The presence of aldehyde in MHSG and MTSG is clearly evident from the peak at 1720 cm\(^{-1}\) (Fig. 3.1B and 3.2B). The amine-glutaraldehyde reaction produces an imine N=\(C\) bond, Schiff-base, seen at 1647 cm\(^{-1}\) while an ethylenic C=\(C\) bond formed by resonance stabilization of the imine appears at 1563 cm\(^{-1}\)[5]. A peak at 794 cm\(^{-1}\)
Results and Discussion

is due to the Si-C bond which demonstrates the incoorporation of alkyl groups into silica.

![Fig 3.2 IR spectra of (a) MCF 35 and immobilized sample (b) silane, glutaraldehyde and enzyme binded samples](image)

The IR spectra of Montmorillonite K-10 clay (Fig 3.3A) have two characteristic regions: (i) 4000–3000 cm\(^{-1}\) and (ii) 1200–400 cm\(^{-1}\). The transmission bands in the first region, which correspond to (O–H), were assigned to the free surface hydroxyl groups of the layer as well as to the adsorbed water which indicated that there were an abundance of free hydroxyl groups on the surface of K-10. The band at 3638 cm\(^{-1}\) is attributed to the stretching vibration of OH groups bonded to Al or Mg. The bands in the region 1200 to 400 cm\(^{-1}\) give more information about the structural characteristics of clay minerals and are attributed to lattice vibration [6, 7]. Bands at 1048, 521, and 688 cm\(^{-1}\) originate from the clay lattice Si–O stretching, Al–O–Si vibrations and coupled Al-O and Si-O out of plane vibrations. The band at 931 cm\(^{-1}\) is due to Al-Al-OH deformation.
Fig 3.3 IR spectra of (a) K-10 and immobilized sample (b) silane bounded samples (c) Glutaraldehyde binded sample (K-10G) and enzyme adsorbed on K-10G (K-10GE)

After immobilization of lipase (Fig 3.3A) there was a shift in the position of adsorption suggesting that there might be intermolecular interaction between enzyme and some specific sites of matrix, and apparently the montmorillonite K-10 was a good immobilization matrix for enzyme loading.
The successful grafting of silane moieties onto clay is evident in the infrared spectra as shown in Fig 3.3B. A new peak at 2964 cm\(^{-1}\) was attributed to antisymmetric stretching of ethyl group of silane, indicating the existence of silane in the grafted products. In the region 1400–1720 cm\(^{-1}\), the spectrum of the silane bound clay exhibits a strong band around 1595 cm\(^{-1}\) associated with the \(-\text{NH}_2\) bending vibrations. In addition a number of bands at lower frequencies attributed to bending vibrations of CH\(_2\) and CH\(_3\) units are resolved. The strong band at 1637 cm\(^{-1}\) is due to adsorbed water deformation.

### 3.3 Thermogravimetric analysis

The thermal stability of enzymes is one of the important criteria for long-term and commercial application. The activity of immobilized enzyme is known to be more resistant against heat than that native state. Thermal gravimetric analysis (TG) provides an important tool for thermal stability studies of macromolecules [8]. This technique has enabled us to determine the temperature range at which a heated sample undergoes a major conformational change by means of monitoring the thermal weight loss profile. In the case of free lipase and immobilized lipase derivatives such temperature range can be related to the protein unfolding and thus to the enzyme denaturation.

![Fig 3.4 TG/DTG spectra of (a) MCF160 (uncal), (b) MCF160 (calc)](image)

![Fig 3.5 TG/DTG spectra of (a) MCF35 (uncal), (b) MCF35 (calc)](image)
Fig 3.6 TG/DTG spectra of (a) K-10 (b) Free lipase (c) Immobilized lipases

Fig 3.7 TG/DTG spectra of functionalized samples of MCF160

Fig 3.8 TG/DTG spectra of functionalized samples of MCF35
Results and Discussion

Fig 3.9 TG/DTG spectra of functionalized samples of K-10

In order to optimise the calcination condition, TG-DTA analysis (Fig. 3.4. and 3.5) for silica synthesized via hydrothermal and room temperature method was carried out. The presence of peak at 100°C in both samples is probably due to the loss of adsorbed water. The sample MCF35 displayed a weight loss: at 280°C. There is virtually no weight loss above 480°C. The low temperature weight loss is partly due to loss of mesitylene and CTAB. The steady TG pattern is observed above 550°C. MCF160 displayed two processes: a weight loss at 50-150 °C due to the desorption of water and another weight loss from 180 to 370°C is due to the decomposition of P123 and other organics. The results from TGA/DTA demonstrate that P123 and TMB are readily removed from the MCFs under mild conditions.

Figure 3.6 (b) shows the TG curve for free lipase which was characterized by two weight loss peaks. In the first one, at temperature range from 30 to 180°C, characterized by a low weight loss due to the dehydration of the interstitial water containing in the free lipase sample. From 180 to 600°C, continuous weight loss was observed indicating a complete decomposition of the organic structure of lipase [9].

After adsorption of lipase (Fig 3.6 (c)), a weight loss is observed at 300° C due to the decomposition of enzyme moieties which confirms the adsorption of enzyme on MHI as these peaks are not observed in the case of calcined silica. The lower values obtained for the weight loss associated with the lipase encapsulated derivatives is the result of an increased matrix thermal stability suggesting that a strong interaction...
between enzyme and all tested supports occurred which enhanced the conformation stability of the native form. Montero et al. [10] have observed similar behaviour after immobilization of C. cylindracea lipase in poly(propylene). When TG curves of both lipase adsorbed on MCF160 and MCF35 are compared a greater amount of enzyme decomposition is seen on MCFA.

All the functionalized samples exhibited weight loss at 60°-90°C due to the loss of adsorbed water. In the case of MHS (Fig 3.7), the weight loss which extends from 190 to 500 °C was substantial, and it is attributed primarily to the removal of water by dehydroxylation and some loss of organic constituents (C, H, O and N) in the form of volatiles either present or formed by the beginning of organics decomposition of silane moieties. This indicates that the silane binded samples are thermally stable at temperatures below 450°C. Above 500°C, little weight loss occurred for the immobilised derivatives on functionalized samples. The weight loss in this region is associated with final dehydroxylation reactions and definitive carbonisation of organic compounds, including the lipase. A greater weight loss due to the adsorbed water is observed at 100°C in the case of lipase immobilized on MHSIG. The weight loss which occurs due to the decomposition of the enzyme at 237°C is not observed in any of the functionalized samples which confirm the strong adsorption of lipase via covalent bonding. In the case of MCF35 (Fig 3.8), the same peaks were observed for the functionalized samples and the strong adsorption via hydrophobic bonding of the lipase is evident from the peak around 500°C.

The DTG curve of MK-10 (Fig 3.6 (a)) displayed peak at 56°C, assigned to the loss of the physically adsorbed water. There is a weight loss in the region 100-300°C which is due to the decomposition of the adsorbed enzyme (Fig 3.6 c). The peaks at 69°, 225°C and 429°C are attributed to the loss of the physically adsorbed water and due to the decomposition of the intercalated silane while the peak at 533°C is corresponding to the decomposition of the grafted silane as illustrated in the Fig 3.9 [11]. An additional peak was recorded at 225°C which was due to the desorption from the external surfaces.
A weight loss is observed at 437°C in the case of K-10SG (Fig 3.9) which is due to the decomposition of silane and glutaraldehyde moieties from the functionalized surfaces. In the case of chemical adsorption of lipase on functionalized surfaces, a single weight loss is observed at 233°C in the case of MHGE, at 276°C in the case of MTGE and at 297°C in the case of K-IOGE which is assigned to the decomposition of amino acid residues of the enzyme as well as the organic groups (silane and glutaraldehyde). Two stages of weight loss were observed for MTGE at 289°C and 485°C while only a single weight loss was observed in the case of MHGE which indicated the enhanced thermal stability of these systems. The weight loss peak is shifted to lower temperature which depicts the enhanced thermal stability of the covalently bound enzyme systems. A much greater loss of water is observed in the case of MHG and K-10G after enzyme adsorption than MTG which also proves the higher amount of loading of enzyme on these supports.

3.4 Small angle X-ray diffraction

XRD studies of MCF160 (Fig 3.11 (a)) exhibits one strong primary peak in the range of $2\theta = 0.88^\circ$ due to the presence of mesopores. Unlike highly ordered mesoporous silica materials such as SBA-15 and MCM-41 [12], a certain plane or space group (e.g., p6mm) or to a lamellar diffraction pattern, cannot be observed in MCF silica which confirmed that the cells in the MCF materials are spherical and quite uniform in size. The appearance of single XRD peak in these samples is well consistent with a previous report [13].

After functionalisation (Fig 3.11 (b and c)) and immobilization (Fig 3.10b) in MCF160 there is a decrease in d spacing (Table 3.1) and intensity of the peaks due to the packing of lipase molecules inside the mesopores without affecting the structural integrity of the adsorbent. The XRD patterns of MCF160 after immobilization are similar to that before the immobilization, which shows that the regular mesoporous structure is retained even after immobilization and functionalisation. The decrease in peak intensities after enzyme binding and functionalisation may be due to the surface binding of the enzyme as well as the
functional groups suggesting the indirect evidence for enzyme incorporation in these particles. The decrease in intensity is probably not due to lower structural order but to the larger contrast in density between the silica walls and the empty pores relative to that between the silica walls and the pores filled with lipase molecules as observed by Marler et al. [14].

![X-ray diffraction patterns](image)

**Fig 3.10** X-ray diffraction patterns of 
(a) MCF-160 (b) MHI

**Fig 3.11** X-ray diffraction patterns of 
(a) MCF-160C (b) MHS (c) MHSG

Surface functionalized materials exhibit XRD patterns at the low-angle region, as well. As Sayari [15] also observed, the overall intensity of the XRD peaks decreased after this treatment. In our study, little structural changing was observed by post-synthesis treatment. Thus, sample functionalized with APTES (MHS) shows larger peaks reflecting a smaller ordered pore structure.

X-ray diffractograms of MCF35 are shown in Fig. 3.12 (a) & (b). They all exhibit low angle reflections characteristic of ordering at the mesoscale. Broad reflections in the low angle region is characteristic of a mesoporous lamellar silicate phase [16]. After calcination (Fig 3.12 b) the silicate phase displays one reflection with a slight contraction of d-spacing. This suggests that whilst ordering remains at the mesoscale this ordering is less than that of a lamellar phase. In other words, the mesophase becomes more disordered upon calcination. The increase in d spacing (Table 3.1) after immobilization and functionalisation probably may be due to the disordering of the pores.
Due to the immobilization of Porcine pancreatic lipase onto the mesoporous channels of SBA-15, a similar widening and broadening of the peaks as well as shift to higher angle was observed [17]. Retention of the hexagonal mesoporous structure of all SBA-15 as well as C_{16}-MCM41 adsorbents after cyt c adsorption at different solution pHs while decrease in intensity of both the lower and higher order peaks with increasing cyt c concentration were observed in the work done by Vinu et al. [18].

**Table 3.1** d-spacing values of the pure, functionalized and immobilized samples of silica and clay

<table>
<thead>
<tr>
<th>Sample</th>
<th>d spacing (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF35</td>
<td>32</td>
</tr>
<tr>
<td>MTI</td>
<td>40</td>
</tr>
<tr>
<td>MTS</td>
<td>39</td>
</tr>
<tr>
<td>MTSG</td>
<td>36</td>
</tr>
<tr>
<td>MCF160</td>
<td>100</td>
</tr>
<tr>
<td>MHI</td>
<td>74</td>
</tr>
<tr>
<td>MHS</td>
<td>96</td>
</tr>
<tr>
<td>MHSG</td>
<td>89</td>
</tr>
<tr>
<td>K-10</td>
<td>9.95</td>
</tr>
<tr>
<td>KI-10</td>
<td>9.95</td>
</tr>
<tr>
<td>K-10S</td>
<td>9.97</td>
</tr>
<tr>
<td>K-10SG</td>
<td>9.94</td>
</tr>
</tbody>
</table>
XRD is the usual method for measuring the structure of layered silicates. The Bragg equation based on XRD patterns is generally used to detect layer expansion of montmorillonites. The basal distances for total dehydrated clays are expected to be between 9.6 and 10 Å [19, 20, 21].

![Fig 3.14 X-ray diffraction patterns of (a) MK-10 (b) KI-10](image)

![Fig 3.15 X-ray diffraction patterns of (a) MK-10 (b) K-10S (c) K-10SG](image)

The parent montmorillonite clay gives a distinct peak around 2θ equal to 8.88°, which corresponds to a basal spacing of 9.95Å (Fig 3.14 (a)). After grafting with 3-aminopropyltriethoxysilane and glutaraldehyde, (Fig 3.15 b & c) the d spacing for the same was 9.97Å and 9.94Å (Table 3.1). Upon immobilization of lipase (Fig 3.14 b) there was no change in d-spacing which confirmed that the texture of MK-10 clay is maintained and the modification takes place only at the external surface, further supported by surface area measurements. Thus, the enzyme did not enter the interlayer space. Mostly lipase inhabited on the external surfaces and at the edges of the interlayer sheets through hydrogen bonding, Van Der Waals and electrostatic force interactions with hydrophilic residues of the enzyme. According to the results of XRD, FTIR and thermal analysis, it can be concluded that the texture of the montmorillonite is maintained and the 3-aminopropyltriethoxysilane is only connected with the surface of clay. After immobilization of Horseradish peroxidase enzyme on aluminum-pillared interlayered clay (Al-PILC), no change appeared in the XRD pattern. The rough dimensions of Horseradish peroxidase were far larger than the basal spacing of the
support, which made HRP diffuse into the Al-PILC galleries impossible [22]. There was not much change in the basal spacing when lipase was adsorbed onto modified and unmodified bentonite [23]. There was not much change in the d spacing after APTES functionalisation with palygorskite due to functionalisation only on the external surface [24]. Naidja and Huang [25] found that large molecules of aspartase (MW 180,000) were intercalated between the montmorillonite layers and expanded the d-spacing of Ca-montmorillonite from 14.7 to 26.5 Å.

3.5 C H N Analysis

Elemental microanalysis results of the enzymatic prepared systems give an indication of the presence of nitrogen, with respect to the pure support. In order to illustrate the superiority of enzyme-support complex obtained from heptane, two set of experiments in aqueous and in heptane were carried out. The carbon, hydrogen and nitrogen levels of the support, free lipase and immobilized derivatives (in both aqueous and organic medium) are presented in Table 3.2. The nitrogen content of the various immobilized supports in heptane increases in the order MCF160>KI-10>MCF35. By comparing the results, the nitrogen incorporation on the immobilized derivative obtained from buffer solution was much lower than the one measured for the preparation obtained from heptane, which is in agreement with the catalytic activities.

Table 3.2 Elemental (CHN) analysis data of the samples immobilized in buffer and heptane

<table>
<thead>
<tr>
<th>Sample name</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Free lipase</td>
<td>26.2</td>
<td>5.6</td>
<td>1.8</td>
</tr>
<tr>
<td>MT-Buffer</td>
<td>2.84</td>
<td>3.22</td>
<td>0.46</td>
</tr>
<tr>
<td>MT-Heptane</td>
<td>3.10</td>
<td>3.67</td>
<td>0.57</td>
</tr>
<tr>
<td>MH-Buffer</td>
<td>2.54</td>
<td>0.36</td>
<td>0.52</td>
</tr>
<tr>
<td>MH-Heptane</td>
<td>2.90</td>
<td>0.56</td>
<td>0.64</td>
</tr>
<tr>
<td>K-10-buffer</td>
<td>3.06</td>
<td>0.64</td>
<td>0.51</td>
</tr>
<tr>
<td>K-10-heptane</td>
<td>3.52</td>
<td>0.81</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Functionalisation of the mesocellular silica foams (MCF160 and MCF35) was carried out in two different solvents (acetone and toluene) in order to find the better solvent in which maximum functionalization took place. As expected, calcined silica has no C, H and N. However, on APTS loading, % of C, % of H and % of N is observed to increase. On further binding of glutaraldehyde to amino functionalised silica, %C and %N are expected to increase whereas overall %N is expected to decrease which is evident from the CHN data given in Table 3.3.

These data show that loading of APTS and glutaraldehyde increases in the order MCF160 > K-10 > MCF35. This data along with FTIR, TG, NMR and surface area results confirmed the incorporation of the amine and the glutaraldehyde moieties on to silica and clay. It was confirmed from the CHN results that acetone was a better solvent for functionalization of MCF-160 since the % of nitrogen incorporated is much greater in this medium. In the case of MCF-35, toluene was chosen as the medium for functionalisation from the CHN results. In the case of K-10 functionalisation was carried out in acetone.

**Table 3.3** Elemental (CHN) analysis data of various samples after functionalisation with APTES and glutaraldehyde

<table>
<thead>
<tr>
<th>Sample name</th>
<th>%C</th>
<th>%H</th>
<th>%N</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHS-A</td>
<td>24.7</td>
<td>7.1</td>
<td>8.1</td>
</tr>
<tr>
<td>MHSG-A</td>
<td>40.3</td>
<td>7.5</td>
<td>5.2</td>
</tr>
<tr>
<td>MHS-T</td>
<td>21.5</td>
<td>5.5</td>
<td>6.9</td>
</tr>
<tr>
<td>MHSG-T</td>
<td>31.1</td>
<td>6.0</td>
<td>5.1</td>
</tr>
<tr>
<td>MTS-A</td>
<td>14.9</td>
<td>4.4</td>
<td>5.1</td>
</tr>
<tr>
<td>MTSG-A</td>
<td>18.8</td>
<td>4.7</td>
<td>3.7</td>
</tr>
<tr>
<td>MTS-T</td>
<td>18.5</td>
<td>4.8</td>
<td>5.7</td>
</tr>
<tr>
<td>MTSG-T</td>
<td>32.8</td>
<td>6.2</td>
<td>4.4</td>
</tr>
<tr>
<td>K-10SG-A</td>
<td>29.3</td>
<td>5.2</td>
<td>5.3</td>
</tr>
</tbody>
</table>

A-acetone, T-toluene
3.6 Nitrogen Adsorption Measurements

Fig. 3.16 shows the N$_2$ adsorption isotherm of MCF160. The material exhibits a type IV adsorption isotherm with a type-H1 hysteresis loop, which is the characteristic of mesoporous material [26]. The isotherms show large hystereses, which suggests that the MCFs possess ink-bottle-type pores in which large cells are connected by narrower windows [27]. The nitrogen adsorption/desorption isotherms exhibited steep hysteresis of type H1 at high relative pressures which is typical for mesoporous materials that exhibit capillary condensation and evaporation and have large pore sizes with narrow size distributions. The as-prepared MCF material has high pore volume and large pore diameter. The sharp rise at high relative pressures ($P/P_0$ near 1) indicates the existence of large mesopores in this material. These unique characteristics of MCF demonstrate that it is a favorable host matrix for immobilization of protein.

To know whether the lipase molecules are adsorbed inside the mesopores of MCF, the adsorbent was characterized by nitrogen adsorption after lipase adsorption. Nitrogen adsorption measurements were carried out on samples immobilized in aqueous (pH-7) (Fig 3.17b) and organic medium (heptane) (Fig 3.17c). The amount of nitrogen adsorbed in MCF160 loaded with lipase from n-heptane was higher as compared to those from aqueous medium. There is a shift in the $P/P_0$ to lower value which shows the encapsulation of enzymes inside the pore in MCF’s, leading to the occlusion of most pores. Second, the increasing weight of silica after adsorption of CRL can also reduce the pore volume and specific surface area. Third, the tight packing of CRL molecules instead of adsorption in the mesopores may also decrease the pore volume and specific surface area. The shape of the adsorption isotherm curves for MCF160 (Figs. 3.2b & c) are very similar before and after encapsulating lipase.

The specific pore volume of MCF 160 after immobilization in n-heptane decreases from 2.49 to 0.99 while in aqueous medium the pore volume
decreases from 2.49 to 1.25. There is a much greater decrease in pore diameter for heptane immobilized samples than the aqueous ones which is evident from the pore size distribution curves [Fig 3.18 (b) & (c)]. The BJH pore size distributions of MCF160 and MHI are relatively narrow. Moreover, the monotonic decrease of specific surface area and pore volume confirms the tight packing of the lipase molecule inside the mesopores of MCF 160 silica (Table 3.3). Table 3.3 summarizes the textural properties of the adsorbents before and after lipase immobilization in aqueous and organic medium. The sharp peaks in the pore size distribution plots confirm the narrow size distributions of both the cells and the windows.

Here, the larger pore size in MCF160 undoubtedly enhanced lipase physical adsorption and functionalisation. The nitrogen adsorption/desorption isotherms as well as the pore size distribution (PSD) of the parent MCF unambiguously reveal the characteristic three-dimensional mesocellular structure of the MCF support.

![Fig 3.16 Nitrogen adsorption isotherms of (a) MCF160, (b) MHI (pH-7) (c) MHI (heptane)](image1)

![Fig 3.17 Nitrogen adsorption isotherms of functionalized samples of MCF160](image2)

After functionalisation (Fig 3.17 (a) & (b)) the shape of the hysteresis shifted from H1 to a H3. A slight pore disordering takes place due to the strain arising in the pore channels due to the high content organic groups and such features are common in mesoporous silicas after organic modifications. These
features are prominent for MCF like silicas due to the flexibility in the pore walls and to their typical morphological structure. The capillary nitrogen condensation was shifted gradually to lower relative pressures, and thus indicating the diminishing of pores size. The surface area decreases from 595 m$^2$/g to 266 m$^2$/g after glutaraldehyde binding (Table 3.5). The decrease in surface area and pore volume is not supposed to result from the structure collapse caused by the immobilization of lipase and the following silylation. The pore diameter decreases from 161 to 126 m$^2$/g for silane binded samples and to 98 m$^2$/g for glutaraldehyde samples. The pore size distribution curves of the silane and glutaraldehyde samples are shown in Fig 3.19 (b) & (c).

The amount of nitrogen adsorbed decreases markedly in the rod-like silica (from 687 cm$^3$ g$^{-1}$ to 472 cm$^3$ g$^{-1}$) and the vesicle-like silica (from 1028 cm$^3$ g$^{-1}$ to 703 cm$^3$ g$^{-1}$) upon CRL adsorption [28]. Similar results have been reported for the adsorption of lysozyme and cytochrome c onto MCM-41 and SBA-15 [18, 29].

![Fig 3.18](image1.png)

Fig 3.18 Pore size distribution of
(a) MCF160 (b) MHI (pH-7) (c) MHI (heptane)

![Fig 3.19](image2.png)

Fig 3.19 Pore size distribution of
(a) MCF160 (b) MHS (c) MHSG

The N$_2$ adsorption/desorption isotherms curves of MCF 35 are shown in Fig. 3.20 & 3.21). The MCF35 materials prepared via room temperature method exhibits a nitrogen adsorption isotherm of type IV with a hysteresis in the region of $0.4 < p/p_0 < 1$, which is a direct evidence of the presence of mesopores. MCF35
exhibited a weak hysteresis indicating a small mesopores. The meso cellular foams exhibit H2 hysteresis. Type H2 hysteresis is attributed to a difference between the condensation and evaporation processes occurring in pores with narrow necks and wide bodies ("ink-bottle" pores). This is consistent with the previous report [30] on meso-cellular foams formed with windows and cells. After two step functionalization [Fig 3.21 (a) & (b)] the material gets completely distorted due to the less stability of the room temperature synthesized sample and thus the insertion of high content of organic groups inside the pore channels can meaningfully distort its pore structure. The mesoporosity is gradually getting reduced due to the functionalisation step. Thus, the materials synthesized under room temperature conditions were not stable enough for functionalisation while the mesocellular silica foams synthesized hydrothermally are stable enough for further modification. As a large number of silanol groups are present on MCF35 compared to MCF160 a higher degree of functionalisation takes place on MCF35. But due to the instability of the material disordered mesostructures are obtained. The surface area decreases from 914 m$^2$/g to 506 m$^2$/g after immobilization of lipase in heptane while the surface area of aldehyde functionalized sample decreases from 914 to 148 m$^2$/g (Table 3.5).

**Table 3.4** Textural parameters of catalysts immobilized in aqueous and organic media

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Surface area (m$^2$/g)</th>
<th>Pore diameter (Å)</th>
<th>Pore volume (cm$^3$/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF160</td>
<td>595</td>
<td>161</td>
<td>2.49</td>
</tr>
<tr>
<td>MHI (pH-7)</td>
<td>266</td>
<td>131</td>
<td>1.25</td>
</tr>
<tr>
<td>MHI (heptane)</td>
<td>246</td>
<td>78</td>
<td>0.99</td>
</tr>
<tr>
<td>MCF35</td>
<td>914</td>
<td>35</td>
<td>0.81</td>
</tr>
<tr>
<td>MTI (pH-7)</td>
<td>514</td>
<td>34</td>
<td>0.47</td>
</tr>
<tr>
<td>MTI (heptane)</td>
<td>506</td>
<td>33</td>
<td>0.44</td>
</tr>
<tr>
<td>MK-10</td>
<td>246</td>
<td>36</td>
<td>0.42</td>
</tr>
<tr>
<td>KI-10 (heptane)</td>
<td>73</td>
<td>34.7</td>
<td>0.17</td>
</tr>
</tbody>
</table>
Results and Discussion

Fig 3.20 Nitrogen adsorption isotherms of
(a) MCF35
(b) MTI (pH-7)
(c) heptane

Fig 3.21 Nitrogen adsorption isotherms of
(a) MTS
(b) MTSG

MCF35 presented a wider pore size distribution (Fig 3.22 a) with a lower pore volume due to its more disordered nature compared to MF160. The physical adsorption of lipase in MCF35 did not significantly change the main pore size, which remained around 34Å [Fig 3.22 (a) and (b)]. Nonetheless, the surface area of lipase loaded MCF35 decreased from 914 to 512 m²/g for heptane immobilized sample and 514 m²/g for pH-7 immobilized sample. The maintenance of the pore size in MCF35 suggests that lipase molecules should mainly occupy the external surface of the particles.

Fig 3.22 Pore size distribution of
(a) MCF35
(b) MTI (pH-7)
(c) heptane

Fig 3.23 Pore size distribution of
(a) MCF35
(b) MTS
(c) MTSG
The pore size distribution shifts to higher value after functionalisation with silane and glutaraldehyde (Fig 3.23 (a) and (b)) which clearly shows that the walls of MCF35 are very thin and hence gets easily disrupted to form larger pores. This observation is also supported by XRD studies in which an increase in d spacing is observed. Table 3.5 summarizes the textural properties of pure silica and the functionalized samples.

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>Surface area (m²/g)</th>
<th>Pore diameter (Å)</th>
<th>Pore volume (cm³/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF160</td>
<td>595</td>
<td>161</td>
<td>2.49</td>
</tr>
<tr>
<td>MHS</td>
<td>309</td>
<td>126</td>
<td>0.21</td>
</tr>
<tr>
<td>MHSG</td>
<td>266</td>
<td>98</td>
<td>0.05</td>
</tr>
<tr>
<td>MCF35</td>
<td>914</td>
<td>35</td>
<td>0.81</td>
</tr>
<tr>
<td>MTS</td>
<td>339</td>
<td>55</td>
<td>0.24</td>
</tr>
<tr>
<td>MTSG</td>
<td>148</td>
<td>39</td>
<td>0.07</td>
</tr>
<tr>
<td>MK-10</td>
<td>246</td>
<td>36</td>
<td>0.42</td>
</tr>
<tr>
<td>K-10S</td>
<td>186</td>
<td>36</td>
<td>0.36</td>
</tr>
<tr>
<td>K-10SG</td>
<td>133</td>
<td>35</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Natural smectite clays display type IV isotherms with a H3 or H4 hysteresis loops. There is neither a high uptake at high P/P₀ values nor a definitive mesoporous region. The pore size distribution is shifted to smaller pore sizes, and indeed the assignment of aggregates of platelet like particles giving rise to slit shaped pores applies to smectites. The phenomenon arises due to the complexity of capillary condensation in pore networks with pore blocking effects. H4 hysteresis loops arise in systems containing microporous slit shaped pores. Fig. 3.24 (a) & (b) shows the N₂ adsorption/desorption isotherms of parent montmorillonite and the immobilized sample. The steep increase in adsorbed volume at low partial pressure is attributed to microporous (pore radius ~10 Å) condensation, while the hysteresis occurring at high partial pressures is attributed to the mesoporous structures (pore radius of 18-200 Å) [31].
Adsorption isotherm of montmorillonite K-10 clay belongs to the type II in the Brunauer, Deming, Deming and Teller (BDDT) classification [32], characteristic of nitrogen adsorption on macroporous adsorbents (with less or no porosity). Furthermore, the hysteresis loops of these isotherms are assigned to type I4 in the IUPAC classification [33], which is representative of the slit-shaped pores in layered materials. The pore size curves Fig 3.26 (a) shows a wide pore size distribution.

After adsorption of lipase in heptane, the amount of N\textsubscript{2} adsorbed decreases while there is not much change in the P/P\textsubscript{0} value as shown in the Fig 3.24 (b), which shows that the adsorption is entirely external and no intercalation is taking place in the clay which is evident from the XRD results. The surface area of pure montmorillonite K-10 is 246 m\textsuperscript{2}/g which decreases to 73 m\textsuperscript{2}/g (Table 3.5) after lipase adsorption while there is not much shift in the pore size distribution curves (Fig 3.26 (b)). The results of the textural characterization for the K10 clay are similar to those reported in the literature. Kawi and Yao [34] obtained a surface area of 197 m\textsuperscript{2}/g for this clay. The K-10 clay has a low microporosity.

After functionalisation with silane and glutaraldehyde, the surface area decreases from 246 to 133 m\textsuperscript{2}/g with no appreciable decrease in the pore diameter and pore volume.
The pore size distributions of the pure clay and the functionalized samples are shown in Fig 3.27 (a) and (b). There is no intercalation taking place upon functionalisation which depicts that the binding of organic groups is entirely external.

### 3.7 Transmission electron microscopy

TEM image (Fig. 3.28) of MCF 35 sample reveals a disordered array of silica struts comprising of uniformly sized spherical cells (20–34 nm) interconnected by windows with a narrow size distribution, which is characteristic structural feature of the MCF materials. The strut-like structure resembles that of aerogels [30, 35]. TEM images of this material exhibited disordered mesopores with wormhole like structure. Uniformly sized cellular pores is evident from the TEM image.

Transmission electron micrographs of MCF160 are shown in Fig. 3.29. It can be easily seen that the hydrothermally synthesized MCF catalysts present the typical three-dimensional and ultralarge pore structures of the pure MCFs.
Fig 3.29 Transmission electron micrographs of MCF 160 at two different magnifications

The TEM image illustrates that MCF has a three dimensional mesocellular arrangement of the MCF frameworks. Mesopore cells in the sample could be observed from the image.

3.8 Scanning Electron Microscopy

Since an enzyme is a highly polymeric material, immobilization on a solid matrix can change the morphology.

Fig 3.30 Scanning electron micrographs of MCF160
**Fig 3.31** Scanning electron micrographs of MCF35

**Fig 3.32** Scanning electron micrographs of K-10
Fig. 3.30 and 3.31 shows the SEM images of MCFs prepared via hydrothermal route and room temperature method. MCFs appear to be bigger particles due to aggregation. The MCFs exhibit cauliflower-type morphology. After immobilization (MHI, MTI), the surface was filled by the rounded structure, which is presumably protein aggregates. Similar enzyme aggregates of chymotrypsin were also observed on polystyrene/polystyrene/maleic anhydride) NFM after covalent binding [36]. After functionalisation with glutaraldehyde the particles appear to be bigger due to aggregation.

The morphologies of montmorillonite K-10 are displayed in SEM photographs (Figure 3.32). Generally, both morphologies shown were uniformly layered structures with a flaky aspect on a smooth surface. The appearance of boulder like structures may be due to the presence of enzyme and an increase in particle size after immobilization is observed. A slight porous nature is observed after functionalization with glutaraldehyde. The polyhedral particles of KIT-6 samples lost their individual nature after lipase immobilization due to aggregation into larger entities [37].

3.9 CPMAS Nuclear magnetic resonance spectroscopy

The $^{29}$Si MAS spectrum of the parent MCF 160 (Fig 3.33) exhibits two broad resonances at -112.3 ppm for a Q$^4$ environment and at -102.6 ppm for a Q$^3$ environment together with a shoulder at -93.1 ppm ascribable to Q$^2$ species. After immobilization of lipase (MHI), the intensity of Q$^2$ and Q$^3$ sites decreased (Fig 3.33). The Q$^4$ peak shifted to -104.1 ppm which showed that the enzyme is interacting with Q$^3$ and Q$^2$ sites than Q$^4$. From specific surface area and $^{29}$Si NMR data, the presence of interactions between the enzyme and the silica network is evident. The solid-state $^{29}$Si CP-MAS NMR spectra of MCF 35 silica showed two chemical shifts at δ = -102.5 (Q$^3$), and -92.5 (Q$^2$) ppm due to the different surroundings (Fig 3.34). There was no Q$^4$ peak detected which means that there are decreased crosslinked silanol groups or else due to the increased content of non-condensed silanol groups. The explanation is that Q$^2+Q^3/Q^4$ ratio of the uncalcined form of MCF35 is 0.09 and it is 1.31 for MCF’s. Upon calcinations this ratio remains the same in the case of MCF35 but it decreases to 0.39 for MCF’s. Hence the walls of the MCF’s
are more strained due to the transformation of Q$^3$ to Q$^4$ by calcination which accounts for the lower stability of MCF 35.

After functionalisation with aminopropyl triethoxysilane (APTES), MHS showed a distinctive chemical shift at d = -65 ppm (T$^3$), which was attributed to APTES grafted on the MCF silica (Fig 3.33). The peak intensity ratio of Q$^3$/Q$^4$ for MCF 160 silica was higher than that for MAS and Q$^2$ peak observed in the MCF silica undergoes a shift from -92 to -85 ppm. A decrease in intensity of the Q$^2$ peak is observed. These results indicate that hydroxyl groups of MCF silica reacted with APTES by covalent bonding. In the case of MTS, in addition to the peaks at -101 ppm (Q$^3$) and -111 ppm (Q$^4$) due to the (Si-O)$_3$-SiOH and (Si-O)$_4$ Si moieties of silica surface, there appears two peaks at -59 and -67 ppm identified with the silane silicons that have two Si-O-Si attachments to the silica surface (or to other silicons) and R-Si silicons with three Si-O-Si linkages [Fig 3.34 (b)]. The presence of $^{29}$Si peaks due to the specified types of silane silicons attached indicates the grafting of silane on the surface of silica. The peaks at -60 ppm and -67 ppm are attributed to $^{12}$ [R-Si (OSi)$_2$-OH] and T$^3$ silicons [ R-Si (OSi)$_3$].

Fig 3.33. $^{29}$Si NMR spectra of MCF160, MHI and MHS

Fig 3.34. $^{29}$Si NMR spectra of (a) MCF35 (b) MTS
Fig 3.35 $^{29}$Si NMR spectra of (a) K-10 (b) K-10S (c) K-10SG

Fig 3.36 $^{27}$Al NMR spectra of K-10

Fig 3.37 $^{27}$Al NMR spectra of K-10 S

Fig 3.38 $^{13}$C NMR spectra of (a) MHI (b) MHS

Fig 3.39 $^{13}$C NMR spectra of MTS
Fig 3.40 $^{13}$C NMR spectra of (a) K-10S (b) K-10SG

Fig 3.41 $^{13}$C NMR spectra of (a) MCF160G (b) MCF160GE

The $^{13}$C NMR spectrum of MHS showed three chemical shifts at $d = 9$ (C1), 24 (C2), and 43 (C3) ppm, while MCF silica exhibited no resonance peak (Fig 3.38 (b)). The three resonance peaks observed in the MHS were attributed to different carbon atoms (C1, C2, and C3) in the APTES. This result indicates that aminopropyl functional groups were successfully grafted on the MCF silica. In the case of MTS also, $^{29}$Si NMR gave characteristic peaks due to the three type of C atoms of APTES at C1 (10.4ppm), C2 (21.7ppm) and C3...
(42.3ppm), which confirmed the grafting of silane onto MCF35 silica. After immobilization (MAI) (Fig 3.38 (a)) peaks due to CH₂ groups, C-N bond, aromatic C, amide and carboxylic acid groups of various amino acids in enzyme appeared in the spectrum which gives further support for the information obtained from IR, TG, surface area and CHN results. After binding with glutaraldehyde (Fig 3.41 (a)(MHG), peaks due to alkane C atom attached to imine appears at 8, 22, 42, 66, 73 and 145 ppm. The peak due to the imine groups formed between enzyme and glutaraldehyde appears at 175 ppm and the aldehyde carbon of glutaraldehyde appears at 214 ppm.

In the case of lipase binded covalently to the functionalized samples (MHGE) (Fig 3.41 (b)), the ¹³C spectra gives peaks due to alkane C of lysine in enzyme at 10, 11.8, 40.7 ppm. Peaks due to C-O and C-N groups present in tyrosine and cysteine appears at 61 and 72 ppm. Aromatic C atoms in tyrosine appear at 99 ppm. The imine group formed between the enzyme and aldehyde appears at 174 ppm and also the peaks due to the carboxylic acid groups in cysteine and lysine appears at 180 ppm.

Clays are aluminosilicates and hence there is ample scope for NMR analysis due to the presence of two NMR nuclei- ²⁷Al and ²⁹Si. Changes in the chemical shift environment of Al can be easily visualized with the help of ²⁷Al NMR. This technique is of particular interest in studying octahedral and tetrahedral sites in the Al framework and so can be easily applied for the study of enzyme immobilization on montmorillonite.

²⁷Al NMR spectra of Montmorillonite K-10 clay (Fig 3.36) showed two resonances around 0 and 63ppm representing Al in octahedral and tetrahedral coordination [38, 39, 40]. The ²⁷Al of K-10S (Fig 3.37) appears much broader, due to the tight binding of organic moieties with the surface of clay. The octahedral resonance undergoes a shift from 0ppm to 2ppm and the tetrahedral resonance undergoes a similar shift from 63.8ppm to 73.2ppm. These shifts
confirmed that both the octahedral and tetrahedral sites are involved in binding with silane.

The $^{29}$Si MAS NMR spectra of montmorillonite K-10 (Figure 3.35 (a)) show a broad resonance line showing several shoulders. This line is centered at -103 ppm, but ranges from -90 to -113 ppm. This is the chemical shift region characteristic of Si atoms surrounded by zero, one, two, three, and four Al atoms. For $^{29}$Si NMR, two peaks originate at -93 and -103 ppm that is assigned to Si in Q$^3$ and Q$^4$ states respectively. Additional peaks due to the presence of APTES were observed at -63ppm due to the T$^2$ species (Fig 3.35 (b)). The Q$^3$ peak disappeared after silanisation which means that the isolating groups are interacting with clay surface. The Q$^4$ intensity increases after silanisation which may be due to the interaction of silane groups of APTES. After glutaraldehyde binding the intensity of the peaks decreased with not much change in the peak position.

Incorporation of silane groups onto clay was confirmed by the presence of peaks at 10.7, 22.6, 43ppm in the $^{13}$C NMR spectrum of K-10S (Fig 3.40 (a)). The incorporation of glutaraldehyde (K-10SG) is evident from the peak at 165ppm and 145ppm due to the imine and C attached to CH=N group (Fig 3.40 (b)).

3.10 Contact angle measurements

Proteins adsorbed to solid surfaces alter the original interfacial properties [41, 42]. To show that the modified supports are hydrophobic in nature, we have carried out the contact angle measurement with the unmodified and modified surfaces at room temperature (25°C).
Fig 3.42 Contact angle photographs of functionalised samples of MCF35

Fig 3.43 Contact angle photographs of functionalised samples of MCF160
Fig. 3.42, 3.43 and 3.44 shows the contact angle of the functionalized surfaces of all the supports. The contact angles of pure supports MCF-160, MCF-35, KI-10 as well as the adsorbed ones remained 0° (water drops spread instantly when placed on the surface of the substrate) indicating that the surfaces are hydrophilic in nature. This is due to the hydroxyl groups of the different supports that make it superhydrophilic.

![Contact angle photographs of the functionalised samples of K-10](image)

The contact angle of MCF-160 increases from 64° to 85° after glutaraldehyde treatment. Adsorption occurring via favorable electrostatic attraction would orient hydrophobic regions of the molecule away from the surface. Indeed, the presence of these hydrophobic domains leads to a higher water contact angle than that measured on bare hydrophilic surfaces [42]. The same increase in pattern of the contact angle values was observed for MTS (40°) to MTSG (61°) and also in the case of K-10S (46°) to K-10SG (58°). The increase in the contact angle [43]
Results and Discussion

indicated an increase in the hydrophobicity of the chemically modified surfaces and also the successful incorporation of the amine and glutaraldehyde moieties on to the surface. The lower contact angle of MCF-35G compared to other supports features its wettability nature and results in lower immobilization.

Table 3.6 Contact angles of the pure, immobilized and functionalized samples

<table>
<thead>
<tr>
<th>SAMPLE</th>
<th>CONTACT ANGLES (°)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF160</td>
<td>0</td>
</tr>
<tr>
<td>MHS</td>
<td>64</td>
</tr>
<tr>
<td>MHSG</td>
<td>85</td>
</tr>
<tr>
<td>MHSGE</td>
<td>55</td>
</tr>
<tr>
<td>MHI</td>
<td>0</td>
</tr>
<tr>
<td>MCF35</td>
<td>0</td>
</tr>
<tr>
<td>MTS</td>
<td>40</td>
</tr>
<tr>
<td>MTSG</td>
<td>61</td>
</tr>
<tr>
<td>MTSGE</td>
<td>21</td>
</tr>
<tr>
<td>MTI</td>
<td>0</td>
</tr>
<tr>
<td>K-10</td>
<td>0</td>
</tr>
<tr>
<td>KI-10</td>
<td>0</td>
</tr>
<tr>
<td>K-10S</td>
<td>46</td>
</tr>
<tr>
<td>K-10SG</td>
<td>58</td>
</tr>
<tr>
<td>K-10SGE</td>
<td>45</td>
</tr>
</tbody>
</table>

The higher contact angle of MHSG signifies the relative hydrophobic surface with respect to MTSG and K-10G. The surface modification of carriers resulted in the variation of the contact angle (Table 3.5). From the static contact angle measurements, the water contact angle of the modified MHSG is about 85° and value decreased to 55° after lipase immobilization, indicating that surface immobilization has changed the surface property of the support to a more hydrophilic one which will be beneficial for carrying out the hydrolysis reaction. The surfaces became more hydrophilic after lipase adsorption in the case of MTSG and K-10G. The order of hydrophilicity being is MTSG>MHSG >K-10G which is also a proof for the higher amount of lipase adsorption. But probably due to the tight packing of enzyme molecules and also the steric hindrances...
imported by the organic groups, only on the surface rather than inside the pores, the activity is much less than the other covalently bound immobilized systems in nonaqueous medium which favors a hydrophobic surface.

Yang et al. [44] used contact angle methods to measure the change in hydrophilic-hydrophobic balance exhibited by a number of different materials following adsorption of protein. They found that adsorption of protein rendered hydrophilic surfaces more hydrophobic and hydrophobic surfaces more hydrophilic.

3.11 Conclusions

Mesocellular silica foams were successfully prepared via hydrothermal route and via room temperature. The resulting carriers were evaluated for the immobilization of Candida rugosa lipase via adsorption and chemical bonding. Various physicochemical characterization techniques were employed for the characterization of the pure silica supports and clay and also for the study of functionalized as well as immobilized samples. The important conclusions from the characterization studies are:

- Mesocellular silica foams were synthesized by oil-in-water microemulsion templating route and were functionalized with silane and glutaraldehyde.
- The experimental results from IR spectroscopy and elemental analysis demonstrated the presence of immobilized lipase and also functionalisation with silane and glutaraldehyde on the supports.
- A slight broadening and lowering of d-spacing values after immobilization and modification was observed in the case of MCF160 and MCF35 but there was no change in the d-spacing in the case of K-10 which showed that the enzymes are adsorbed only on the external surface.
- The surface area, pore diameter and pore volume decreased drastically in the case of MCF160 due to the incorporation of the organic groups into
the mesopores. In the case of MCF35 a disordered nature was observed while in the case of K-10 there was only a decrease in the amount of nitrogen adsorbed after functionalisation without any change in pore diameter revealing that the functionalisation took place on the external surface in conformity with the XRD results.

- The thermal stability of the systems improved after immobilization and functionalisation when compared to the pure supports.
- SEM micrographs showed aggregation due to protein immobilization.
- TEM micrographs revealed the mesocellular structure of silica foams.
- $^{29}$Si NMR of K-10 clay shows broadening after functionalisation which shows the strain imparted by the organic groups.
- $^{29}$Si NMR and $^{13}$C studies revealed the incorporation of the organic groups onto the supports which was further confirmed from the CHN results. There was an increase in contact angle with surface functionalisation in all the supports which can be correlated with the increased activity observed for the covalently bound systems. These studies illustrated that hydrophobicity could explain the difference of enzyme activity of the functionalized samples.

References


