CHAPTER 6

BIOMEDICAL APPLICATIONS OF SOME SYNTHESIZED COMPOSITE ION EXCHANGERS

Studies on application of different composite exchangers revealed that these are promising candidates of significant analytical utility and will surely contribute much to find solutions for various environmentally related problems. Other than the conventional use of composite exchangers as detoxicants, some biomedical applications of the exchanger as drug carrier and their antimicrobial properties are discussed here.

6.1 DRUG DELIVERY APPLICATIONS

The design and construction of suitable drug carrier vehicles is the main focus in the biomedical field. The drug carrier is an important component of controlled and targeted drug delivery system and it must be bio compatible, non toxic and have the capability to release therapeutic molecules at the site of action [263]. Most therapeutic agents do not capably accumulate in the desired sites due to their nonspecific delivery throughout the body and as a result conventional therapeutic agents require high dosages [264]. The development of controlled delivery system can overcome this issue by increasing the patient compliance and therapeutic efficiency of pharmaceutical agents and it delivers the drug at a relevant in-vivo location with minimal side effects. A variety of materials such as biodegradable polymers [265], natural clay minerals [266] and porous inorganic matrix [267] etc., are mainly employed as drug carriers. One of the attractive methods for modified drug release is the use of ion exchange materials as carriers for therapeutic agents.

In the past decades, organic ion exchanger/ion exchange resins (IER) have received considerable attention from scientific community due to their versatile properties as drug delivery vehicles. Apart from conventional applications of separation, purification and processing, IER are equally suitable for different areas of drug delivery technologies, including controlled release, transdermal, nasal, topical and taste masking. A major drawback of controlled release using traditional drug carrier is dose dumping which results
in increased risk of toxicity. The use of IER as sustained drug release systems plays a
significant role because of their drug retarding properties and prevention of dose dumping.
Drug loaded IER, called drug resinates can moreover be used as drug reservoirs, which
cause a change in drug release characteristics. The mechanism involved during the release
of loaded drug from the resinates is shown below (eqn 6.1),

\[
\text{IER-Drug}^+ + X^+ \rightleftharpoons \text{IER-X}^+ + \text{Drug}^+ \quad (6.1)
\]

Where, \(X^+\) is the ion present in the gastro intestinal tract. The drug molecule
attached to the resins reaches the site of delivery; the exchange of drug with the highly
activated appropriate counter ion present in the gastro intestinal tract takes place by the
reversal of the exchange process which results in the liberation of free drug ions. The IER
free of drug is eliminated or biodegraded from or at the site of delivery [268]. Thus ionic
strength and pH at the site of delivery plays key role in the release of drug from the
immobilized resinate.

Although IER have been used for long time for a variety of applications in drug
delivery research, they suffer certain limitations. The main drawback existing with organic
ion exchangers (IER) is their very low chemical and thermal stability and they were found
to be less stable in highly acidic and basic media. So, further applications of IER are
limited by its high solubility and less adsorption efficiency of organic drugs. Nowadays,
organic-inorganic composite exchangers have received considerable attention because of
their better mechanical, chemical, thermal and radiation stabilities [269]. Composite
exchanger can combine the advantageous properties of both organic and inorganic
components and may offer special properties through reinforcing or modifying each other
and it can retain many beneficial features of both components. In many cases, synthetic
composite ion exchange materials were introduced as a more flexible alternative to the use
of natural or artificial zeolites. Synthetic composite exchangers mimic the properties of
artificial zeolite and have been used in different fields of analytical chemistry. Detailed
literature survey reveals that, though composite ion exchange materials has been
extensively used for different environmental applications, their use as drug carriers has not
been studied much and till now no formulation is available in the market as synthetic
composite ion exchange material as drug carrier. In view of this, it may be beneficial to
consider the use of synthetic composite ion exchange as drug carrier in pharmaceutical research.

Application of different synthesized composite exchanger as drug delivery matrix has been investigated using salicylic acid as model drug. Salicylic acid (SA) is a β-hydroxy acid, belonging to the category of non steroidal anti inflammatory drug (NSAID), chemically classified as monohydroxy benzoic acid. It is commonly used to treat warts, corns, acne, psoriasis [270] etc. Also, it is a major component of aspirin and is recognized by its anti-inflammatory, analgesic and antipyretic effects. Recently it has been suggested that use of anti-inflammatory drug could reduce the development of different types of cancer, including breast cancer [271]. Studies on application ion exchangers as drug delivery system for anti inflammatory drug will be promising because many NSAID loaded material prove their efficacy in cancer therapy [272].

In the present section, SA drug was loaded on synthesized composite exchangers by ion exchange mechanism inorder to evaluate its application as drug carrier. Pectin–Zirconium (IV) molybdosilicate exchanger showing comparatively higher uptake (higher than 80% within 2.5 h) of SA drug, denoted as SA@ Pc-ZrMoSi, was chosen from all synthesized composite exchangers inorder to verify their potential viability as drug carrier. In-vitro drug release studies were carried out on the drug loaded exchangers, these test demonstrates the release pattern of a drug from resinate preparation dosage form. These data are required as a guide to formulation during the development stage earlier to clinical testing.

6.1.1 Experimental

6.1.1.1 Materials

Salicylic acid (E.merck), Hydrochloric acid (HCl), potassium chloride (KCl), potassium dihydrogen orthophosphate (KH2PO4), sodium chloride (NaCl) and sodium hydroxide (NaOH) were purchased from Loba chemie and were used as received.

6.1.1.2 Preparation and characterization of drug carrier

Composite ion exchange material Pectin–Zirconium (IV) molybdosilicate showing comparatively higher uptake (higher than 80% within 2.5 h) of drug was chosen from all
synthesized exchangers to prepare complexes with model drug Salicylic acid (SA). A detailed route for the synthesis and characterization of the exchanger Pc-ZrMoSi has been given in Chapter 3. Pc-ZrMoSi having a composition of 2 % pectin and 1:2:1 ZrMoSi was used for intercalation of anti inflammatory drug Salicylic acid (SA).

6.1.1.3 Cell viability test

i) In vitro cytotoxicity analysis of Pc-ZrMoSi

In-vitro cytotoxic effect of Pc-ZrMoSi was determined by MTT assay method. L929 (Mouse Lung Fibroblast) cell line was initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium (DMEM) ( Gibco, Invitrogen).

The cell line was cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphoteracin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator (NBS Eppendorf, Germany).

The viability of cells was evaluated by direct observation of cells by Inverted phase contrast microscopy and followed by MTT assay method.

a) Preparation of compound stock:

1.0 mg of each compound was added to 1.0 mL of DMEM and dissolved completely by cyclomixer. After that the extract solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility.

b) Cytotoxicity Evaluation:

After 24 h the growth medium was removed and freshly prepared each compounds in 5% DMEM were five times serially diluted by two fold dilution (200 µg, 100µg, 50µg, 25µg nd 12.5µg in 100mL of 5% DMEM). Each concentration of 100µL was added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO₂ incubator.

c) Cytotoxicity Assay by Direct Microscopic observation:

The entire plate was observed at an interval of 5 days in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and
microscopic observations were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

d) Cytotoxicity Assay by MTT Method:

15 mg of MTT (Sigma, M-5655) was reconstituted in 3 mL PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells was removed and 30µL of reconstituted MTT solution was added to all test and cell control wells. The plate was gently shaken well, then incubated at 37°C in a humidified 5% CO₂ incubator for 4 h. After the incubation period, the supernatant was removed and 100µL of MTT Solubilization Solution (DMSO) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 570 nm [273].

The percentage of growth inhibition was calculated using the formula (eqn 6.2):

\[
\text{% of viability} = \frac{\text{Mean OD of sample}}{\text{Mean OD of control group}} \times 100
\]  

(6.2)

ii) Activity studies of SA loaded Pc-ZrMoSi exchanger

Cell viability of different cancer cell with SA loaded Pc-ZrMoSi were determined by direct observation of cells by Inverted phase contrast microscopy and followed by MTT assay method. For this MCF-7 and Ca Ski cell lines were cultured in DMEM media as explained above. And cultured cell lines were incubated with SA loaded Pc-ZrMoSi exchangers with gradient concentration of 12.5, 25, 40, 50 µg/mL. Then % cell viability was calculated using eqn 6.2.

6.1.1.4 Drug loading studies

0.05g of the cation exchanger Pc-ZrMoSi was dehydrated by heating upto 125 °C, in order to remove interstitial water molecule and was suspended in a 1000mL solution of 1.44 mmol of SA in 1:10 ethanol: water mixture. The resulting suspensions were stirred for 4 h at a temperature of 30°C and then they were filtered off, and dried exchangers were kept in a dessicator. The SA loaded exchanger was designated as SA@ Pc-ZrMoSi, and this complex formation is further confirmed by analyzing the exchanger using instrumental
techniques such as UV-DRS and FTIR. The concentration of SA was determined by analyzing the filtrate using UV-Visible spectrometry at a $\lambda_{\text{max}}$ of 303 nm. The percentage of drug adsorbed was calculated using the equation **(eqn 6.3)**,

$$\text{SA adsorbed (\%)} = \left( \frac{C_0 - C_e}{C_e} \right) \times 100$$  \hspace{1em} (6.3)

The amount of drug loaded on the exchanger was calculated using the mass balance equation **(eqn 6.4)**,

$$q_e = \frac{(C_0 - C_e) V}{m}$$  \hspace{1em} (6.4)

Where $q_e$ is the amount of SA adsorbed at equilibrium (mg/g), $C_0$, the initial drug concentration in solution (mg/L), $C_e$, the SA concentration in solution at equilibrium (mg/L), $m$, the mass of Pc-ZrMoSi used (g) and $V$, the volume of SA solution (L).

Effect of parameters such as temperature, pH, time and concentration of SA on drug loading capacity were evaluated. The effect of contact time on the adsorption efficiency of SA onto Pc-ZrMoSi exchanger was evaluated by suspending 0.05g of exchanger in 1000 mL aqueous SA solution of 200 ppm concentration at pH 4.3 over a period of 0.25, 0.5, 0.75, 1, 2, 3 and 5 h on a water bath shaker. Effect of pH on the adsorption efficiency of SA on Pc-ZrMoSi, was investigated by treating aqueous SA solution with the exchanger in the pH range of 1 to 12 and allowed to stand on a water bath shaker for a period of 2.5 h. Further, effect of temperature on adsorption of SA was optimized by varying the temperature between 25-65°C, keeping other parameters constant. Effect of initial concentration of SA on adsorption efficiency was analysed by treating the exchanger in a solution of SA having initial concentration between 40-450 mg/L.

### 6.1.1.5 In-vitro Drug release studies

**In vitro** drug release behavior was studied using two different dissolution mediums, consisting of simulated gastric fluid (SGF, HCl buffer solution of pH 1.2) and simulated intestinal fluid (SIF, Phosphate buffer solution of pH 7.4). Both SGF and SIF were prepared as per the reported method [274], SGF buffer solution of pH 1.2 was prepared by mixing 250 ml of 0.2 M HCl and 147 mL of 0.2 M KCl and SIF buffer solution of pH 7.4 was prepared by mixing 250 mL 0.1 M KH$_2$PO$_4$ and 195.5 mL of 0.1 M NaOH. The
studies were performed using water bath shaker at 37± 0.5 °C. The release test of SA from Pc-ZrMoSi was carried out by suspending 0.05 g of SA@ Pc-ZrMoSi in 1000 mL simulated gastric and intestinal fluids with continuous shaking. Samples were withdrawn at different time intervals, filtered, and analyzed for concentration of SA by UV-Visible spectroscopy.

6.1.2 Results and discussions

UV-Visible spectra of SA solution before and after treatment with exchangers are shown in Fig.6.1. SA solution shows an absorption peak at $\lambda_{\text{max}}=303$ nm and was found to decrease gradually with increase of time, indicating proper loading of the SA cationic drug into the matrix of composite exchanger Pc-ZrMoSi.

![Fig.6.1 UV-Vis spectra of SA solution before and after treatment with Pc-ZrMoSi](image1)

![Fig.6.2 UV-Vis spectra of Pc-ZrMoSi before and after loading of SA](image2)

6.1.2.1 Drug loading confirmation

UV-Visible DRS of solid Pc-ZrMoSi and SA@ Pc-ZrMoSi were depicted in Fig.6.2. Additional absorption maxima near 550-650 nm region is observed in the spectra of drug loaded exchanger, SA@Pc-ZrMoSi. This indicates the strong intercalation of drug molecule in the matrix of ion exchanger Pc-ZrMoSi. According to this UV spectra SA molecule was not degraded using the exchanger Pc-ZrMoSi.

FTIR spectra of Pc-ZrMoSi exchanger and SA @ Pc-ZrMoSi exchanger shows clear evidence for interaction between SA molecule and the composite exchanger and are depicted in Fig.6.3. Spectra of SA loaded exchanger retains all the characteristic bands (3300 cm$^{-1}$, 1622 cm$^{-1}$, 1379 cm$^{-1}$, 1089 cm$^{-1}$, 956 cm$^{-1}$, 901 cm$^{-1}$, 797 cm$^{-1}$) of pristine
PC-ZrMoSi with slight shift in intensity and position of the peaks. In addition to these, a strong band is observed at 1667 cm\(^{-1}\), 1502 cm\(^{-1}\) region which is characteristic of \(-\text{C}=\text{O}, \ -\text{C}-\text{C}-\) stretching vibrations of SA molecule [275].

Fig. 6.3 FTIR spectra of Pc-ZrMoSi before and after loading of SA

6.1.2.2 Cytotoxicity of Pc-ZrMoSi

The cytotoxicity of Pc-ZrMoSi was evaluated by direct microscopic observation followed by MTT assay on L929 cell lines. Inverted phase contrast microscopic images of cells after interaction with different gradient concentration of Pc-ZrMoSi, 200 µg, 100µg, 50µg, 25µg nd 12.5µg/mL, are shown in Fig. 6.4. Any significant detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity. No considerable change in morphology was observed upto 50µg/mL of Pc-ZrMoSi exchanger.

Fig. 6.4 Inverted phase contrast microscopic images of cells after interaction with Pc-ZrMoSi
Percentage viability test results by MTT assay method are depicted in Table 6.1. Almost 86% and 54% of cell viability was found for 12.5 and 200 µg/mL, respectively, of Pc-ZrMoSi composite exchanger. i.e, it shows more than 50% viability for all concentration tested. This signifies the composite exchanger Pc-ZrMoSi has no considerable toxicity and this assures the applicability of Pc-ZrMoSi as a safe drug carrier.

<table>
<thead>
<tr>
<th>Conc. (µg/mL)</th>
<th>Mean OD at 570nm</th>
<th>% viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>0.54875</td>
<td>100</td>
</tr>
<tr>
<td>12.5</td>
<td>0.4744</td>
<td>86.45103</td>
</tr>
<tr>
<td>25</td>
<td>0.43215</td>
<td>78.75171</td>
</tr>
<tr>
<td>50</td>
<td>0.40595</td>
<td>73.97722</td>
</tr>
<tr>
<td>100</td>
<td>0.3234</td>
<td>58.93394</td>
</tr>
<tr>
<td>200</td>
<td>0.2965</td>
<td>54.03189</td>
</tr>
</tbody>
</table>

A safer concentration of 50µg/mL of Pc-ZrMoSi exchanger was used throughout for in-vitro drug intercalation and release studies in which it shows more than 70% cell viability at this concentration.

6.1.2.3 Intercalation conditions

a) Effect of contact time

About 86% of initial concentration used of SA drug was adsorbed on to Pc-ZrMoSi exchanger within 2.5 h of interaction time. % of SA adsorbed with increasing contact is shown in Fig.6.5 (a). It seems that no more amount of SA was adsorbed after interaction of Pc-ZrMoSi with drug molecule upto 5 h. This suggests that equilibrium is achieved within 2.5 h of interaction and therefore it was selected as the optimum time for the intercalation reaction.

b) Effect of pH

Fig.6.5 (b) shows the effect of pH on the sorption of drug SA on Pc-ZrMoSi composite exchanger. It was observed that % of SA adsorbed increased upto pH 4.0 and decreased with further increase of pH of SA solution. Below pH 4.0, $H^+$ ions and SA molecule compete for exchangeable sites in Pc-ZrMoSi which results in lower adsorption of SA on to the matrix of Pc-ZrMoSi. The decrease in adsorption of SA above pH 4.0 may be due to the presence of $Na^+$ ions at higher pH, which compete with the drug SA.
maximum sorption of 90% was observed at pH 4.0 as pK_a value, corresponding to its protonated form was 4.0, there exist equilibrium between ionized and unionized form of SA at pH 4.0.

**Fig.6.5 Effect of a) Contact time b) pH c) Temperature d) Initial concentration of SA parameters on % sorption on Pc-ZrMoSi exchanger**

c) Effect of temperature

**Fig.6.5 (c)** shows the effect of temperature of reaction media on the % sorption of SA drug on to the matrix of composite exchanger. Initially, adsorption % increases with increase of temperature upto 40°C and then shows a slight decrease. Also, % sorption of SA was found almost constant around 50-65 °C of temperature.

d) Effect of concentration of SA

Effect of initial concentration of SA on the sorption is shown in **Fig.6.5 (d)**. The % of intercalation increases as initial concentration of SA in the solution increases. This may
be attributed to greater concentration gradient at the initial stage. The maximum amount was 88% for an initial concentration of 400 mg/L.

### 6.1.2.4 In-vitro release studies

The in-vitro controlled release test were carried out by suspending the drug loaded exchanger SA@Pc-ZrMoSi in simulated gastric and intestinal fluids under continuous shaking on a water bath at 35°C and are shown in Fig.6.6. The percentage of SA released was measured at 30 min time interval by measuring the absorbance. In the release profile of SA in gastric fluid (pH 1.2), 61.5 % of SA was released within 3 h. The slow and sustained release may be interpreted in terms of ion exchange process between the drug and alkali metal ions of the buffer. Where as in the case of intestinal fluid (7.4) release rate was very fast and 79 % of SA was released within 3 h. It was observed that the release of SA from the drug loaded exchanger is much more pronounced in basic medium than in the acidic medium, this higher release at pH 7.4 may be due to higher Na⁺ concentration which competes with SA molecule.

![Fig.6.6 Control release profile of SA from SA@ Pc-ZrMoSi composite at SGF (pH-1.2) and SIF (pH-7.4)](image)

The release amount did not reach 100 % due to the presence of electrostatic interaction between the drug SA and the composite exchanger Pc-ZrMoSi. Moreover, these
are characteristics of ion exchange reaction. As it is an equilibrium process all exchangeable cations may not react completely which leads to incomplete release [276]. The overall results confirm the suitability of drug loaded Pc-ZrMoSi for the controlled/sustained release of anti-inflammatory drug.

6.1.2.5 In-vitro drug release kinetics

To the better understanding of release mechanism of drug molecule, different kinetic models were analysed using in-vitro release data of SA from the exchanger at different physiological pH. The best model is the one with highest correlation coefficient, $R^2$ and was used to describe the release profile of SA from the composite exchanger. The kinetics of in-vitro release of drug from carrier materials is usually described using Zero order, First order, Higuchi and Korsmeyer-peppas mathematical models.

a) Zero order model[277]

Zero order describes the system where the release rate of drug from the formulation is independent of concentration. In its simplest form it is represented as (eqn 6.5),

$$Q = Q_0 + k_0 t \quad (6.5)$$

Where $Q$ is cumulative amount of drug release, $Q_0$ is the initial amount of drug in solution, $k_0$ is the zero order rate constant and $t$ is the time in hours. For release kinetic study, the graph is plotted (Fig.6.6(a)) between % cumulative drug release (% CDR) versus time.

b) First order model[278]

First order model describes the concentration dependent drug release from the system, and is formulated as (eqn 6.6),

$$\log Q_1 = \log Q_0 - \frac{k_1 t}{2.303} \quad (6.6)$$

Where $Q_1$ is cumulative percentage drug remaining, $Q_0$ is the initial concentration of drug and $k_1$ is the first order constant. The data obtained from the release study is plotted (Fig.6.7 (b)) as log cumulative % drug remaining (% CDR) vs time.
c) **Higuchi model**[279].

Higuchi’s model describes the release of drug as a square root of time-dependent process and its simplest form is formulated as *(eqn 6.7)*,

\[
Q = k_H t^{1/2}
\]  
*(6.7)*

Where \(k_H\) is the constant reflecting the design variables of the system. The data obtained were plotted *(Fig.6.7(c))* as cumulative percentage of drug released versus square root of time. It describes the release as a diffusion process based on the Fick’s law, which is dependent on square root of time.

d) **Korsmeyer-Peppas model** [280]

This is a semi-empirical model with diffusion based mechanism and implies that the fractional release of the drug is exponentially related to the release time and is given by the equation *(eqn 6.8)*,

\[
\frac{M_t}{M_{\infty}} = k_{kp} t^n
\]  
*(6.8)*
Where $M_t/M_{\infty}$ is the fraction of drug released at time $t$, $k_{KP}$ is the rate constant, $n$ is the diffusion/release exponent indicative of mechanism of drug release. The data obtained were plotted as log cumulative percentage drug released versus log time (Fig. 6.7 (d)) and it gives a straight line with slope $n$ and intercept $\log k_{KP}$.

<table>
<thead>
<tr>
<th>Models</th>
<th>Parameters</th>
<th>pH-1.2</th>
<th>pH-7.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero order</td>
<td>$R^2$</td>
<td>0.9426</td>
<td>0.9379</td>
</tr>
<tr>
<td></td>
<td>$k_0 (h^{-1})$</td>
<td>8.0965</td>
<td>11.478</td>
</tr>
<tr>
<td>First order</td>
<td>$R^2$</td>
<td>0.9562</td>
<td>0.9461</td>
</tr>
<tr>
<td></td>
<td>$k_1 (h^{-1})$</td>
<td>0.1576</td>
<td>0.3382</td>
</tr>
<tr>
<td>Higuchi</td>
<td>$R^2$</td>
<td>0.9690</td>
<td>0.9529</td>
</tr>
<tr>
<td></td>
<td>$k_p (h^{-1/2})$</td>
<td>19.734</td>
<td>27.956</td>
</tr>
<tr>
<td>Korsmeyer-Peppas</td>
<td>$R^2$</td>
<td>0.9753</td>
<td>0.9625</td>
</tr>
<tr>
<td></td>
<td>$n$</td>
<td>0.2298</td>
<td>0.2369</td>
</tr>
<tr>
<td></td>
<td>$k_{KP} (h^{n})$</td>
<td>44.15</td>
<td>59.42</td>
</tr>
</tbody>
</table>

Based on the $R^2$ values, the Korsmeyer-Peppas model gives best fit to the drug release data (Table 6.2). The value of $R^2$ is 0.9753 for pH-1.2 and 0.9625 for pH 7.4. The $n$ value is used to characterize different release mechanism, if it is less than 0.45 it indicates the overall solute diffusion mechanism is quasi-Fickian, 0.45 indicates Fickian diffusion, 0.45<$n<$0.89 indicates non Fickian diffusion, 0.89-1 indicates case 2 relaxation and if it is >1 it is non Fickian super case 2. The value of release exponent, $n$ obtained from the slope of the Korsmeyer-Peppas plot was 0.2298 for pH 1.2 and 0.2369 for pH 7.4. This obtained value of $n$ indicates that the drug release process could be described as quasi-Fickian diffusion controlled mechanism.

### 6.1.2.6 Activity studies of SA@Pc-ZrMoSi exchanger

The potential of salicylic acid loaded Pc-ZrMoSi exchanger towards cancer cell viability were evaluated using MCF-7 and Ca Ski cell lines. The results obtained from microscopic observation and MTT assay are presented in Fig. 6.8. A decrease in cell viability of more than 50% is observed for both MCF-7 and Ca Ski cell line compared to their respective control. Also, as concentration of SA loaded Pc-ZrMoSi exchanger
increases from 12.5 to 50µg/mL, reduction in % cell viability is increased. This indicates the potential activity of released anti-inflammatory drug SA to inhibit the growth of cancer cells and act as anticancer agents. Thus SA loaded Pc-ZrMoSi exchanger will be a promising material in cancer therapy. Though, further detailed in-vitro and in-vivo studies are needed for better practical application of this material as drug carrier.

6.8 Cell viability of MCF-7 and Ca Ski cell lines using SA @Pc-ZrMoSi

6.2 ANTIMICROBIAL APPLICATIONS

Deterioration of existing fresh water resources is largely observed in developing countries mainly due to human activities. Excessive sewage generation and its discharge into nearby lakes and rivers are the main cause of water pollution. A major reason of ground water contamination is the existence of enteric pathogens, which have been found to survive in septic tanks and then move along with percolating waste water through soil. Nowadays human beings are at high risk of infections from their surrounding water bodies by microorganisms such as bacteria, fungi, moulds [281] etc. So clean water, i.e., water free of toxic chemicals and pathogens is necessary to human health. Therefore, the removal or inactivation of pathogenic microorganism is a vital step in the treatment of waste water [282]. Today a number of materials such as inorganic nano particles, activated carbon, halogen and its derivatives etc., are employed as anti bacterial agents and recently, scientific community gives much attention to develop new and cost effective materials as anti bacterial agents. Several investigations have been carried out for the use of natural and synthetic ion exchange material as bactericidal agents for water disinfection [283, 284].
The development of new ion exchanger with the ability to inhibit pathogenic microbial growth will be hopeful for environmental remediation. In the present section, the details of inhibitory power of some synthesized composite exchangers on the growth of *E.coli* (gram-negative bacteria) and *Staphylococcus aureus* (gram-positive bacteria) were investigated and compared with standard antibacterial drugs such as Gentamycin.

### 6.2.1 Experimental

The antimicrobial effects of some synthesized composite exchangers were determined for two bacterial strains such as *E.coli* and *S. aureus* by Agar- well diffusion method. In this method, the materials are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in millimeters. For this Muller Hinton Agar Medium and Nutrient broth were prepared as mentioned below. Gentamycin and streptomycin were used as standard antibacterial agent (concentration: 20mg / mL).

**Muller Hinton Agar Medium (1 L):** The medium was prepared by dissolving 33.9 g of the commercially available Muller Hinton Agar Medium (HiMedia) in 1000 mL of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100 mm petriplates (25-30 mL/plate) while still molten.

**Nutrient broth (1L):** One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient medium (HiMedia) in 1000 mL distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Petriplates containing 20 mL Muller Hinton medium were seeded with 24 h culture of bacterial strains such as *E.coli* and *S. aureus*, wells of approximately 10 mm were bored using a well cutter and 25 µL, 50 µL and 100 µL of sample was added to the well from a stock concentration of 0.1g/mL. The plates were then incubated at 37°C for 24 h. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Gentamycin was used as a positive control.
6.2.2. Results and discussions

The antimicrobial effect of PANI-CeMoSi, PANI-ZrMoSi and Pc-ZrMoSi were determined for two bacterial strains such as *E.coli* and *S. aureus* by Agar-well diffusion method. The area of zone of inhibition is used as a criterion to ascertain the bactericidal activity of composite exchangers. If the bacteria are susceptible to the particular exchanger used as bactericidal agent from a disc, an area of clear media where bacteria are not able to grow surrounds the disc, is known as zone of inhibition. Thus larger the zone of inhibition, more sensitive is the bacteria to the anti bacterial agent. According to this criterion, 10 mm to 17 mm zone of inhibition zone would represent significant antibacterial activity [285]. Details of antibacterial activity studies of PANI-CeMoSi composite exchangers are given in Table.6.3. It is found inactive against *E.coli* and *S.aureus* at low concentration of 25 µg/L. Significant antibacterial activity is observed for *E.coli* but activity is negligible for *S.aureus* at higher concentrations of 100 µg/L. The photographic images of antibacterial study of PANI-CeMoSi towards *E.coli* and *S.aureus* shown in Fig.6.9, clearly shows formation of inhibition zone.

![Fig.6.9 Images of zone of inhibition for E.coli and S.aureus for PANI-CeMoSi](image)

<table>
<thead>
<tr>
<th>Table.6.3 Test results of antimicrobial screening of PANI-CeMoSi</th>
</tr>
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<tbody>
<tr>
<td>Sample</td>
</tr>
<tr>
<td>Gentamycin</td>
</tr>
<tr>
<td>PANI-CeMoSi</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Fig. 6.10 and Table 6.4 clearly indicate that PANI-ZrMoSi has significant antibacterial activity against both gram-negative and gram-positive bacteria. The resulting zone of inhibition was 16 mm for *E. coli* and 12.5 mm for *S. aureus* at a concentration of 100 µg/L. Also, it shows moderate activity against *E. coli* at very low concentration of 25 µg/L of exchanger.

![Fig. 6.10 Images of zone of inhibition for E. coli and S. aureus for PANI-ZrMoSi](image)

**Table 6.4 Test results of antimicrobial screening of PANI-ZrMoSi**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume of sample (µL)</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>S. aureus</em></td>
</tr>
<tr>
<td>Gentamycin</td>
<td>20</td>
<td>33.6</td>
</tr>
<tr>
<td>PANI-ZrMoSi</td>
<td>25</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Antibacterial screening of Pc-ZrMoSi was performed and analysis data are depicted in Table 6.5. Fig. 6.11 shows the zone of inhibition generated for *E. coli* and *S. aureus* pathogens by Pc-ZrMoSi exchanger. It seems that the exchanger is totally inactive against *S. aureus* at low concentrations and very poor activity at higher concentrations. But it exhibited better antibacterial activity against the growth of gram negative bacteria *E. coli*. The property of antibacterial activity can make the exchanger kill microorganisms, thereby making the Pc-ZrMoSi exchanger bio compatible. This will be advantageous for its use as a drug carrier in a controlled release system. Also, the exchanger existing within the body after drug release is beneficial to human metabolism as it offers resistance to the pathogenic microorganism.
The mechanism responsible for bactericidal activity of ion exchangers towards *E. coli* and *S. aureus* involves the rupturing of bacterial cell wall due to binding of composite exchanger by release of ions through ion exchange interaction. The reactive functional groups such as -OH, -NH₂ are present in the composite ion exchanger bonds to capture pathogenic bacteria for its degradation. Also, the metal ion in the ion exchange matrix inhibits the active transport and enzyme activity, which results in plasmolysis of the cell wall, cytoplasm separation from the bacterial cell wall or inhibition of synthesis of bacterial cell wall [286]. All exchangers studied show higher anti bacterial activity towards gram negative bacteria *E. coli* than *S. aureus*. This could be explained based on the nature of the material present in the cell wall of gram-positive and gram-negative bacteria. *E. coli* (gram-negative) is surrounded by a thin peptidoglycin cell wall and *S. aureus* (gram-positive) by layers of peptidoglycin and is thicker than cell wall of *E. coli*. Also, cell wall of *E. coli* has a thickness of 7-8 nm and that of *S. aureus* has around 20-80 nm. Thus an easier permeability and easier interaction of composite exchanger with *E. coli* could be achieved which leads to easy destruction and death of the cell [287].
Conclusion

A very interesting possibility of composite exchanger in drug delivery research has been investigated by the use of Pc-ZrMoSi exchanger towards salicylic acid delivery. The incorporation of SA on to the composite exchanger has been achieved and release studies were performed through *in-vitro* experiments. Cytotoxicity analyses of Pc-ZrMoSi exchanger was carried out by MTT assay method and support its use as a safe drug carrier. Drug release kinetics are analysed to understand the release mechanism. Activity of SA loaded Pc-ZrMoSi exchanger was tested by evaluating the cell viability of MCF-7 and Ca Ski cancer cell lines. A reduction in cell viability is observed and it supports the promising use of this material in cancer therapy. However, further detailed studies are needed for better practical applicability of this exchanger as drug delivery carrier. Antimicrobial activity study using synthesized exchanger showed that all the tested composite ion exchangers are effective against bacterial strains such as *E.coli* and *S. aureus*. Also, comparatively higher antibacterial activity is observed for *E.coli* than *S. aureus* using all the exchangers. The performance of ion exchangers as bactericidal agents along with better ion exchange properties can be applied in wastewater treatment, in order to control the growth of microbial in wastewater as well as the removal of toxic pollutant from it.