Surface-Enhanced Biocompatibility and Adsorption Capacity of a Zirconium Phosphate-Coated Polyaniline Composite

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ABSTRACT: The present study deals with the synthesis, characterization, and testing of a novel composite, zirconium(IV) phosphate-coated polyaniline (ZrPO₄@PANI), toward the adsorption- and surface-controlled toxicity applications. Following the synthesis of the ZrPO₄@PANI composite using the sol–gel route, various characterization techniques such as Fourier transform infrared spectroscopy, scanning electron microscopy, and powder X-ray diffraction were employed to confirm its surface functionality, morphology and agglomeration, and crystallinity and crystal nature, respectively. The composite was found to be effective toward the adsorptive removal of the methylene blue dye (an organic pollutant) as against the changes in the dye concentration, dose, pH, and so forth. Also, to understand the MB adsorption kinetics, the experimental data were evaluated using the Langmuir and Freundlich models and the results were described in accordance with the Langmuir isotherm model (an adsorption capacity of 120.48 mg/g at ambient temperature). In addition, the tests conducted using pseudo-first- and pseudo-second-order kinetic models confirmed the existence of pseudo-second-order rates. Furthermore, the calculation of thermodynamic parameters for the MB adsorption, namely, changes in enthalpy, entropy, and Gibbs’ free energy, exhibited a spontaneous, feasible, and exothermic nature. Finally, the comparative studies of in vitro toxicity and flow cytometry confirmed that the copresence of ZrPO₄ along with PANI significantly improved the biocompatibility. The outcome of the experimental results implies that the composite is capable enough of serving as the safe and low-cost adsorbent, in addition to supporting the effective capping of the surface toxicity of PANI.

1. INTRODUCTION

The recent increase in the usage of synthetic dyes for the product development in various industries such as paint, food, paper, plastic, textile, and so forth and at the same time the inadequate purification of industrial and household sewages lead to the water, air, soil, and ecological pollutions. In general, the water color is a primary indicator of its quality, and it is a fact that even a minute amount of dye (below the 1 ppm range) in water becomes evident by its color and therefore can be considered as not suitable for drinking or maintenance of ecosystems. Because of the increased pollution of synthetic dyes in the environment, the most prominent one to suffer is the aquatic system as the increased levels of dyes in water cause the inadequate passage of natural sunlight. Under such conditions, the photosynthetic metabolism of aquatic life gets strongly interrupted and cannot sustain in the presence of toxic dye constituents. Among many different organic dyes responsible for environmental pollutions, methylene blue (MB) is one common industrial dye of synthetic origin, which enters the human body through water and air sources. The common effects of this dye on humans include the increased heart rate, tremors, vomiting sensation, Heinz body formation, hemoglobin depletion, methemoglobinemia, and micturition and also causes jaundice, cyanosis, quadriplegia, and so forth. Therefore, considering the severe effects of the MB dye on humans, it is highly required to treat the MB dye-containing water before it essentially gets discharged into the environment. The various methods employed for the treating of effluents include filtration, chemical precipitation, reverse osmosis, ion exchange, electrochemical deposition, ultrafiltration, and coagulation. All these methods are different from each other in terms of efficiency of removing the dyes, cost-effectiveness, the complexity of handling the machines, need of trained personnel, and so forth. Among such methods, the adsorption technique is a more prominent and favorable

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one for treating the effluents because of its different features namely cost-effectiveness, simplicity in design, easy operating method, no need for manpower, and so forth. It also provides the best results without giving out any harmful degradation products and even liberates high-quality effluents. In the adsorption technique, a solid support with a porous architecture is employed for the effective separation of dye molecules and the operating mechanism between the adsorbate and the adsorbent is mostly physicochemical. In a study, for example, a composite of self-assembled monolayers from chitosan, carbon nanotubes, and octa-amino polyhedral silsesquioxanes has been developed for the isolation of Congo red and methyl orange dyes. The other composites in the same category include the natural adsorbents manufactured from nanofibers containing the cellulose nanocrystals, graphene oxide-polyethylenimine hydrogels, and Fe₃O₄/poly(allylamine)/carboxylate graphene oxide sheets formed from layer-by-layer self-assembly-mediated formation, to name a few.

Polyaniline (PANI) is an excellent conductive polymer as it contains the −NH groups in addition to the aromatic unsaturation in its structure, and it is widely used in the manufacturing of printed circuit boards, antistatic coatings, and also as the corrosion protector. Similarly, zirconium oxide (ZrO₂) maintains some attractive features such as optical, dielectric, thermal, and chemical properties and so it is useful in many different applications to incorporate resistance and biocompatibility. Taking advantage of the PANI’s cage-like structural formation capacity linked to the thermochemical resistance offered by ZrO₂, the PANI/ZrO₂ nanocomposite was found to be very much useful for the monolayer adsorption and its adsorption capacity toward the MB dye was found to be 77.5 mg/g.

By considering the inbuilt properties offered by the PANI and zirconium derivatives, the present work is aimed to develop some non-toxic and stable adsorbents for the removal of the MB dye from the aqueous samples. The biocompatibility of the ZrPO₄@PANI composite was studied by making use of an in vitro cell culture model over a 24 h period. Following the in vitro studies, the adsorption capacity of the composite for removing the MB dye was tested, where the results confirmed that the composite has lots of practical merits when used for industrial wastewater treatment. The adsorption capability of the ZrPO₄@PANI composite was evaluated toward the MB removal from the aqueous media against the changes in various parameters such as adsorption capacity, optimization of different physicochemical parameters, and adsorption kinetics. Different adsorption models were employed to brief out the equilibrium isotherms and then the isotherm constants were illustrated. The thermodynamic factors, namely changes in enthalpy, entropy, and the free energy were determined to get the information on the reaction mechanism and even elaborate the scientific concept of using it traditionally.

2. RESULTS AND DISCUSSION

In order to come up with the ZrPO₄@PANI composite with the best possible ion-exchange capacity (IEC), we have prepared various composites using different concentrations of PANI and tabulated the IEC results in Table 5. Also, it was found from the results that PANI−ZrPO₄ has shown superior ion exchange ability toward Na⁺ ions as compared to the ZrPO₄ precipitate. In addition, among all the different combinations tested, the A-6 sample prepared with 0.6 M PANI provided the highest IEC value (1.98 meq/g) and so this is selected as the suitable material for carrying out the sorption studies.

Figure 1 provides the comparison of the scanning electron microscopy (SEM) images of (a) pure PANI, (b) ZrPO₄, and (c) ZrPO₄@PANI and (d) the corresponding energy-dispersive X-ray analysis (EDAX) spectrum of the ZrPO₄@PANI sample. From the images, it can be observed that the rough surfaces of the ZrPO₄ (Figure 1a) particles are becoming soft and are getting coated fully with the PANI (Figure 1c). In addition, a closer look of the ZrPO₄@PANI’s surface (the inset of Figure 1c) indicates the maintenance of porous structures with several uniform pores that can provide suitable binding sites and complete localization toward the trapping of adsorbent molecules when used for the adsorption purposes.
Furthermore, the EDAX spectrum in Figure 1d provides visual evidence for the persistence of ZnPO$_4$ in the composite of ZrPO$_4$@PANI, and the elements investigated are Zr, P, O, and C, thereby confirming the efficient synthesis procedure.

Figure 2a compares the Fourier transform infrared (FTIR) spectrum of pure PANI with those of the ZrPO$_4$@PANI composite and of the pure PANI sample, where the band observed around 3310 cm$^{-1}$ is due to the NH$_2$ stretching and the sharp peak at 2893 cm$^{-1}$ is from the $\text{-CH}$ group of the polymer chain. Similarly, the 1730 cm$^{-1}$ peak is from the carbonyl bond of the carboxyl group, the 1635 cm$^{-1}$ band is attributed to the NH$_2$ bending vibration, and the 526 cm$^{-1}$ band is attributed to the NH$_2$ wagging. Also, the band located at 1552 cm$^{-1}$ can be linked to the C═C stretching vibrations of the benzenoid rings, the 1410 cm$^{-1}$ band is from the C−N stretching vibration, and the 1040 cm$^{-1}$ band is due to the in-plane C−H bending mode. The characteristic peak around 3152 cm$^{-1}$ for the composite is from the symmetric stretching vibration and the peak at 1620 cm$^{-1}$ is due to the deformed vibrations of O−H bonds. The peak at 1252 cm$^{-1}$ is linked to the out-of-plane bending vibration of P−OH and the 3455 cm$^{-1}$ peak of the−NH bond stretching indicates the formation of a very strong bond. The bands observed at 2954 cm$^{-1}$ and 1190 cm$^{-1}$ correspond to the in-plane C−H stretching vibrations, while the band at 1190 cm$^{-1}$ corresponds to the in-plane C−H bending vibrations formed as quinoid rings doped with the PANI. Also, the bands observed at 1454 and 1520 cm$^{-1}$ confirm a benzenoid and quinoid stretching mode. The bands obtained were truly coherent toward the emeraldine salt of PANI.

Comparison of the X-ray diffraction (XRD) patterns of the ZrPO$_4$@PANI composite with that of pure PANI is shown in Figure 2b, where the typical reflection patterns observed for the composite are at 2$\theta$ of 11.7, 19.6, 34.2, and 37.98° corresponding to ZrPO$_4$, while the diffraction patterns at 14.8, 20.3, 25.2, 27.4, and 29.9° are related to PANI. However, for the pure PANI sample, the patterns are observed at 20.3°, 25.2°, and 27.4°, which indicate a highly ordered structure and crystallinity of the conducting polymer due to the continuous repetition of quinoid and benzenoid rings. Also, for the composite, the observation of two reflection patterns at 14.8 and 29.9° indicates that the PANI chain maintains the parallel and perpendicular periodicity. The results indicate that the polymer bonding with metal phosphate does not affect the crystalline structure of PANI. Because some of the diffraction patterns of PANI coincide with the ZrPO$_4$ patterns, all the patterns related to individual components are not seen and in addition, no impurity patterns are observed, thereby confirming the purity. The outcome of this diffractogram clarifies the formation of the ZrPO$_4$@PANI composite in a semicrystalline phase. In addition, the mean crystallite size of the ZrPO$_4$@PANI composite was calculated to be 35 nm using the Scherrer equation. Furthermore, the zeta potential comparison (in the pH range of 2–11) of pure PANI and the ZrPO$_4$@PANI composite provided in Figure 2c indicates
the changes occurring in the total potential value of the pure PANI polymer following its conjugation with ZrPO₄. The observed changes include the conversion of the positive charge of PANI’s surface toward negative zeta potential values, and these slight changes may have the capacity to significantly alter the total adsorption capacity of the composite.

2.1. Influence of the Adsorbent Dose and Contact Time. The analysis of the effect of the adsorbent dosage provides information relating to the effectiveness of an adsorbent and even the capability of a dye that is to be adsorbed in the least dosage in accordance with the economic factors. The dosage is considered as the main aspect among other parameters, which enhances the adsorption process by mainly limiting the adsorption capacity of the adsorbent. For studying the influence of the enhanced adsorption dosage on the percentage (%) removal ability of the dye, the adsorbent dose was selected in the range of 0.01 to 0.09 g per 100 mL. The pH was maintained at 4, the initial concentration of the dye was 50 mg/L, and the contact time was 1 h, and the results are shown in Figure 3a,b. The outcome illustrated in Figure 3a clarifies that there is an increase in the removal efficiency of the ZrPO₄@PANI composite compared to the other two adsorbents of pure PANI and ZrPO₄ compounds and this adsorption capacity seems to be increased with an increase in the adsorbent dose. This enhanced adsorption of the ZrPO₄@PANI composite can be linked to the extra support offered by means of the composite formation and stability in the aqueous solvents. Also, the results shown in Figure 3b made it clear that about 95.77% removal efficiency was achieved at a dose of 0.09 g/100 mL, and it is considered as the favorable efficiency liberating inexpensive materials. The observation of such a high efficiency from the composite can be linked to the availability of more active sites for dye adsorption. The same phenomenon was also explained in terms of MB adsorption through ficus carica bast. Similarly, the adsorption capacity seems to be increased with an increase in the adsorbent dosage until 0.05 g dosage. A further increase of the dosage beyond 0.05 g (per 100 mL) did not bring many changes to the % removal of the dye. As such, there was an adsorbent particles’ congregation that brought about an increase in the effective material’s surface area. Therefore, 0.05 g/100 mL was recognized as the optimum dose to carry out further experiments.

To use any material as a potential adsorbent, understanding the effective contact time is of basic significance. The equilibrium time agrees with the mechanism of diffusion control because it moves toward the surface of sorption. Figure 3c shows the influence of the contact time on the MB removal, where about 94.68% removal occurs during the first 60 min and after a time period of 90 min, the equilibrium seems to be achieved. The adsorption rate changes due to the vacant nature of all the adsorbent sites initially, and the solute concentration gradient was also high. Later, it was observed that the vacant site of the adsorbent got decreased leading to a decrease in the adsorption rate as well as the concentration. At the end of the experiment, the decrease in the adsorption rate implies the monolayer formation, which is mainly because of the lack of active sites needed for the uptake mechanism just after maintaining the equilibrium state. The observation of such a high efficiency from the composite can be linked to the availability of more active sites for dye adsorption. The same phenomenon was also explained in terms of MB adsorption through ficus carica bast. Similarly, the adsorption capacity seems to be increased with an increase in the adsorbent dosage until 0.05 g dosage. A further increase of the dosage beyond 0.05 g (per 100 mL) did not bring many changes to the % removal of the dye. As such, there was an adsorbent particles’ congregation that brought about an increase in the effective material’s surface area. Therefore, 0.05 g/100 mL was recognized as the optimum dose to carry out further experiments.

Figure 3. (a) Comparison of the dye adsorption behavior of ZrPO₄@PANI along with pure ZrPO₄ and PANI, (b) influence of ZrPO₄@PANI on dye adsorption capacity, and (c) influence of the contact time on the adsorption capacity of the ZrPO₄@PANI composite.

Figure 4. Influence of (a) initial MB concentration and (b) solution pH on the adsorption behavior of the ZrPO₄@PANI composite.
eco-friendly, and cost-effective material was investigated with the varied dye concentrations in the range of 10 to 100 mg/100 mL. The adsorption mechanism was influential because of the solute-solution since the adsorptive reactions are directly proportional to the solute concentration. The outcome showed that the % removal tends to decrease with an increase in the concentration as shown in Figure 4a. It is also inferred that a very limited amount of high energy sites is present on the adsorbent’s surface. At a high dye concentration, the availability of adsorption sites tends to become less for maintaining the constant dosage of the adsorbent. As such, the removal of MB is dependent on the concentration, and the unoccupied active sites can be observed on the surface of the adsorbent at the low concentrations. The interaction between the dye and adsorbent also increases with an increase in the concentration. The outcomes showed a decreased % removal with an increase in the concentration similar to that of the MB adsorption on a magnesite-halloysite composite and an increased adsorption capacity was also said to be observed for it. The observation of such behavior of our ZrPO₄@PANI composite can be mainly linked to the concentration gradient that serves as the main driving force along with an increase in the concentration.

With the changes in the pH, the adsorption process is also affected to a large extent. The influence of solution pH is dependent on the number of ions present in the mixture and on the electrostatic interactions along with the adsorption surface. The solution pH affects the aqueous chemistry as well as the surface-binding sites. For the studies, the pHs in the range of 2–11 were adjusted by making use of 0.1 M HNO₃ and 0.1 M NaOH solutions. Figure 4b shows the dye adsorption onto the surface of the ZrPO₄@PANI composite, where the results indicate that there is no much change in the % removal of the dye from the solution. From the analysis, we observed only a little change, that is, a 92.67 to 95.12% increase in the % removal with an increase in the solution pH from 2 to 11, which means that the adsorption onto the ZrPO₄@PANI composite is also dependent on pH. At low pHs, the adsorbent surface is positively charged and is generally suitable for ionic contaminants. Because the MB dye is cationic, the positive charges occupying the appropriate adsorption sites contended the similar positively charged dye molecules, which finally leads to a decreased dye adsorption. At high solution pHs, the negatively charged surface supports more for the adsorption of cationic dye contaminants.

2.3. Adsorption Mechanism. The mechanism of removing the MB dye from the solution can occur either in an electrostatic or non-electrostatic way, where the active sites present at the adsorbent surface play a critical role. The non-electrostatic interaction involves Vander Waal’s forces, π-π stacking, and hydrophobic interactions, and the schematic representation of the interaction between MB and the adsorbent surface is shown in Scheme 1. It is quite obvious that MB shows a planar structure comprising of many aromatic rings, which can easily form π-π stacking along with the rings on the copolymer of PANI. The electrostatic attraction among cationic MB molecules and phosphate groups plays a significant role in improving the adsorption capacity. In the process of dissolution of MB molecules, Cl⁻ ionizes first and liberates out in a detached state, whereas the remaining cationic part gets adsorbed onto the surface that is negatively charged by means of electrostatic forces.

2.4. Influence of Ionic Strength. The influence of ionic strength on the dye uptake was put under study by the addition of the NaCl concentration that ranges between 0.01 to 0.5 mol/L. It was observed that with an increase in the ionic strength, there was a decrease in the MB removal capacity as shown in Figure 5. The observation of such a result can be due to the availability of NaCl in the solution that safeguards the electrostatic interaction between the opposing charges mainly at the solid sorbent surface and the dye molecules, leading to a decrease in the adsorbed amount with respect to an increase in the NaCl concentration.

2.5. Adsorption Kinetics and Thermodynamics. The adsorption kinetic studies were carried out for estimating the contact time required for experimental adsorption to attain an equilibrium state. Studies based on adsorption kinetics provide important knowledge and an understanding of the adsorption rate on the adsorbent surface and how it controls the residual time during the overall adsorption process. The study of adsorption kinetics is also performed to determine the favorable model that fits well to elaborate the experimental outcome. The two kinetic models namely pseudo-first-order and pseudo-second-order were used to evaluate the time dependency of the adsorption process and also to infer the corresponding relation to the adsorption data. The non-linear forms for the pseudo-first-order and pseudo-second-order are shown in eqs 1 and 2.

\[
\log(q_e - q_t) = \log q_e - \frac{k_1 t}{2303}
\]  

Figure 5. Influence of NaCl concentrations on the adsorption of the ZrPO₄@PANI composite.
The value of $R^2$ for the pseudo-second-order reaction (0.996) is more than the value of $R^2$ for the pseudo-first-order reaction (0.986). It shows that the rate-limiting step is the chemisorption process, which includes valence force by the mechanism of either exchange or sharing of electrons.37

The thermodynamic parameters such as changes in entropy ($\Delta S$), free energy ($\Delta G$), and enthalpy ($\Delta H$) were studied in order to find out the thermodynamic model of intermediate dye adsorption onto the ZrPO$_4$@PANI composite. The parameters were estimated on the basis of equations given below:

$$\ln(K_d) = \frac{\Delta S}{R} - \frac{\Delta H}{RT}$$

(3)

$$\Delta G = -RT \ln(K_d)$$

(4)

$$K_d = \frac{(C_0 - C_e)}{C_e} \frac{V}{W}$$

(5)

where $K_d$ is the distribution coefficient, $R$ is the universal gas constant, and $T$ is the temperature in Kelvin.

For determining the thermodynamic parameters, the dye adsorption onto the specific material was calculated at a range of temperatures. The thermodynamic parameters were found to be transfer unit mole of solution from the solution into the solid–liquid interface. The values of $K_d$ and $\Delta G$ at certain specific temperatures were measured based on eqs 3 and 4.

From the table, the negative value of $\Delta G$ specifies the spontaneous nature and feasibility of the adsorption process.38 As the value of $\Delta G$ decreases at high temperatures, it clarifies that the higher temperature is suitable for the adsorption process. The positive values of $\Delta H$ and $\Delta S$ show that the adsorption process is an endothermic process as well as arbitrary at the adsorbent–solution interface. The accuracy of the values of $\Delta S$ and $\Delta H$ is confirmed by the unity of the $R^2$ value from the plots. The chemisorption of $\Delta G$ was in between the range of $-80$ to $-400$ kJ/mol, whereas the physisorption of $\Delta G$ was in the range between $-20$ and $0$ kJ/mol.39

### Table 2. Thermodynamic Adsorption Parameters of the Dye Onto the ZrPO$_4$@PANI Composite at Different Temperatures

| temp (K) | $\Delta G$ (kJ/mol) | $\Delta H$ (kJ/mol) | $\Delta S$ (J/mol K) |
|---------|---------------------|---------------------|---------------------|
| 293     | $-1.72$             | 35.77               | 127.96              |
| 298     | $-2.36$             |                     |                     |
| 303     | $-3.00$             |                     |                     |
| 308     | $-3.64$             |                     |                     |
| 313     | $-4.28$             |                     |                     |
| 318     | $-4.92$             |                     |                     |

2.6. Adsorption Isotherms. The adsorption isotherm explains the interaction of adsorbents and the adsorbate molecules when the whole system attains an equilibrium state. The Langmuir isotherm model is in accordance with the assumption related to the monolayer adsorption on the homogeneous surface without underlying through the interaction of adsorbates and adsorbents.40 On the other hand, the Freundlich isotherm model presumes multilayer adsorption on the heterogeneous surface.41 Both isotherms are given in the non-linear form in eqs 6 and 7. The theoretical model describing the experimental data was taken from the correlation coefficient.

$$\frac{C_e}{q_e} = \frac{1}{q_m} + \frac{1}{K_q q_m}$$

(6)

$$q_e = K_q C_e^{1/n}$$

(7)
The logarithm of both sides in the relation 7 is explained in the linear form for the Freundlich isotherm.

$$\log q_e = \log K_F + \frac{1}{n} \log C_e$$

(8)

where $C_e$ is the equilibrium concentration of the adsorbate, $q_e$ is the amount of adsorption at the equilibrium, $q_m$ is the monolayer adsorption capacity, $n$ is the Freundlich intensity constant, and $K_L$ and $K_F$ are the Langmuir and Freundlich constants, respectively. The values of $q_m$ and $K_L$ were calculated from the slope and the interception of straight lines of the plot $1/q_e$ versus $1/C_e$ in Figure 7a and the estimated parameters are shown in Table 3. The $K_F$ and $n$ values were determined from the linear plot of $\log q_e$ versus $\log C_e$, which is shown in Figure 7b and the estimated parameters are shown in Table 3. As per the study (Table 3), the Langmuir isotherm fitted well with the experimental data based on $R^2$. The study suggests that monolayer sorption continues on the surface, which contains a limited number of adsorption sites and constant strategies required for adsorption without adsorbate transmigration on the surface. The heterogeneity factor ($n$) is employed to find out whether the adsorption process is linear, chemical, or physical. In the current study, Table 3 shows that the value of the heterogeneity factor is greater than 1 showing that the adsorption of the MB dye onto the composite is a physical process.

2.7. Studies of Desorption and Reusability. The most significant aspect for reducing the cost of material is recycling the adsorbent. This study is mainly in accordance with the framework of the sustainable development concept, so it is necessary to conduct the desorption test for water rinsing. Although the use of chemical agents is considered as more effective, they are prone to generate dangerous leachates, where the released byproducts are needed to be disposed of very carefully.

The studies of desorption were carried out with 1% each of NaOH, H$_2$SO$_4$, and HCl. The ZrPO$_4$@PANI composite saturated with the MB dye was kept in varied desorption media and kept in a rotatory shaker to stir regularly for 1 h at 150 rpm. Then, the adsorbent was separated and washed thoroughly with the distilled water. The results of our composite’s behavior in the presence of three different solutions are shown in Figure 8a. From the figure, it can be
observed that HCl can be recognized as a good, favorable desorption medium as compared to H₂SO₄, followed by NaOH. The studies under the HCl as a desorption medium indicated that about 94% of MB dye could be desorbed in 1 h. The main advantage of the adsorbent material is its reusability. The material’s reusability at the time of MB removal from the solution is shown in Figure 8b. It is found that the material had about 85% MB dye removing capacity even after its simultaneous reuse five times, which confirms the stability offered by PANI as a surface-coating material in aqueous solutions. We observed an apparent decrease in the efficiency of the adsorbent per cycle, and the involvement of non-electrostatic forces limited the complete desorption process. At the end cycle, HCl was employed in the form of a desorption medium in order to completely remove the adsorbed MB ions from the surface of the ZrPO₄@PANI composite.

2.8. Comparison of Adsorption Capacity with Other Available Adsorbents. The adsorption capacity of the sample was compared with those of selected other adsorbents as listed in Table 4. The analysis of the results provided the information that our synthesized composite is more efficient for removing the MB dye or its ions from the aqueous phase.

2.9. Biological Significance. The biological significance of the composite formation with the ZrPO₄ and PANI was studied using the in vitro cell culture systems and for that the rat liver cells were incubated with different concentrations (25–250 μg/mL) of PANI, ZrPO₄, and the ZrPO₄@PANI composite for a 24 h period. The results of cell viability and proliferation studies shown in Figure 9 indicate that there is a significant reduction in the viability of cells (as compared to the untreated controls) following their exposure to the PANI samples over the 100 μg/mL concentration and this percentage loss in the viability increases gradually upon increasing the dosage up to 250 μg/mL. However, the other two samples, ZrPO₄ and ZrPO₄@PANI, at similar concentrations did now show much loss in the viability of cells and this can be due to the presence of ZrPO₄ on the PANI surface. The significant reduction in the viability of cells following the surface loading of ZrPO₄ onto the PANI polymer clearly depicts its influence on controlling the toxic responses of PANI or its intermediates. The structure of the PANI polymer maintains two phenyl rings that are conductive, which can easily form some physiologically active and/or harmful intermediates by taking advantage of the aniline or aromatic amine moieties. Thus, the initiated toxic intermediates can further generate the free radical species by making use of the intracellular proteins and associated mechanisms, which all lead to cell death finally. We observed almost no effects of cell viability losses on the ZrPO₄ exposed cells, meaning that they are the safer particles. In addition, the presence of these particles on the surface of PANI also enhances the biocompatibility of the PANI polymer as the losses in the cell viability values are intermediate between pure PANI and ZrPO₄.

Table 4. Comparison of ZrPO₄@PANI Composite’s Capacity for MB Adsorption with That of Other Composites

| No. | Adsorbent                       | MB Adsorption Capacity (mg/g) | Refs |
|-----|---------------------------------|-------------------------------|------|
| 1   | yellow passion fruit peel       | 6.8                           | 42   |
| 2   | graphene oxide                  | 19.3                          | 43   |
| 3   | eucalyptus saw biochar          | 29.94                         | 44   |
| 4   | carbon nanotubes                | 35.4                          | 45   |
| 5   | ruthenium nanoparticle-loaded AC | 41.60                         | 46   |
| 6   | Fe₃O₄@SiO₂-EDA-COOH              | 43.15                         | 47   |
| 7   | peanut hull                     | 68                            | 48   |
| 8   | reed-derived biochar            | 77.35                         | 49   |
| 9   | palm kernel fibre               | 95.4                          | 50   |
| 10  | acid-activated kaolin           | 101.5                         | 51   |
| 11  | kaolin                          | 102.04                        | 52   |
| 12  | ZrPO₄@PANI                      | 120.48                        | present study |

Figure 9. Comparison of the cell viability studies of PANI and ZrPO₄ along with ZrPO₄@PANI composite over the concentration range of 25–250 μg/mL during a 24 h period.
Following the cell viability study, the materials are tested toward their mechanism of toxicity and cell death using a flow cytometer, where the cells are stained with propidium iodide (PI) and Annexin V-FITC and the corresponding results are shown in Figures 10 and 11, respectively. From Figure 10, upon staining of cells with PI as it can bind to the cell’s DNA, about 8% of the cells were found to be alive, 49% of cells to be early apoptotic, and 42% to be late apoptotic or early necrotic (Figure 10b). In a similar way for the ZrPO₄@PANI composite-treated cells, these values get shifted to 30% of living, 63% for early apoptosis, and only 6% for late apoptosis or early necrosis (Figure 10d). Such an observation of a shift in these values is considered to be highly significant as the incorporation of PANI with ZrPO₄ brings a number of early necrotic cells to life and early necrosis. Also, we observed that almost a similar percentage of healthy normal cells (93%; Figure 10c) for the ZrPO₄ sample was exposed compared with that of controls (99%; Figure 10a), meaning that this compound does not induce any intracellular mechanisms toward the cells, thereby confirming the safe application of the particles. The same property of biocompatibility for ZrPO₄ also seems to be persisted when we exposed the cells to the ZrPO₄@PANI composite, that is, the availability of ZrPO₄ on the surface of PANI reduced the number of dying cells by masking the highly toxic effects of the PANI polymer.

Similarly, the flow cytometry results following the staining of cells with Annexin V-FITC shown in Figure 11 confirmed again the importance of having ZrPO₄ on the surface of the ZrPO₄@PANI composite. From the figure, M1 and M2 correspond to the viable and apoptotically dying cell’s fluorescence intensities, where 15% of live cells of the PANI-treated cells (Figure 11b) increased to 87% following the exposure to the ZrPO₄@PANI composite (Figure 11d). Also, 84% of cells experiencing the apoptotic pathway (Figure 11b) decreased to only 12% (Figure 11d), which is exclusively due to the availability of ZrPO₄ on the PANI polymer. The sustainable behavior of the ZrPO₄ compound can be confirmed by the observation of more than 90% live cells (Figure 11c), which is similar to the number of cells from the control measurements (98%; Figure 11a). Thus, from the adsorption and biological analysis of various samples, it can be confirmed that the presence of ZrPO₄ on the PANI polymer not just improves the adsorbing capacity of PANI but also enhances the biocompatibility of PANI by protecting it as a shield from the toxic-induced responses.

### 3. CONCLUSIONS

In conclusion, we confirmed from this study that the ZrPO₄@PANI composite is efficient enough for the successful removal of the MB dye from the aqueous solutions possessing optimal pHs and time intervals, where the composite shows an
4. EXPERIMENTAL SECTION

4.1. Materials and Methods. The chemicals used in synthesizing ZrPO₄@PANI are aniline (99.98%), zirconium oxychloride (99.98%), ammonium persulphate (99.98%), and orthophosphoric acid (99.98%). All the chemicals were of analytical grade and high purity from Sigma-Aldrich (Mumbai, Maharashtra, India) and they did not need any further purification.

4.2. Synthesis of PANI. PANI in the form of a gel was synthesized by the chemical polymerization of aniline in the presence of HCl and ammonium persulphate and the detailed procedure is described elsewhere. Briefly, 10% of aniline was added with 100 mL of 1 M HCl in a double-wall flask maintained at a temperature of 48 °C upon stirring continuously. Now, 150 mL of ammonium persulphate solution was added dropwise to the flask with continued stirring. After 6 h, the solution was filtered using a Buchner funnel and the leftover residue was washed thoroughly three to four times with the demineralized water (DMW). The resultant residue was dried in a desiccator and was preserved there itself until later use.

4.3. Synthesis of ZrPO₄. For the synthesis of ZrPO₄, a solution of 0.1 M H₃PO₄ and 0.1 M ZrOCl₂·8H₂O was taken in a beaker and was mixed thoroughly with the maintenance of pH 1 at ambient temperature. The mixing was continued for at least 60 min to obtain Zr(IV)PO₄ precipitates and the precipitates were filtered and washed with ethanol at least three to four times to remove the unreacted reagents used. The white precipitate was separated by centrifugation and finally dried in an air oven at 120 overnight, and further preserved in the desiccator.

4.4. Synthesis of the ZrPO₄@PANI Cation-Exchanger. The ZrPO₄@PANI cation-exchanger was synthesized through a sol–gel mixing of ZrPO₄ in the PANI precipitate in a 1:1 weight ratio. The material obtained as a result turned as greenish-black slurry and then it was kept for another 24 h, at ambient temperature. The gel-based on the PANI was filtered and then washed with DMW to discard the excess acids from it or the adhering material present in the trace form of ammonium persulphate. It was then dried at 60 °C in an air oven and the obtained powder is added with DMW to obtain the small-size granules, where they were converted into their acidic (H⁺) form by the soaking of granules in 1 mg L⁻¹ HNO₃ solution for a continuous 24 h with shaking to randomly exchange the supernatant liquid. On washing with DMW, excess acid was easily removed. It was later dried at 60 °C and then ground down through a mortar and pestle to obtain the powder form of the composite. Table 5 shows the comparison of IEC for various samples of ZrPO₄@PANI formed by changing the molar ratio of aniline. The tentative structure of the composite material is shown in Scheme 2.

4.5. Instrumentation. The surface morphology was studied by scanning electron microscopy (SEM) using the FEI QUANTA 250 ESEM operated at 15.00 kV. For the elemental analysis, an EDAX detector connected to the SEM instrument was used. The powder XRD studies were performed on an XRD 6000 instrument, Shimadzu, with Cu Kα radiation λ = 1.5418 Å. For the UV–vis spectroscopic analysis, a SPECORD PLUS double beam spectrophotometer was used, and for the FTIR spectroscopy studies, a PerkinElmer FTIR instrument was used, where the samples were prepared using the KBr pellet method (wavenumber range used was 400–4000 cm⁻¹). Also, for the zeta-potential studies, the Malvern zeta sizer 2000 & Mastersizer Micro instrument was used.

### Table 5. Various Combinations of the ZrPO₄@PANI Composite Formed by Using Different Molar Ratios of PANI

| sample | volume ratio (v/v) | Zr(IV) (M) | PO₄⁻³⁻ (M) | aniline (M) | IEC (meq/g) |
|--------|-------------------|------------|------------|------------|-------------|
| A-1    | 1:1:1             | 0.1        | 0.1        | 0.01       | 1.56        |
| A-2    | 1:1:1             | 0.1        | 0.1        | 0.05       | 1.67        |
| A-3    | 1:1:1             | 0.1        | 0.1        | 0.1        | 1.78        |
| A-4    | 1:1:1             | 0.1        | 0.1        | 0.2        | 1.87        |
| A-5    | 1:1:1             | 0.1        | 0.1        | 0.4        | 1.93        |
| A-6    | 1:1:1             | 0.1        | 0.1        | 0.6        | 1.98        |
| A-7    | 1:1:1             | 0.1        | 0.1        | 0.8        | 1.92        |
| A-8    | 1:1:1             | 0.1        | 0.1        | 1.0        | 1.89        |
| A-9    | 1:1:0             | 0.1        | 0.1        | 0           | 0.87        |
where $C_0$ and $C_e$ are the initial and equilibrium concentrations of MB in the solution (mg L$^{-1}$), respectively, $m$ is the dosage of the adsorbent (mg), and $V$ is the volume of the MB solution (L).

4.7. Biological Assays. For testing the biocompatibility of the ZrPO$_4$@PANI composite, the samples were tested using the in vitro BRL 3A rat liver cell culture systems toward their viability and associated cell cycle. For that, the BRL 3A cells of the rat liver origin obtained from the American type culture collection (ATCC, Manassa, VA, USA) were tested for the changes in the viability using the MTT dye (3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazolium bromide) and apoptosis by means of Annexin V/FITC kits, where the fluorescence intensities of fluorescence isothiocyanate (FITC) and PI were measured, and the detailed experimental procedures were explained in our earlier publications.$^{56}$ Briefly, the rat liver BRL 3A cells were maintained in Eagle’s minimum essential medium (EMEM) supplemented with 10% (v/v) FBS (fetal bovine serum) and 1% antibiotics (penicillin, 100 units/mL; streptomycin, 100 μg/mL) kept at 37 °C incubators having 5% CO$_2$ and 95% humidity.

For the MTT assay, about $10^4$ cells/well were seeded onto 96-well plates, allowed to grow for 24 h, and when the cell growth reaches an 80% confluence level, the growth medium was replaced with fresh EMEM containing 2% (v/v) FBS and the test compounds of varying concentrations ($25$–$250$ μg/mL). The test compounds include ZrPO$_4$@PANI and pure forms of PANI and ZrPO$_4$, where the incubation was continued for another 24 h and following that period, the MTT reagent was added as per the manufacturer’s instruction and incubated for another 4 h. After that, the solution was replaced with 100 μL DMSO (dimethyl sulfoxide), and the formation of formazan crystals was tested by measuring the absorbance in the microplate reader at a 570 nm wavelength (BioTek Instruments, Winooski, VT, USA). The well of cells with no treatment to any external agent was selected as the control measurements and based on these controlled values, the results are calculated as the percentage decrease in the cell viability, where the values represented are the mean ± SD (standard deviation) of three replicate experiments.

To further investigate the role of ZrPO$_4$ in controlling the surface-induced toxic mechanisms of the PANI material, the ZrPO$_4$@PANI composite along with its precursors (ZrPO$_4$ and PANI) have been subjected to the cell cycle analysis by making use of the flow cytometry studies.$^{57}$ It is to be noted here that the weights of materials used for the preparation of sample solutions are selected in such a way that the final amount of PANI is expected to be the same in both pure PANI and the ZrPO$_4$@PANI composite (as the nanocomposite was formed from an equal amount of individual components). For the testing, the BRL 3A cells at a density of $2.5 \times 10^5$ cells/well in 6-well plates were seeded and when the cell growth reaches 80% confluence, the medium was replaced with a fresh medium containing 2% FBS along with the corresponding test compounds (ZrPO$_4$@PANI, ZrPO$_4$, and PANI) at their selected concentrations ($250$ μg/mL) for a 24 h incubation period. After that period, the cells were stained with PI as this agent has the capacity to intercalate the cell’s DNA and quantitatively assess the extent of replication that occurs during

\[ q_e = \frac{(C_0 - C_e) V}{M} \] (9)

\[ R = \frac{C_0 - C_e}{C_0} \times 100\% \] (10)
the cell cycle by means of the fluorescence emission that was measured. Similarly, the apoptotic assay with the use of Annexin V-FITC was performed for investigating the influence of the ZrPO₄@PANI composite on the BRL 3A liver cells. Similar to the cell cycle analysis, the apoptosis assay also involves the treatment of cells grown in the 6-well plate when the confluence levels were reached with the test compounds (ZrPO₄@PANI, ZrPO₄, and PANI) at selected concentrations (250 μg/mL; 24 h). After that, the cells were trypsinized and obtained the cell pellet using centrifugation at 3500 rpm binding V-FITC (100 μL) first and then Annexin V-FITC (5 μL) was added and incubated for another 30 min. Now, the cell cycle stage was analyzed using a flow cytometer maintained at the excitation and emission wavelengths of 490 and 670 nm, respectively, using Cell Quest Software.

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Notes

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