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Preparation of novel aminated chitosan schiff's base derivative for the removal of methyl orange dye from aqueous environment and its biological applications



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ABSTRACT

A novel, eco-friendly aminated chitosan Schiff's base (ACSSB@ZnO) was developed and utilized to remove MO from aqueous environment. The impact of different significant parameters, for example, pH (3–11), adsorbent dose (0.1–0.6 g), contact time (0–120 min), and temperature (303–323 K) have been explored by batch process. Kinetic data was illustrated by pseudo-second-order model and the isotherms fitted well with Langmuir isotherm model. The highest sorption capacity of ACSSB@ZnO was observed to be 111.11 mg/g at 323 K. Positive enthalpy and entropy values demonstrated that the MO adsorption procedure was an endothermic. Negative Gibbs free energy values implied the spontaneous nature of the adsorption system. Moreover, reusability experiments were studied and it can be regenerated by using NaOH as effluent.

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1. Introduction

Lot of contaminations are discharged into the earth with the advancement of industrialization. Among the diverse sorts of contamination, wastewater stream is one of the serious issues because of a lot of water utilized in our day by day life. Dye wastewater is the primary thing for water contamination [1]. Dye wastewater can be released into the environment through various activities and may show adverse effects on human health. Dyes are an important class of synthetic organic compounds used in many industries, like the coloring, printing, and related enterprises prompted a lot of color containing wastewater and their consequent releases into eco-system that might be dangers for human well-beings [2]. Methyl orange is an aromatic and azorelated anionic dye. These group of dyes can causes severe ecological issues even they are at low concentration [3]. Long exposure of MO leads to cause, troubles in breathing, heaving, looseness of the bowels, and queasiness. Hence it is vital to take out the colors from the wastewater before it is released into nature.

A wide scope of wastewater treatment strategies like ozonation, membrane filtration, ion exchange, oxidation, degradation by biological and adsorption have been utilized by various analysts to refine and decontaminate the wastewater from these toxic compounds [4–8]. Adsorption is currently considered an efficient and cost-effective method for the removal of pollutants among the methods used for water treatment [9]. However, improved sorbent materials with greater capacity for adsorption and selectivity towards waterborne contaminants need to be developed. Choice of appropriate adsorbents ought to be founded on their sorption ability, bottomless nearby accessibility, minimal effort

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and eco-accommodating nature. Adsorbent prepared from natural polymer and biopolymer is attractive because of their minimal effort and simple access. Numerous approaches to developing cheaper and more efficient adsorbents involving natural polymers have been studied recently. Additionally, chitosan has been considered as a perfect adsorbent due to bottomless free amino and hydroxyl bunches on its spine [10]. Chitosan and its derivate forms has potential applications in the fields of biotechnology, biomedicine and cosmetics because of their unique properties such as hydrophilicity, biocompatibility, biodegradability and anti-bacterial property.

A considerable amount of research on the development of effective, low-cost and easily available alternative sorbents has been done in recent years. Distinctive modified chitosan, and agriculture basis adsorbents for example, quaternary ammonium salt altered chitosan attractive composite adsorbent [11], chitosantripolyphosphate/TiO₂ nanocompsite [12], rice husk and egussi peeling [13], aloe vera leaves wastes [14], seed powders [15], chitosan/Al₂O₃/magnetite nanoparticles composites [16], chitosan/bentonite composite [17], chitosan/maghemite [18], poly HEMAchitosan-MWCNT nano-composite [19], CoFe₂O₄/MgAl-LDO [20], AC/NiFe₂O₄ [21], chitosan/organic rectorite composite [22], heterogenized manganese nanoadsorbents [23], chitosan spheres [24], sorel's cement [25], MCGO [26], and porous chitosan doped with graphene oxide [27] have been recently striven for the expulsion of colors, yet high successful and progressively prudent sorbent materials are yet to be expected to search. In addition, some synthetic adjustments have been made to improve the adsorption limit of chitosan composites. Crosslinking is an essential advance against corrosive, soluble base and synthetic substances and mechanical quality of the chitosan [28]. Various types of crosslinking agents, such as, glutaraldehyde, and epichlorohydrin [29-31] have been utilized by different analysts in chitosan reactions. Zinc oxide nanoflakes were incorporated into aminated chitosan Schiff's base (ACSSB@ZnO) was prepared and utilized as a productive sorbent material to remove MO from aqueous solution. Because of its large surface area, nanoparticles are having high sorption capacity. Low toxicity and biodegradability are some of the most significant characteristics of ZnO particles. ZnO particles possess superior characteristics of UV blocking, antibacterial and antimicrobial properties. In biomedical applications, ZnO particles have gained enormous interest. In this connection, utilization of nanoparticles has greater attention in removal process.

The objective of this investigation was to explore ACSSB@ZnO to remove MO from the aquatic environment. In this study chitosan was crosslinked with terephthalaldehyde and it can acts as a crosslinking as well as complexing agent. Crosslinking can increase the additional functional groups and these functional groups plays an important role to attract the dye molecules. Crosslinked sorbents have many desirable characteristics like chemically stable, insoluble acidic environmental and comparatively low price [30]. After preparation ACSSB@ZnO was undergone through structural (XRD), spectral (FTIR and ¹HNMR), and surface morphological (SEM) analyses. The impacts of different working conditions in particular, pH, adsorbent portion, contact time, and temperature were explored in mode of batch operation. Kinetic, equilibrium and thermodynamics of MO onto ACSSB@ZnO was investigated systematically.

2. Materials and methods

2.1. Chemicals and reagents

Chitosan powder with a molecular weight of 310,000–375,000 Da was purchased from the Tianjin umbrella science and technology Co., Ltd, China. Terephthalaldehyde, SOCl₂, thiosemicarbazide, MO, HNO₃, HCl, CH₃COOH, and NaOH were purchased from Tianjin guangfu technology development Co., Ltd, China. 1.25 g of MO was used to prepare dye stock solution. Scheme 1A showed the structure of methyl orange.

2.2. Adsorbent preparation

In acetic acid solution 2 g of chitosan (2 percent v/v) was dissolved by stirring. The blend was retained for 3-6 h until a definite solution could be achieved because of the poor solubility of chitosan. Terephthaldehyde (2.81 g) dissolved in 30 mL of C₂H₅OH and added drop wise at ambient temperature with constant stirring. At room temperature, the mixture was then stirred for 16 h, followed by 18 h of reflux resulting in a pale yellow gel. The mass was almost dry and focused under vacuum. 10 mL of DMF was appended and cooled to 0-5 °C to the residue weight. Schiff's base formation can take place through the free amino groups (C₂ position) with the terephthalaldehyde. A special characteristic of this newly synthesized material is that it need not any extra cross linker like glutaraldehyde, while the complexing agent acts as a crosslinking agent. SO₂Cl was added and refluxed for 45 min. The -OH group at C₃ was turned into the chlorinated chitosan Schiff's base. Then thiosemicarbazide was mixed and stirred at an ambient temperature for 7 h with a mechanical agitator at 600 rpm. Thiosemicarbazide was replaced by the chlorine in the chlorinated chitosan base to achieve the Schiff's base (ACSSB). At last 2 g of ZnO nanoflakes [32] (prepared according to our previous study) were introduced into ACSSB and correctly refluxed. The solution was dropped into 0.1 N NaOH after



Fig. 1. XRD spectra of (A) pure chitosan, (B) ZnO, and (C) ACSSB@ZnO.



Fig. 2. FTIR spectra of (A) pure chitosan, (B) ZnO, (C) ACSSB@ZnO, and (D) MO loaded ACSSB@ZnO.

30 min. The desirable product was isolated by filtration and washed with methanol and subsequently deionized water in order to remove unnecessary chemicals. For further depiction, it was labelled as ACSSB@ZnO. In our earlier article, we addressed detailed adsorbent preparation [32].

2.3. Adsorption experiments

All MO sorption experiments were conducted in batch procedure. The pH effect was initially investigated from 3.0 to 11.0 and the adsorbent dose experiments were conducted with doses varying from 0.1 g to 0.6 g. Contact time effect was evaluated at periods ranging from 0 min to 120 min and dye concentration ranging from 50 to 150 mg/L at a fixed adsorbent (0.5 mg) concentration. Equilibrium experiments were studied with various concentrations. All these experiments were done in 150 mL of flasks by adding 100 mL of MO (with known concentration and pH) with a known quantity of ACSSB@ZnO, and then mechanically agitated in a shaker until equilibrium was achieved. The suspensions were filtered by whattsman filter paper after the specified time periods, 5 mL aliquots were taken from the supernatant and a spectrophotometer (Unico, UV-2100 spectrophotometer) was used to determine the concentration of MO. How much amount of MO was adsorbed by ACSSB@ZnO was determined from the following equation.

$$q_e = \frac{(C_i - C_e)V}{M} \tag{1}$$

where $q_e \text{ (mg/g)}$ is the sorption capacity at equilibrium, C_i and C_e are initial and equilibrium concentration (mg/L) of MO, M (g) is quantity of the adsorbent, and V (L) is the volume MO.

2.4. Biological activity of ACSSB@ZnO

2.4.1. Antibacterial assay

The antibacterial activity of CS, ACSSB and ACSSB@ZnO were assessed by zone of inhibition method by using *S. aureus* (ATCC strain 29523) and *E. coli* (ATCC Strain 29522). The bacteria suspension (50 μ L of 10⁸ CFU/mL) was first distributed on a LB agar plate, followed by the loading of 60 μ L of the newly prepared compound onto a sterile blank platter on the agar surface. The zone of inhibition diameter was assessed after 24 h.



Fig. 3. SEM images of (A) pure chitosan, (B) ZnO, (C) ACSSB@ZnO and (D) MO loaded ACSSB@ZnO.



Fig. 4. ¹HNMR spectra of (A) pure chitosan, (B) CSSB, (C) chlorinated CSSB, and (D) ACSSB.

2.4.2. In vitro anti-tumorigenic assay

In vitro anti-tumorigenic property of CS, ACSSB, and ACSSB@ZnO was estimated by the MTT assay. MG63 osteosarcoma cell line and MCF7 human breast cancer cell line were cultured in Dulbecco's minimum essential medium (α -MEM) with 10% fetal bovine serum (FBS), 10 µg/mL penicillin, 10 µg/mL streptomycin, and 50 µg/mL fungi zone. Every three days, the medium was altered. Cells were harvested using 0.25% trypsin and 0.1% EDTA, seeded onto96-well plates at 5000 cells/ well. After 24 h, medium was replaced with fresh medium containing CS, ACSSB, and ACSSB@ZnO in 20, 100 and 250 µg/ml concentration.

Incubation of cells was carried at 48 h, and then 20 μ L MTT (5 mg/mL) were added. Cells were incubated with MTT solution for 4 h at 311.15 K. MTT has been removed with care and 150 μ L of dimethyl sulfoxide has been added to each source. At 490 nm the absorbance was assessed by an enzyme-linked immunosorbent assay micro plate reader.

2.4.3. Wound healing and in vivo antibacterial effects

In vivo animal study was approved by the animal experimental center of Tianjin University. Twenty four Kunming mice (Male, 7 weeks old







Fig. 5. TEM images of (A) pure ZnO, (B) ACSSB@ZnO and (C) MO loaded ACSSB@ZnO.



Fig. 6. (A) Effect of pH, (B) adsorbent dose, (C) contact time, (D) Pseudo-Second-Order kinetic plot, (E) Weber-Morris plot at different MO concentrations, (F) Langmuir isotherm, (G) Thermodynamic analysis for the removal of MO onto ACSSB@ZnO, (H) Desorption % of different eluents and (I) Adsorption-Desorption of MO onto ACSSB@ZnO.

and around 20 g of weight), were evenly divided into four groups: control group, CS group, ACSSB group, and ACSSB@ZnO. Mice were anaesthetized by using isoflurane, then a partial thickness wound with a length of 1 cm, was created on the back skin. Freshly prepared compound paste was applied every day (upto 7th) on the wound surface. In the control group mice wound PBS was employed. Mice were sacrificed at day 8, and skin sample including wound and surrounding the wound was collected and fixed in 4% formaldehyde. Histological sections were stained with hematoxylin and eosin (H & E) staining and wound healing was observed microscopically. To analyze the in vivo antibacterial properties of the compounds prepared, wound pus swab was collected at day 3 and cultured for bacterial growth in LB agar. Pus sample inoculated LB agar plates were in incubated for 48 h at 311.15 K and bacterial colonies were observed.



Fig. 6 (continued).

2.4.4. Statistical analysis

The results were reported as the mean \pm standard deviation. A single way variance analysis (ANOVA) followed by bonferroni correction was conducted for statistical analysis. Difference at p < 0.05 was considered to be significant.

3. Results and discussion

3.1. BET analysis

BJH adsorption/desorption method was applied to find out the surface area, pore volume and diameter of ZnO nanoflakes and ACSSB@ ZnO. ZnO nanoflakes having 6.3, 0.028 and 0.32 mm of surface area, pore volume and pore sizes. Whereas ACSSB@ZnO having 11.2, 0.066 and 0.62 of surface area, pore volume and pore sizes.

3.2. XRD analysis

Pure chitosan (Fig. 1A) reveals the peaks at $2\theta = 10$ and 20° . ZnO (Fig. 1B) represents hexagonal lattice structure. XRD spectra of ACSSB@ZnO (Fig. 1C), exhibits new peaks at 30.1°, 35.5°, 43.1°, 53.5°, 57.0° and 62.6°. This shows the distribution of ZnO nanoparticles across the whole ACSSB. Aromatic functional groups (terephthaldehyde) was successfully incorporated on the pure chitosan polymer structure. Hence XRD results reports that ACSSB@ZnO express the less crystalline than the chitosan.

3.3. FTIR analysis

Functional groups were identified from the FT-IR analysis. Chitosan [32] shows (Fig. 2A) the peaks at 3443, 2924, 1652, 1598, 540, 1420 and 1147, 1377 and 1421, 1076 and 1155, 680, 1162 and 987 cm⁻¹ represents the —OH and —NH₂, —CH, —C=O, —NH, N—H angular, C—N axial, N—H angular deformation, —CH₃, C—O stretching from the $\beta(1 \rightarrow 4)$ glycosidic bonds. ZnO (Fig. 2B) having the bands at 3432, 1596, 1404 and 453 cm⁻¹. Generally metal oxide peaks obtained below 1000 cm⁻¹. The peaks located at 453 and 520–750 cm⁻¹ represents the Zn—O. ACSSB@ZnO shows the peak at 1635 belongs to C=N peak. CH₂-Cl absorption peak at 780 cm⁻¹ indicates that chlorination was done in chitosan. The peak at 1136 cm⁻¹ represents formation of C—NH bond in ACSSB@ZnO. After MO loaded the intensity in the wavenumbers were changed into lower and upper wavenumbers. The functional groups like hydroxyl, sulphur and amine groups might be participated in MO sorption.

3.4. Surface morphological analysis

SEM examination gives an adequate surface morphology of any material. Morphological assessment of pure chitosan, ZnO, ACSSB@ZnO [32] and methyl orange loaded ACSSB@ZnO were depicted in Fig. 3A– D. Chitosan having smooth surface. Whereas ZnO exhibits the flakes morphology. After ZnO was integrated in the ACSSB, notable surface morphology was observed. SEM image of ACSSB@ZnO shows a smooth plate like structure. The surface of the ACSSB@ZnO had undergone remarkable change after MO sorption.

3.5. ¹HNMR analysis

The ¹H NMR spectra of chitosan, CSSB, chlorinated CSSB and ACSSB were shown in Fig. 4A-D. Chitosan exhibits the peaks in between 4.80 and 1.80 ppm. Peaks of 3.4–4.0 δ relative to the hydrogen atoms bonded to the glucopyranose unit's carbon atoms 3, 4, 5 and 6. There is a peak at 1.99 ppm correlates to the hydrogen atoms of acetamido methyl groups. Chitosan schiff's base (CSSB) ¹H NMR spectra (Fig. 4B) exhibits the characteristic peaks of four benzene ring hydrogens at 7.21-7.95 ppm, whereas the peaks at 8.82 ppm belongs to HC==N. The broad signal at 8.82 ppm is assigned to the CSSB. In CSSB the aromatic peaks appeared in the range of 7.21–7.95 ppm. Whereas the ¹H NMR spectra of 4b exhibited the extra peaks at around 2.3-4.9 ppm belongs to CH₂-Cl group indicated that formation of chlorinated chitosan schiff's base (CH₂—OH converted into CH₂-Cl groups) and it was shown in Fig. 4C. Chlorinated chitosan schiff's base reacts with thiosemicarbazide and forms the final product of ACSSB. The peak at 2.2–4.4 ppm belongs to methylene group indicates that there was no CH₂-Cl bond and it was converted as CH₂-NH bond.

3.6. TEM analysis

The size, form, structure and morphology of nanoparticles was identified through transmission electron microscopy (TEM). Morphology structure of pure ZnO, ACSSB@ZnO and MO loaded ACSSB@ZnO were shown in Fig. 5A–C. Fig. 5A and B shows the transmission electron micrographs of pure ZnO, ACSSB@ZnO appeared in flakes like structure (with the scale of 100 and 50 nm). The appearance of uniformly dispersed dark dots (Fig. 5B) with uniform particle size shows that ZnO flakes are well distributed over the ACSSB surface. The surface of the ACSSB@ZnO had undergone remarkable change after MO sorption.

3.7. Effect of pH

Medium acidity is one of the most important parameter in adsorption process [33–35]. This can be clarified based on change in surface property of ACSSB@ZnO with the adsorbate atom. The experiments were conducted by varying pH from 3.0 to 11.0 to assess the impact of pH for MO removal and the removal efficiency gradually decreased with increasing pH and the results were shown in Fig. 6A. The main mechanism involved in MO sorption is electrostatic interaction. The adsorbent has a positive charge in acidic solution and attracts more negative molecules of MO dye. In addition, hydrogen particles can form bridging ligands between the adsorbent and dye molecule [36]. Maximum removal of MO was obtained at lower pH values and was mainly due to the electrostatic attractions. Chitosan amine groups are protonated at lower pH. As the pH increases, the percentage of MO removal decreases. The main reason may be due to anionic dye solution competition with excess OH^{-} [19]. As the pH increases (pH > 3), the adsorbent density decreases and the adsorption capacity decreases as a consequence. On the basis of above results, pH 3.0 was chosen for subsequent batch studies.

Table	1

Kinetic evaluation of MO onto ACSSB@ZnO.

MO Conc. (mg/L)	Lagergren-first-order			Pseudo-second-order			Weber and Morris		
	K ₁ (1/min)	\mathbb{R}^2	SSE	K ₂ (g/mg min)	\mathbb{R}^2	SSE	K_{id} (mg/g min ^{-0.5})	R ²	SSE
30	0.029	0.991	0.979	0.0135	0.999	0.0341	0.198	0.977	0.8694
50	0.032	0.981	0.984	0.0134	0.999	0.0184	0.248	0.957	0.8841
70	0.034	0.989	0.988	0.0086	0.999	0.022	0.365	0.963	0.8803
90	0.039	0.979	0.992	0.0078	0.999	0.0106	0.488	0.938	0.9044

 Table 2

 Isotherm evaluation of MO onto ACSSB@ZnO

Dye	Temp. K	Langmuir Constants				Freundlich Constants	
		$q_m (mg/g)$	$K_{L}\left(L/mg\right)$	\mathbb{R}^2	$K_{f}(mg/g)$	1/n	R ²
MO	303 313 323	58.82 71.42 111.11	34.46 21.71 11.88	0.999 0.999 0.999	23.12 18.70 12.53	0.415 0.513 0.695	0.991 0.992 0.992

3.8. Effect of adsorbent dose

Adsorbent dose affects the adsorption system and determines the ability of the sorbent by limiting the amount of destinations accessible to expel hazardous dyes at a predefined initial concentration. The impact of adsorbent measurement on MO removal was examined from 0.1 to 0.6 g and the outcome reports were displayed in Fig. 6B. As observed in Fig. 6B, with an increase in the adsorbent volume, the removal level of MO increased to a specific value and then reached the equilibrium (0.5 g). This could be due to the rapid involvement of active sites with the MO. As the adsorbent portion increases, surface area and accessible destinations have been automatically increased to interact with dye molecules and thereafter better adsorption occurs [37]. There is no significant amount of MO removal after achieving an equilibrium. This might be because of the way that the surface active sites present in the adsorbent gets totally involved by the MO particles and prompted the immersion of active sites. In view of the outcomes the experiments were performed at 0.5 g of adsorbent dose.

3.9. Effect of contact time

Contact time was another important parameter in adsorption processes and obtained results were displayed in Fig. 6C. In order to determine the contact time effects, MO sorption was studied from 0 to 120 min. Initial rapid adsorption was done with accessibility of active sites present in ACSSB@ZnO. A growing number of functional groups have been involved in MO adsorption until equilibrium has been achieved. A short time later with the slow inhabitance of these sites, the adsorption turned out to be less productive. After this period, the removal of MO does not change because the repulsive forces of equilibrium occurred between ACSSB@ZnO and MO. After 105 min of contact time balance has been observed and there is no additional upgrade in the sorption limit with further increase in contact time. In this way, 105 min was considered sufficient time for ACSSB@ZnO to remove MO.

3.10. Adsorption kinetics

Kinetic studies give the clear idea and conditions of sorbate-sorbent interactions. Pseudo-first-order [38], Pseudo-second-order [39] and intra-particle diffusion models [40] were used to determine the kinetic reaction mechanism and their reactions were shown in Eqs. (2)–(4).

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303}t$$
 (2)

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e} t$$
(3)

$$q_t = K_{id} t^{0.5} + c \tag{4}$$

where q_e (mg/g) and q_t (mg/g) are the amounts of MO sorbed at equilibrium and at time *t*. K₁, K₂ and K_{id} represents the rate constants for first-order, second-order and intra particle diffusion process. The values K₁ and K₂ were acquired from log (q_e - q_t) versus t (Figure not shown) and t/qt versus t (Fig. 6D) and were shown in Table 1. K_{id} obtained from the q_t vs. t^{0.5} plot (Fig. 6E). The pseudo-first-order correlation

coefficient values (R^2) appear to be low. This suggests that MO adsorption is not pursued with pseudo-first-order model. The pseudo-second-order kinetic model provides the best correlation to remove MO and the rate-limiting step might be chemical sorption by exchanging electrons of the dye molecule with the adsorbent. Therefore, it can be concluded that sorption system followed well with pseudo-second-order reaction instead of pseudo-first-order reaction. The R^2 values are closer to 1 (>0.999) and demonstrating that the pseudo-second-order model can perfectly fitted for the removal of MO onto ACSSB@ZnO.

The sorption takes place in three steps in the intra-particle diffusion model. The first sharper part is the transfer of mass of solvent molecules to the adsorbent surface from the bulk solution; this is also referred to as instant adsorption. The second part is the gradual adsorption phase where the intraparticle diffusion is a limiting rate. The third part is the final stage of equilibrium where diffusion will start slowly, this is because of the extremely low level of MO [41]. Obviously the regression of q_t versus t^{1/2} was inclined to be linear and the plots did not go through the inception, proposing that the intraparticle dissemination isn't the main rate-controlling. This clearly shows that it is not a rate control step for MO sorption.

3.11. Adsorption isotherms

Isotherm experiments were conducted to determine the sorption system's nature and mechanism. Langmuir [42], and Freundlich [43] isotherm were used to know the equilibrium data.

Langmuir isotherm assumes that the adsorbed molecules do not interact with others once a dye molecule occupies a site [44].

$$\frac{1}{q_e} = \frac{1}{q_m b} \left[\frac{1}{C_e} \right] + \frac{1}{q_m} \tag{5}$$

where C_e (mg/L) is the equilibrium solution concentration, q_e (mg/g) is the amount of adsorbent per unit adsorbent mass. q_{max} and b are the constants of Langmuir. We can observe that the maximum sorption capacity of MO onto ACSSB was found to be 111.11 mg/g at 323 K. The R^2 values showed that the experimental data fitted with the Langmuir model (Fig. 6F) better than Freundlich model.

Affinity of the adsorbent was studied with the help of dimensionless separation factor R_I and it was represented by the following equation.

$$R_L = \frac{1}{(1+b\ C_0)} \tag{6}$$

where $C_0 (mg/g)$ is the dye concentration, b is the Langmuir constant. R_L value indicates the adsorption process to be irreversible (RL = 0), favorable ($0 < R_L < 1$), linear ($R_L = 1$) or unfavorable ($R_L > 1$). Estimations of R_L in the present framework range from 0 to 1, showing a very good MO sorption onto ACSSB@ZnO and this demonstrates that there is a good

Table 3	
Comparison the adsorption capacity of methyl orange dye with various adsorbents.	

Adsorbent	рН	Temperature (K)	Qmax	Ref. No
Y-Fe ₂ O ₃ /SiO ₂ /chitosan composite	-	310	34.29	[46]
Acid modified carbon coated monolith	6	303	147.06	[47]
Maghemite/chitosan nanocomposite films	3	330	29.41	[48]
PPy nanofibres	7	298	169.55	[49]
GO-IPDI-CDs	6	318	83.40	[50]
m-CS/c-Fe ₂ O ₃ /MWCNTs	3.14-6.5	297	66.09	[51]
CCG/TNC	4.0	313	416.1	[52]
ACSSB@ZnO	3.0	323	111.11	Present study



Fig. 7. Antimicrobial activity of (A) pure chitosan, (B) ACSSB and (C) ACSSB@ZnO onto S. aureus and E. coli.

interaction of MO with the adsorbent surface sites. This implies that the Langmuir model can depict the isotherm data very well.

Freundlich isotherm express that the sorption was done on heterogeneous surface and the equation can be expressed as follows,

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \tag{7}$$

where K_f (mg/g) and 1/n are Freundlich constants and the adsorption intensity-related empirical parameter. Under the conditions studied, the value of 1/n was between 0 and 1 indicating the appropriate MO sorption onto ACSSB@ZnO. The R^2 values for Freundlich isotherm were found to be 0.991, 0.992 and 0.992. These results showed that the Freundlich model was unable to adequately describe the relationship between the quantity of sorbed MO onto ACSSB@ZnO and its equilibrium concentration. Langmuir isotherm model possesses higher R^2 values (0.999) than the Freundlich model. This indicates that monolayer sorption of MO occurs on the homogeneous surface of the adsorbent. Additionally SSE test was done and the results were kept in Table 2.

3.12. Effect of temperature

Temperature is a crucial parameter in MO sorption and the experiments were conducted at various temperatures (303, 313 and 323 K) with a pH value of 3. By increasing the temperature the sorption capacity of the sorbent increases gradually. Increasing temperature may result in expanding within the internal structure of ACSSB@ZnO allowing further penetration of the dye molecule [45]. The maximum MO sorption capacity of the ACSSB@ZnO increases from 58.82 to 111.11 mg/g. This could be because the interaction between the MO with the active functional groups present in ACSSB@ZnO was stronger at higher temperatures. This means that MO sorption should be in endothermic nature. The following equations have been used to determine thermodynamic properties of $((\Delta G^{\circ}), (\Delta H^{\circ})$ and $(\Delta S^{\circ}))$ MO onto ACSSB@ZnO.

$$\Delta G^{\rm o} = -RT \,\ln K_L \tag{9}$$

$$\Delta G^{\circ} = \Delta H^{\circ} - T \Delta S^{\circ}$$
(10)

$$\ln K_L = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} \tag{11}$$

where *R* is the universal gas constant $(8.314 \times 10^{-3} \text{ kJ/mol K})$, *T* is the temperature (*K*) and *K* is obtained from *b* and *q*_m. Enthalpy (Δ H^o) and entropy (Δ S^o) changes were calculated from the graph of ln K_L vs 1/T (Fig. 6G). The value of the Δ G^o is calculated from Eq. (10). The negative values at a given temperature (-8.917, -8.009 and -6.647 kJ/mol) express the spontaneous nature and confirm the feasibility of the reaction. Positive Δ H^o (28.29 kJ/mol) and Δ S^o (0.1163 J/mol K) suggests the endothermic and increased randomness of MO adsorption onto ACSSB@ ZnO.

3.13. Comparison of ACSSB@ZnO for MO removal with other adsorbents

The maximum sorption capacity of MO was compared with the various adsorbents [46–52] and the data were presented in Table 3. At the same time, surface area is the exclusive factor in determining the adsorbent capacity. Large surface area can provide more active sites to attract dye molecule. The value of q_{max} for MO adsorption onto ACSSB@ZnO is much higher than the adsorbents listed in Table 3. Other sorbents ' adsorption capacity is much lower than ACSSB@ZnO, which may be due to less surface area, pore size, pore volume, and less availability of functional groups in the sorbent. Consequently, ACSSB@ZnO appears to be more competitive than other adsorbents and it may be worth noting for MO removal from aqueous solution.



Fig. 8. Anticancer activity of pure chitosan, ZnO and ACSSB@ZnO onto Mg63 and MCF7.

ACSSB@ZnO

ACSSB

в

А

Day 0

Day 3

Day 7

PBS

CS



Blank





Fig. 9. (A) Mice skin wound healing ability. (B) Histological evaluation of wound healing. (C) Bacterial growth form wound pus.

3.14. Reusability

In order to assess financial efficient and environmental sustainable use, the reusability of material is essential. A successful adsorbent should possess high sorption capability and good re-usable properties and it can reduces wastewater treatment costs significantly. In perspective of this criterion, ACSSB@ZnO was explored with the reusability studies for MO removal. Various eluents like HCl, H₂O, NaOH and NH₄OH were utilized as desorbing agents with different concentration levels (0.1 to 0.4 M). The obtained data were kept in Fig. 6H and 6I. In this study NaOH solution was acted as good eluent to desorb MO from the MO-loaded ACSSB@ZnO. The reusability experiments were conducted after adsorption of MO and the adsorbent was taken out, rinsed well with the distilled water. From the Fig. 6I, it has been noted that the desorption capability of MO significantly reduces with the rise of reuse times. It was shown that the elimination efficiency of MO decreased slowly from 94.36% (first cycle) to 81.32% in the final regeneration cycle (fourth cycle). The main reason for this one is there is no effective adsorbate-adsorbent interaction, and unavailability of sorption sites. Hence all the reusability studies were performed with the 0.1 M of NaOH solution. This suggests that ACSSB@ZnO is extremely reusable sorbent, and it is essential for real polluted solutions also.

4. Biological applications

4.1. In vitro antibacterial and anti-tumorigenic property of ZnO-ACSSB

Wounds are frequently create a favorable atmosphere for microorganism colonization that can retard healing and lead to cause infection. Disc diffusion method is widely used to test antibacterial activity. The antibacterial activity of CS, ACSSB and ACSSB@ZnO is shown in Fig. 7. Inhibition zone diameter on E. coli culture (gram negative bacteria) of 10 mm in CS (chitosan), 15 mm in ACSSB and 20 mm in ACSSB@ZnO (Fig. 7). Likewise in zone of inhibition on S. aureus culture (gram positive bacteria) was 12 mm in CS, 16 mm in ACSSB and 22 mm in ACSSB@ZnO. We found clear and wider zone of inhibition in ACSSB@ ZnO onto E. coli and S. aureus culture compare to CS, and ACSSB. Antitumorigenic effect of CS, ACSSB, and ACSSB@ZnO on MG63 and MCF7 cells were shown in Fig. 8. The CS at 20, 100, and 250 µg/mL enhanced MG63 cell viability by 22, 24, and 25% respectively (Fig. 8). The ACSSB, at all the concentration treated, did not affect MG63 cell viability. Interestingly, ACSSB@ZnO at 100, and 250 µg/mL reduced MG63 cell viability by 62% and 92% respectively (Fig. 8). The CS at all concentration treated did not affect MCF7 cell viability (Fig. 8). The ACCSB at 100 µg/mL enhanced MCF7 cell viability by 14%. The ACSSB@ZnO at 100, and 250 µg/mL inhibited MCF7 cell viability by 62% and 92% respectively (Fig. 8). Low-molecular-weight chitosan derivatives and zinc complexes inhibits liver cancer cells grows in vitro. Our findings showed concentration dependent anti-tumorigenic property of ACSSB@ZnO.

4.2. Wound healing and in vivo antibacterial properties of ACSSB@ZnO

The wound healing ability of ZnO-ACSSB is shown in Fig X. The CS, ACSSB and ACSSB@ZnO groups demonstrated superior cure than the control group at day 3 and 7 (Fig. 9). In ACSSB and ACSSB@ZnO organizations more pronounce was noted early wound cure and closure (Fig. 9A). H & E staining of histological section also showed strong wound healing property of ACSSB@ZnO (Fig. 9). The pus culture from the mice wound was assessed on day 3 of the treatment by the in vivo antibacterial activity of the newly formulated CS, ACSSB and ACSSB @ ZnO (Fig. 9C). Exudate culture control group showed apparent bacterial colonial development. CS, ACSSB and ACSSB@ZnO cultures demonstrates that there was no growth of bacteria in the exudates (Fig. 9B). Similar effects on wound healing and in vitro antibacterial impact of chitosan and Zn products were investigated. In this research, ACSSB@ZnO accelerated wound cure and regulated the development of bacteria in the wound surface.

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5. Conclusion

A novel, cost-effective and environmentally friendly ACSSB@ZnO has been synthesized, characterized and utilized to remove MO from aqueous solution. The adsorption capacity of ACSSB@ZnO was highly dependent on various operating variables such as basic pH (3.0), adsorbent dose (0.5 g), contact time (105 min) and temperature (323 K). The pseudo-second order kinetic reaction model was found to be the best for kinetic evaluation. Additionally, the adsorption rate increased with increasing temperature, which indicated that the adsorption process could be accelerated by high temperature. The isothermal data was well correlated with the Langmuir model and the highest sorption capacity was found to be 111.11 mg/g at 323 K. The thermodynamic parameters were calculated and the negative values of ΔG° and positive value of ΔH° indicate the spontaneous and endothermic nature of the adsorption processes. The results shows that ACSSB@ZnO can act as a potential sorbent to remove MO from aqueous environment.

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