

The Effect of Competing Ions on the Sorption of Amoxicillin, Ciprofloxacin, and Sulfamethoxazole on Chitosan-Carbon Nanotube Hydrogel Beads

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Abstract – The present work pertains to the synthesis of chitosan-carbon nanotube (CCNT) hydrogel beads using a two-step process for the uptake of amoxicillin (AMX), ciprofloxacin (CIP) and sulfamethoxazole (SMX) in the presence of varying concentration of sodium chloride (NaCl) and humic acid (HA) from 0 mg/L to 40 mg/L as competing ions. From the results obtained it was concluded that the increase in NaCl or HA concentration demonstrated antagonistic effects in the uptake of AMX, CIP and SMX on the synthesised CCNT hydrogel beads due to the formation of aggregates with an increase in ionic strength. Moreover, NaCl demonstrated the least effects on the uptake of the model antibiotics as compared to HA, indicating that NaCl ions exhibit minimal competitive effects with adsorbate molecules for active adsorption sites on CCNT hydrogel beads. Similarly, from the single factor analysis of variance results p-values of less than 0.05 were recorded for the uptake of AMX, CIP and SMX on CCNT hydrogel beads, explicitly indicating that there was a statistical difference between the means for the independent and dependent variables, thus cementing the negative effect of increasing ionic strength on the uptake of model adsorbates. Moreover, the findings of the present work suggest that there is need for a pretreatment stage aimed at eliminating co-existing contaminants prior to the application of solid-liquid adsorption for complete eradication of contaminants of emerging concern particularly antibiotics.

Keywords: chitosan, carbon nanotube, amoxicillin, ciprofloxacin, sulfamethoxazole, humic acid, sodium chloride.

1. Introduction

Recently, there has been a significant increase in the use of antibiotics for human consumption and agricultural use particularly in poultry farming, animal husbandry, beekeeping, aquaculture as well as livestock as growth promoters [1, 2]. The comprehensive use of antibiotics can be ascribed to their ability to minimize the burden of common infectious diseases qualifying them to be crucial for medical interventions as elucidated by Manjunath, et al. [1]. Sadly, there has been a noticeable global increase in the consumption of antibiotics of 65% between year 2000 and 2015 from 21.1 to 34.8 billion defined daily dose, while the antibiotic consumption rate increase has been reported to move from 11.3 to 15.7 daily dose per 1000 inhabitants per day as elucidated by Manjunath, et al. [1] and Klein, et al. [3]. Due to the increase in the consumption rate of antibiotics, they have been detected in water bodies at minute concentrations ranging from ng/L to µg/L [2]. The occurrence of antibiotics in water receiving bodies is ascribed to human secretion through urine and faeces, from original compounds, as its metabolites and/or as conjugates glucuronic and sulfuric acid, as well as the use of animal manure (i.e., mixture of faeces, urine and bedding material) [2, 4, 5]. It is worth noting that approximately 50 – 90 percent of antibiotics administered by humans or animals are excreted via urine and faeces as a mixture of the parent compound as well as in its metabolite form [5], which are then sent into wastewater treatment plants (WWTPs) as urban wastewater. However, the occurrence of antibiotics in water receiving bodies is evident enough that the current WWTPs cannot completely eradicate these emerging contaminants of environmental concern as reported in literature [6, 7]. Moreover, antibiotics are characterised as pseudo-persistent compounds, as such their occurrence in aquatic environment triggers antimicrobial resistance against pathogens subsequently leading to ecotoxicity. Available literature [8] suggests that, about one-third of children under the

age of five years have succumbed to infection with antibiotic-resistant organisms. Therefore, there is an urgent need for antibiotics removal from water bodies on the basis that antibiotic resistance is being considered as one of the emerging health challenges.

A number of techniques have been investigated for the removal of antibiotics from wastewater ranging from advanced oxidation [9], membrane technology [10], biodegradation [11] as well as solid-liquid adsorption [1]. However, hitherto, solid-liquid adsorption has been one of the most preferred technologies for antibiotic removal from aqueous environments as compared to the other aforementioned technologies. The growing appetite in the application of solid-liquid adsorption is ascribed to its advantages i.e., cost effective, easy operation without any sludge nor byproducts generation, reusability as well as online operation [1, 12]. There has been a paradigm shift in the application of conventional adsorbents derived from non-renewable resources such as polymers since they render the adsorption process expensive. As such, most researchers within the scientific community are exploring new avenues in the application of low-cost adsorbents derived from agricultural waste for complete eradication of emerging contaminants of environmental concern. The current study focuses on the application of chitosan-based adsorbent for the removal of amoxicillin (AMX), ciprofloxacin (CIP) and sulfamethoxazole (SMX) from aqueous solutions. Chitosan is characterised as a natural polysaccharide that can be easily obtained from crustacean (i.e., lobster, crabs, shrimps as well as insects and fungi) chitin by thermochemical deacetylation [13]. Owing to its environmentally compliant properties such as being inexpensive, biocompatible, non-toxicity, hydrophobicity as well as biodegradability, it has cemented its application as a biosorbent in wastewater treatment processes [14]. According to Khumalo, et al. [14] the presence of active amino and hydroxyl functional groups within the chitosan structure allows it to be an excellent biosorbent with chelating sites for targeted compounds in aqueous environments. Chitosan can be easily modified to gels, beads and films with enhanced mechanical strength, diffusion capacity as well as swelling [13]. The versatility of chitosan makes it an excellent material to develop novel composites with a wide range of functional groups.

Due to the lack of studies focusing on the uptake of antibiotics from solution in the presence of competing ions, herein chitosan-carbon nanotube (CCNT) hydrogel beads were synthesised via precipitation in an alkaline solution as discussed by Khumalo, et al. [14]. The adsorption efficacy of CCNT hydrogel beads was investigated for the uptake of AMX, CIP and SMX in the presence of sodium chloride (NaCl) and humic acid (HA) as competing ions.

2. Materials and Methods

2.1. Materials

All chemicals used in the present study were not subjected to any purification process they were used as received from respective suppliers. Amoxicillin ($\geq 95\%$ anhydrous basis), ciprofloxacin ($\geq 98\%$ pure), sulfamethoxazole (HPLC grade), and multi-walled carbon nanotubes ($>98\%$ carbon basis) were supplied by Lasec laboratories, Durban, South Africa. Chitosan powder from shrimp shells ($\geq 75\%$ deacetylation), sodium hydroxide (NaOH) pellets ($\geq 99.5\%$ pure), methanol (CH_3OH) ($\geq 99.9\%$ pure), 98% pure sulfuric acid (H_2SO_4), HPLC grade NaCl salt and humic acid ($\text{C}_{187}\text{H}_{186}\text{O}_{89}\text{N}_9\text{S}_1$) salt were supplied by Sigma-Aldrich, South Africa. Glacial acetic acid ($\text{C}_2\text{H}_4\text{O}_2$) ($\geq 99.7\%$ pure) was supplied by Shalom Laboratories, South Africa.

2.2. Adsorbent synthesis

CCNT hydrogel beads were synthesized by adopting the procedure as outlined in our previous work [14]. Multiwall-CNTs were first modified by soaking a specific amount of CNTs in a solution of concentrated sulfuric acid (99%) and nitric acid (65%) at a volume ratio of 1:2, respectively for 24 hours. The acid modification of CNTs was imperative for the attachment of functional groups such as carboxylic and hydroxyl groups on the CNTs surface. Functionalized CNTs were rinsed with ultra-pure deionized water until a filtrate pH of 7 was achieved. Thereafter, 100 g of chitosan powder was dissolved in 400 mL of 1 %v/v of $\text{C}_2\text{H}_4\text{O}_2$ solution on the basis that chitosan has a relatively high solubility in weak acidic aqueous environments [15]. The chitosan- $\text{C}_2\text{H}_4\text{O}_2$ mixture was then vortexed using a magnetic stirrer bar at 200

rpm for 24 hours at room temperature to allow for complete dissolution of chitosan due to its low solubility. Thereafter, 5 wt% with respect to chitosan of functionalized multiwall-CNTs were added into the chitosan-C₂H₄O₂ mixture and vortexed at 200 rpm for 2 hours to obtain a homogeneous distribution of CNTs in the mixture. CCNT hydrogel beads were synthesized by adding the viscous CCNT gel dropwise in a solution of 15 wt% NaOH and 95 %v/v methanol at a volume ratio of 4:1, respectively, using a 10 mL syringe which precipitated into CCNT hydrogel beads. The CCNT hydrogel beads were soaked in the NaOH-methanol solution for 24 hours, then rinsed with deionised water until a pH of 7 was obtained from the filtrate prior to being utilised for adsorption studies.

2.3. Adsorption batch studies

The adsorption efficacy of the model adsorbent was investigated by conducting batch adsorption experiments using 50 mL clear bottles with screw caps. A sample size of 50 mL for a predetermined initial concentration 40 mg/L of AMX, CIP and SMX at a fixed solution pH of 7 was used. The solution pH was adjusted using 1.0 M NaOH and 1.0 M H₂SO₄. A predetermined quantity of 1.5 g/L of CCNT hydrogel beads was added into respective AMX, CIP and SMX aqueous solutions and in a shaker for 24 hours at a rate of 150 rpm at 293 K. Thereafter, samples were drawn and filtered using a 0.45 µm syringe filter, thereafter, transferred into a 10 mL sample tube. Filtered samples were centrifuged at 5000 rpm for 10 minutes. Thereafter, the supernatant solution of the centrifuged sample was analysed for the residual of AMX, CIP and SMX concentration using a Uv-vis spectrophotometer (UV-1900i, Shimadzu, South Africa). The amount of antibiotics adsorbed on CCNT hydrogel beads and adsorption efficacy were calculated using Eqs. (1) - (2) below.

$$q_{e,i} = \frac{(C_0 - C_e)}{m} \times V \quad (1)$$

$$\text{Adsorption \%} = \left[\frac{C_0 - C_e}{C_0} \right] \times 100 \quad (2)$$

Where $q_{e,i}$ is the adsorption capacity at equilibrium of component i measured in mg/g; C_0 is the initial adsorbate concentration of component, i measured in mol/L; C_e is the concentration of the adsorbate in solution after adsorption equilibrium measured in mol/L; m is the mass of adsorbent on a dry basis measured in g; and V is the volume of the adsorbate solution with the initial concentration measured in L.

3. Results and Discussion

3.1. Effect of NaCl and HA on the uptake of AMX, CIP and SMX on CCNT hydrogel beads.

Salts are abundant from natural as well as anthropogenic sources, as such the salinity of surface water and groundwater may vary considerably. NaCl is one of the most detected salts in surface water due to its application as a road de-icing agent subsequently enters water receiving bodies. On the other hand, human acid (HA) is ubiquitous in aquatic environments highly concentrated in sediments of terrestrial [16], thus playing a role on the fate of co-existing organic contaminants through interaction by cation exchange, complexation, and/or hydrogen bonding. Herein, the influence of varying NaCl and HA concentration (i.e., 0 mg/L to 50 mg/L) on the uptake of AMX (Fig. 1), CIP (Fig. 2) and SMX (Fig. 2) on CCNT hydrogel beads was investigated at a fixed adsorbate concentration of 40 mg/L. From the findings of the present work, it can be inferred from Figs. (1) – (3) that NaCl had a minimal negative effect on the uptake of the model adsorbate as compared to HA. In the context of NaCl, it is worth noting that the uptake of AMX, CIP and SMX did not change for a NaCl concentration of less than 15 mg/L. However, the decrease in the uptake of the model adsorbates (i.e., AMX from 53 mmol/kg to 43 mmol/kg; CIP from 64 mmol/kg to 50.14 mmol/kg; and SMX from 75 mmol/kg to 52.09 mmol/kg) with increasing NaCl concentration can be attributed to the mere fact that an increase in ionic strength weakens the electrostatic attraction between the adsorbate and adsorbent thus compromising the efficacy of the adsorbent. Moreover, the decrease in the uptake of the model adsorbate

with increasing NaCl concentration suggests that they were competitive effects between the adsorbate and Na⁺ on the adsorbate active sites.

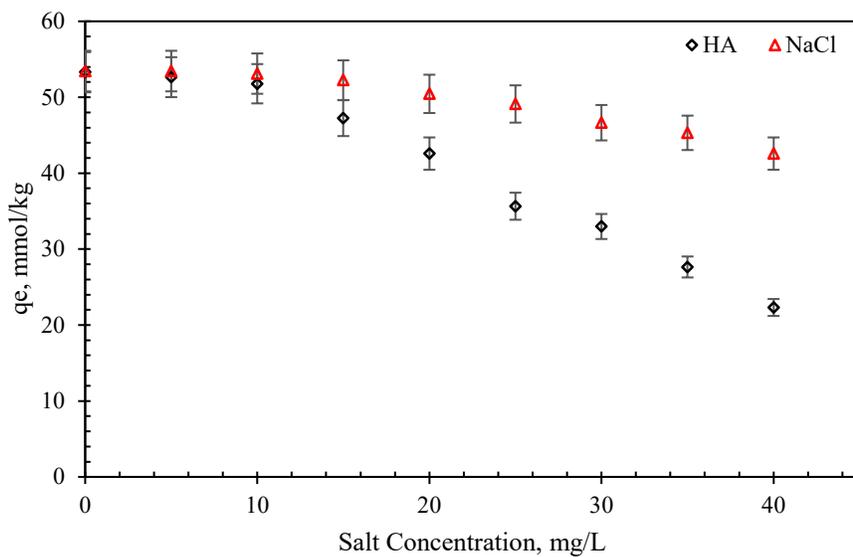


Fig. 1: Effect of HA and NaCl on the sorption of AMX on CCNT.

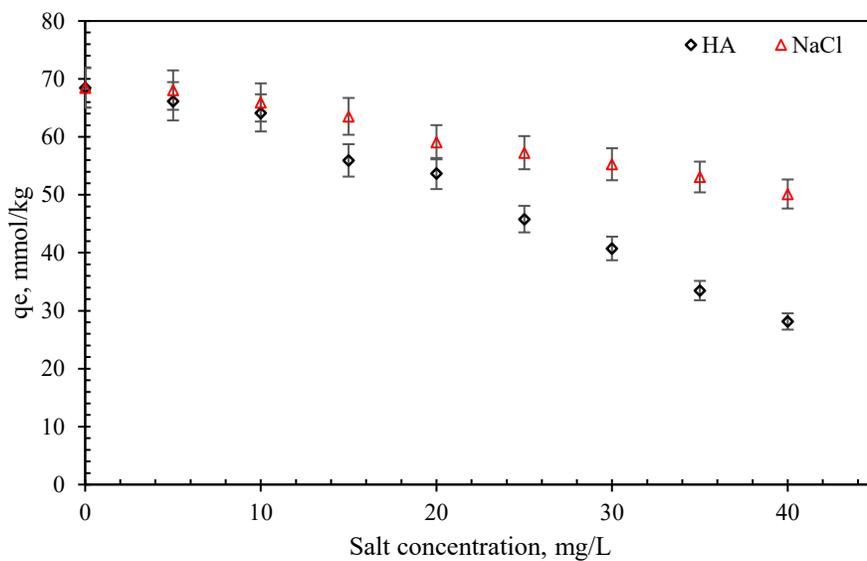


Fig. 2: Effect of HA and NaCl on the sorption of CIP on CCNT.

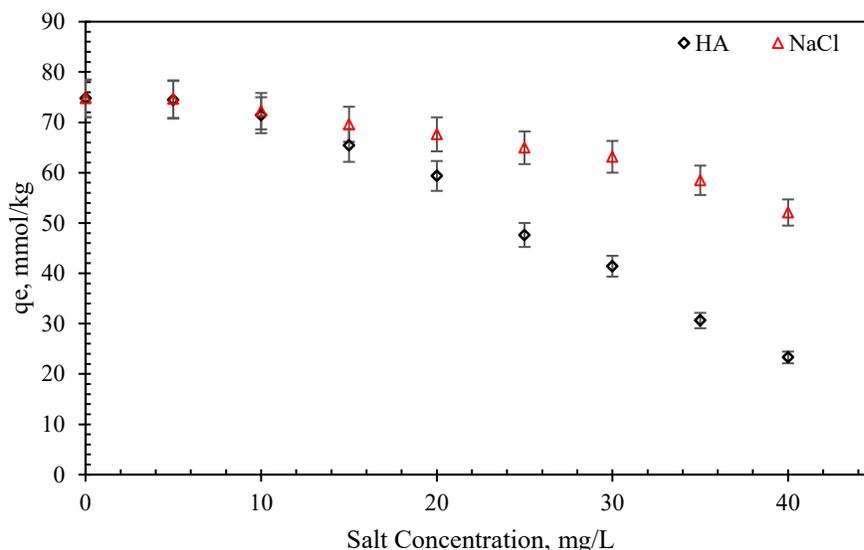


Fig. 3: Effect of HA and NaCl on the sorption of SMX on CCNT.

It is worth noting that, the results obtained suggest that the adsorption system in the presence of NaCl and HA exhibit intermolecular aggregation. Generally, intermolecular forces such as van der Waals forces, ion-dipole and dipole-dipole forces exist between organic molecules in solution [17]. These forces increase upon an increase in salt concentration facilitating the aggregation of organic substances. However, for the present work it can be inferred from Figs. (1) – (3) that the uptake of AMX, CIP and SMX on CCNT hydrogel beads is not promoted by the addition of NaCl and HA due to aggregation. In the context of HA, the significant decrease in the adsorption of the model adsorbates can be attributed to the formation of HA aggregates in solution which are adsorbed on CCNT active sites. The adsorption of large HA aggregates hinders any further adsorption of isolated adsorbate molecules on CCNT hydrogel beads active sites, subsequently resulting to a significant decrease on the efficacy of the adsorbent. Moreover, the significant decrease in the uptake of AMX, CIP and SMX with increasing HA concentration can also be ascribed on cation bridging which is a crucial mechanism that triggers the aggregation of humic molecules into large colloidal structures as elucidated by Zhang, et al. [17]. The findings of the present study suggest that humic molecules aggregates were adsorbed on CCNT hydrogel beads, initiating charge neutralization of negatively charged adsorbate molecules subsequently suppressing the adsorption process through electrostatic attraction. The findings of the present study are congruent to similar studies reported in literature [18]. However, it is worth noting that, in some cases an increase in ionic strength can improve the facilitate the uptake of organic adsorbates on carbonaceous adsorbents due to the effect of salting-out. An increase in ionic strength can result to a decrease in the solubility of the adsorbate thus allowing the dominance of the uptake of model adsorbate by hydrophobic interaction between the adsorbate and adsorbent [19].

3.2. Analysis of variance

The percentage removal of AMX, CIP and SMX on CCNT hydrogel beads with varying HA (Fig. 4) and NaCl (Fig. 5) concentration were statistically examined using the single factor analysis of variance (ANOVA). In the ANOVA, a single continuous dependent variable (i.e., adsorbate percentage removal) was compared with the mean of the initial concentrations of NaCl and HA as independent variable. Herein, two hypotheses were formulated i.e., 1) null hypothesis: assuming that there is no statistically significant difference that exist between the means of the independent and dependent variables, and 2) alternative hypothesis: assuming that there is a statistical difference between the means. For both model competing ions adsorption systems, the findings of the current study recorded p-values of less than 0.05 indicating that there was a statistical difference between the means of the independent and dependent variables rejecting the null hypothesis.

Table 1: Single factor ANOVA results for the percentage removal of AMX, CIP and SMX on CCNT hydrogel beads

Compound	Humic acid			Sodium chloride		
	SS	F-value	p-value	SS	F-value	p-value
AMX	5750	26.29	0.000101	10 349	95.32	3.83E-08
CIP	8 334	32.33	3.38E-05	13 454	104.67	2.0E-08
SMX	4476	17.02	0.000792	8 342	69360	3.21E-07

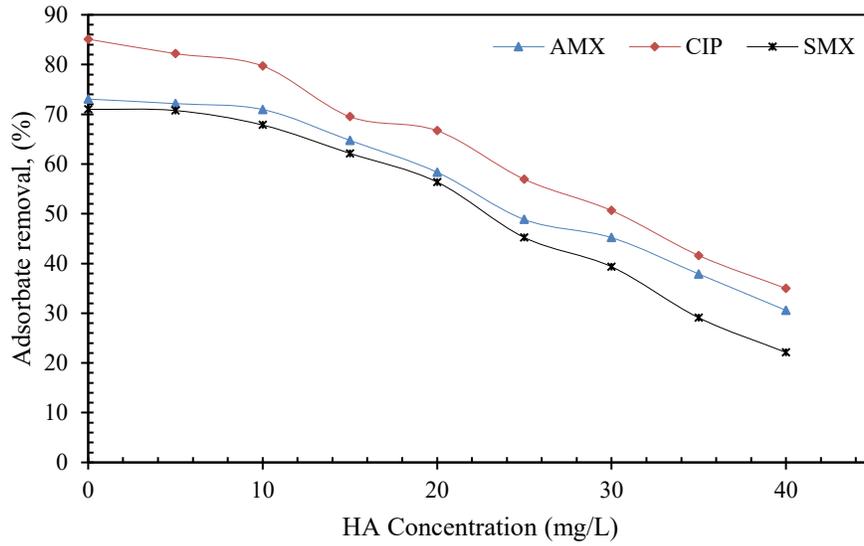


Fig. 4: Effect of HA on the percentage removal of AMX, CIP and SMX.

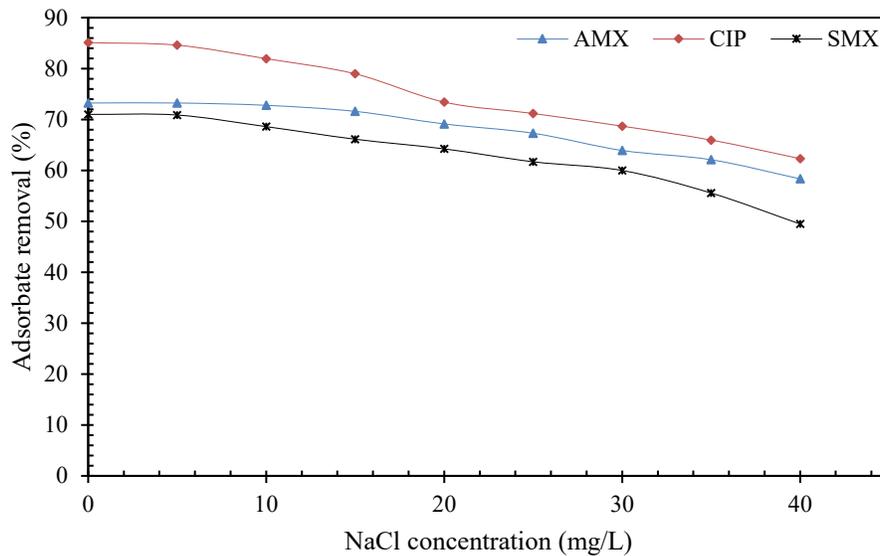


Fig. 5: Effect of NaCl on the percentage removal of AMX, CIP and SMX.

Therefore, the ANOVA results suggest that there was a variation on the uptake of the model adsorbates with varying HA and/or NaCl concentration. Moreover, the mean differences were further confirmed at 95% confidence level by the recorded F-values of less than 1 as presented in Table 1. Similarly, the uptake of AMX, CIP and SMX in the presence of NaCl recorded relatively high sum of squares (SS) values when compared to the uptake of the model antibiotics in the presence of HA. The ANOVA results on the recorded SS values suggests that the uptake of AMX, CIP and SMX in the presence of NaCl was relatively high regardless of the increase in concentration as compared to the uptake of the aforementioned adsorbates in the presence of HA. The relatively low SS values in the presence of HA suggest that HA had significant antagonistic effects in the uptake of AMX, CIP and SMX with increasing concentration. As such, it can be inferred from the ANOVA results that there was a statistical decline in the percentage uptake of AMX, CIP and SMX with an increase in HA or NaCl concentration.

4. Conclusion

From the findings of the current work, it can be inferred that the presence of competing ions in the form of NaCl and HA have antagonistic effects in the uptake of antibiotics on CCNT hydrogel beads from aqueous solutions. Moreover, it is apparent that the uptake of AMX, CIP and SMX is not facilitated by an increase in ionic strength particularly in the presence of NaCl and HA due to the formation of aggregates with increasing NaCl and HA concentration subsequently compromising the efficacy of carbonaceous adsorbents. Therefore, there is a need for a pretreatment stage aimed at eliminating co-existing contaminants prior to the application of solid-liquid adsorption for complete eradication of contaminants of emerging concern particularly antibiotics.

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