

IN-VITRO KINETICS, ADSORPTION ISOTHERM, AND EFFECT OF PH ON ANTIDOTAL EFFECT OF ACTIVATED CHARCOAL IN TRAMADOL HYDROCHLORIDE INTOXICATION

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ABSTRACT

Tramadol overdose has been one of the most frequent causes of drug poisoning in the recent years, especially in young adult males. In the current work, the in-vitro study on adsorption kinetics and the effect of pH on antidotal effect of activated charcoal (AC) in tramadol hydrochloride intoxication were carried out. For adsorption study tramadol hydrochloride solutions of various concentrations were prepared in both simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) and analyzed by UV spectrophotometer. For kinetics study tramadol hydrochloride and charcoal in ratio 1:5 was kept in 6 different flasks and sonicated for 5, 10, 15, 20, 25 and 30 minutes and analyzed spectrophotometrically. The data were plotted among two most commonly used adsorption isotherm, Langmuir isotherm and Freundlich isotherm and their coefficient of determination (R^2) was compared to get the best adsorption isotherm equation. The kinetics study was done in both SGF and SIF. The result showed that AC 50 gm can adsorb 4802.692 mg tramadol hydrochloride at gastric environment and 8064.516 mg tramadol hydrochloride at intestinal environment. The R^2 value in the current study is found to be more in SIF (0.986) than in SGF (0.985). In accordance to the value of R^2 , the pseudo second order kinetics model fit best for this study with R^2 value of 0.9997 in SGF and 0.9994 in SIF. From the current study it can be concluded that 50g AC has the capacity to adsorb sufficient amount of tramadol hydrochloride and the kinetics followed during the adsorption was pseudo-second order.

Keywords: Activated charcoal, adsorption kinetics, Langmuir isotherm, Freundlich isotherm, pseudo-first order, pseudo-second order

INTRODUCTION

A potential option in the medical management of ingestion of a dangerous amount of a drug is administration of oral activated charcoal (AC) in conjunction with other measures. AC comes in direct contact with the drug and can adsorb certain drugs to a varying extent, thereby reducing their systemic absorption and toxicity [1]. The effectiveness of AC as an adsorbent is attributed to its unique properties including large surface area, high degree of surface reactivity, universal adsorption effect, and favorable pore size [2]. AC is known as a universal antidote which is able to adsorb from 100 - 1000 mg of poison per gram, inhibiting the absorption of orally ingested compounds as well as increasing the systemic clearance of drugs through the gastrointestinal tract [3]. The mechanism for the latter may involve interruption of the enterohepatic recycling and/or promotion of drug movement from the systemic circulation into the gut lumen (i.e. gastrointestinal dialysis). Variables that may alter the efficacy of charcoal therapy include the preparation and dose of charcoal used, toxins ingested, nature of the stomach contents, gastrointestinal pH and time from toxin ingestion to charcoal administration [4]

The mechanism of adsorption of various drugs on AC has been extensively studied and known to proceed by surface phenomenon. The high binding capacity of AC has been attributed to its high surface area. Activation creates a highly developed internal pore structure and small particle size. These factors determine the extent of adsorption at equilibrium. In vitro adsorption to AC in aqueous solutions is a nonspecific process that reaches equilibrium in less than 30 minutes. Desorption of poison may occur because substance adsorption to AC is a reversible process but the extent and clinical impact of this phenomenon have not been determined [5]

For many drugs (or poison as well as pesticides), the adsorption onto activated charcoal gets varies with respect to pH [6,7,8,9,10,11] and most of them attributed to the adsorption to activated charcoal of only the unionized form of the drug [12]. Adsorption kinetics study is the study of rate and mechanism of the adsorption process. The adsorption kinetic studies describe the solute uptake rate which in turn controls the residence time of adsorbate uptake at the solid-liquid interface. Adsorption kinetics are one of the important characteristics in the defining the efficiency of an adsorption process and to understand the behavior of the adsorbent.

Tramadol is an opioid analgesic that acts on the neurotransmission of noradrenalin and serotonin [13]. It has been novel centrally acting analgesic used for the treatment of mild to severe pain. Tramadol binds weakly to the μ -opioid receptor and also inhibits reuptake of monoamines such as serotonin and norepinephrine [14]. Tramadol overdose has been one of the most frequent causes of drug poisoning in the recent years, especially in young adult males with a history of substance abuse and mental disorders [15]. The number of poisoning cases admitted to tertiary hospital as well as lower level hospital is increasing [16]

In the current work, the in-vitro study on adsorption kinetics and the effect of pH on antidotal effect of activated charcoal in tramadol hydrochloride intoxication were carried out.

MATERIALS AND METHOD

Materials

Standard of tramadol hydrochloride was obtained from Lomus pharmaceuticals private limited, Kathmandu. Tramadol hydrochloride was supplied by the retail pharmacy. Activated Charcoal powder (Detox) was obtained from community pharmacy. All the reagents such as potassium dihydrogenphosphate (KH_2PO_4); Sodium Hydroxide (NaOH); orthophosphoric acid was of analytical grade. The simulated gastric and intestinal fluid environments (pH 1.2 and pH 6.8) without enzyme were prepared as per pharmacopoeial method described in United State Pharmacopoeia 2002).

Simulated Gastric Fluid pH 1.2 (SGF): was prepared by taking NaCl 2gm, Conc. HCl

7ml in 1000ml distilled water.

Simulated Intestinal Fluid pH 6.8 (SIF): was prepared by taking 68.05 gm potassium dihydrogen phosphate, NaOH 8.96g in 10L water.

Preparation of standard calibration curve:

Tramadol hydrochloride stock solution (500 g/ml): 50mg standard tramadol hydrochloride was dissolved in 100ml each of gastric fluid and intestinal fluid. Then different concentration of tramadol hydrochloride standard solutions was obtained by dilution such as 500 $\mu\text{g/ml}$, 250 $\mu\text{g/ml}$, 125 $\mu\text{g/ml}$, 62.5 $\mu\text{g/ml}$, 31.25 $\mu\text{g/ml}$, and 15.625 $\mu\text{g/ml}$. Each concentration of tramadol hydrochloride standard solution was filtered by watman filter paper and placed into individual, capped vial. The UV_a (UV absorbance) at 278 nm for intestinal fluid-stock solution and gastric fluid-stock solution was measured for each one. UV_a was plotted against the concentration of tramadol hydrochloride and respective calibration curve for Tramadol hydrochloride was obtained for two different pH (1.2 and 6.8) solutions.

Adsorption study:

Then weight of 12.5mg containing tramadol hydrochloride powder was taken and dissolved in 100ml in both gastric and intestinal fluid one by one and various amount of activated charcoal ranging from 12.5mg to 350mg was weighed then added. The sample was sonicated for 15 minute at 37°C. After sonication the solution was filtered through watman filter paper. The filtrate was then analyzed by UV spectrophotometer.

Kinetics study:

The drug of tramadol hydrochloride and charcoal in ratio 1:5 was kept in 6 different flasks and kept for sonication. Then each flask was taken out of the sonicator in subsequently 5, 10, 15, 20, 25 and 30 min. After filtration these six solutions are analyzed as before in adsorption studies. The data obtained were analyzed with pseudo-first order and Pseudo-second order [17]. The kinetics study was also done in both simulated gastric and intestinal fluid.

Tramadol hydrochloride analysis

The amount of tramadol hydrochloride in each trial of the study was calculated from calibration curve

Estimation of Langmuir adsorption parameter (Data analysis)

It is the simplest theoretical model for monolayer adsorption which was developed from either kinetic derivation or thermodynamic derivation. A derived equation of the Langmuir adsorption isotherm was used to estimate the maximum adsorption capacity (Q_m), defined as the maximal quantity of the drugs (in mg) adsorbed per gram activated charcoal. Q_m was calculated from the following equation.

$$\frac{C_f}{Q} = \frac{1}{Q_m \times k} + \frac{C_f}{Q_m}$$

Where C_f (mg/L) is the drug concentration at equilibrium, calculated as the average of the three results, and b is a constant.

The Langmuir isotherm adsorption parameter, Q_m , was calculated by linear least square fitting of the experimental data. A plot of C_f/Q versus C_f yields a straight line with the slope $1/Q_m$. Q_e is the adsorption density [mg of adsorbate per g of adsorbent; calculated as $Q_m = V(C_0 - C_f)/W$, where V is total volume in liter

and W the quantity of AC in grams; C_0 (mg/l) is the initial quantity of tramadol hydrochloride and C_f is the concentration after the adsorption]

Comparison of Q and C_f by graph:

The plateau obtained corresponds to the formation of a monolayer of adsorbent on the drug.

Statistical analysis of data

Statistical analysis was done by using analysis software SPSS 17 version and Microsoft excel.

RESULT

Adsorption saturation of AC with Tramadol hydrochloride

The final concentration of tramadol hydrochloride C_f (mg/l) was plotted against amount of tramadol hydrochloride adsorbed per gram of charcoal Q (mg/g AC) for each sample. Plots of Q vs C_f in SGF and SIF are shown in figure 1 and 2 respectively.

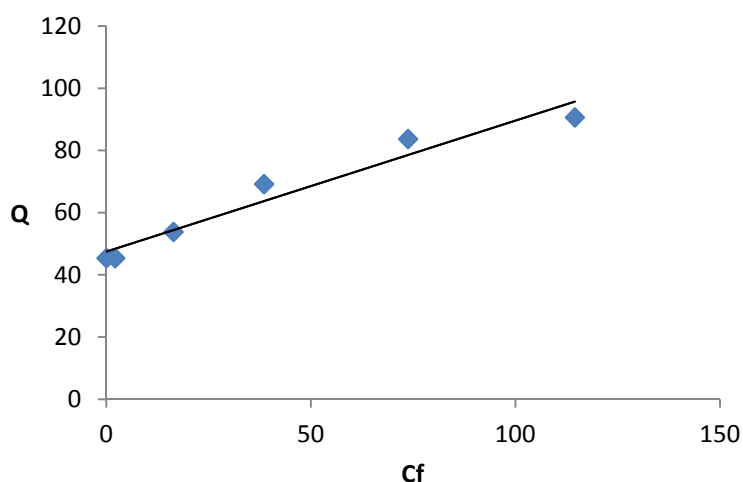


Fig 1: Adsorption saturation curve of AC with tramadol hydrochloride in SGF

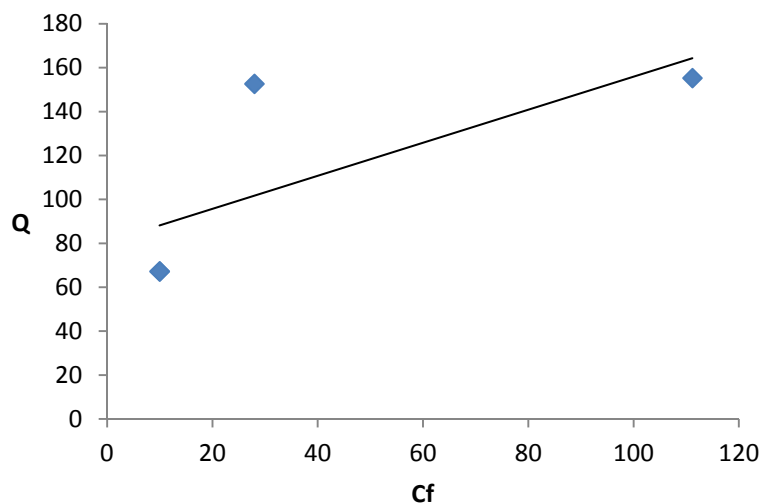


Fig2: Adsorption saturation curve of AC with tramadol hydrochloride in SIF

Maximal Adsorption Capacity

Langmuir adsorption isotherm was used to calculate the maximal adsorption capacity q_m (mg tramadol hydrochloride adsorbed/g of AC). The result is shown in table 1.

Table 1: Maximal adsorption capacity, Q_m ((mg tramadol hydrochloride adsorbed/g of AC), of tramadol hydrochloride to AC in SGF and SIF

Simulated fluid	Q_m (mg of tramadol hydrochloride adsorbed/g of AC)
Simulated gastric fluid (pH 1.2)	96.153846 [102.352, 86.325, 99.7845]
Simulated intestinal fluid(pH 6.8)	161.29032 [140.521, 181.348, 162.00196]

The maximum adsorption capacity of AC for tramadol hydrochloride in SGF and SIF was different from each other. AC can adsorb 4807.6923mg tramadol hydrochloride (48capsules) at gastric environment and.8064.516 mg tramadol hydrochloride (80 capsules) at intestine environment at its standard dose of 50 g.

Effect of pH on MAC

Table 2: Effect of pH on MAC

		p value
pH 6.8	pH 1.2	0.007

The p value from independent sample t test is significant at 95% confidence interval level. This shows that there is significant effect of pH in the MAC.

Equilibrium isotherm

Equilibrium isotherm equations were used to describe the experimental adsorption data. The two most common isotherms for describing solid–liquid adsorption systems are Langmuir and Freundlich adsorption isotherms. Langmuir’s plots showed excellent coefficient of determination (R^2) at both pH (1.2 and 6.8) of simulated fluid and their mixture with ethanol, indicating the excellent fitting model to the experiment data. The data plotted according to Freundlich and Langmuir for two different pH along with ethanol at different concentrations are shown in figure 3,4,5 and 6.

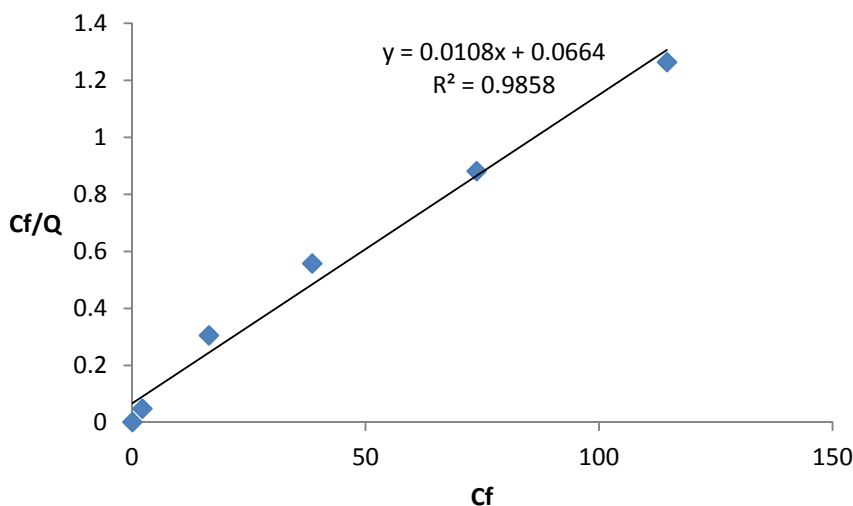


Fig 3: Langmuir plot of tramadol hydrochloride on to AC on SGF

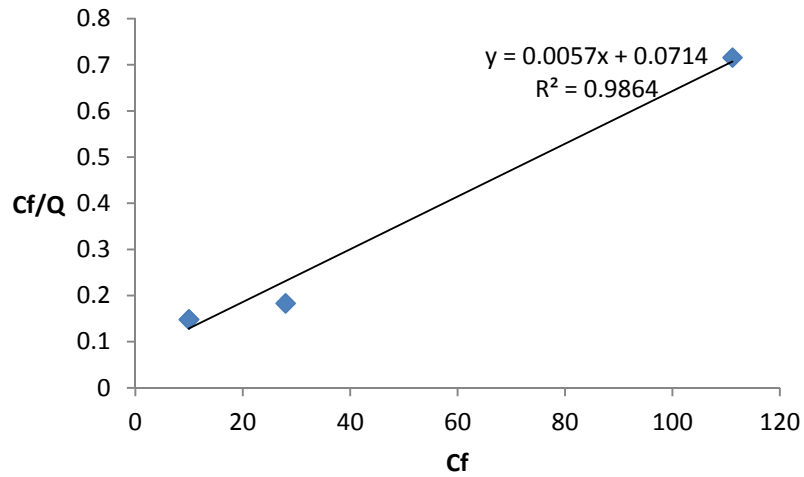


Fig 4: Lagmuir plot of tramadol hydrochloride on to AC on SIF

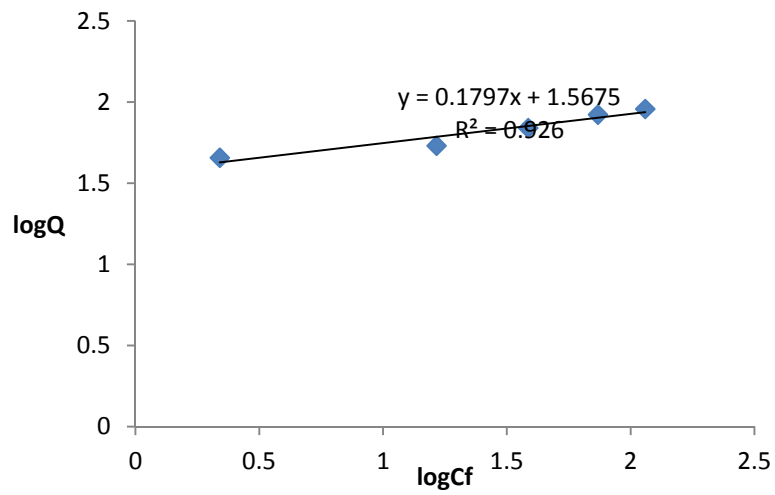


Fig5: Freundlich plot for tramadol hydrochloride onto AC in SGF

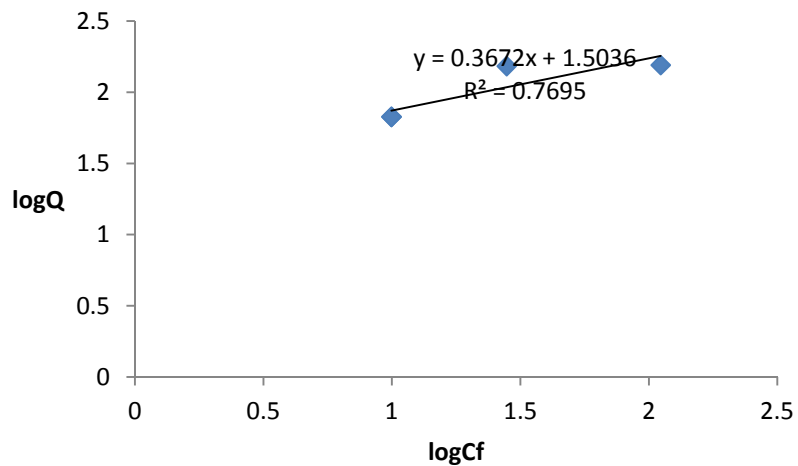


Fig 6: Freundlich plot for tramadol hydrochloride onto AC in SIF

Comparison of linear coefficient of determination, R² for different adsorption isotherm.

Table 3: Comparison of coefficient of determination, R²

Adsorption isotherm	R ²	
	SGF	SIF
Langmuir isotherm	0.985	0.986
Freundlich isotherm	0.926	0.769

The data were fitted on both Langmuir and Freundlich adsorption isotherm. The Langmuir isotherm plot showed excellent coefficient of determination for each trail of study. Thus all result was analyzed by fitting the data on Langmuir isotherm equation.

Adsorption kinetics study

Kinetic studies for the adsorption of Tramadol hydrochloride onto AC were studied using pseudo-first order and pseudo-second order model. It was observed experimentally from the present studies that the adsorption kinetics behavior of tramadol hydrochloride onto AC was found to follow only pseudo-second order kinetic model but not pseudo-first order model. (17 Putra et al., 2009)

For pseudo 1st order model the plot of log(q_e-q_t) versus 't' should be a straight line with -ve slope value and when we plot this values we get a line with -ve slope but R² value is less than the pseudo 2nd order plot. When we plot a graph for 't/q_t' vs 't', according to pseudo second order model we get a straight line with slope having +ve value and good R² value (>0.9961). So adsorption studies of tramadol hydrochloride on AC follow pseudo-second order kinetic model. The result is shown in figure 7, 8, 9 & 10.

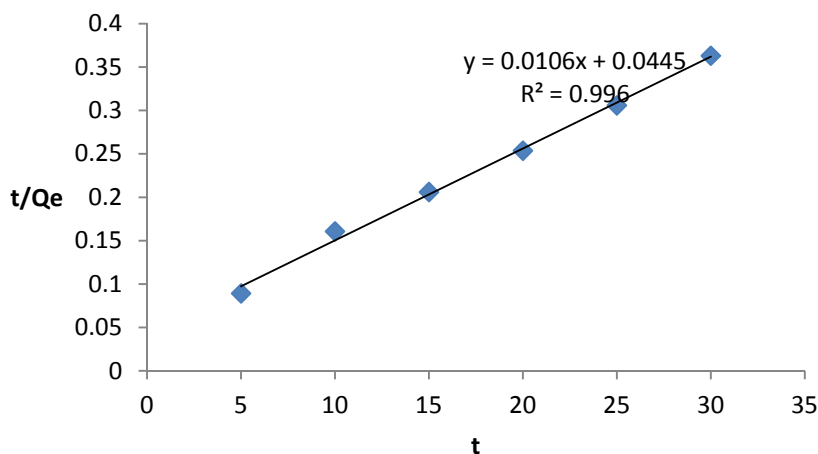


Fig7: Pseudo 2nd order plot for adsorption of tramadol hydrochloride On AC in SGF

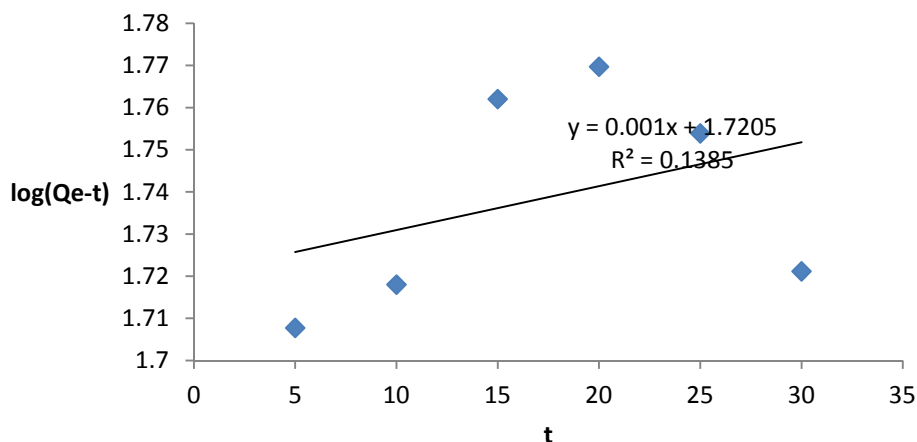


Fig 8: Pseudo 1st order plot for adsorption of tramadol hydrochloride on AC in SGF

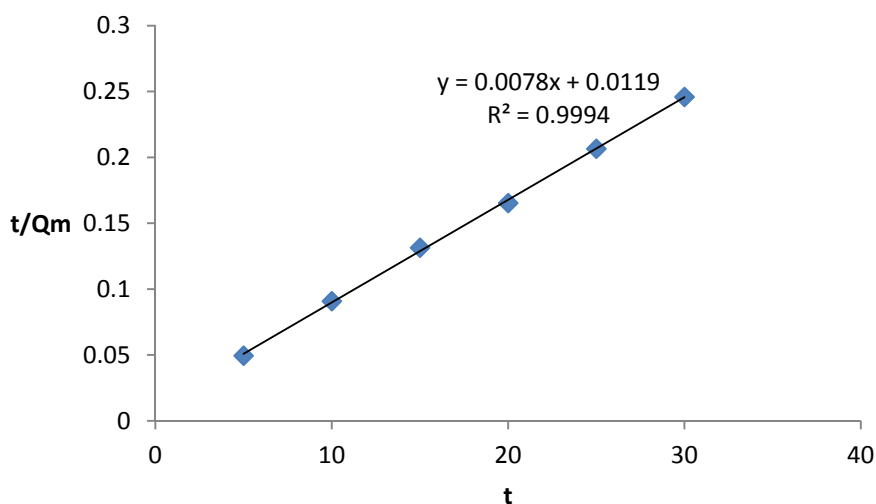


Fig 9: Pseudo 2nd order plot for adsorption onto AC in SIF

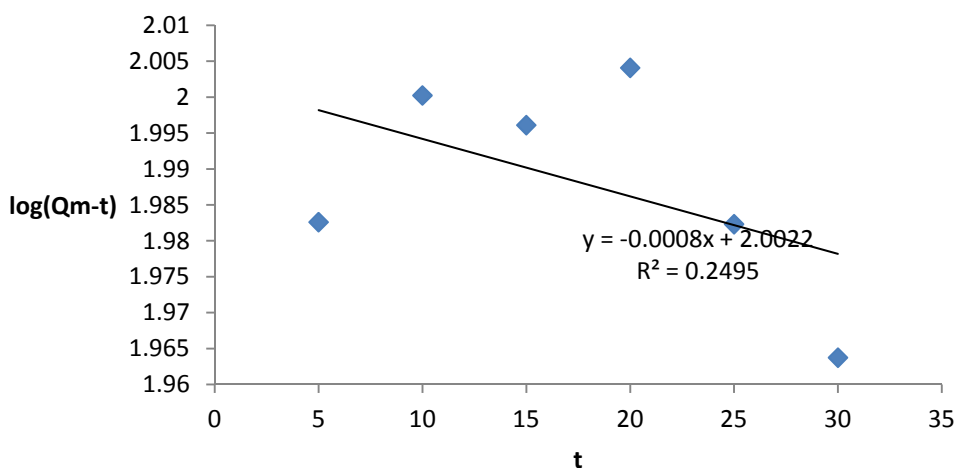


Fig10: Pseudo 1st order for adsorption onto AC in simulated intestinal fluid

Comparison of linear coefficient of determination, R² for different adsorption kinetic model.

Table 8: Comparison of coefficient of determination, R²

Adsorption kinetics	R ² SGF	R ² SIF
Pseudo first order	0.138	0.249
Pseudo second order	0.996	0.999

The data were fitted on both pseudo 1st and pseudo 2nd order model. The pseudo 2nd order has valid from this result. Thus all the results were analyzed by fitting the data on pseudo second order equation.

DISCUSSION

The adsorption studies were performed at two different pH values to simulate the environment in GI tract (1.2 and 6.8). To achieve saturation of AC with study drug, the amount of AC in each of the test was varied so that the ratios of the mass of AC: study drug varied from 25:1 to 1:1. Study drug was added in powder form from capsules in order to simulate in vivo intoxication conditions when the patient takes the capsule available in the market. The use of AC in poison management to adsorb poisons and drugs have been widely studied and applied.

The maximum adsorption capacity of AC for tramadol hydrochloride in SGF and SIF was found to be significant. AC 50 gm can adsorb 4802.6923 mg tramadol hydrochloride (48 capsules) at gastric environment and 8064.516 mg tramadol hydrochloride (80 capsules) at intestine environment.

In a similar adsorption study of tramadol hydrochloride on AC, the drug adsorbed was found to be 98.571mg/gm in simulated gastric fluid and 142.363mg/gm in simulated intestinal fluid [18] A small difference in adsorption capacity of AC for tramadol hydrochloride has been found at the two different pH in the current study. Similar result was found in a study done by Raffa *et al.* [18]

The adsorption of tramadol hydrochloride (Ultram tablets) by AC was determined in vitro and in vivo. The study showed that the amount of tramadol bound to AC, measured as percentage of control was a dose related function of amount of AC. When Ultram tablet (50 mg tramadol Hydrochloride) was studied, it was found that no tramadol was detected in slurries in which 62 tablets were exposed to 50 g of AC. When 100 tablets (each tablet contain 50 mg tramadol) were mixed with 50g AC, only 4.6% unbound tramadol was detected .The amount of tramadol completely adsorbed per gram of AC was 96.4 mg [18]. In the present study, 100mg tramadol hydrochloride capsules were studied. When 48 capsules were adsorbed at simulated gastric fluid and 80 capsules were adsorbed at simulated intestinal fluid at AC standard dose of 50g. The amount of tramadol hydrochloride adsorbed per gram of AC was 96.153846 at simulated gastric fluid and 161.29032 at simulated intestinal fluid.

Earlier investigations have demonstrated that the adsorption of drugs primarily occurs in their un-ionized form [12]. Tramadol hydrochloride is a weak basic or neutral drug. As the unionized fraction of tramadol hydrochloride is a little higher at pH 6.8 than at pH 1.2. In adsorption study [18] the drug adsorption study has been 98.571mg/gm in simulated gastric fluid and 142.363 mg/gm in simulated intestinal fluid. In another similar study performed to determine maximal adsorption of different drugs, the amount of diazepam adsorbed on AC was found to be 136mg/g [9]. The current study and the previous studies can make easily light the fact that the adsorption capacity of AC depend on the characteristic of drug.

Equilibrium isotherms were used to describe the experimental adsorption data. The data were plotted among two most commonly used adsorption isotherm, Langmuir isotherm and Freundlich isotherm and their coefficient of determination (R^2) was compared to get the best adsorption isotherm equation to produce highly accurate result. As accordance of the value of R^2 , Langmuir isotherm was used. To fit the data into the Langmuir adsorption isotherm, the value R^2 should range from 0.87-0.99 indicating that the experimental data fits strongly into the individual regression [19]. In the present study, all the R^2 values satisfied this condition and greater value of R^2 was found in Langmuir than that of Freundlich. The R^2 value in the current study is found to be more in simulated intestinal fluid than in simulated gastric fluid. This was found to 0.985 at simulated gastric fluid and 0.986 in simulated intestinal fluid respectively. This can be explained on the basis of excellent logarithmic relationship was observed between adsorption affinity and solubility of tramadol hydrochloride in simulated intestinal fluid.

In the adsorption kinetics models study were used to describe the experimental data of tramadol hydrochloride. The data were plotted among two most commonly used adsorption kinetics models, pseudo first order and pseudo second order model and their coefficient of determination (R^2) was compared to get the best adsorption kinetics model to produce result with a higher accuracy. In accordance to the value of R^2 , pseudo second order model showed the best result in both SGF and SIF. To fit the data the R^2 value should range from 0.87-1. The pseudo second order kinetics model fit best for this study with good R^2 value both in gastric and intestinal pH this was found to 0.9997 at simulated gastric fluid and 0.9994 at simulated intestinal fluid respectively. This infers that the rate of drug adsorbed at given time is proportional to the square of the difference of the drug adsorbed at certain time and that is adsorbed at equilibrium. Higher the change in adsorption capacity of the AC the rate will increase with square of it.

Administration of adsorbents such as AC with cathartics or agents promoting intestinal motility is made as methods to promote rapid removal in the case of drugs such as tricyclic antidepressants, anticholinergic drugs, antihistamines, and opiates because an overdose of these drugs decreases the bowel motility. In overdose of these drugs it is difficult to discharge drug-charcoal complexes from the GI-lumen since the complexes tend to remain in the lumen for a long time. The consequence of prolonged retention time may lead to desorption of the poisoned drugs from adsorbents [19]. AC should not be administered simultaneously with ipecac, since it binds with ipecac [20].

CONCLUSION

The extrapolation of this in vitro studies show that if AC is given within short time after tramadol hydrochloride intoxication, a standard treatment dose of 50g AC has the capacity to adsorb sufficient amount of drug. AC should be given soon after most significant ingestions because of the low frequency of serious complications and the absence of effective techniques to enhance the elimination of most toxins once absorbed. The kinetics followed during the adsorption was pseudo-second order.

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