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Interaction Characteristics and Thermodynamic Behaviour of Gatifloxacin by Aluminium Hydroxide

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ABSTRACT The interaction pattern of gatifloxacin was temperature-dependent Langmuir isotherm, and the Langmuir coefficients increased as the temperature was raised. The perturbation experiment conducted on this system showed that the nature of interaction was irreversible. The enthalpy change is a positive value, indicating the existence of increased activation energy as the temperature is raised. The entropy value, 24.21 e.u. obtained in this system, indicated that the hydration shells of the ions were rather tightly bound. Intestinal permeation study also revealed the decreased bioavailability of gatifloxacin relatively to the presence of aluminium hydroxide. The strong adsorption of gatifloxacin by aluminium hydroxide is due to formation of complexes with cations of aluminium hydroxide through carboxyl and carbonyl groups of gatifloxacin.

KEYWORDS Gatifloxacin, Interaction, Aluminium hydroxide, Thermodynamic behavior, Intestinal permeation

INTRODUCTION

Gatifloxacin is a broad-spectrum flouroquinolone with a 3-methyl-piperazinyl side chain at position 7 and a methoxy group at position 8 of the quinolone ring. It offers enhanced activity against gram positive cocci and anaerobic coverage compared to other fluoroqinolones (Wakabayashi et al., 1994; Bauernfeind, 1997; Wise et al., 1997). The good antibacterial activity is of clinical interest for the treatment of community-acquired respiratory infections, especially of pneumonia besides others. Hoeffken et al. (1985) first reported the reduced enteral absorption of quinolones in presence of antacids. Yamanaka-Yuen & Gantu (1990) showed a reduction of bioavailability of norfloxacin, if aluminium magnesium hydroxide was given 5 min before or 2 hr after norfloxacin. Lazzaroni et al. (1993) found a substantial decrease of the relative bioavailability after administration of the antacid within 5min before the intake of rufloxacin. In a study involving trovafloxacin (Teng et al., 1997), the AUC values were significantly decreased, when administration of aluminium magnesium hydroxide was 30 min before or 2 hr after trovafloxacin.

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Other authors observed interaction between ciprofloxacin and ofloxacin and the antacid (Lode, 1988; Nix et al., 1989).

The principle mechanism of the interaction between antacid containing polyvalent cations and fluoroquinolones is thought to be chelation of the antibiotics by the ions (Hoeffken et al., 1988; Lode 1988; Nix et al., 1989; Radandh et al., 1992). No comprehensive work on the adsorption/interaction of gatifloxacin on aluminium hydroxide has been reported. The objective of the present study was to evaluate the interaction characteristics and mechanism of interaction of gatifloxacin with aluminium hydroxide. Further, thermodynamic parameters for the sorption process of gatifloxacin by aluminium hydroxide were obtained utilizing van't Hoff equation. To better understand the effect of aluminium hydroxide on the oral bioavailability of gatifloxacin, the study was also aimed to estimate the intestinal permeation using isolated everted goat intestine. Although in vivo tests undoubtedly represent the most reliable techniques to study enteral drug permeability, the increasing demand for a rapid bioavailability estimation and cost connected to in vivo test led us to consider a simplified approach represented by the everted animal intestine (Grassi et al., 1999; Wilson et al., 1954; Balimane et al., 2000; Turner et al., 1970) technique.

MATERIALS AND METHODS

Materials

Aluminium hydroxide was procured from SD Fine-Chem. Ltd., Mumbai, India [MW =78.00; minimum assay 47% (Al_2O_3); pH of solution NMT 10.0; maximum limits of impurities chloride 0.5%, sulfate 0.25%, arsenic 0.0005%]. Gatifloxacin was obtained as a gift sample from Cipla Ltd., Mumbai, India.

Intestinal permeation experiments were performed using as donor and receiver phases a Ringer buffer (pH = 7.4; 37°C) and oxygenated by aeration, able to maintain the homeostasis of the intestine cells of the isolated tissue of goat intestine (collected from slaughter house not later than 1 hr; Schilling et al., 1990).

To measure gatifloxacin permeability through goat intestine in presence of aluminium hydroxide in three different concentrations and one reference (gatifloxacin solution), four different kinds of experimental tests were performed. In the reference case, the donor environment was filled with 1000 mL buffer pH 7.4 solution (37°C) in which 100 mg gatifloxacin has been dissolved.

Uniformity in the donor environment was ensured by means of a stirrer (speed 50 rpm). In the remaining sets a proper amount of aluminium hydroxide (200, 300, 400 mg) was dispersed additionally in the reference medium containing gatifloxacin and the system was left under same agitated condition for 1 hr at 37°C. Regardless the four different sets considered, four intestine sections (6 mm exposed for permeation) were hoisted in each of the donor environment.

Estimation of Adsorption Equilibrium Time of Gatifloxacin by Aluminium Hydroxide

In vitro adsorption studies were performed in distilled water of pH 6.4 (not adjusted) at 30°C. Aluminium hydroxide, 250 mg was placed in a 200 mL stoppered glass bottle containing 100 mL of a specified concentration of 30.905, 61.810, and 154.525 μM gatifloxacin solution with constant agitation. At 0.25, 0.5, 1.0, 4.0, 8.0, and 24.0 hr incubation, 5 mL solution was removed and rapidly filtered (Millipore filter, pore size 0.22 μ) to separate the drug–aluminium hydroxide complex. The drug concentration in the supernatants was measured spectrophotometrically (Systronics UV-Vis Spectrophotometer-108) at λ_{max} = 289 nm to estimate the adsorption equilibrium time. The amount of gatifloxacin adsorbed on the aluminium hydroxide was calculated from the difference in the initial concentrations of the solution examined before and equilibrium concentration after adsorption per unit mass of adsorbent.

Determination of the Extent of Gatifloxacin Adsorption by Aluminium Hydroxide

Aluminium hydroxide, 250 mg was placed to a 200 mL stoppered bottle containing 100 mL of a specified concentration ranging from 30.905 to 186.372 μM gatifloxacin solution. The bottles were agitated in a water bath for 1 hr at a constant temperature. Then the solution was filtered and assayed for the gatifloxacin

contain in order to determine the amount of gatifloxacin adsorbed per 1 g of aluminium hydroxide.

Perturbation Experiments

Glass bottles containing 100 mL of 46.357 μM gatifloxacin solution and 250 mg of aluminium hydroxide were agitated in a water bath for 1 hr at 30°C and then the temperature was shifted from 30 to 60, 50, 37, and 30°C with 1 hr intervals. At the end of each interval 5 mL solution was withdrawn and filtered and the drug contents were assayed for gatifloxacin contents.

Intestinal Permeation Study

A suitable length of small intestine of goat was collected from slaughter house, separated from the mesentery, rinsed with the buffer using a 10 mL disposable syringe, then cut in different sections. Each section was everted on a Teflon rod, and fixed on its location by means of thread. The experimental set up, as illustrated by Meriani et al. (2004) briefly, intestinal holder was cylindrical glass vessel connected to a "U" glass tube whose one portion was represented by intestine. Intestine holders, volume (four in one set), filled by buffer fluid represented the receiver environment (4 × 12 mL) and the holder placed in the donor environment. Both receiver and donor phases were continuously aerated to keep the intestine cells alive during experimentation. At regular interval of time after beginning of the permeation test 4 mL of the receiver phase were sampled from each intestine holder and replaced with pure buffer, for a total time of 120 min. Gatifloxacin concentration in each of four liquid phases sampled was estimated by UV spectrophotometer (Systronics UV-Vis Spectrophotometer-108).

RESULTS AND DISCUSSION

Adsorption Equilibrium

The results of the experiments to see the amount of gatifloxacin adsorbed on aluminium hydroxide from aqueous solution at various time indicated that the maximum adsorption took place up to 1 hr (Fig. 1). Based on this result, the following adsorption isotherm experiments to calculate apparent thermodynamic functions were conducted for 1 hr.

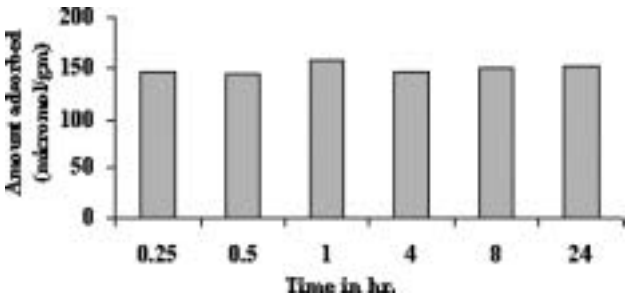

FIGURE 1 **Adsorption of Gatifloxacin on Aluminium Hydroxide With Time From Aqueous Solution. Maximum Adsorption Took Place Less Than 1 hr.**

Adsorption Isotherm

Adsorption isotherms of gatifloxacin on aluminium hydroxide from aqueous solution in the temperature range of 30 to 60°C are presented in Fig. 2. All the adsorption isotherms exhibit the plateau, which is characteristic of complete monolayer adsorption. Shi et al. (2001) also observed plateau adsorption isotherms when they studied the detoxification of endotoxin by aluminium hydroxide adjuvant. However there were differences in the concentration of gatifloxacin in solution in monolayer adsorption region as the temperature varied from 30–60°C. The difference indicates that the aluminium hydroxide had a relative higher affinity for gatifloxacin at lower temperature than the higher when it varied 30–60°C.

Interaction Characteristic and Thermodynamic Behavior

When the interaction process is ionic and chemical in nature, the interaction of the system should be

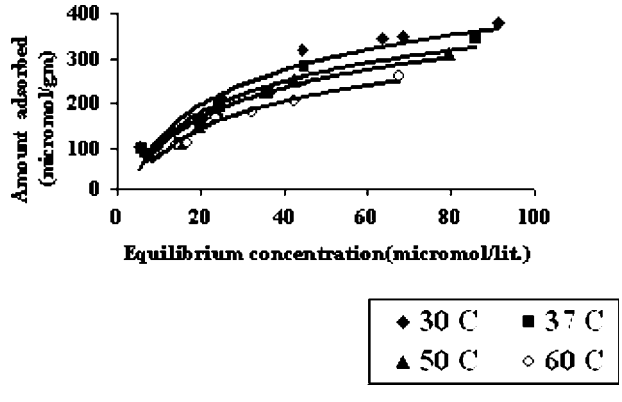

FIGURE 2 **Adsorption Isotherm of Gatifloxacin on Aluminium Hydroxide From Aqueous Solution at Various Temperature.**

irreversible (Ohkura et al., 1980). The perturbation experiment of the present system was conducted by the method described by Ohkura et al. (1980). When the temperature of the gatifloxacin solutions with aluminium hydroxide was shifted from 30 to 60, 50, 37, and 30°C, the amount of gatifloxacin adsorbed to aluminium hydroxide was observed to change (Fig. 3). Although a slight decrease in the amount adsorbed was observed between 30 and 60°C initially and finally again a slight increase when shifted from 37 to 30°C. Therefore it was proposed that the adsorption of gatifloxacin to aluminium hydroxide was irreversible and the type of gatifloxacin adsorption by aluminium hydroxide is chemisorptions (Ohkura et al., 1980). They were temperature dependent Langmuir isotherm in nature which is represented as:

$$\frac{C}{x/m} = \frac{1}{ab} + \frac{C}{a} \qquad (1)$$

x/m is the amount of gatifloxacin adsorbed per gram of aluminium hydroxide; *C*, equlibrium concentration; *a* Langmuir constant for the amount adsorbed at saturation or the adsorption capacity and *b*, Langmuir equilibrium constant related to the enthalpy of adsorption. A plot of

$\frac{C}{x/m}$ Versus *C* should give a straight line with a slope of $\frac{1}{a}$

and intercept of *1/ab* (Fig. 4). When the data from the adsorption isotherm were plotted by the linearized form of the Langmuir equation, good-fit straight line resulted, as seen by the high R^2 values (Table 1). The adsorption capacity and adsorption coefficients (Langmuir constants) were calculated from the slope and the intercept of the trend lines at 30, 37, 50, and 60° C. In Fig. 4 difference in values at different temperatures was observed. Langmuir coefficients were increased as the temperature was raised. Since adsorption is often an irreversible process, affinity constants derived from the Langmuir equation should only be considered apparent values. For the same reason, any thermodynamic parameters derived from the apparent affinity constant should also be considered as apparent value.

Plaut et al. (1981) reported that the standard enthalpy associated with the sorption process of chlorhexidine digluconate from aqueous solution by Poly (2-hydroxyethyl methacrylate) powder was zero over the temperature range 20 ~ 50°C. They concluded that the sorption process of this system was associated with ion exchange interaction whose standard enthalpies are generally zero or very small (Heard & Ashworth, 1968; Plaut et al., 1980). To make this type of thermodynamic assumption for the sorption process of gatifloxacin by aluminium hydroxide in the present system, the following equations were applied.

$$\ln b = -(\Delta H/R)1/T + \Delta S/R \qquad (2)$$

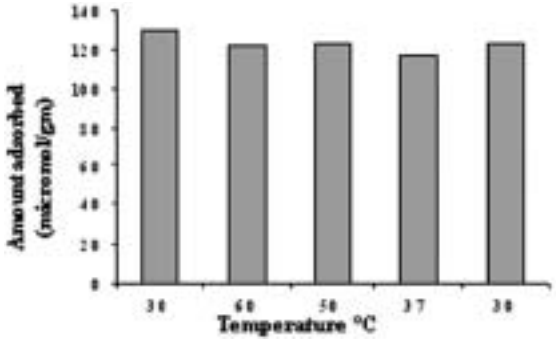

FIGURE 3 Perturbation Experiments on the Adsorption of Gatifloxacin by Aluminium Hydroxide From Aqueous Solution (Initial Drug Concentration 57.15 μM). The Temperature was Shifted From 30°C to 60, 50, 37, and 30°C at 1 hr Interval.

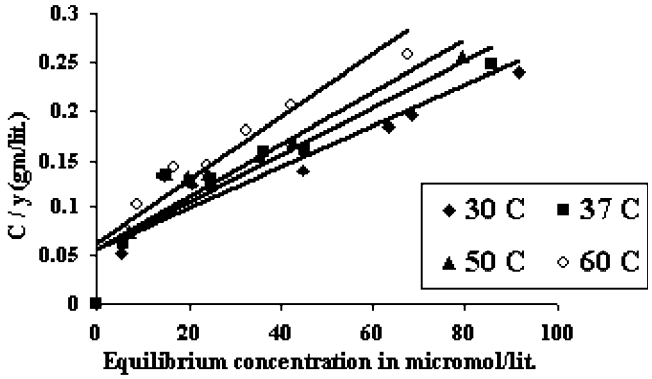

FIGURE 4 Langmuir Plot of Adsorption of Gatifloxacin by Aluminium Hydroxide From Aqueous Solution at Various Temperatures (30, 37, 50, 60°C).

TABLE 1 Apparent Langmuir Constants of Adsorption of Aluminum Hydroxide From Aqueous Solution Containing Gatifloxacin

Temperature (°C)	Slope	Intercept	Adsorption capacity *a* (μmol/g)	Adsorption coefficient *b* (Liter/μmol)	R^2
30	0.0018	0.0763	555.55	0.0236	0.8837
37	0.002	0.0789	500.00	0.0253	0.9045
50	0.0022	0.0804	454.54	0.0274	0.9431
60	0.0026	0.0909	384.61	0.0286	0.9734

TABLE 2 Apparent Thermodynamic Functions of Adsorption of Aluminium Hydroxide From Aqueous Solution Containing Gatifloxacin From van't Hoff Plot

Temperature (°C)	Enthalpy ΔH (kcal/mole)	Entropy, ΔS (*ε.υ.*)	*Φρεε ενεργψ* ΔF (kcal/mole)	R^2
30	1.267	24.210	–6.068	0.9825
37	1.267	24.210	–6.238	
50	1.267	24.210	–6.553	
60	1.267	24.210	–6.795	

In which $\Delta S/R$ is intercept on the ln*b* axis and $-(\Delta H/R)$ is slope of a plot of ln*b* versus *1/T*

$$\Delta F = \Delta H - T.\Delta S \qquad (3)$$

ΔF is free energy exchange; ΔH, enthalpy change; *T*, absolute temperature; and ΔS, entropy change. Plot of ln*b* vs. *1/T* resulted an excellent straight line (R^2 = 0.9825), which reflected a good correlation between Langmuir equilibrium constant and temperature. Table 2 shows the results of apparent thermodynamic functions calculated from Langmuir constant. Inczedy has reported that the standard enthalpies of ion exchange interaction are generally within the range –0.024 to 2.4 kcal/mole (Inczedy, 1966). The enthalpy (ΔH) in the present study obtained by van't Hoff plot (Fig. 5), was 1.267 kcal/mole which fell within the range reported by Inczedy (1966) and was indicative of ion-ion interaction. ΔH is a positive value that indicates that the heat is absorbed by the system (endothermic) and correspondingly activation energy should increase as the temperature is elevated. The increasing value of *b* shows this to be a fact. Plaut et al. (1980) also calculated the entropy by using poly (2-hydroxyethyl methacrylate) obtained the value of 5.02 e.u. and referred it as the driving force behind ionic interactions associated with the disruption of the hydration shells of the ions. Akaho and Fukumori (2001) obtained the value

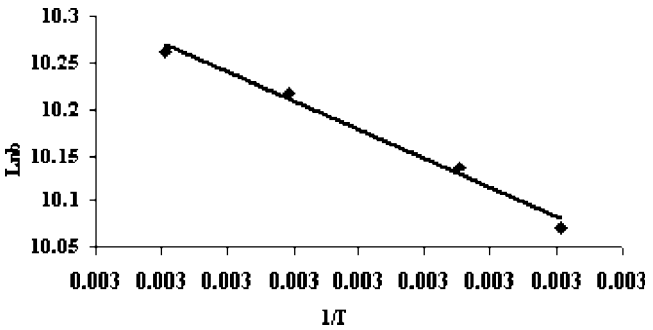

FIGURE 5 van't Hoff Plot of Apparent Thermodynamic Functions of Gatifloxacin Adsorbed on Aluminium Hydroxide From Aqueous Solution.

of 27.4 to 28.2 and suggested that hydration shell of the ions in the carbon black-chlorhexidine system was more tightly formed. The small enthalpy value and the positive entropy value of 24.21 e.u. in the present system may suggest that the interaction of gatifloxacin with aluminium hydroxide was ionic interaction in nature, and its sorption process was similar to that reported by Akaho & Fukumori (2001); Plaut et al. (1981); Ohkura et al. (1980); Fitzgerald & Russel (1992); Daltrey & Hugo, (1974). The aluminium hydroxide has an isoelectric point of 11.4 (Rinella et al., 1998; Shirodkar et al., 1990). As such aluminium hydroxide in distilled water will have an alkaline pH not more than 10 and the surface of the aluminium hydroxide will be positively charged. Thus, gatifloxacin

being a weak acid is electrostatically attracted to aluminium hydroxide.

Adsorption of chemical on the surface of other chemicals and particles continues to be a topic of considerable interest and application (Eckert et al., 1994; Klein et al., 1994; Rojo et al., 1994). Investigation of its adsorption from the molecular standpoint will help clarify not only chemical interaction with other substances but also mode of action of a compound in question. The metal cation of aluminium hydroxide can interact to form complex through carboxyl and carbonyl groups of gatifloxacin (Lober et al., 1999). The possible assumption is also suggested by some literatures that the interaction between antacids containing polyvalent cations and fluoroquinolones is due to chelation of the antibiotic by the ions (Hoeffken et al., 1988; Lode 1988; Nix et al., 1989; Radandt et al., 1992). Aluminium, in particular, forms a very stable complex with quinolones, which are not easily soluble (Timmers & Sternglanz 1978). This type of interaction leads to the adsorption phenomenon in which a larger area is occupied by gatifloxacin on the surface of aluminium hydroxide. Fig. 6 shows the variation of gatifloxacin concentration in the receiver environment relatively to the presence of aluminium hydroxide in the donor. Gatifloxacin in the presence of aluminium hydroxide presented decreased bioavailability of the drug compared to the drug alone. Curve-A refers to the drug in aluminium hydroxide 1:2 system in donor, Curve-B to 1:3, and Curve-C to 1:4 systems in donor. The data relative to reference (gatifloxacin solution alone) are represented as Curve-D. Drug concentration in the receiver environment decreased with the increased concentration of aluminium hydroxide in the donor. Drug-aluminium hydroxide in 1:4 system showed minimum bioavailability compared to 1:2 and 1:3 systems.

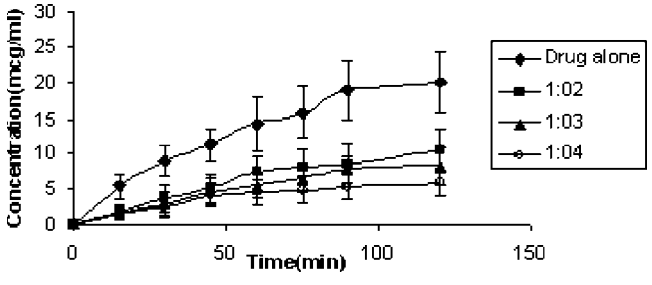

FIGURE 6 **Effect of Aluminium Hydroxide on Permeation of Gatifloxacin (Concentration in Everted Intestinal Lumen Versus Time) From Different Dispersion Systems. Vertical Bars Indicate Standard Error (*n* = 4).**

CONCLUSION

Gatifloxacin showed a Langmuir adsorption isotherm on the surface of aluminium hydroxide and the equilibrium state was achieved within 1 hr. The adsorption of gatifloxacin on aluminium hydroxide from aqueous solution showed temperature dependent Langmuir isotherms. Since the Langmuir equilibrium constant increased as the temperature was raised from 30 to 60°C, it indicates that the mechanism of adsorption was chemisorption in nature.

Since the entropy value in the system was rather large, in the order of 24.21 entropy units, the hydration shells in the system should be tightly bound. The result of perturbation experiment, in which the amount adsorbed not appreciably changed regardless of temperature changes from 30 to 60, 50, 37, and 30°C, demonstrated that the interaction of the system was irreversible. In conclusion, the extensive and strong adsorption of gatifloxacin by aluminium hydroxide is due to irreversible binding or formation of complexes with metal cations of aluminium hydroxide through carboxyl and carbonyl groups of gatifloxacin. This is an important factor affecting the intestinal absorption and bioavailability of gatifloxacin upon concomitant use of aluminium hydroxide with gatifloxacin. Intestinal permeation experiments through everted goat intestine proved that the bioavailability is significantly affected in presence of aluminium hydroxide.

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