

Supramolecular Dynamics of Thalidomide and its Derivatives in Water-Sediment System

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ABSTRACT The contamination of drug residues, including chiral ones, is not acceptable in earth's ecosystem. The dynamicity of enantiomers of thalidomide and its derivatives (3-methyl thalidomide, 3-ethyl thalidomide, and 3-butyl thalidomide) was ascertained at supramolecular level in water-sediment system using solid phase extraction (SPE) and stereoselective HPLC. Enantiomeric separation of these drugs was carried out on Ceramosphere RU-2 (25 cm × 0.46 cm, particle size 50 μm) chiral column using pure ethanol (1.0 ml/min) as eluent at 230 nm detection. Retention times, capacity, separation, and resolution factors of the enantiomers of these drugs were in the range of 20.0–36.0, 2.08–3.93, 1.35–1.57, and 1.0–2.0 min, respectively. Percentage recoveries of the enantiomers in SPE were in the range of 90.0 to 95.0 in water-sediment system. Langmuir and Freundlich model were best fitted for dynamic equilibrium concentrations at different experimental parameters. Thalidomide and its derivatives follow first-order kinetics at dynamic equilibrium. The rate constants of chiral interconversions were 0.390 and 0.385 days⁻¹ for *S*- and *R*-enantiomers, respectively. The uptake of thalidomide by sediment is quite good and of endothermic nature indicating good self-purification capacity of the nature for such toxic species. *Chirality* 00:000–000, 2009. © 2009 Wiley-Liss, Inc.

KEY WORDS: chiral separations; supramolecular chiral dynamics; thalidomide; water-sediment system

INTRODUCTION

Thalidomide [α -(*N*-phthalimido)-glutarimide] is a sedative, hypnotic, and anti-inflammatory medication, which was sold from 1957 to 1961 in almost all European countries under different trade names. But it was banned in 1961 after a calamity called Thalidomide tragedy¹; because of its teratogenic nature of malformations, including phocomelia, in about 12,000 newly born babies of women in 46 different European countries, who had taken thalidomide during their pregnancies.² Later researchers concluded that this tragedy was due to *S*(-)-enantiomer of thalidomide.³ Basically, thalidomide [α -(*N*-phthalimido)-glutarimide] (Fig. 1A) consists of a two ringed structure with an asymmetric carbon in the glutarimide ring, which exists as an equal mixture of *S*(-) and *R*(+) enantiomers. Recently, thalidomide is used for the treatment of various diseases such as rheumatoid arthritis, graft-versus-host, Behcet's syndrome, cutaneous lupus, cancer, and refractory aphthous ulcerations in patients with AIDS in various countries.^{4–9} In 1998, thalidomide has been approved specifically for treating erythema nodosum leprosum (ENL) in leprosy (Hansen's Disease) in USA¹⁰ and on investigational basis for the treatment of above-cited disease in some North American countries.^{11–14} It has

been observed that thalidomide showed teratogenic effect even after the administration of safe *R*(+)-enantiomer because of its rapid conversion into toxic *S*(-)-form. Some studies have been carried out in vitro on interconversion and stereoselectivity of thalidomide. Besides, in vitro pharmacokinetics of thalidomide have also been established in human beings.^{15–22}

In natural conditions, thalidomide is degraded to more than 20 byproducts because of rapid pH-dependent hydrolysis in aqueous solution. The plasma concentration of thalidomide is 10–15 h depending on the age, race, and sex of the patients. After this period, thalidomide and its metabolites are excreted to the environment through urine and feces. Therefore, there are good chances of contamination of our natural water resources due to teratogenic thalidomide and its derivatives, which may enter into the

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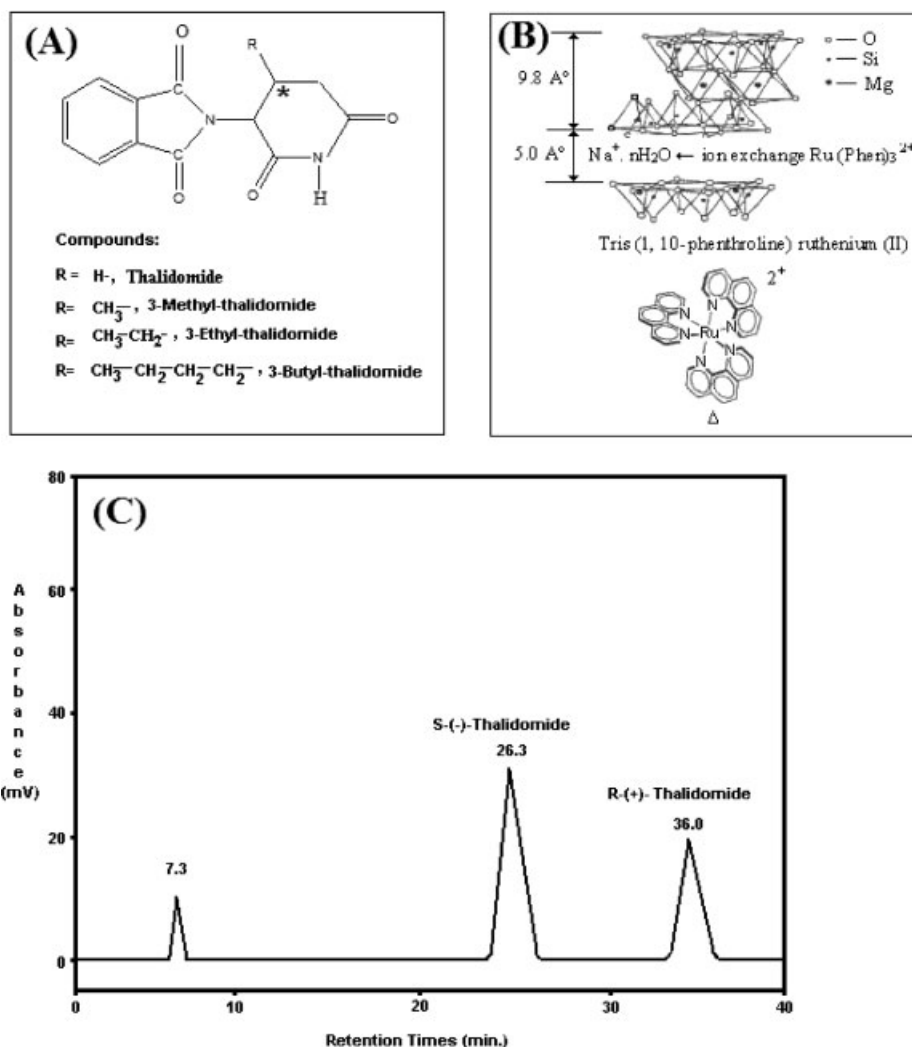


Fig. 1. Structures of (A) thalidomide and its derivatives, (B) Ceramosphere RU-2 chiral stationary phase based on a sodium magnesium silicate, and (C) chiral separation of racemic thalidomide.

human body resulting in serious side effects. In view of this, the study of the fate and dynamics of chiral thalidomide and its derivatives in water and the sediment of rivers, the most probable targets of contamination, are important and essential. The article investigates the dynamics of chiral thalidomide and its derivatives in water and sediment samples at supramolecular level and the results are presented in this article.

EXPERIMENTAL

Chemicals and Reagents

The racemic mixtures and optically active *R*-(+)- and *S*-(-)-forms of thalidomide and its derivatives (3-methyl thalidomide, 3-ethyl thalidomide, and 3-butyl thalidomide) were obtained from Ciba-Geigy (Basle, Switzerland). The solutions (0.1 mg/ml) were prepared in methanol. Methanol, ethanol, ethyl acetate, acetone, diethylether, chloroform, hexane, and dichloromethane of HPLC grade were purchased from Fisher Scientific (Fairlawn, New Jersey). Tri-

fluoroacetic acid, triethylamine, sodiumdihydrogen phosphate, and phosphoric acids were of AR grade and were purchased from Sigma Chemical, USA. Purified water was prepared using a Millipore Milli-Q (Bedford, MA) water purification system. pH meter of Hach, USA, was used to measure pH of the solutions. C₁₈ Sep-Pak Vac (1 cc) cartridge was obtained from Waters, USA. The vacuum was generated by the homemade vacuum pump assembly. Zymark Turbo Vap LV evaporator was obtained from Hopkinton, USA. The chiral column Ceramosphere RU-2 (25 cm × 0.46 cm, particle size 50 μm) was obtained from Shiseido (Tokyo, Japan). The determination of thalidomide and its derivatives was carried out by stereoselective HPLC.

Chromatographic Conditions

An aliquot of 10 μl of each solution was injected on to a HPLC system consisting of Waters solvent delivery pump (model 510), Waters injector (model WISP 710B), Waters tunable absorbance detector (model 484), and Waters integrator (model 740). The order of elution of the enantio-

mers was confirmed by using optically active *R*(+)-form of each compounds. The column used was Ceramosphere RU-2 (25 cm × 0.46 cm, particle size 50 μm) [Sodium magnesium silicate with optically active (1,10-phenanthroline) ruthenium II complex, Δ configuration, Fig. 1B]. The mobile phase was ethanol, which was filtered and degassed before use. The flow rates of the mobile phase were 1.0 ml/min with detection at 230 nm. The chart speed was kept constant at 0.1 cm/min. All the experiments were carried out at 23°C ± 1°C. The chromatographic parameters such as capacity factor (*k*), separation factor (α), and resolution factor (Rs) were calculated.

Solid Phase Extraction

Solid phase extraction (SPE) methodology was developed by spiking 10 ml of racemic and optically pure thalidomides and its derivatives (0.1 mg/l) separately to 90 ml riverine waters; already filtered through Whatman filter papers No. 24. The spiked water samples were shaken for about 5 min and pH was adjusted to 7.0 with concentrated sodium hydroxide. The spiked water samples were kept at room temperature overnight. C₁₈ cartridges were preconditioned using methanol (1.0 ml) followed by water (1.0 ml). After equilibration, 100 ml of the spiked water samples were passed through these cartridges at 0.5 ml/min flow rate. Cartridges were washed with 2.0 ml of deionized water and then dried under vacuum for 5.0 min. Racemic and optically pure thalidomides and its derivatives were eluted from cartridges by using 5.0 ml acetonitrile at 0.5 ml/min flow rate. Acetonitrile extracts were dried under air at 40°C in a Zymark Turbo Vap LV evaporator and redissolved in 100 nl acetonitrile separately and respectively. Racemic and optically pure thalidomides and its derivatives (0.1 mg/l) were also mixed with 90 ml filtered riverine waters; containing 1.0 g of riverine sediment separately and respectively; shaken 5 min and kept overnight for 24 h. The sediments were removed by centrifugation and thalidomides and its derivatives were extracted using acetonitrile (50 ml) by sonicator. The extracted thalidomides and its derivatives were dried under air at 40°C in a Zymark Turbo Vap LV evaporator and redissolved in 100 nl acetonitriles separately and respectively. These acetonitrile solutions obtained from water and sediment samples were used to analyze chiral thalidomides and its derivatives using above-cited stereoselective HPLC conditions.

Dynamics and Kinetics

To determine the dynamics of thalidomide and its derivatives, chiral adsorption studied were also carried out using riverine water and sediment. The effect of various parameters such as concentration of racemates, contact time, pH, temperature, and particle size of riverine sediment was studied. The dynamics of these racemates between sediment and water was carried out using 0.01–0.1 mg/ml concentrations, 5–120 min contact times, 2–10, 20–40°C temperatures, 1–10 g/l doses, and 0–75, 75–150, 150–210, 210–250, 250–300, 300–425 μm particle size. The experimental protocol was adopted and used as described elsewhere.²³ The dynamics and kinetics of chiral thalidomide and its derivatives in natural conditions were also

TABLE 1. Retention times, capacity, separation, and resolution factors for chiral resolution of thalidomide and its derivatives in standard solutions

Chiral compounds	t_{r1}	t_{r2}	k_1	k_2	α	Rs
	<i>S</i> (–)	<i>R</i> (+)	<i>S</i> (–)	<i>R</i> (+)		
Thalidomide	26.3	36.0	2.60	3.93	1.51	1.95
3-Methyl thalidomide	25.2	35.4	2.45	3.85	1.57	2.00
3-Ethyl thalidomide	22.8	28.3	2.26	3.05	1.35	1.00
3-Butyl thalidomide	20.0	25.1	2.08	2.86	1.38	1.00

Experimental conditions: column, Ceramosphere RU-2; mobile phase, pure methanol; flow rate, 1.0 ml/min; detection, 230 nm; concentration, 0.1 mg/ml; injected amount, 5.0 μl.

evaluated by using well-known models and equations.¹⁷ The concentrations of enantiomers of thalidomide and its derivatives in water and sediment samples were analyzed using above-cited SPE and HPLC conditions.

RESULTS AND DISCUSSION

Chiral Separations

Chromatographic parameters such as capacity (*k*), separation (α), and resolution (Rs) factors for the resolved enantiomers of thalidomide and its derivatives in standard solutions and riverine water and on sediment samples are given in Tables 1 and 2, respectively. A typical chromatogram of chiral separation of thalidomide under reported conditions is shown in Figure 1C. A perusal of Tables 1 and 2 clearly indicates that enantiomers of thalidomide and its derivatives were resolved successfully. The slight lower values of chromatographic parameters in riverine water and sediment may be due to interference of some other species present in the sediment. Retention times, capacity, separation, and resolution factors of the enantiomers of these drugs were in the range of 20.0–36.0, 2.08–3.93, 1.35–1.57, and 1.0–2.0 min, respectively. The order of the elution was confirmed by using optically active pure *R*(+)-forms of each compound. It has been observed that *S*(–)-enantiomer eluted first followed by *R*(+)-enantiomer of all the studied racemates. A variation in the chromatographic parameters was carried out to obtain the best resolution. To optimize the chromatographic conditions, ethanol, acetonitrile, sodium perchlorate, and several buffers were tested but no good resolution could be achieved. Ethanol containing 0.1% trifluoroacetic acid and triethylamine was also tested as the mobile phases but no good resolution could be achieved. As a result of extensive experimentation, the optimized chromatographic conditions were developed and reported herein.

Chiral Separation at Supramolecular Level

The supramolecular structure of the used CSP is shown in Figure 1B and it contains a spherical clay (sodium magnesium silicate) as the basic packing material with a ruthenium complex [(1,10-phenanthroline) ruthenium II complex, Δ configuration] as chiral selector. It has alternative sheets of octahedron of magnesium and tetrahedron of silica sandwiching chiral selector resulting in chiral cav-

TABLE 2. Retention times, capacity, separation, and resolution factors for chiral resolution of thalidomide and its derivatives in river water and sediment

Chiral compounds	t_{r1} S(-)	t_{r2} R(+)	k_1 S(-)	k_2 R(+)	α	Rs
Thalidomide	26.2 (26.1)	35.7 (35.6)	2.58 (2.56)	3.90 (3.88)	1.51 (1.50)	1.92 (1.90)
3-Methyl thalidomide	25.1 (25.0)	35.2 (35.1)	2.42 (2.40)	3.81 (3.80)	1.57 (1.55)	2.00 (2.00)
3-Ethyl thalidomide	22.6 (22.0)	28.1 (28.0)	2.24 (2.22)	3.01 (3.00)	1.35 (1.33)	1.00 (1.00)
3-Butyl thalidomide	19.8 (19.5)	25.0 (25.0)	2.06 (2.02)	2.82 (2.80)	1.37 (1.35)	1.00 (1.00)

The values of chromatographic parameters inside and outside parenthesis correspond to water and sediment, respectively. Experimental conditions: as in Table 1.

ities. Therefore, the tetrahedral and octahedral cavities having the ruthenium complex serve as chiral baskets and racemates can be separated once they enter these cavities. The exact chiral recognition mechanism on this phase is not known but it can be rationalized that the enantiomers form transient diastereomeric complexes with ruthenium complex, which are stabilized mainly by π - π interactions with π electron of phenanthroline ring system. Enantiomeric separation of these racemates is due to their penetration into the tetrahedron and octahedral cavities of silica and magnesium clay where they form transient diastereomeric complexes with chiral ruthenium complex which are stabilized by π - π interactions between aromatic rings and 1,10-phenanthroline. Besides, other interactive forces such as steric, van der Waals forces may contribute toward the chiral resolution on the reported CSP. Briefly, aromatic ring of each enantiomers fits stereogenically in different fashion into chiral cavities of stationary phase which is stabilized by the π - π interactions of different magnitude for both R(+) and S(-)-enantiomers and, hence, the resolution of enantiomers occurred. However, stronger hydrogen bondings are formed between hydrogen atom, attached to N-H amide group and oxygen atoms of clay. It is important to mention that the nature of hydrogen bonding is achiral, which binds the enantiomers strongly to the clay and, hence, enantiomers are eluted at high retention times. However, achiral hydrogen bondings provide sufficient time to the enantiomers to rest onto ruthenium complex, which may be helpful in chiral resolution.

Solid Phase Extraction

The SPE of thalidomides and its derivatives was optimized by adjusting different pHs of riverine water and flow rates. The flow rates of the eluting solvent (acetonitrile) were also varied. Besides, other eluting solvents such as ethanol, ethyl acetate, acetone, diethylether, chloroform, hexane, dichloromethane were also tried. As a result of exhaustive experimentation, the optimized SPE conditions were developed and used as mentioned in the experimental section. The percentage recoveries of thalidomide and its derivatives were good and ranged from 95.0 to 90.0% during dynamic equilibrium, kinetic and chiral interconversions experiments. It has also been observed that percentage recoveries of racemic and optically active thalidomides were slightly higher than those of its derivatives, which may be due to poor binding of later with C₁₈ material. The poor adsorption of thalidomide derivatives on reversed phase material of cartridges may be explained at

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supramolecular level by considering steric effect of bulky alkyl groups present in thalidomide derivatives.

Dynamics and Kinetics

To understand the fate of thalidomide and its derivatives in nature, the dynamic equilibrium studies were carried out in riverine water-sediment system. The study involves adsorption on river bed sediment, dynamic equilibrium between riverine water and sediment, kinetics and chiral interconversions. The results of these experiments are discussed in the following sections.

Equilibrium Dynamics

The equilibrium and adsorption of thalidomide and its derivatives were carried out at different contact time, concentrations, doses, pHs, particle sizes, and temperatures. The effects of these parameters for thalidomide enantiomers only are shown in Figures 2A to 3A. These figures indicate that a dynamic equilibrium existed at 40 mg/l concentration with 8 h contact time. The equilibrium pH was 7.0 with 8 g/l sediment dose. The effect of temperature on dynamic equilibrium was also studied and it was observed that the larger amount of thalidomide was on sediment at 40°C indicating endothermic nature of adsorption. Among various particle sizes studied, the maximum uptake was on 0–75 μ m size but its percentage is poor in riverine bed sediment and, hence, 210–250 μ m particle size was used to carry out all studies. Basically, dynamic equilibrium was studied for both R- and S-enantiomers of thalidomide and it was observed that S-enantiomers adsorbed slightly higher on sediment in comparison to R-antipode.

To find out the mechanistic parameters associated with thalidomide and its derivatives, the results obtained from the above plots were analyzed by the well-known models of Langmuir and Freundlich involving the following equations.

$$\frac{1}{q_e} = \frac{1}{Q_0} + \frac{1}{bQ_0C_e} \quad (1)$$

where, q_e is the amount adsorbed (mg/g), C_e is the equilibrium concentration of the adsorbate (mg/L), and Q_0 and b are the Langmuir constants related to maximum adsorption capacity and energy change in adsorption, respectively (Fig. 3B). Similarly, eq. 2 was used for Freundlich model.

$$\log q_e = \log K_F + \frac{1}{n} \log C_e \quad (2)$$

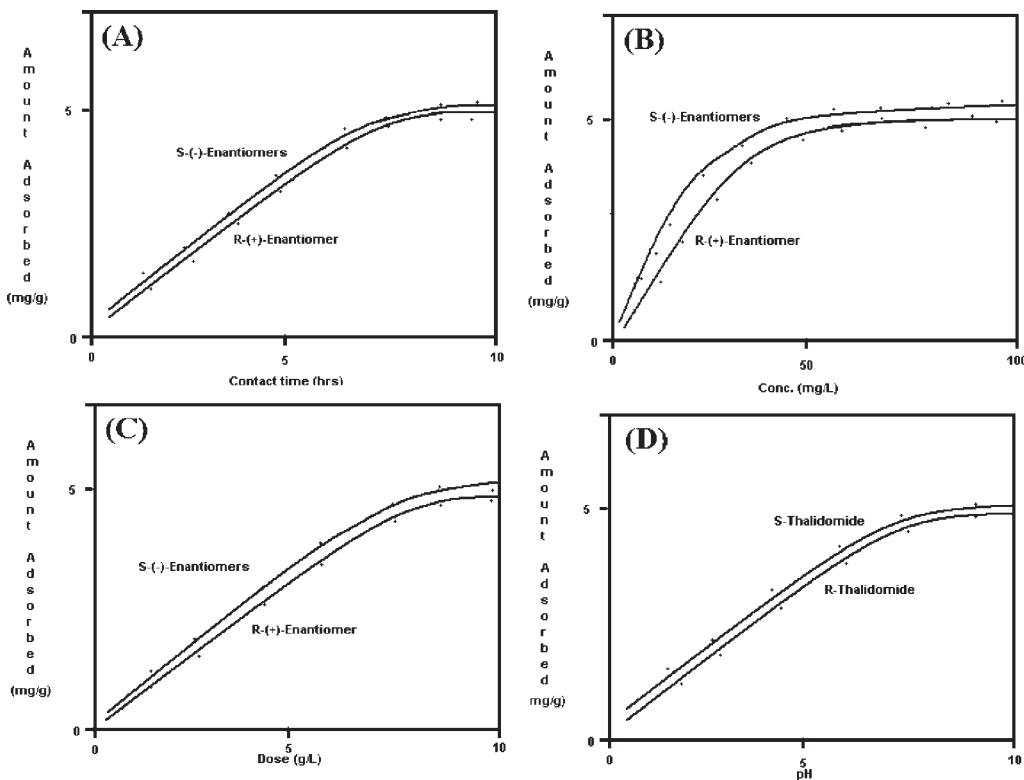


Fig. 2. Effect of (A) contact time, (B) concentrations, (C) dose, and (D) pH on uptake of S- and R-thalidomide.

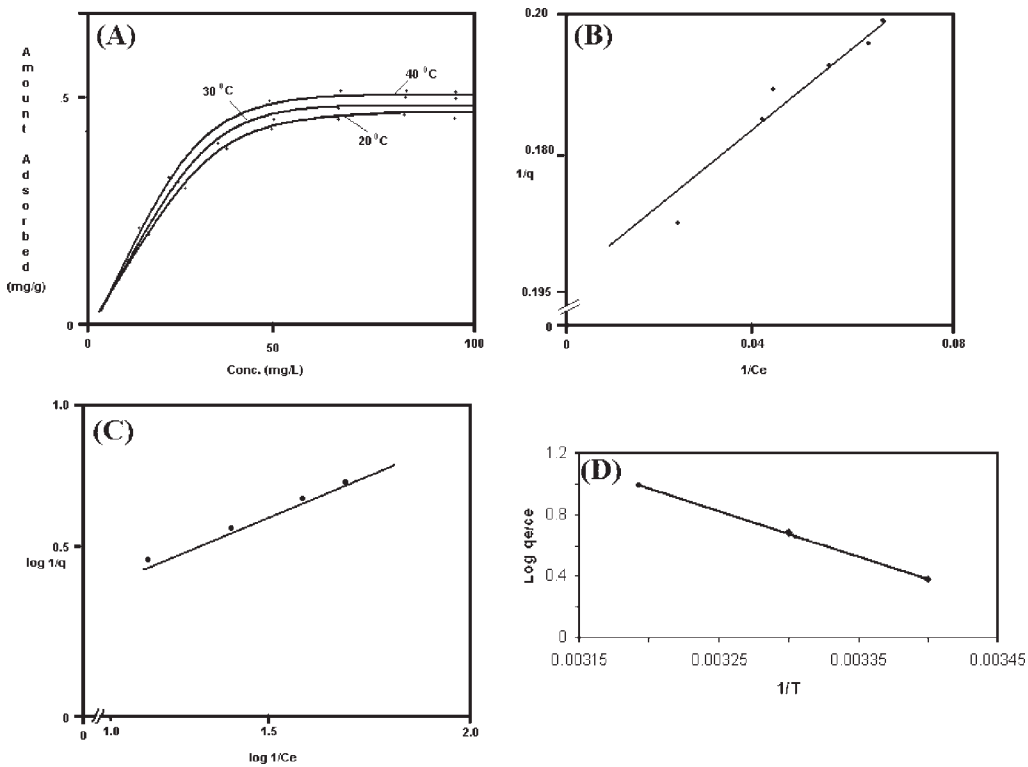


Fig. 3. Effect of (A) temperature on uptake of S-thalidomide, (B and C) Langmuir and Freundlich isotherms for S-thalidomide, and (D) plots of $\log(q_e/C_e)$ for adsorption of S-thalidomide on reverine sediment against $1/T$ for different concentrations.

TABLE 3. Langmuir constants of *S*-thalidomide and its derivatives

Compounds	Q_0 (mg/g)			b (l/mg)		
	20°C	30°C	40°C	20°C	30°C	40°C
Thalidomide	5.11	5.21	5.26	2.1×10^{-3}	1.95×10^{-3}	1.90×10^{-3}
3-Methyl thalidomide	5.00	5.11	5.15	2.0×10^{-3}	1.91×10^{-3}	1.88×10^{-3}
3-Ethyl thalidomide	4.91	4.96	5.00	1.92×10^{-3}	1.85×10^{-3}	1.80×10^{-3}
3-Butyl thalidomide	4.89	4.94	4.99	1.90×10^{-3}	1.82×10^{-3}	1.77×10^{-3}

where, q_e is the amount adsorbed (mg/g), C_e is the equilibrium concentration of the adsorbate (mg/L) and K_F and n are Freundlich constants related to the adsorption capacity and adsorption intensity, respectively (Fig. 3C). Langmuir and Freundlich constants are given in Tables 3 and 4, respectively. An evaluation of Figures 3B and 3C and Tables 3 and 4 indicates the applicability and validity of the experiments conducted.

Equilibrium Kinetics

The kinetics of thalidomide and its derivatives in nature (riverine water-sediment system) was also studied by calculating free energy, enthalpy, and entropy. The changes in enthalpy (ΔH) and entropy (ΔS) were determined from the slope ($-\Delta H/2.303R$) and intercept ($-\Delta S/2.303R$) of the vant-Hoff's plot [$\ln(q_e/C_e)$ vs. $1/T$], respectively (Fig. 3D only for *S*-Thalidomide). The values of ΔH and ΔS for thalidomide derivatives were also calculated in the same way (figures are not given). The free energy change (ΔG) was calculated from the following equation:

$$\Delta G = \Delta H - T\Delta S \quad (3)$$

$$\log(q_e/C_e) = -\Delta H/2.303RT + \Delta S/2.303R \quad (4)$$

where (q_e/C_e) is called the adsorption affinity and it is the ratio of q_e , the amount adsorbed per unit mass at equilibrium to C_e , the equilibrium concentration of the adsorbate. The thermodynamic parameters obtained for the adsorption of thalidomide and its derivatives under investigation are given in Table 5. Positive values of free energy indicate that the adsorption of thalidomide and its derivative is not spontaneous but rather slow.

The adsorption of thalidomide and its derivatives can be considered as a reversible reaction with equilibrium established between two phases. The Lagergren first-order rate eq. 6 can be applied for the determination of the adsorption rate constant.

$$\log(q_e - q_t) = \log q_e - \frac{K_{ad}}{2.303}t \quad (5)$$

TABLE 4. Freundlich constants of *S*-thalidomide and its derivatives

Compounds	n			K_F (mg/g)		
	20°C	30°C	40°C	20°C	30°C	40°C
Thalidomide	6.25	6.30	6.34	4.47	4.50	4.55
3-Methyl thalidomide	6.20	6.25	6.30	4.45	4.48	4.52
3-Ethyl thalidomide	6.16	6.20	6.26	4.40	4.45	4.50
3-Butyl thalidomide	6.12	6.15	6.20	4.36	4.40	4.46

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where q_e and q_t are concentrations of thalidomide and its derivatives at equilibrium and time t , and K_{ad} is the rate constant. Experimental data, measured at the optimal pHs for these molecules are plotted (figures are not given) and plots give straight lines with a slope $-K_{ad}/2.303$, confirming the applicability of the first-order Lagergren rate expression. Kinetic data were also analyzed by the procedure given by Reichenberg¹⁸ using eqs. 7–10.

$$F = 1 - \frac{6}{\pi^2} \sum_{n=0}^{n=\infty} \frac{1}{n^2} \exp \left[\frac{-D_i t \pi^2 n^2}{r_0^2} \right] \quad (6)$$

$$\text{Or } F = 1 - \frac{6}{\pi^2} \sum_{n=0}^{n=\infty} \frac{1}{n^2} \exp[-n^2 Bt] \quad (7)$$

$$F = \frac{Q_t}{Q^0} \quad (8)$$

$$B = \frac{\pi^2 D_i}{r_0^2} \quad (9)$$

where F is the fractional attainment of equilibrium at time t , Q_t is the amount of adsorbent taken up at time t and Q^0 is the maximum equilibrium uptake (at infinite time), D_i is the effective diffusion coefficient of these molecules in the adsorbent phase, B is the time constant, r_0 is the radius of the adsorbent particle (sediment), assumed to be spherical, and n is an integer that defines the infinite series solution.

The Bt values were obtained for each observed value of F , from Reichenberg's eq. 8. The linearity test of Bt versus time plots was used to distinguish between the film diffusion and particle-diffusion-controlled adsorption. If the plot of Bt vs. time (having slope B) is a straight line passing through the origin the adsorption rate is governed by the particle diffusion mechanism; otherwise, it is governed by film diffusion. For all drugs the plots are

TABLE 5. Equilibrium thermodynamic parameters for *S*-thalidomide and its derivatives

Compound	ΔG° (kJ/K mol)			ΔH° (kJ/K mol)	ΔS° (kJ/mol)
	20°	30°	40°		
Thalidomide	1.83	3.82	5.81	56.48	0.20
3-Methyl thalidomide	1.85	3.85	5.90	50.89	0.22
3-Ethyl thalidomide	1.90	3.91	5.98	47.04	0.24
3-Butyl thalidomide	1.96	3.95	6.09	45.10	0.28

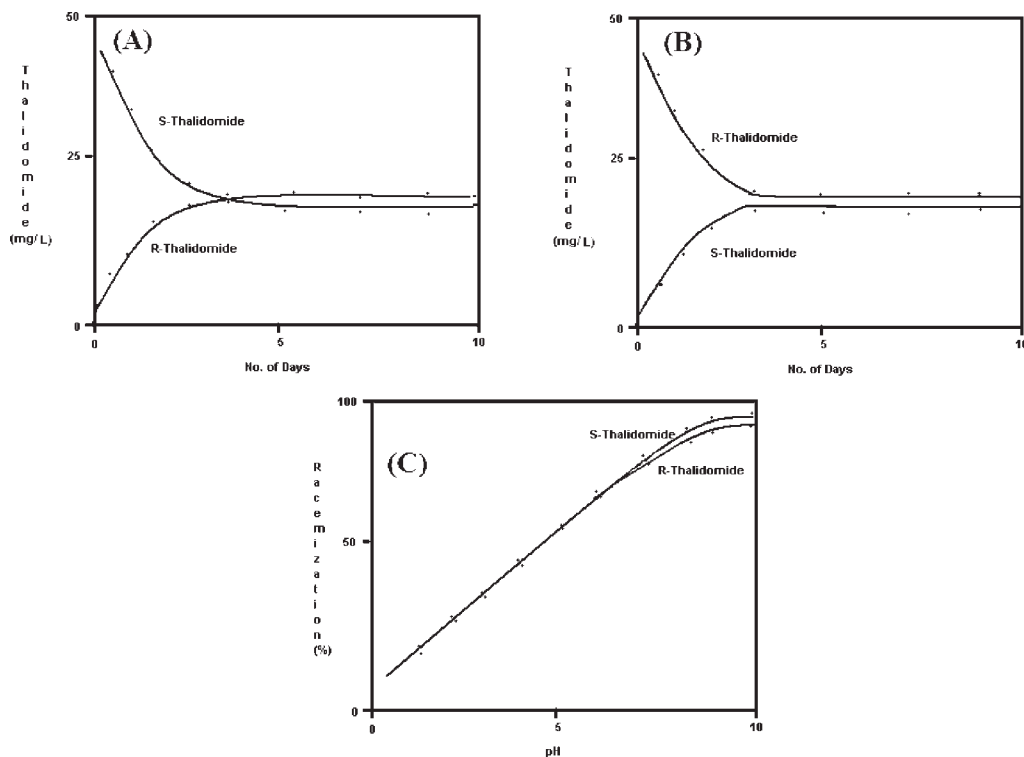


Fig. 4. Chiral interconversion of (A) *S*-thalidomide and (B) *R*-thalidomide enantiomers in water-sediment systems and the effect of pH on the chiral interconversions of *S*- and *R*-thalidomide.

linear but do not pass through the origin indicating a film diffusion mechanism.

Chiral Interconversions

The chiral interconversions of *R*- and *S*-thalidomide were studied under natural conditions. The solubilities of thalidomide and its derivatives in water are poor and, hence, the probability of their occurrence on sediment is high. Therefore, the chiral interconversions were studied on sediment after their adsorption on it. Optically active pure enantiomers of thalidomide and its derivatives were used for chiral interconversions. The chiral interconversions of *S*- and *R*-thalidomide are shown in Figures 4A and 4B and the rate constants of the chiral interconversions were calculated by the following equation.

$$k = (2.303/t) \log a/(a - X) \quad (10)$$

where t is the time in days in which x amount of racemate is formed from initial concentration (a) of pure enantio-

mers. The values of rate constants of the chiral interconversions for thalidomide and its derivatives are given in Table 6, which indicates higher rate of chiral interconversions of *S*-enantiomers of thalidomide and its derivatives. The values of rate constants were 0.390 and 0.385 day⁻¹ for *S*- and *R*-enantiomers respectively, indicating slightly higher tendency of racemization of *S*-thalidomide. The effects of pH and temperature were studied on the chiral interconversions of thalidomide enantiomers. pH was varied from 2.0 to 10 and the results are shown in Figure 4C, which shows that racemization was higher at high pH (up to 9.0) and becomes constant after this pH. The effect of temperature was carried out at three different values, i.e., 20, 30, and 40°C and slightly higher rate of chiral interconversions was observed at 40°C.

Mechanism of Dynamics at Supramolecular Level

To ascertain the toxicities of thalidomide and its derivatives, it is important to understand the mechanism of dynamic equilibrium of thalidomide and its derivatives

TABLE 6. Rate constants (day⁻¹) of chiral interconversions of thalidomide and its derivatives at 30°C at in water of pH 7.5

Compounds	<i>S</i> -Thalidomide	<i>R</i> -Thalidomide
Thalidomide	1.88/day (2.17×10^{-5} /second)	1.84/day (2.13×10^{-5} /second)
3-Methyl thalidomide	1.03/day (1.19×10^{-5} /second)	1.00/day (1.15×10^{-5} /second)
3-Ethyl thalidomide	0.99/day (1.14×10^{-5} /second)	0.95/day (1.10×10^{-5} /second)
3-Butyl thalidomide	0.94/day (1.06×10^{-5} /second)	0.90/day (1.04×10^{-5} /second)

TABLE 7. Regression analysis data for chromatographic and SPE methods of thalidomide and its derivatives

Chiral compounds	HPLC (peak areas)			SPE (% recoveries)		
	SD	CC	CL	SD	CC	CL
Thalidomide	±0.10	0.9998	99.5	±0.05	0.9999	99.5
3-Methyl thalidomide	±0.10	0.9998	99.4	±0.05	0.9999	99.4
3-Ethyl thalidomide	±0.10	0.9998	99.5	±0.05	0.9999	99.5
3-Butyl thalidomide	±0.10	0.9999	99.4	±0.05	0.9999	99.4

SD, standard deviation; CC, correlation coefficients; CL, confidence limit.

under natural conditions. pK_a values of thalidomide and its derivatives are more than 10.0 and, hence, these drugs exist as neutral molecules at the experimental conditions reported in this work. The dynamic equilibrium was found to be film diffusion at supramolecular level and is controlled by certain forces such as van der Waal, hydrogen bondings, steric effects. These drugs contain electronegative atoms such as oxygen and nitrogen and on the other hand sediment has hydroxyl and silicon groups. These groups and atoms form hydrogen bondings between these drugs and sediment. The adsorption of these drugs is endothermic indicating a slow process at natural conditions. The order of adsorption is thalidomide > 3-methyl thalidomide > 3-ethyl thalidomide > 3-butyl thalidomide, indicating the role of the steric effects of the alkyl groups, i.e., methyl, ethyl, and butyl in thalidomide derivatives; in comparison to thalidomide. In summary, various physical forces and the structures of these drugs at supramolecular level are responsible for different dynamic equilibrium concentrations of the studied racemates under the reported experimental conditions. Both thalidomide enantiomers are not stable under natural conditions and, hence get racemized. The chiral interconversions is favored at low pH indicating a mechanism through proton exchange phenomenon as discussed by other authors.^{15-18,24,25-28}

VALIDATION

Validation of the developed methods was ascertained by carrying out five sets ($n = 5$) of the chromatographic and SPE procedures under identical conditions. The regression analysis was carried out using Microsoft Excel program and the results are given in Table 7 for chromatographic and SPE procedures, respectively. Table 7 shows that standard deviation was ± 0.10 and ± 0.05 for chromatographic and SPE methods while the correlation coefficients (R^2) and confidence levels were 0.9999–0.9998% and 99.4–99.5%, respectively.

CONCLUSIONS

The presence of thalidomide and its derivatives in nature is hazardous and this study indicates that most of these drugs remain on sediment with poor concentration in aqueous phase. Sediment has the capacity to hold these toxic drugs and, hence, we are thankful to nature for its self purification power. However, there is a limit of the

sediment to hold these drugs when present at high concentration (more than 40 mg/l), consequently, thalidomide and its derivatives are supposed to be present in water, which can enter into human body. The chiral interconversions of the safe *R*-enantiomers to the *S*-toxic enantiomer under natural condition is a serious phenomenon. The present work describes the dynamics of the chiral thalidomide at supramolecular level, in natural conditions. This study may be useful to control the contamination of earth ecosystem because of these toxic drugs. Therefore, one should be careful before releasing waste effluents, containing thalidomide, in the nature. Such type of effluent discharges should be treated properly before their release into the environment.

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