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Research Article

Thermodynamic and kinetic study of chiral separation of some non-steroidal anti-inflammatory drugs on dinitrobenzamido tetrahydrophenanthrene stationary phase

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ABSTRACT

The chiral separation of three arylpropionic acid-based non-steroidal anti-inflammatory drugs: ibuprofen, ketoprofen and ketorolac is thermodynamically and kinetically studied on dinitrobenzamido tetrahydrophenanthrene (Whelk-O 2) stationary phase in the reversed phase mode. This study aims to investigate the chromatographic behavior of these compounds on Whelk-O 2 and to better understand this stationary phase and the mechanistic details of its chiral recognition. Statistical moments are used to calculate thermodynamic and kinetic parameters. The thermodynamic study shows that upon increasing temperature from 15 to 35 °C, both retention and enantioselectivity decrease and the enantioseparation process is enthalpy-controlled. The retention mechanism is independent of temperature and no conformational changes occur in the Whelk-O 2 phase as indicated by the linear van't Hoff plots. The investigated drugs have the same retention mechanism on Whelk-O 2 as demonstrated by the linear enthalpy-entropy compensation plots. The chiral recognition mechanism involves π-π interaction and hydrogen bonding with ibuprofen having the strongest interactions. The kinetic study involves the investigation of sorption and desorption rate constants using Arrhenius plots. The rate of sorption is always greater than the rate of desorption for the three drugs. Ibuprofen has sharper peaks and shorter retention time compared to ketoprofen and ketorolac due to its faster rates and lower activation energy of sorption and desorption.

Keywords: Enantioseparation; Whelk-O 2; Non-steroidal anti-inflammatory drugs; Thermodynamic parameters; Kinetic parameters.

1. INTRODUCTION

Brush-type chiral stationary phases which have small molecules as chiral selectors were developed by Pirkle in the late 1970s.[1-3] Since then, brush or Pirkle-type chiral stationary phases have been extensively utilized in both analytical and preparative chiral separations. One of these phases is Whelk-O chiral stationary phase which was originally designed for the enantioseparation of non-steroidal anti-inflammatory drugs in the early 1990s.[4,5] The chiral selector of Whelk-O phase is 1-(3,5-dinitrobenzamido)-1,2,3,4-tetrahydrophenanthrene comprising an electron-deficient dinitrophenyl group, an electron-rich phenanthryl group, and an amide linkage connecting them. There are two versions of this stationary phase, namely Whelk-O 1 and Whelk-O 2. Both have the same chiral selector but differ in the way of attachment to silica where Whelk-O 2 has a trifunctional

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linkage rather than a monofunctional one. This modification makes Whelk-O 2 more stable against hydrolysis while using strong mobile phase additives such as trifluoroacetic acid. The chemical structures of Whelk-O 1 and Whelk-O 2 are shown in **Figure 1**. In addition to non-steroidal anti-inflammatory drugs, Whelk-O is applied for the chiral separation of many other families including amides, epoxides, esters, ureas, carbamates, ethers, aziridines, phosphonates, aldehydes, ketones, carboxylic acids, and alcohols. The Whelk-O stationary phase can be used with organic, aqueous, and supercritical solvents in either analytical or preparative applications.[6] It has proven to be the most successful charge transfer selector.[7]

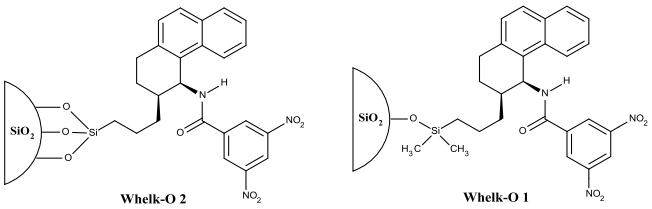

Figure 1. Chemical structures of Whelk-O 1 and Whelk-O 2.

The chiral recognition mechanism of Pirkle-type stationary phases is usually explained by the three-point interaction model.[8-10] The main interactions for these stationary phases are π-π stacking and hydrogen bonding as indicated by crystallographic [11,12] and NMR [13,14] studies. The chiral resolution mechanism may also involve other types of interactions such as dipole-dipole interactions and steric hindrances [15,16] For Whelk-O chiral stationary phase, the three interactions are: 1. π-π stacking between an aryl group in the analyte and the Whelk-O dinitrophenyl group. 2. hydrogen bonding between a hydrogen bond acceptor in the analyte and the Whelk-O amide hydrogen. 3. either CH–π interactions [17] or edge-to-face π-π interactions [12,14,18,19] between the analyte and the Whelk-O phenanthryl group. Accordingly, an analyte having an aromatic ring and a hydrogen bond acceptor near a chiral center is a good candidate for separation on Whelk-O. Furthermore, the cleft formed by the dinitrophenyl group and the phenanthryl group has a crucial function in resolving enantiomers. The more retained enantiomer fits into the cleft by forming the three interactions simultaneously, whereas the less retained enantiomer docks outside of the cleft undergoing two interactions without forming the edge-to-face π-π interaction.[12,14] This mechanism is reinforced by the findings of studying the co-crystallization between Whelk-O as a chiral selector and N-(1-(4-bromophenyl)ethyl)pivalamide as a chiral analyte.[12] NMR studies [14,19] of mixtures of various analytes and soluble Whelk-O selector also support this mechanism. Due to the shielding effect of the aromatic rings of the cleft, the protons which are close to the main interaction sites of the more retained enantiomers are shifted upfield compared to those of the less retained enantiomers.

Thermodynamic and kinetic studies play an essential role in liquid chromatographic chiral separations. Thermodynamic parameters are used to figure out the energetically favored interactions between the analyte and the mobile and stationary phases, which are reflected on retention and enantioselectivity. Kinetic parameters are used to figure out the rates of mass transfer processes (sorption and desorption), which are reflected on the efficiency or plate height. Thus, the valuable information provided by thermodynamic and kinetic studies can be used to elucidate the retention mechanism and enhance resolution for successful chiral separations.[20-22]

In this study, the effect of temperature on the enantioseparation of arylpropionic acid derivatives is investigated on Whelk-O 2 chiral stationary phase in the reversed phase mode. The purpose of this thermodynamic and kinetic study is firstly to explore the chromatographic behavior of these compounds on Whelk-O 2 including their structure-retention relationship and secondly to better understand this stationary phase and the mechanistic details of its chiral recognition.

2. Materials and methods

2.1. Chemicals

Arylpropionic acid-based non-steroidal anti-inflammatory drugs, consisting of (RS)-2-(4-(2-Methylpropyl)phenyl)propanoic acid (ibuprofen, purity: 99.48%), (RS)-2-(3-benzoylphenyl)propanoic acid (ketoprofen, purity: 99.18 %) and (RS)-5-benzoyl-2,3-dihydro-1H-pyrrolizine-1-carboxylic acid (ketorolac, purity: 99.12 %), are selected as model analytes for this study. The structures of these drugs are shown in **Figure 2**. Ibuprofen and ketoprofen are kindly supplied by Sigma Pharmaceutical Industries, Quesna, Egypt. Ketorolac tromethamine is kindly supplied by Amriya Pharmaceutical Industries, Alexandria, Egypt. 2-propanol (HPLC grade, Romil Ltd, Cambridge, UK) is used as organic modifier. Sodium acetate anhydrous (Iso-Chem fine chemicals, Cairo, Egypt) is used as mobile phase additive.

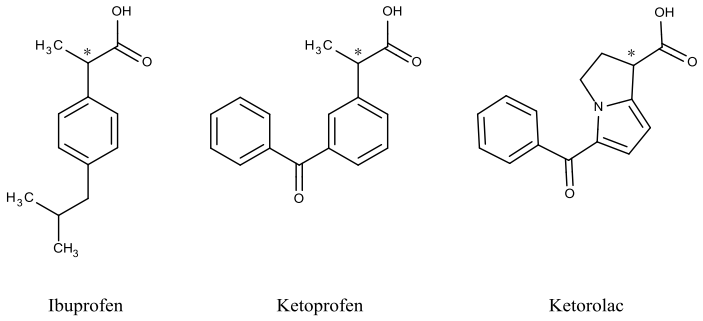

Figure 2. Structures of arylpropionic acid-based non-steroidal anti-inflammatory drugs.

2.2. Standard solutions

A stock standard solution of 1 mg mL^{-1} of each drug is prepared by transferring accurately weighed 25 mg powder into 25 mL volumetric flask, dissolved in 2-propanol by sonication for 15 minutes and the volume is completed with the same solvent. The solution is subsequently used to prepare working standard solution of 100 µg mL^{-1} for HPLC analysis by dilution with 0.1 M sodium acetate.

2.3. High performance liquid chromatographic system

A Dionex UltiMate 3000 RS system is used, (Thermo Scientific™, Dionex™, Sunnyvale, CA, USA), equipped with quaternary RS pump, RS auto-sampler injector, thermostated RS column compartment and RS diode array detector. The instrument is connected to a Dell compatible PC, bundled with Chromeleon® 7.1 Chromatography Data System software. The HPLC column is (R,R)-Whelk-O 2 (4.6 mm diameter × 250 mm length, 10 µm particles), (Regis Technologies, Inc. Morton Grove, IL, USA).

The mobile phase is a mixture of 2-propanol and 0.1 M sodium acetate aqueous solution in a ratio of (20:80, v/v). The aqueous solution is filtered through Nylon membrane filter with 0.22 µm pore size prior to use. The flow rate is set at 1.0 mL min^{-1}. The column is equilibrated for 45 minutes prior to injection. The temperature is varied between 15 and 35 °C at 5 °C intervals. The injection volume is 20 µL and UV detection is performed at 221 nm for ibuprofen, 260 nm for ketoprofen and 323 nm for ketorolac.

2.4. Data analysis

Data are processed according to the approach adopted by Gebreyohannes and McGuffin [22] where thermodynamic and kinetic parameters are calculated from statistical moments. Non-linear regression (TableCurve 2D, v5.01, SYSTAT Software, Inc.) is used for fitting the individual chromatographic peaks. Pearson IV is found to be the optimum function for fitting with a coefficient of determination ($r^2 = 0.999$). The Pearson IV function is

$$C(t) = \frac{a_0\left[1+\frac{\left(t-\frac{a_2a_4}{2a_3}-a_1\right)^2}{a_2^2}\right]^{-a_3} exp\left[-a_4\left[tan^{-1}\left(\frac{t-\frac{a_2a_4}{2a_3}-a_1}{a_2}\right)+tan^{-1}\left(\frac{a_4}{2a_3}\right)\right]\right]}{\left[1+\frac{a_4^2}{4a_3^2}\right]^{-a_3}} \quad (1)$$

where C(t) is the concentration as a function of time, a_0 is the peak amplitude, a_1 is the peak center, a_2 describes the peak width, and a_3 and a_4 describe the peak shape. a_4 is positive for fronted peaks and negative for tailed peaks. The fitting parameters from Pearson IV function are then used to reproduce the peaks which in turn are used to calculate the first (M_1) and second (M_2) statistical moments.

$$M_1 = \frac{\int C(t)t dt}{\int C(t)dt} \quad (2)$$

$$M_2 = \frac{\int C(t)(t-M_1)^2 dt}{\int C(t)dt} \quad (3)$$

The first statistical moment denotes the retention time (t_R) and is employed to calculate the retention factor (k) and then the enantioselectivity (α).

$$k = \frac{(M_1 - t_0)}{t_0} \quad (4)$$

$$\alpha = \frac{k_2}{k_1} \quad (5)$$

where k_1 and k_2 are the retention factors of the first and second eluted enantiomers, respectively.

The second statistical moment denotes the peak variance and is employed to calculate the plate height (H) and then the kinetic rate constants.

$$H = \frac{M_2 L}{M_1^2} \quad (6)$$

where L is the column length. The desorption rate constant (k_{sm}) is calculated as

$$k_{sm} = \frac{2ku}{(1+k)^2 H_{corr}} \quad (7)$$

where u is the linear velocity and H_{corr} is the corrected plate height. The sorption rate constant (k_{ms}) is calculated from equation (8) which represents the relationship between thermodynamic and kinetic parameters.[23]

$$k = \frac{k_{ms}}{k_{sm}} \quad (8)$$

$$k_{ms} = \frac{2k^2 u}{(1+k)^2 H_{corr}} \quad (9)$$

To determine the corrected plate height (H_{corr}), naproxen is injected into the chromatographic system as it always has small plate height values due to its adsorption onto sites with faster kinetics. The corrected plate height is

calculated by subtracting the plate height of naproxen from the total plate height (H) calculated from equation (6). This eliminates fast mobile phase kinetics leaving only the slow kinetic contribution from the stationary phase.

3. Results and discussion

3.1. *Thermodynamic effects*

The chromatograms of the enantioseparation of ibuprofen, ketoprofen, and ketorolac on Whelk-O 2 in reversed phase mode are shown in **Figure 3**. The retention factor and enantioselectivity values at 20 °C are shown in **Table 1**. These data indicate that ibuprofen is the least retained drug while ketoprofen is the most retained one.

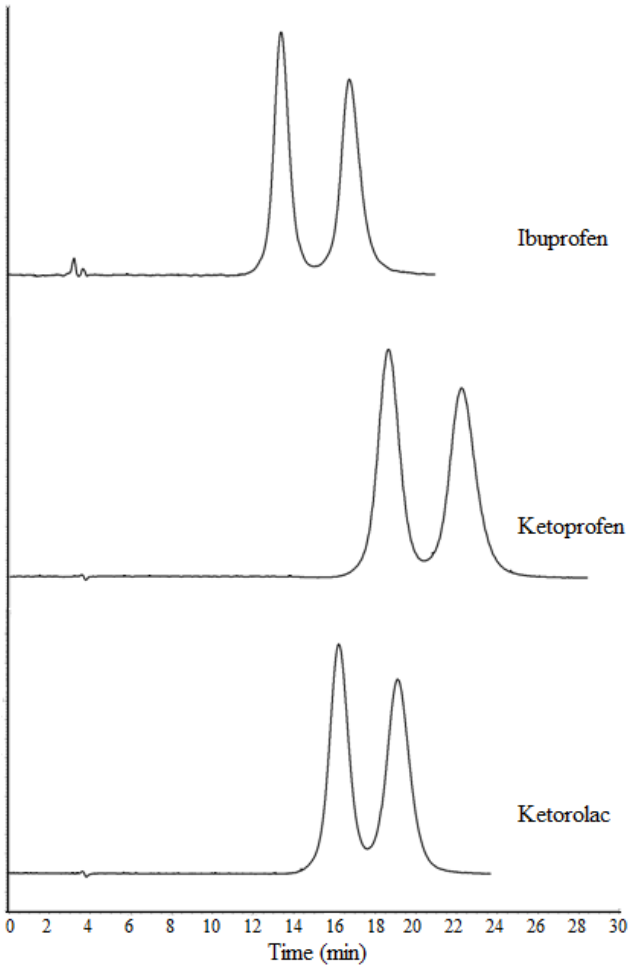

Figure 3. Chromatograms of non-steroidal anti-inflammatory drugs. Column: Whelk-O 2; mobile phase: 2-propanol:0.1 M sodium acetate (20:80, v/v); temperature: 20 °C; flow rate: 1 mL min^{-1}.

Table 1. Retention factor (k) and enantioselectivity (α) for non-steroidal anti-inflammatory drugs at 20 °C.

Solute	k_1[a]	k_2[a]	α
Ibuprofen	3.48	4.63	1.33
Ketoprofen	5.28	6.53	1.24
Ketorolac	4.41	5.39	1.22

[a] Subscripts denote the first (1) and second (2) eluted enantiomers.

3.1.1. *Effect of temperature on retention and enantioselectivity*

van't Hoff plots are used to study the effect of temperature on retention factor and enantioselectivity in the temperature range of 15-35 °C. Equation (10) shows the relationship between retention factor and temperature.[24]

$$\boldsymbol{ln\, k = \frac{-\Delta G}{RT} - ln\, \beta = \frac{-\Delta H}{RT} + \frac{\Delta S}{R} - ln\, \beta} \qquad (10)$$

where ΔG is Gibbs free energy change, ΔH is the enthalpy change, ΔS is the entropy change, R is the gas constant, T is the absolute temperature, and β is the phase ratio. If ΔH, ΔS, and β are independent of temperature, a linear relationship is obtained upon plotting the natural logarithm of the retention factor against the reciprocal of the absolute temperature with a slope of (−ΔH/R) and an intercept of (ΔS/R – ln β).[25] For covalently bonded stationary phases in the reversed phase mode, the natural logarithm of the phase ratio (ln β) usually contributes by less than 2% to the intercept.[26] The relationship between enantioselectivity and temperature is described by equation (11) which is derived from equation (5) and equation (10).

$$\boldsymbol{ln\, \alpha = \frac{-\Delta\Delta G}{RT} = \frac{-\Delta\Delta H}{RT} + \frac{\Delta\Delta S}{R}} \qquad (11)$$

where ΔΔG, ΔΔH, and ΔΔS are the differences in Gibbs free energy, enthalpy, and entropy changes, respectively, between the two enantiomers.[27] If ΔΔH and ΔΔS are independent of temperature, a linear relationship is obtained upon plotting the natural logarithm of enantioselectivity against the reciprocal of the absolute temperature with a slope of (−ΔΔH/R) and an intercept of (ΔΔS/R).

The temperature at which $k_1 = k_2$ (i.e. α = 1) and the co-elution of the two enantiomers occurs is termed the isoenantioselective temperature (T_{iso}) and expressed by equation (12). T_{iso} can be graphically determined either from the intersection point of the van't Hoff (ln k vs. 1/T) lines of the two enantiomers or from the x-intercept of the van't Hoff (ln α vs. 1/T) plot.

$$\boldsymbol{T_{iso} = \frac{\Delta\Delta H}{\Delta\Delta S}} \qquad (12)$$
$$\boldsymbol{where\ \Delta\Delta G = 0}$$

At temperatures lower than T_{iso}, enantioseparation is enthalpically dominated and enantioselectivity decreases as the temperature increases while at temperatures higher than T_{iso}, enantioseparation is entropically dominated, enantioselectivity increases as the temperature increases, and the elution order of enantiomers is reversed.[28]

The three investigated non-steroidal anti-inflammatory drugs exhibit linear van't Hoff plots for both the retention factor (ln k versus 1/T) and the enantioselectivity (ln α versus 1/T) as shown in **Figure 4** and **Figure 5,** respectively. The linearity of these plots implies that the retention mechanism is independent of temperature and that the stationary phase does not undergo conformational changes or deformations in the temperature range studied. The thermodynamic parameters calculated from the van't Hoff plots of the three drugs are listed in **Table 2** and **Table 3**.

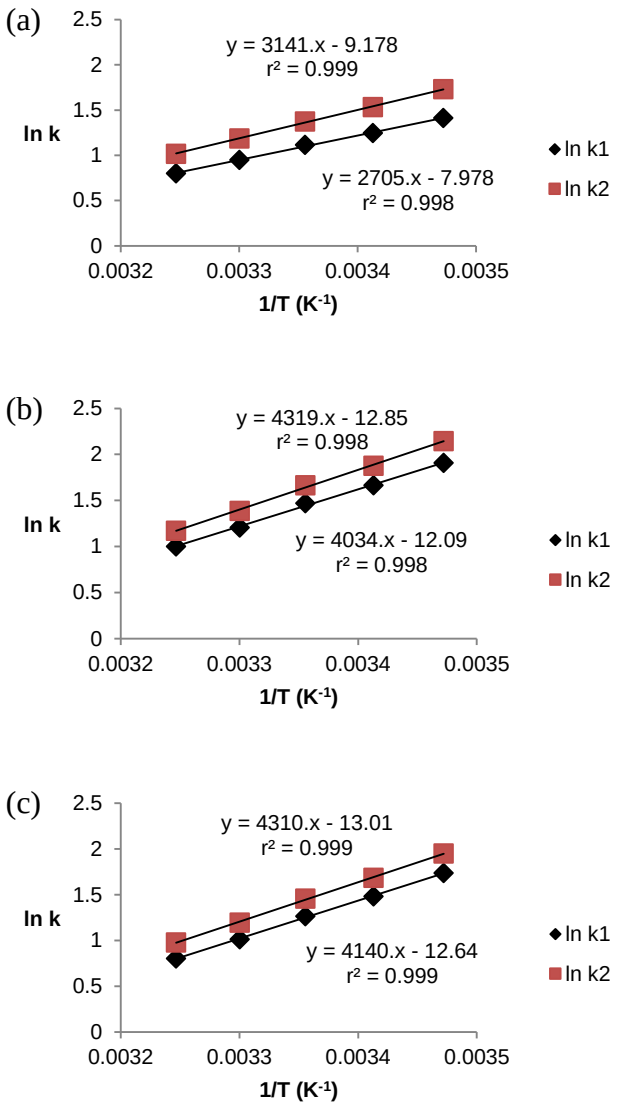

Figure 4. The van't Hoff plots: (ln k) versus (1/T) for the enantiomers of (a) ibuprofen, (b) ketoprofen and (c) ketorolac. Other experimental conditions as given in **Figure 3**.

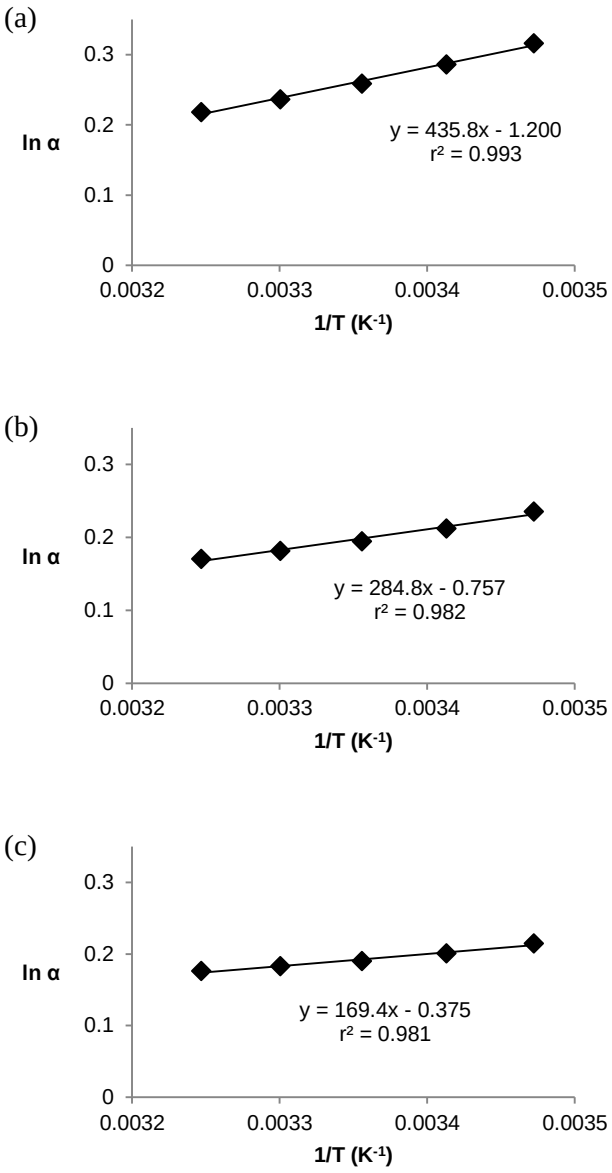

Figure 5. The van't Hoff plots: (ln α) versus (1/T) for the enantiomers of (a) ibuprofen, (b) ketoprofen and (c) ketorolac. Other experimental conditions as given in **Figure 3**.

The negative values of ΔH and ΔS for the three drugs imply that the enantioseparation process is enthalpically favorable but entropically unfavorable. The enantiomers of ketoprofen and ketorolac have similar ΔH values which are higher than those of ibuprofen enantiomers (**Table 2**). Higher ΔH values are usually associated with stronger interactions between the analyte and the stationary phase and hence longer retention. Likewise, ΔS values are comparable for the enantiomers of ketoprofen and ketorolac while ibuprofen has lower ΔS values. The comparable ΔH and ΔS values for ketoprofen and ketorolac may be attributed to the structural similarity of the two drugs (**Figure 2**). The diaryl ketone moiety shared by ketoprofen and ketorolac interacts in a similar manner with the stationary phase.

As shown in **Table 2**, the ΔΔH value of ketoprofen is almost twice as that of ketorolac and ΔΔH of ibuprofen is almost three times as that of ketorolac. The same pattern is observed in the ΔΔS values of the three drugs due to the enthalpy-entropy compensation which will be discussed in section 3.1.3. The absolute value of ΔΔG is an indication of the extent of enantioselectivity (equation 11). Ibuprofen has the highest ΔΔG at all temperatures while there is no significant change in ΔΔG between ketoprofen and ketorolac. This is consistent with the enantioselectivity of the three drugs listed in **Table 1**. As ibuprofen has lower ΔH and ΔS values for its enantiomers but higher ΔΔH and ΔΔS values than their corresponding values for ketoprofen and ketorolac, it can be concluded that ketoprofen and ketorolac have stronger non-chiral interactions but weaker chiral interactions with the stationary phase compared to ibuprofen. In other words, ibuprofen has greater difference between its more and less retained enantiomers in terms of the strength of the enantioselective interactions with Whelk-O 2. Due to this greater difference, ibuprofen has lower isoenantioselective temperature as shown in **Table 2**.

Table 2. Thermodynamic parameters for non-steroidal anti-inflammatory drugs.

	ΔH_1[a,b] (kJ mol^{-1})	ΔH_2[a,b] (kJ mol^{-1})	$\Delta\Delta H$[c] (kJ mol^{-1})	ΔS_1[a,b] (J mol^{-1} K^{-1})	ΔS_2[a,b] (J mol^{-1} K^{-1})	$\Delta\Delta S$[c] (J mol^{-1} K^{-1})	T_{iso}[d] (K)
Ibuprofen	-22.49	-26.12	-3.62	-66.33	-76.31	-9.98	363
Ketoprofen	-33.55	-35.91	-2.37	-100.58	-106.87	-6.30	376
Ketorolac	-34.43	-35.84	-1.41	-105.12	-108.24	-3.13	451

[a] Subscripts denote the first (1) and second (2) eluted enantiomers.

[b] Calculated from the slope and intercept of equation (10)

[c] Calculated from the slope and intercept of equation (11)

[d] Temperatures calculated from equation (12) and reported to the nearest 1 K

Table 3. ΔG and ΔΔG values for non-steroidal anti-inflammatory drugs.

Drug	Temp. (K)	ΔG_1[a,b] (kJ mol^{-1})	ΔG_2[a,b] (kJ mol^{-1})	$\Delta\Delta G$[c] (kJ mol^{-1})
Ibuprofen	288	-3.39	-4.14	-0.76
	293	-3.04	-3.73	-0.70
	298	-2.77	-3.41	-0.64
	303	-2.39	-2.99	-0.60
	308	-2.05	-2.61	-0.56
Ketoprofen	288	-4.57	-5.14	-0.56
	293	-4.06	-4.57	-0.52
	298	-3.64	-4.12	-0.48
	303	-3.04	-3.49	-0.46
	308	-2.57	-3.00	-0.44
Ketorolac	288	-4.16	-4.67	-0.51
	293	-3.61	-4.10	-0.49
	298	-3.14	-3.61	-0.47
	303	-2.55	-3.01	-0.46
	308	-2.06	-2.51	-0.45

[a] Subscripts denote the first (1) and second (2) eluted enantiomers.

[b] Calculated from equation (10)

[c] Calculated from equation (11)

3.1.2. Investigation of chiral recognition mechanism:

The linearity of Van't Hoff plots (ln α versus 1/T) indicates the predominance of one mechanism rather than a competition between multiple retention mechanisms.[29] The chiral recognition mechanism for the enantioseparation of an arylpropionic acid drug on Whelk-O 2 is supposed to include π-π interactions between the dinitrophenyl group of the selector and the aryl group of the drug, hydrogen bonding between the amide group of the selector and the carboxylic group of the drug and steric interactions. Based on the aforementioned results, there is a significant difference among the three drugs in terms of the individual contributions of these interactions to the retention mechanism. The involvement of hydrogen bonding in the chiral resolution mechanism is usually evidenced by large ΔΔH values because hydrogen bonding is a highly temperature dependent process.[30]

Küsters et al. [31] classified the ΔΔH values into three categories, each of which represents certain types of chiral discriminating interactions. The first category includes low ΔΔH values between -0.05 and -0.1 kcal mol^{-1} suggesting an inclusion mechanism where enantioseparation is exclusively due to steric interactions. The second category involves ΔΔH values between -0.5 and -1.0 kcal mol^{-1} where chiral discrimination is due to steric hindrance in addition to another type of interaction such as weak π-π interactions or weak hydrogen bonding. The third category has absolute ΔΔH values greater than l.0 kcal mol^{-1} as the most retained enantiomer possesses an additional strong π-π interaction or strong hydrogen bonding. Ibuprofen has the largest ΔΔH value (- 3.62 kJ mol^{-1} ≈ - 0.9 kcal mol^{-1}), then ketoprofen (- 2.37 kJ mol^{-1} ≈ - 0.6 kcal mol^{-1}) and finally ketorolac has the smallest ΔΔH (- 1.41 kJ mol^{-1} ≈ - 0.3 kcal mol^{-1}). From these values, it can be deduced that the forces driving chiral recognition may consist of steric hindrance, π-π interactions and hydrogen bonding with significant differences among the three drugs in the strength of these interactions. Ibuprofen has relatively strong π-π interactions and hydrogen bonding. Ketoprofen has moderate interactions and ketorolac has very weak interactions. The difference in the enantioselective hydrogen bonding ability of the three drugs may be attributed to the difference in the partial negative charge distribution on the carboxylic oxygen atoms as shown in **Table 4**. Moreover, ketoprofen and ketorolac have another hydrogen bond

acceptor (keto group) which forms non-enantioselective hydrogen bonds. The keto group competes with the carboxylic group for the hydrogen bond donor sites in Whelk-O 2. Consequently, ibuprofen has the largest ΔΔH value as it lacks this competition. The nitrogen atom in ketorolac increases the partial negative charge on the oxygen atom of the keto group compared to ketoprofen (**Table 4**). Therefore, ketorolac has the weakest enantioselective hydrogen bonding and the smallest ΔΔH value.

Table 4. Partial negative charge distribution on the oxygen atoms of the three drugs.*

Drug	Carboxylic group		Keto group
	C = **O**	**O** – H	C = **O**
Ibuprofen	- 0.32	- 0.28	N/A[a]
Ketoprofen	- 0.30	- 0.26	- 0.39
ketorolac	- 0.27	- 0.23	- 0.42

*Calculated by the charge plug-in in Marvinsketch [32]

[a] Not applicable (N/A)

3.1.3. Enthalpy-entropy compensation

A global enthalpy-entropy compensation is demonstrated by a linear relationship between ΔH and ΔS

$$\boldsymbol{\Delta H = T_c \Delta S + \Delta G_{T_c}} \qquad (13)$$

while an enantioselective enthalpy-entropy compensation is represented by a linear relationship between ΔΔH and ΔΔS.

$$\boldsymbol{\Delta\Delta H = T_c \Delta\Delta S + \Delta\Delta G_{T_c}} \qquad (14)$$

where T_c is the compensation temperature at which ΔΔH is exactly compensated by ΔΔS and $\Delta\Delta G_{Tc}$ is the same for all solutes. Having similar T_c values for different solutes indicates that these solutes have the same retention mechanism.[33,34] The Enthalpy–entropy compensation study of the chiral recognition mechanism for ibuprofen, ketoprofen and ketorolac on Whelk-O 2 stationary phase is shown in **Figure 6**. The linear correlation between ΔΔH and ΔΔS is an indication of the mechanistic similarity of the enantioseparation of the three drugs. The compensation temperature, T_c = 333 K = 60 °C. At this temperature, $\Delta\Delta G_{Tc}$ = - 0.31 kJ mol^{-1} and α = 1.12 for the three investigated drugs.

The linearity of (ΔH versus ΔS) or (ΔΔH versus ΔΔS) plots may result from propagation of experimental errors, instead of a real enthalpy-entropy compensation effect. Alternatively, linear plots of ΔG versus ΔH are usually more representative of enthalpy-entropy compensation arising from a chemical rather than a statistical effect.[35,36] Substitution of equation (13) into (ΔG = ΔH − TΔS) results in

$$\boldsymbol{\Delta G = \Delta H\left[1 - \frac{T}{T_C}\right] + \frac{T\Delta G_{T_C}}{T_C}} \qquad (15)$$

When equation (15) is substituted into equation (10),

$$\boldsymbol{ln\, k = \frac{-\Delta H}{R}\left[\frac{1}{T_{hm}} - \frac{1}{T_C}\right] - \frac{\Delta G_{T_C}}{RT_C} - ln\, \beta} \qquad (16)$$

where k values are calculated at T_{hm} which is the harmonic mean of the experimental absolute temperatures. According to equation (16), enthalpy-entropy compensation is represented by a linear plot of ln k versus – ΔH and the compensation temperature can be calculated from the slope. **Figure 7** shows the enthalpy–entropy compensation plot of ln k versus – ΔH for the enantiomers of the three arylpropionic acid drugs. The mechanistic similarity of ibuprofen, ketoprofen, and ketorolac on Whelk-O 2 is demonstrated by the linearity of the plot (r^2 = 0.936) with a compensation temperature of 336 K = 63 °C which is very close to that obtained from the slope of the graph in **Figure 6**.

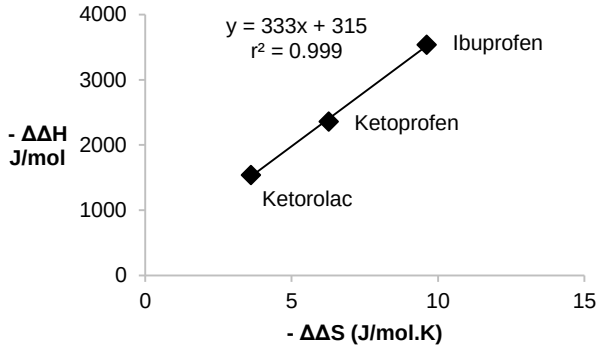

Figure 6. Enthalpy-entropy compensation plot (-ΔΔH versus -ΔΔS) for the enantioselective part of the retention mechanism of non-steroidal anti-inflammatory drugs. Other experimental conditions as given in **Figure 3**.

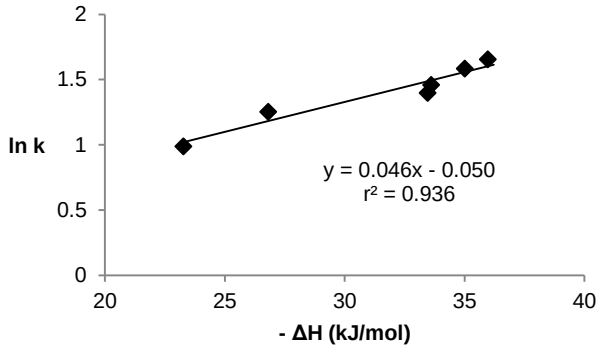

Figure 7. Enthalpy–entropy compensation plot of (ln k) versus (-ΔH) for the enantiomers of non-steroidal anti-inflammatory drugs. Other experimental conditions as given in **Figure 3**.

3.2. Kinetic effects

3.2.1. Sorption and desorption rate constants

k_{ms} is the sorption rate constant which describes the mass transfer from mobile to stationary phase while k_{sm} is the desorption rate constant which describes the mass transfer from stationary to mobile phase. The sorption and desorption

rate constants of the three non-steroidal anti-inflammatory drugs on Whelk-O 2 are calculated from equation (9) and (7), respectively and listed in **Table 5**. The sorption rate constant is generally greater than the desorption rate constant as long as the retention factor (k) is greater than one according to equation (8). **Table 5** shows that ibuprofen has faster desorption rate for the first eluted enantiomer and faster sorption and desorption rates for the second eluted enantiomer. This may account for the sharper peaks and shorter retention time of ibuprofen compared to ketoprofen and ketorolac.

Table 5. Sorption (kms) and desorption (ksm) rate constants for the enantiomers of non-steroidal anti-inflammatory drugs at 15 °C.

Solute	$(k_{ms})_1$[a] (s^{-1})	$(k_{sm})_1$ (s^{-1})	$(k_{ms})_2$[a] (s^{-1})	$(k_{sm})_2$ (s^{-1})
Ibuprofen	9.71	2.36	7.83	1.39
Ketoprofen	10.91	1.62	7.23	0.85
Ketorolac	10.16	1.79	7.22	1.03

[a] Subscripts denote the first (1) and second (2) eluted enantiomers.

3.2.2. Effect of temperature on rate constants

During the sorption and desorption processes, the solute undergoes a transient, high-energy activated complex or transition state (‡) which is the rate determining step in the retention mechanism. The relationship between the kinetic rate constant and the activation energy is given by Arrhenius equation [37],

$$ln\, k_{ms} = ln\, A_{\ddagger m} - \frac{\Delta E_{\ddagger m}}{RT} \qquad (17)$$

where $A_{\ddagger m}$ is the pre-exponential factor and $\Delta E_{\ddagger m}$ is the activation energy required for the sorption process. If $A_{\ddagger m}$ and $\Delta E_{\ddagger m}$ are temperature-independent, $\Delta E_{\ddagger m}$ can be obtained from the slope of the linear plot of ln k_{ms} versus 1/T. Similarly, for the desorption process, if $A_{\ddagger s}$ and $\Delta E_{\ddagger s}$ are temperature-independent, $\Delta E_{\ddagger s}$ can be obtained from the slope of the linear plot of ln k_{sm} versus 1/T. Symmetric and asymmetric peak broadening is mainly due to slower sorption and desorption processes relative to the mobile phase velocity **[22]**. In case of non-linear plots of (ln k_{ms}) or (ln k_{sm}) versus (1/T), activation energy can be calculated at any temperature as follows **[37]**

$$\Delta E_{\ddagger m} = RT^2 \left(\frac{dlnk_{ms}}{dT}\right) \qquad (18)$$

Likewise,

$$\Delta E_{\ddagger s} = RT^2 \left(\frac{dlnk_{sm}}{dT}\right) \qquad (19)$$

then the average $\Delta E_{\ddagger m}$ and $\Delta E_{\ddagger s}$ are calculated in the temperature range investigated. The activation energy of sorption ($\Delta E_{\ddagger m}$) and desorption ($\Delta E_{\ddagger s}$) processes for the enantiomers of the three drugs are summarized in **Table 6**. **Figure 8** shows the Arrhenius plots for the sorption process (ln k_{ms} versus 1/T) of the three non-steroidal anti-inflammatory drugs. **Figure 9** shows the Arrhenius plots for the desorption process (ln k_{sm} versus 1/T) of the three non-steroidal anti-inflammatory drugs.

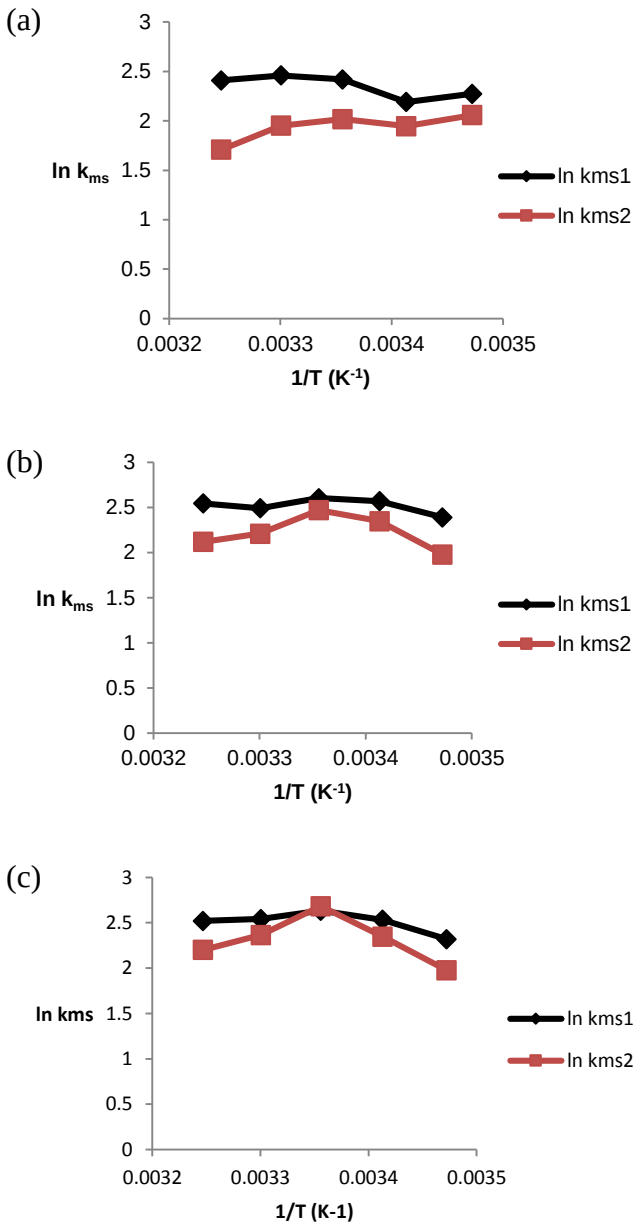

Figure 8. Arrhenius plots of the sorption process (ln kms versus 1/T) for the enantiomers of (a) ibuprofen, (b) ketoprofen and (c) ketorolac. Other experimental conditions as given in **Figure 3**.

Figure 8 shows that the Arrhenius plots for the sorption process are non-linear for the enantiomers of ibuprofen (r^2 = 0.573 and 0.641), ketoprofen (r^2 = 0.204 and 0.018) and ketorolac (r^2 = 0.336 and 0.091) indicating that $A_{\ddagger m}$ and $\Delta E_{\ddagger m}$ for the three drugs are temperature-dependent. The $\Delta E_{\ddagger m}$ values listed in **Table 6** are less than their corresponding $\Delta E_{\ddagger s}$ values matching with the trend observed in **Table 5** where k_{ms} values are greater than their corresponding k_{sm} values. A low $\Delta E_{\ddagger m}$ indicates that k_{ms} is not strongly dependent on temperature as demonstrated in **Figure 8**. All $\Delta E_{\ddagger m}$ values are positive except for the second eluted

enantiomer of ibuprofen which has a negative $\Delta E_{‡m}$ indicating that the sorption rate constant decreases as the temperature increases for this enantiomer (**Figure 8a**).

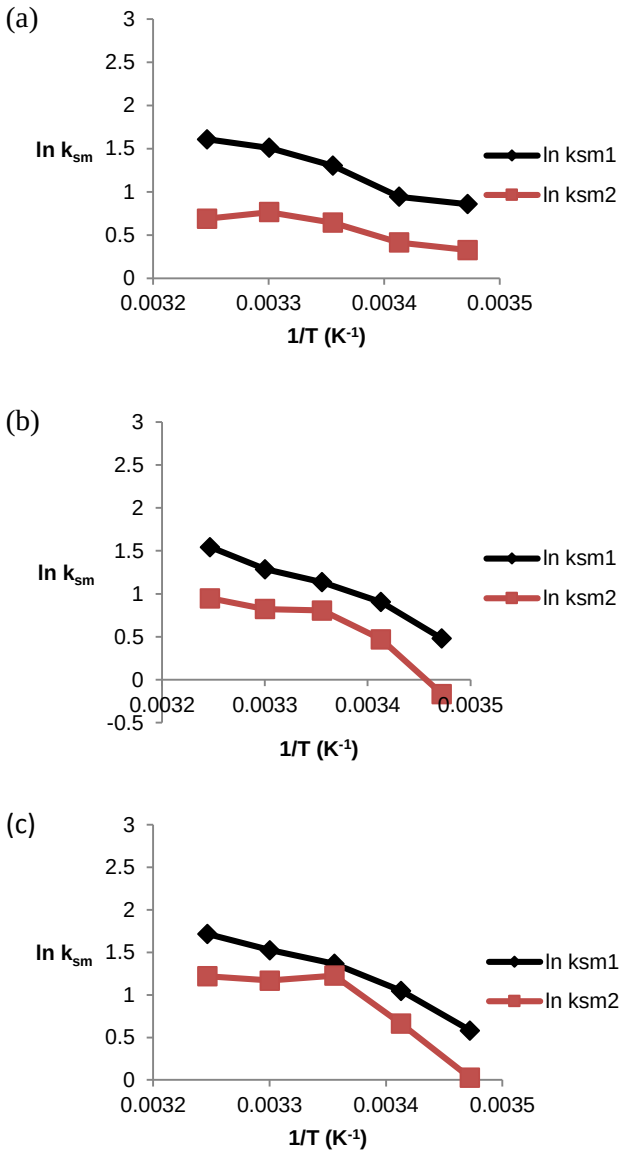

Figure 9. Arrhenius plots of the desorption process (ln ksm versus 1/T) for the enantiomers of (a) ibuprofen, (b) ketoprofen and (c) ketorolac. Other experimental conditions as given in **Figure 3**.

Figure 9 shows that the Arrhenius plots for the desorption process are linear for the first eluted enantiomer of ibuprofen (r^2 = 0.959), ketoprofen (r^2 = 0.972) and ketorolac (r^2 = 0.957). Thus, $(A_{‡s})_1$ and $(\Delta E_{‡s})_1$ for the three drugs do not depend on temperature. Furthermore, $(\Delta E‡s)_1$ calculated from equation (19) is approximately the same as that calculated from the slope of Arrhenius plot. The Arrhenius plots for the desorption process are non-linear for the second eluted enantiomer of ibuprofen (r^2 = 0.833), ketoprofen (r^2 = 0. 0.832) and ketorolac (r^2 = 0.782) indicating that $(A_{‡s})_2$ and $(\Delta E_{‡s})_2$ for the three drugs are temperature-dependent. High values of $\Delta E_{‡s}$ listed in **Table 6** indicate that k_{sm} is strongly dependent on temperature as shown in **Figure 9**. The positive values of $\Delta E_{‡s}$ imply a direct relationship between the desorption rate constant and temperature for the three drugs (**Figure 9)**. For both enantiomers, ketoprofen and ketorolac have comparable $\Delta E_{‡s}$ values which are higher than those of ibuprofen. Again, this may be due the structural similarity of ketoprofen and ketorolac.

The non-linear behavior of some Arrhenius plots may be due to the quantum mechanical phenomenon, proton tunneling which is usually associated with processes involving proton transfer like hydrogen bonding especially at low temperatures where it is difficult to surmount the activation energy barrier.[37] Ibuprofen has the highest differential change of $\Delta E_{‡m}$ and $\Delta E_{‡s}$ between its two enantiomers ($\Delta\Delta E_{‡m}$ = -18.51 kJ mol^{-1} and $\Delta\Delta E_{‡s}$ = -14.86 kJ mol^{-1}) compared to ketoprofen ($\Delta\Delta E_{‡m}$ = -1.30 kJ mol^{-1} and $\Delta\Delta E_{‡s}$ = 1.11 kJ mol^{-1}) and ketorolac ($\Delta\Delta E_{‡m}$ = -0.04 kJ mol^{-1} and $\Delta\Delta E_{‡s}$ = 1.40 kJ mol^{-1}). These kinetic data ($\Delta\Delta E_{‡m}$ and $\Delta\Delta E_{‡s}$) along with the thermodynamic data ($\Delta\Delta G$, $\Delta\Delta H$ and $\Delta\Delta S$) explain the higher enantioselectivity and higher resolution of ibuprofen.

4. Conclusion

This manuscript involves studying the effect of temperature on the enantioseparation of arylpropionic acid-based drugs on Whelk-O 2 stationary phase. The van't Hoff plots are linear for the three investigated drugs. Retention and enantioselectivity decreases as the temperature increases, indicating that the enantiorecognition process is dominated by the differential enthalpy of interaction of the enantiomers with the chiral stationary phase. Ibuprofen, ketoprofen and ketorolac have the same chiral recognition mechanism on Whelk-O 2 as confirmed by the linear plots of ($-\Delta\Delta H$ versus $-\Delta\Delta S$) and (ln k versus $-\Delta H$) for the three drugs indicating the occurrence of enthalpy-entropy compensation where the three drugs have the same $\Delta\Delta G$ and α at the compensation temperature (T_c). Ibuprofen has stronger π-π interactions and hydrogen bonding with Whelk-O 2 than ketoprofen which in turn has stronger interactions than ketorolac according to Küsters theory.

The kinetic data reveal that the rate of sorption is always faster than that of desorption for the three non-steroidal anti-inflammatory drugs. The Arrhenius plots for the desorption process are linear for the first eluted enantiomer but non-linear for the second eluted enantiomer of the three drugs. The Arrhenius plots for the sorption process are non-linear for both enantiomers of the investigated drugs. The sorption and desorption rates increase as the temperature increases for the enantiomers of the three drugs except for the desorption rate of the second eluted enantiomer of ibuprofen where a reversed effect is observed. Ibuprofen has faster rates and lower activation energy of sorption and desorption resulting in sharper peaks and shorter retention time compared to ketoprofen and ketorolac.

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Dedication: This research is dedicated to the soul of Prof. Hamed M. El-Fatatry.

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