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Differential scanning calorimetric and powder X-ray diffraction studies on a homologous series of *N*-acyl-L-alanine esters with matched chains ($n = 9$-18)

D SIVARAMAKRISHNA and MUSTI J SWAMY*
School of Chemistry, University of Hyderabad, Hyderabad 500 046, India
e-mail: mjswamy@uohyd.ac.in; mjswamy1@gmail.com

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Abstract. A homologous series of two chain derivatives of L-alanine, namely *N*-acyl L-alanine alkyl esters (NAAEs), bearing matched, saturated, acyl and alkyl chains ($n = 9$-18) have been synthesized. The thermotropic phase transitions and supramolecular structure of NAAEs were investigated by differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD). Results obtained from DSC studies indicate that the transition temperatures (T_t), enthalpies (ΔH_t) and entropies (ΔS_t) exhibit odd-even alternation with compounds bearing odd acyl and alkyl chains showing higher values of T_t, ΔH_t and ΔS_t as compared to NAAEs with even acyl and alkyl chains. However, the transition enthalpies and entropies of the odd- and even chain length series independently exhibit a linear dependence on the chain length. The *d*-spacings obtained from PXRD increase linearly with chain length with an increment of 1.76 Å/CH_2, suggesting that NAAEs adopt either a tilted bilayer structure or a bent structure. The present results provide a thermodynamic and structural basis for investigating the interaction of NAAEs with other membrane lipids, which in turn can shed light in understanding how they can enhance the transdermal permeability of stratum corneum.

Keywords. *N*-acyl-L-alanine esters; DSC; odd-even alteration; tilted chain packing; PXRD; *d*-spacing.

1. Introduction

The stratum corneum, which is the outermost layer of the skin, is composed of 15-20 layers of flattened, dead cells with no nuclei and cell organelles.[1] The main composition of the stratum corneum is free fatty acids, sterols and ceramides.[2] Ceramides are considered to be the key molecules for lipid lamellar organization, resistance to chemical and physical stress and environmental changes.[3] Transdermal drug action depends on the percentage of the permeable drug through the stratum corneum. *N*-acyl amino acid esters are a new class of lipids, which are structurally similar to the ceramides with a small polar head group and two saturated, unbranched and long *N*-acyl chain and alkyl ester. *N*-acyl glycine alkyl esters (NAGEs) and *N*-acyl serine alkyl esters (NASEs) have been investigated to evaluate their ability to enhance the permeability of the stratum corneum.[4] When present in the drug formulation, such compounds can facilitate transdermal drug delivery by increasing the permeability of stratum corneum. It has been found that while NAGE with matched acyl and alkyl chains bearing 12 C-atoms enhanced the permeability by ~12.5 fold, the corresponding serine analog showed <3 fold increase in the permeability. The lower ability of the serine analog to increase permeability of stratum corneum was explained due to its hydrogen bonding (through the hydroxyl group) with the hydroxyl group of ceramides present in the stratum corneum.[4] Replacing the hydroxyl group with a hydrogen (which would convert the serine analogs to alanine analogs) would remove the hydrogen bonding ability of these molecules which in turn increase their ability to enhance the permeability of the stratum corneum. In view of this, in the present study, we have synthesized *N*-acyl L-alanine esters (NAAEs) with matched acyl and alkyl chains (n = 9-18), which are homologous to NAGEs and exhibit similar hydrogen bonding ability with a slightly increased head group size as compared to the glycine derivatives.

The above observations suggest that NAAEs may be useful in the formulation of transdermal drug delivery systems. In order to understand how NAAEs can be used in such an application it is necessary to explore the properties of these molecules in a systematic manner. Most useful in this context would be studies aimed at understanding the phase behavior and supramolecular

*For correspondence

organization and intermolecular interactions exhibited by them. In the present study the thermotropic phase transitions of NAAEs have been characterized by differential scanning calorimetry (DSC), which revealed an unusual odd-even alternation in the transition temperatures, enthalpies and entropies. In addition, powder X-ray diffraction studies suggest that NAAEs are most likely packed either in a tilted bilayer structure, similar to the organization found in the crystal structure of phosphatidylglycerols, or a bent structure with the acyl and alkyl chains facing each other as seen in the 3-dimensional structure of *N*, *O*-diacylethanolamines (DAEs).

2. Experimental

2.1 *Materials*

Long chain alcohols (C9-C18), *p*-toluenesulphonic acid monohydrate and long chain fatty acids (C9-C18) were purchased from Aldrich (Milwaukee, WI, USA). L-alanine and oxalyl chloride were purchased from Merck (Germany). Other chemicals and solvents (analytical grade) were purchased from local chemical suppliers.

2.2 *Synthesis of N-acyl-L-alanine esters*

N-acyl-L-alanine esters were synthesized in two steps. First L-alanine was converted to the ester, and the product was *N*-acylated to yield the *N*-acyl-L-alanine ester (scheme 1).

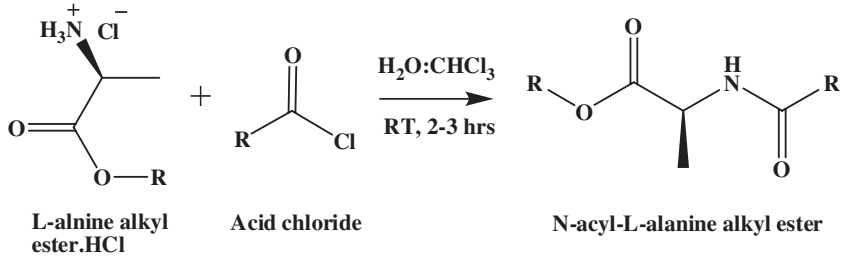
Ester preparation: L-alanine esters were prepared according to a procedure reported earlier.[5,6] L-alanine (1.0 mmol), 1-alcohol (1.0 mmol) and *p*-toluenesulphonic acid monohydrate (1.2 mmol) were taken in about 30 mL of toluene and refluxed at 110°C. After 5-6 h of refluxing, the solvent was removed by rotary evaporation under reduced pressure. The residue was dissolved in chloroform and washed with 10% sodium carbonate and then dried with anhydrous Na_2SO_4. The product obtained was recrystallized from acetone containing HCl followed by only acetone at −20°C.

N-acylation of L-alanine ester: L-alanine esters were prepared according to the procedure reported by Sivaramakrishna *et al*.[7] First, the fatty acyl chlorides were prepared from the corresponding fatty acids by reacting with oxalyl chloride.[8] The acid chloride was then reacted with L-alanine ester hydrochloride (1.0 mmol) in the presence of sodium bicarbonate (2 mmol) dissolved in a mixture of chloroform and water (2:1). After stirring for 2-3 h, the chloroform layer was washed successively with the following: double distilled water, saturated brine solution, 0.1 M HCl and double distilled water. The product obtained was recrystallized from dichloromethane at −20°C. Overall yields of different NAAEs ranged around 80-90%. The final product thus obtained was filtered and dried by vacuum desiccation, and characterized by FTIR, ^{1}H-NMR, ^{13}C-NMR and HRMS.

IR spectra were recorded on a Jasco FTIR 5300 Spectrometer using KBr pellets of the samples. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance NMR spectrometer operating at 400 and 100 MHz, respectively, using $CDCl_3$ as the solvent. Capillary melting points of the NAAEs were recorded on a Superfit (Mumbai, India) melting point apparatus as described earlier.[7]

2.3 *DSC of dry NAAEs*

DSC studies with dry NAAEs were carried out on a Perkin–Elmer Pyris Diamond differential scanning calorimeter. Samples (1-2 mg) were weighed accurately into aluminum sample pans and covered with aluminum lids and sealed by crimping. Reference pans were prepared similarly, but without any sample in them. For long chain NAAEs (n = 11-18), heating scans were performed between 25 and 100°C, whereas for *N*-nonanoyl-L-alanine nonyl ester (NNANE) and



Scheme 1. Synthesis of NAAEs.

N-decanoyl-L-alanine decyl ester (NDADE) heating scans were performed between 10 and 65°C. A scan rate of 2.0°/min was used in all DSC experiments. For each sample three heating and two cooling scans were recorded. Except for NNANE and NDADE, all heating scans gave similar results; therefore, in all cases the first heating scan was considered for further analysis. Transition enthalpies (ΔH_t) were determined by integrating the peak area under the transition curve. Transition entropies (ΔS_t) were determined from the transition enthalpies assuming a first order transition according to the expression:[9]

$$\Delta H_t = T_t.\Delta S_t \quad (1)$$

where T_t is the transition temperature and the ΔH_t values were taken at this temperature in order to calculate ΔS_t values.

2.4 *Powder X-ray diffraction studies*

Powder X-ray diffraction patterns of NAAEs were recorded on a Bruker SMART D8 Advance powder X-ray diffractometer (Bruker-AXS, Karlsruhe, Germany) with Cu-Kα radiation at 40 kV and 30 mA. Fine powders of NAAEs, obtained by grinding with mortar and pestle, were placed on a circular rotating disk of the sample holder. Diffraction patterns were collected at room temperature (~25°C) using a LynxEye PSD data collector over a 2θ range of 1-50° with a step size of 0.0198° and a measuring time of 1.5s for each step.

3. Results and Discussion

A large number of amphiphiles derived from amino acids such as *N*-acyl amino acids bearing both saturated and unsaturated chains in acyl moiety (e.g., *N*-arachdonyl alanine, *N*-palmitoylglycine, *N*-oleoylserine) have been reported to be present in mammalian tissues.[10–12] In view of this, it is of interest to investigate their physicochemical properties and interaction with other membrane constituents. In previous work, we have synthesized and characterized the self assembly, supramolecular organization and thermotropic phase behavior of *N*-acylalanines and *N*-acylglycines.[7,13] While *N*-acylation of glycine and alanine yields anionic amphiphiles, esterification of these amino acids with long chain alcohols would give cationic amphiphiles which may find use in developing catanionic lipid based drug delivery systems. Therefore, we have also synthesized a homologous series of L-alanine alkyl esters and characterized them with respect to aggregation properties in aqueous dispersion, 3-dimensional structure as well as interaction with sodium dodecyl sulfate.[6] Interestingly, *N*-acyl amino acid esters such as *N*-acyl glycine esters and *N*-acyl serine esters were found to increase the permeability of stratum corneum to topically applied drugs, which increases the drug efficacy. In particular, *N*-acyl glycine ester with matched, saturated acyl and alkyl chains was reported to exhibit the highest effect in enhancing the permeability.[4] In view of this, in the present study we have synthesized a homologous series of *N*-acyl L-alanine alkyl esters with matched alkyl and acyl chains and characterized their phase transitions and structural properties in detail. The results obtained are presented below.

3.1 *Synthesis and characterization of NAAEs*

FTIR spectrum of *N*-decanoyl-L-alanine decyl ester (NDADE) is shown in figure S1, in Supplementary Information. The spectra obtained with other NAAEs were qualitatively very similar. IR spectra of NAAEs contained absorption bands due to ester carbonyl group at 1736-1747 cm^{-1}, amide linkage at 1638-1654 cm^{-1} and C-H stretching at 2843-2958 cm^{-1}. C-H scissoring and rocking bands were observed at 1468-1473 cm^{-1} and 717-723 cm^{-1}, respectively. The N-H stretching bands were observed at 3314-3320 cm^{-1}. The IR resonances obtained for the homologous series of NAAEs investigated here are listed in table S1.

^{1}H-NMR spectrum of *N*-decanoyl-L-alanine decyl ester (NDADE) is shown in figure S2. Since all the NAAEs investigated here are chemically very similar and differ only in the number of methylene units in the acyl/alkyl chain, their NMR spectra were almost identical except for the integration value of the peak corresponding to a part of the polymethylene moiety. ^{1}H NMR spectra of the NAAEs gave the following resonances: 0.869-0.903δ (6H, t), 1.256-1.281δ (nH, m), 1.391-1.413δ (3H, d), 1.59-1.65δ (4H, m), 2.172-2.22δ (2H, t), 4.13-4.161 δ (2H, t), 4.59-4.618δ (1H, q), 6.01-6.151δ (1H, d). These resonances are consistent with the structure of NAAEs. The ^{1}H NMR chemical shifts obtained for the NAAEs with matched chains are listed in table S2.

A ^{13}C NMR spectrum of NDADE is given in figure S3. Resonances corresponding to the *N*-acyl chain and *O*-alkyl chains are seen in the spectrum at: ~14.09δ (both terminal methyls), ~22.66δ (methylenes α to terminal methyls), 36.58δ (C-atom α to the amide carbonyl), 65.63δ (C-atom adjacent to the ester oxygen), and 4-5 closely spaced resonances of varying intensity between 25 and 32δ for the remaining methylene groups. Resonances of the alanine moiety are seen at ~47.97δ (chiral C-atom) and ~18.70δ

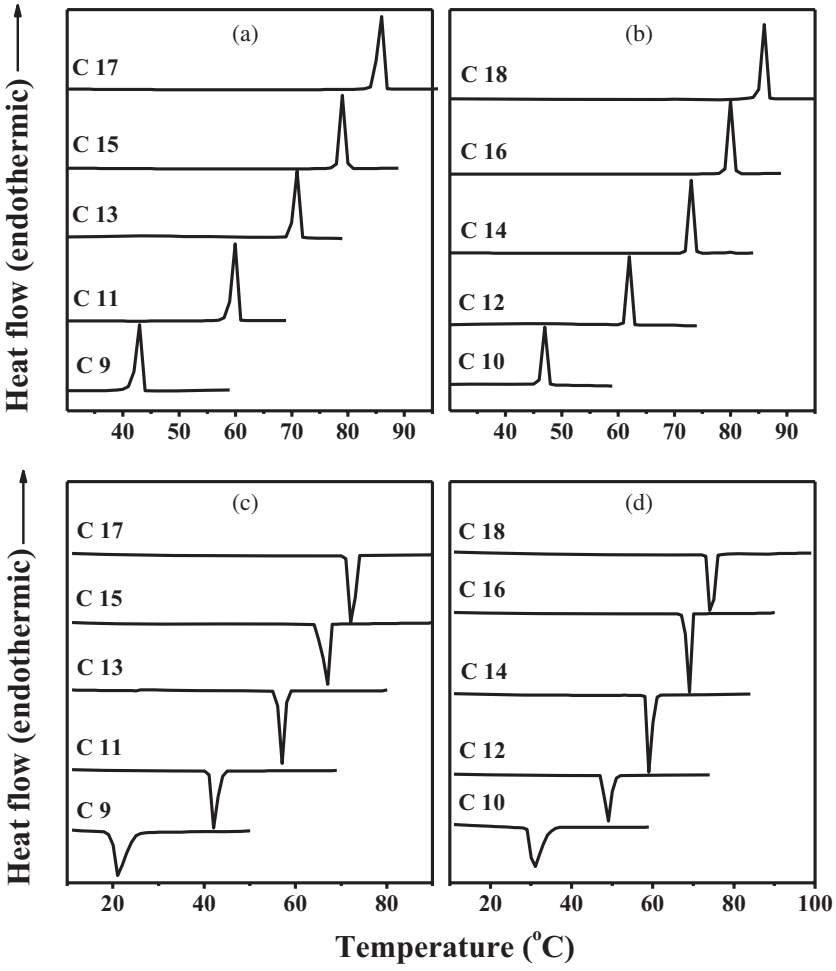


Figure 1. DSC heating (a, b) and cooling (c, d) thermograms of dry NAAEs with odd (a, c) and even (b, d) number of C-atoms in the acyl chain. The number of C-atoms is indicated against each thermogram.

(methyl group). The ester and amide carbonyl resonances are seen around 172.69δ and 173.35δ. The ^{13}C NMR spectra of other NAAEs were qualitatively very similar and were consistent with the expected structures. The ^{13}C NMR chemical shifts of different NAAEs are listed in table S3.

A high resolution mass spectrum of NDADE is presented in figure S4. The two most intense peaks seen at m/z = 384.3462 and 406.3296 match well with the molecular ion of the compound ($[M+H]^+$, calculated mass = 384.61) its sodium adduct ($[M+Na]^+$, calculated mass = 406.60). Other NAAEs with different matched acyl and alkyl chains also yielded essentially similar results and the mass spectrometric data for them are listed in table S4.

3.2 *Differential scanning calorimetry*

Heating thermograms of dry NAAEs with odd and even acyl chains are shown in figures 1a and 1b and the corresponding cooling thermograms are shown in figures 1c and 1d, respectively. This figure shows that both odd and even chain NAAEs exhibits single sharp transitions, which matched well with the capillary melting points of the compounds. When the same samples were subjected to second and third heating scans, except the compounds with 9 and 10 C-atoms in the matched chains, all heating scans gave similar results with minor decreases being observed in the transition enthalpies. Therefore, in all cases the first heating scan was considered for further analysis and the transition temperatures (T_t), enthalpies (ΔH_t) and entropies (ΔS_t) obtained from the first heating thermograms are presented in table 1.

3.3 *Chain length dependence of transition enthalpy and transition entropy*

The effect of varying the chain length on the transition enthalpies (ΔH_t) and transition entropies (ΔS_t) of dry

Table 1. Average values of transition temperatures (T_t), transition enthalpies (ΔH_t) and transition entropies (ΔS_t) of NAAEs in the dry state. Values in parentheses correspond to standard deviations from three independent measurements.

Acyl chain length (n)	Dry NAAEs		
	T_t (°C)	ΔH_t (kcal/mol)	ΔS_t(cal/mol/k)
9	43.4 (0.4)	12.43 (0.01)	39.3 (0.1)
10	47.9 (0.1)	13.33 (0.15)	41.5 (0.4)
11	59.9 (0.1)	16.28 (0.29)	48.9 (0.9)
12	62.8 (0.2)	17.68 (0.24)	52.8 (0.8)
13	71.2 (0.1)	20.88 (0.18)	60.6 (0.5)
14	72.9 (0.4)	21.33 (0.39)	61.7 (1.1)
15	79.5 (0.2)	24.14 (0.23)	68.5 (0.7)
16	80.6 (0.2)	25.01 (0.09)	70.7 (0.2)
17	85.7 (0.2)	28.59 (0.14)	79.7 (0.4)
18	86.2 (0.1)	28.46 (0.19)	78.8 (0.6)

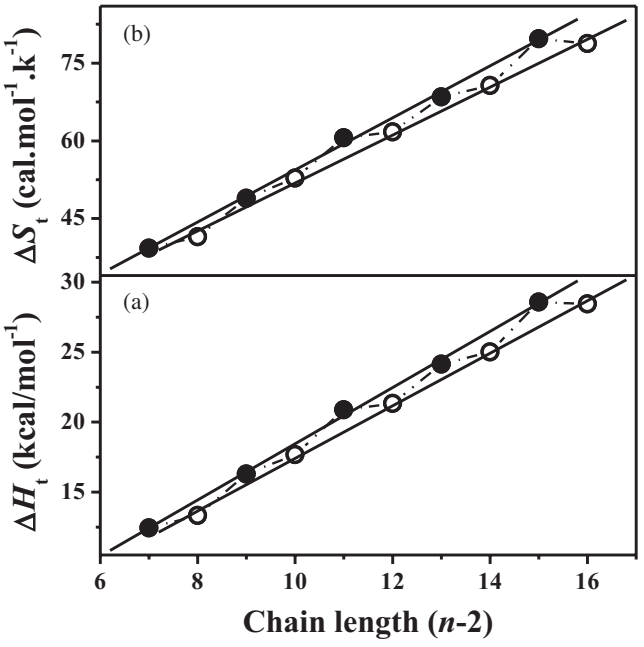


Figure 2. Chain length dependence of transition enthalpy (a) and transition entropy (b) of dry NAAEs. Values of ΔH_t and ΔS_t were plotted against the number of methylene (CH_2) units (n-2). Solid lines correspond to linear least squares fits of the data.

NAAEs is shown in figures 2a and 2b, respectively. It is seen that, both ΔH_t and ΔS_t exhibit an essentially linear dependence on the chain length independently for even and odd chain length series. However, when all the data of odd and even chain NAAEs are viewed together, a zig-zag pattern is seen in the enthalpy and entropy values. The ΔH_t and ΔS_t for the even chain length series are found to be slightly lower than those of odd chain length series, i.e., the calorimetric data exhibit odd-even alteration. This sort of odd-even alteration is a rare observation since in many other homologous

Table 2. Incremental values ($\Delta H_{inc}, \Delta S_{inc}$) of chain length dependence and end contributions ($\Delta H_o, \Delta S_o$) to phase transition enthalpy and entropy of NAAEs in the dry state. Values in parentheses correspond to fitting errors obtained from the least squares analysis.

Thermodynamic parameter	Dry NAAEs	
	Odd chain length	Even chain length
ΔH_{inc} (kcal/mol)	2.01 (0.05)	1.88 (0.05)
ΔH_o (kcal/mol)	−1.64 (0.59)	−1.39 (0.57)
ΔS_{inc} (cal/mol/K)	5.02 (0.15)	4.62 (0.16)
ΔS_o (cal/mol/K)	4.18 (1.67)	5.60 (2.02)

series such as long chain fatty acids, hydrocarbons, alcohols, *N*-acylethanolamines, *N*-acyldopamines, *N*-acylserotonins and *N*, *O*-diacylethanolamines values of thermodynamic parameters for the even chain-length series are higher as compared to those of the odd chain length series.[14–18] However, we recently reported this kind of odd-even alteration in a homologous series of *N*-acyl-L-alanines.[7] Similar results were observed earlier with phosphatidylcholines containing ω-tertiary-butyl fatty acyl chains and *dl*-methyl anteisobranched fatty acyl chains in the hydrated state.[19,20] The ΔH_t and ΔS_t data for odd and even acyl chain length NAAEs could be fit well to expressions (2) and (3) given below[14] as reported previously with *N*-acylethanolamines, *N*-acyldopamines, and *N*-acylserotonins with even and odd acyl chain lengths, *O*-acylcholines with even chainlengths as well as *N*-, *O*-diacylethanolamines with matched as well as mixed acyl chains.[15–18,21–23]

$$\Delta H_t = \Delta H_o + (n-2)\Delta H_{inc} \quad (2)$$

$$\Delta S_t = \Delta S_o + (n-2)\Delta S_{inc} \quad (3)$$

where ΔH_o and ΔS_o are the end contributions to ΔH_t and ΔS_t, respectively, arising from the terminal methyl group of the acyl/alkyl chain and the head group region. ΔH_{inc} and ΔS_{inc} are the incremental values of ΔH_t and ΔS_t per CH_2 group. Values of ΔH_{inc}, ΔS_{inc}, ΔH_o and ΔS_o corresponding to NAAEs with odd and even acyl chains, obtained from linear least squares analysis, are given in table 2.

When the transition enthalpies and transition entropies of NAAEs are compared with those corresponding to the single chain amphiphiles derived from L-alanine (*N*-acyl-L-alnines and L-alanine alkyl esters),[6,7] it is seen that NAAEs exhibit higher values of ΔH_t and ΔS_t for the solid to liquid transition, which could be attributed to the high molecular weight of the NAAEs. On the other hand, when compared to the *N*, *O*-diacylethanolamines (DAEs, which differ from the NAAEs

only in the head group region, due to the presence of the α-methyl group and the reversal of the connectivity between the ester carbonyl and oxygen) the corresponding values are lower, which could be due to the bulkier head group (due to methyl substitution), which could prevent tight packing of the NAAE molecules in the crystal lattice.

A linear chain length dependence of the ΔH_t and ΔS_t observed here for the dry NAAEs of odd and even chain lengths indicate the structures of NAAEs within each series are likely to be very similar in the solid state. Therefore, three dimensional structure determination of any one molecule in each series of NAAEs can give a good idea of the molecular packing and intermolecular interactions present in the crystal structure in each series. Odd-even alternation in the ΔH_t and ΔS_t can be explained based on differences in molecular packing between even and odd chain length compounds. When the long alkyl/acyl chains are tilted with respect to methyl end group planes then the van der Waals interactions between methyl groups from opposite layers are usually different for the even- and odd chain length series.[24,25] Therefore, odd-even alternation is clearly observed when the alkyl chain packing is inclined with respect to the methyl end planes, but not when they are packed perpendicular to the methyl end planes.[14,25] The odd-even alternation in the calorimetric properties of dry and hydrated NAAEs as observed here suggests that the acyl and/or alkyl chains are tilted with respect to the respective methyl end planes in them. In addition, our results suggest that the terminal methyl groups of opposing layers of the acyl and/or alkyl chains are more closely packed in the NAAEs with odd chain lengths as compared to those with even chain lengths.

3.4 *Chain length dependence of transition temperature*

A plot depicting the chain length dependence of transition temperatures of NAAEs in the dry state is given in figure 3. This plot shows a zig-zag pattern with the odd-chain length NAAEs displaying higher T_t values as compared to the even-chain length NAAEs, i.e., the T_t values also exhibit odd-even alternation. The T_t values increase in a smooth progression in each series but the increments between successive values in each series decrease with the increase in the chain length. Although it has been observed that compounds in the even-chain-length series exhibit higher values of the calorimetric properties (T_t, ΔH_t and ΔS_t) compared to the odd chain length molecules in many cases, it is quite interesting to note that for NAAEs, the odd-chain length series exhibits higher values of calorimetric properties as compared to the even-chain-length series as seen with *N*-acyl-L-alanines and L-alanine alkyl esters.[6,7]

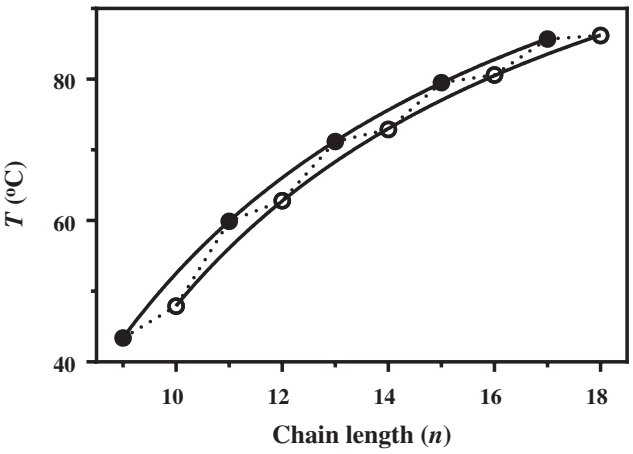


Figure 3. Chain length dependence of chain-melting phase transition temperatures of NAAEs in the dry state. Solid lines correspond to nonlinear least-squares fit of the transition temperatures to eq. 7.

As the acyl/alkyl chainlength increases, the end contributions towards the total enthalpy and entropy of the phase transition will be very small as compared to the contributions due to the polymethylene portion, such that the former are negligible in comparison. Therefore, at infinite acyl chain length, equations 2 and 3 can be reduced to equations (4) and (5):[14]

$$\Delta H_t = (n-2)\Delta H_{\text{inc}} \tag{4}$$

$$\Delta S_t = (n-2)\Delta S_{\text{inc}} \tag{5}$$

Then the transition temperature at infinite chain-length, T_t^{∞}, can be obtained from:

$$T_t^{\infty} = \Delta H_{\text{inc}}/\Delta S_{\text{inc}} \tag{6}$$

Incorporating the values of ΔH_{inc} and ΔS_{inc} from table 1 into eq. (6), the T_t^{∞} values for the NAAEs of odd and even acyl chain lengths have been estimated as 400.4 and 406.9 K, respectively.

For a variety of one-chain and two-chain amphiphiles/lipids, whose transition enthalpy and transition entropy exhibit linear dependence on the chain length, it has been shown that the ΔH_t and ΔS_t Values can be fit to the following equation:[26]

$$T_t = \Delta H_t/\Delta S_t = T_t^{\infty}\,[1-(n_o - n_o')/(n-n_o')] \tag{7}$$

where n_o ($=-\Delta H_o/\Delta H_{\text{inc}}$) and n_o'($=-\Delta S_o/\Delta S_{\text{inc}}$) are the values of n at which ΔH_t and ΔS_t extrapolate to zero. It can be seen from figure 3 that the T_t values of dry NAAEs containing even as well as odd number of C-atoms in the acyl/alkyl chains independently fit quite

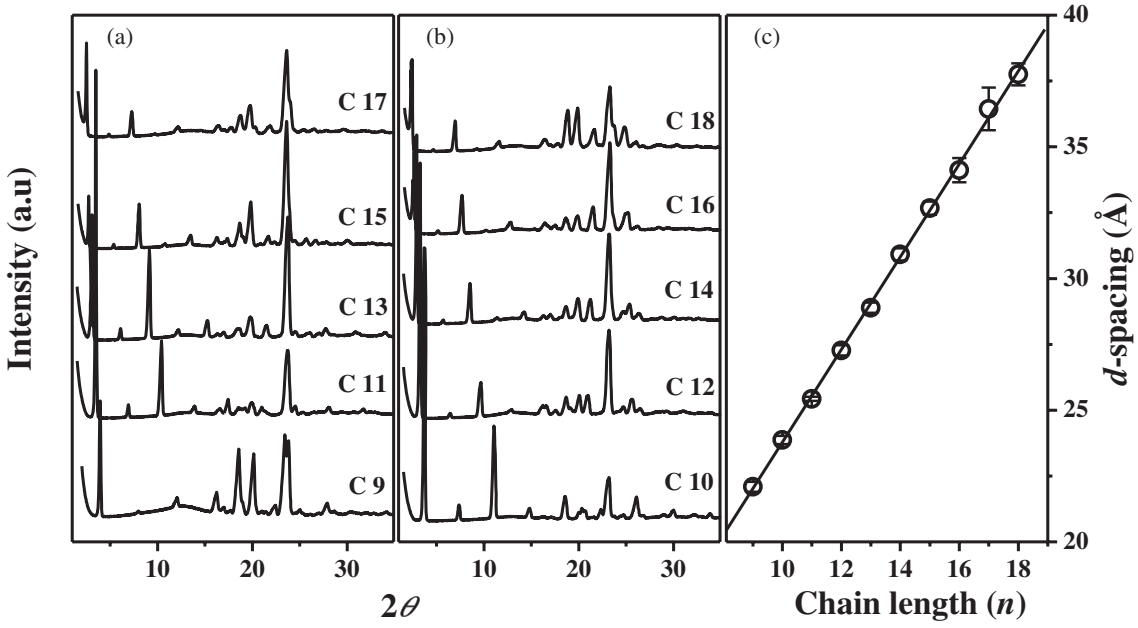


Figure 4. Powder X-ray diffraction patterns of *N*-acyl-L-alanine esters with different saturated acyl chains (a, b) and dependence of *d*-spacings on the chain length (c). The number of C-atoms in the saturated, unbranched acyl chain is indicated against each PXRD profile. The solid line in C represents a linear least squares fit of the data. The slope of this line yielded increase in the d-spacing per each additional CH_2 group as 0.88 Å.

well with eq. (7). In addition, the fitting parameters also yielded the T_t^{∞} values for NAAEs of both odd and even chain lengths in the dry state as 404.8 K and 402 K. These values are in good agreement with the T_t^{∞} values estimated using eq. (6), presented above.

3.5 *Powder X-ray diffraction*

Our attempts to obtain good quality single crystals of NAAEs using different solvents/combinations did not yield much success as we got only crystalline flakes, which showed very weak diffraction. Therefore, we carried out X-ray diffraction data on finely powdered samples in order to gather some insights on the structural aspects of NAAEs in the solid state. The PXRD data obtained for NAAEs of different chain lengths are shown in figure 4a, b (2θ range $= 1 - 30°$). All NAAEs (n=9-18) gave several sharp diffraction peaks. It was observed that the diffraction peaks move toward lower 2θ values with the increase in the length of the acyl/alkyl chains from 9 C-atoms to 18 C-atoms (3.95° to 2.39° for $n = 1$). In each case 4-5 peaks were used to calculate the *d*-spacings. The average *d*-spacing values obtained are given in table 3 and a plot of chain length dependence of the *d*-spacing is shown in figure 4c. The plot shows that the *d*-spacings exhibit a linear dependence on the acyl/alkyl chain length with a slope of 1.76 Å/CH_2, which corresponds to an increment of 0.88 Å per additional CH_2 moiety in each acyl chain.

Table 3. *d*-Spacing values of NAAEs determined from PXRD data.

Acyl chain length (n)	*d*-spacing (Å)
9	22.09 (0.19)
10	23.87 (0.16)
11	25.43 (0.08)
12	27.27 (0.19)
13	28.89 (0.19)
14	30.91 (0.23)
15	32.67 (0.25)
16	34.11 (0.46)
17	36.44 (0.81)
18	37.75 (0.42)

If the molecules were to pack in a normal bilayer format, with the acyl as well as alkyl chains oriented parallel to each other, then the incremental increase in the *d*-spacing corresponding to the addition of a CH_2 group in each chain would be 2.54 Å. This would then correspond to an increase in the *d*-spacing by 1.27 Å for each CH_2 group since there will be two molecules in the two leaflets of the bilayer. The increment in *d*-spacing observed for the NAAEs are 0.88 Å, which is significantly smaller than the above value, suggesting that the bilayer must be tilted similar to that seen in the structure of dimyristoylphosphatidylglycerol (DMPG) and cerebroside, in which the hydrocarbon chains are tilted with respect to the bilayer normal by 29° and

49°, respectively.[27,28] From the above slope the chain tilt angle with respect to the bilayer normal was calculated as 46.1°. This value is closer to the tilt angle observed in cerebroside and considerably higher than that found in DMPG.

An alternative packing arrangement for the NAAEs would be similar to thatobserved in *N*, *O*-diacylethanolamines with matched chains, which adopt a bent structure with the *O*-acyl chains being oriented at an angle with respect to the *N*-acyl chains.[16] In the supramolecular organization of DAE molecules in the crystal, the *N*-acyl and *O*-acyl chains face each other.[16] The present PXRD data on NAAEs would be consistent with both these packing arrangements and further studies are required to distinguish between them.

3.6 *Conclusions*

In the current work, we have synthesized and characterized a homologous series of *N*-acyl-L-alanine esters bearing saturated acyl and alkyl chains with matched chainlengths (n = 9-18). An unusual odd-even alternation was observed in the transition temperatures, enthalpies and entropies of NAAEs in the dry state with the ΔH_t and ΔS_t values corresponding to the odd-chain-length series being higher than those obtained for the even-chain-length compounds. Values of T_t, ΔH_t and ΔS_t for both odd- and even-chain-length series exhibit linear dependence on the acyl chain length, suggesting that the molecular packing and intermolecular interactions would be rather similar for all the NAAEs in each series. Powder X-ray diffraction studies suggest that all NAAEs with matched acyl and alkyl chains (n = 9-18) adopt either a tilted bilayer structure or a bent structure. These observations provide a thermodynamic and structural perspective for understanding the phase behavior of NAAEs and for investigating their interaction with other membrane lipids, which in turn can shed light on how these amphiphies can enhance the transdermal permeability of stratum corneum.

Supplementary Information

Representative FTIR, ^{1}H-NMR, ^{13}C-NMR and HRMS spectra of *N*-decanoyl-L-alanine decyl ester are given in Figures S1–S4. Corresponding spectral data for all NAAEs (n = 9-18) are given in tables S1–S4. Supplementary Information is available at www.ias.ac.in/chemsci.

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