Thermally Stimulated Depolarisation Current (TSDC) as a Novel Analytical Approach for the Study of Anhydrous Phospholipids

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INTRODUCTION

Phospholipids (PLs) are important biological compounds that have application in various industries. It is well known that the acyl chain length of PLs influences the crystal-to-liquid transition temperature (Tm) [1], which is an important property that influences uses of PLs.

Understanding the molecular dynamics of anhydrous PLs below the Tm can potentially shed light on their intrinsic behaviour in living systems and in industrial applications. TSDC is an important technique that can be used to obtain such information, by means of molecular relaxation processes, at temperatures below the Tm.

MEASUREMENT PRINCIPLE

TSDC measures movement of molecular dipoles as a function of temperature, in response to the application of a static electrical field (Fig.1). The sample is placed between two parallel electrodes and polarised (1) using static electrical field at a temperature (T_p). This is followed by quench cooling to a temperature well below the polarisation temperature (T_0) at which point oriented dipoles are "frozen in" (2). The electrical field is short circuited and the sample is heated to a final temperature (T_f) $>T_p$. Dipoles move back during linear heating generating a small current that is measured using a sensitive ammeter, which contains information about molecular mobility. The complex dipole relaxation current in TSDC output can be deconvoluted into discrete elementary relaxation modes by the application of thermal windowing (TW) experiments. For TW step (2) is modified so that the sample is initially cooled just below T_p and held isothermal with the electrical field switched off, before cooling to T_0. From TW results, the distribution of relaxation times (τ) is obtained and kinetic information e.g. the Activation energy (Ea) which is used to characterise molecular relaxation behaviour [2].
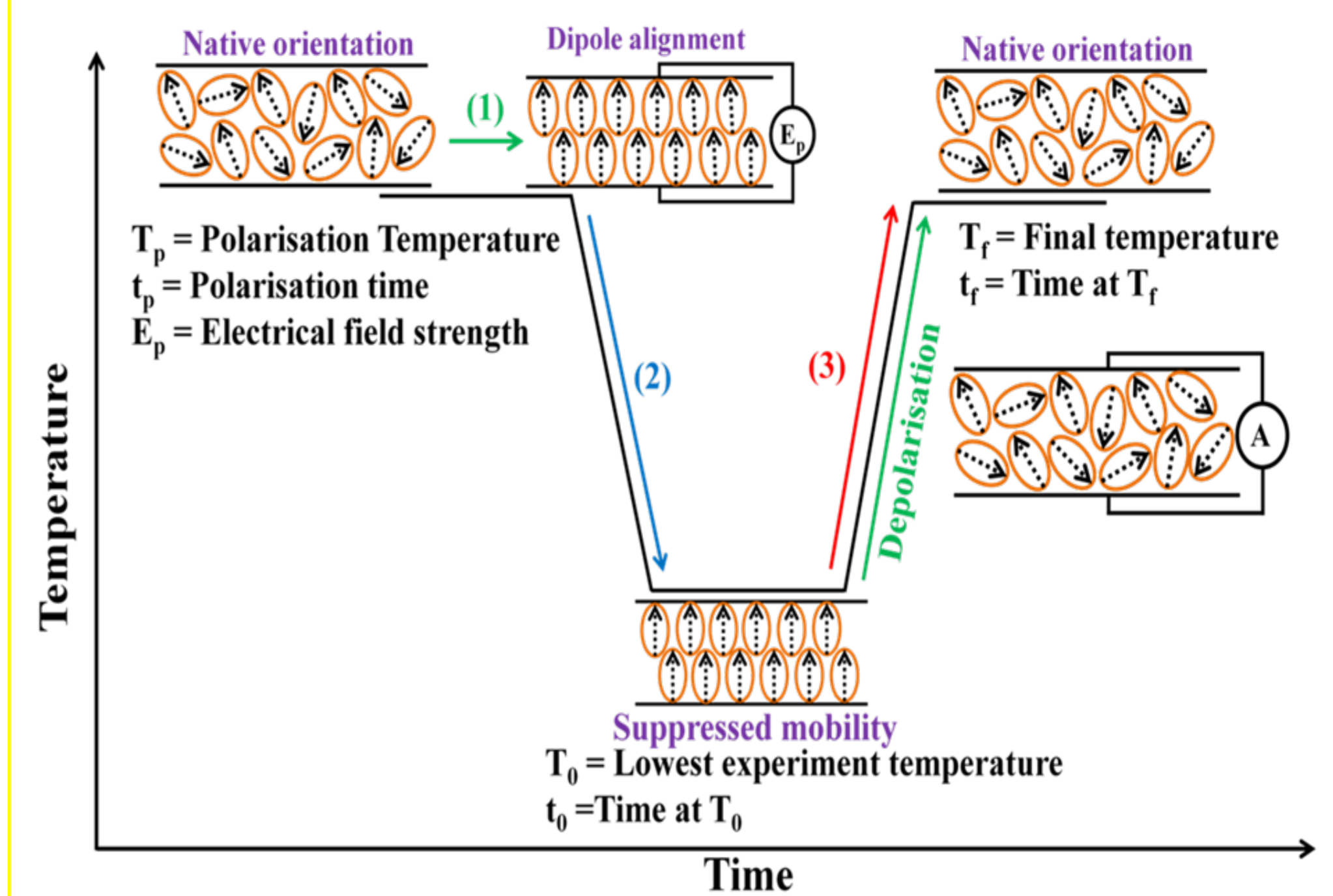

Fig.1. Schematic of the response of molecules during a typical TSDC experiment.

MATERIALS and METHODS

The phosphatidylcholines (PCs) 1,2-dilauryl-sn-glycoro-3-phosphocholine (DLPC), 1,2,-dimyristol-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) of >99% were kindly provided by Lipoid (Germany), and dehydrated in-situ (Dehydration was confirmed by thermogravimetric analysis (TGA). Samples are placed between two parallel electrodes and polarised at 90°C for 2 minutes using 350 V/mm in the TSDC experiments covering the temperature -25 to 100°C. For TW experiments conditions were Ep = 400 V/mm at Tp of -20 to 60°C in increments of 3°C (Tw = 3°C, tw = 2 min).

RESULTS and DISCUSSION

The global TSDC curves (Fig.2 (a)) contain information of the overall molecular relaxation process which is indicative of molecular mobility. The results demonstrate, for the first time, that anhydrous PCs undergo significant molecular mobility at temperatures below their Tm. The temperature of relaxation current maxima was found to increase with increasing carbon chain length (Fig. 2 (b)). This shows that increasing carbon chain length requires an increase in heat energy to initiate molecular motions in PCs. Both, molecular mobility below Tm and Tm for series of PCs show correlation between measured values (Tmax in TSDC and TM in DSC) implying that carbon chain length plays important role in structural arrangements, flexibility and mobility of PCs molecules (Table 1).
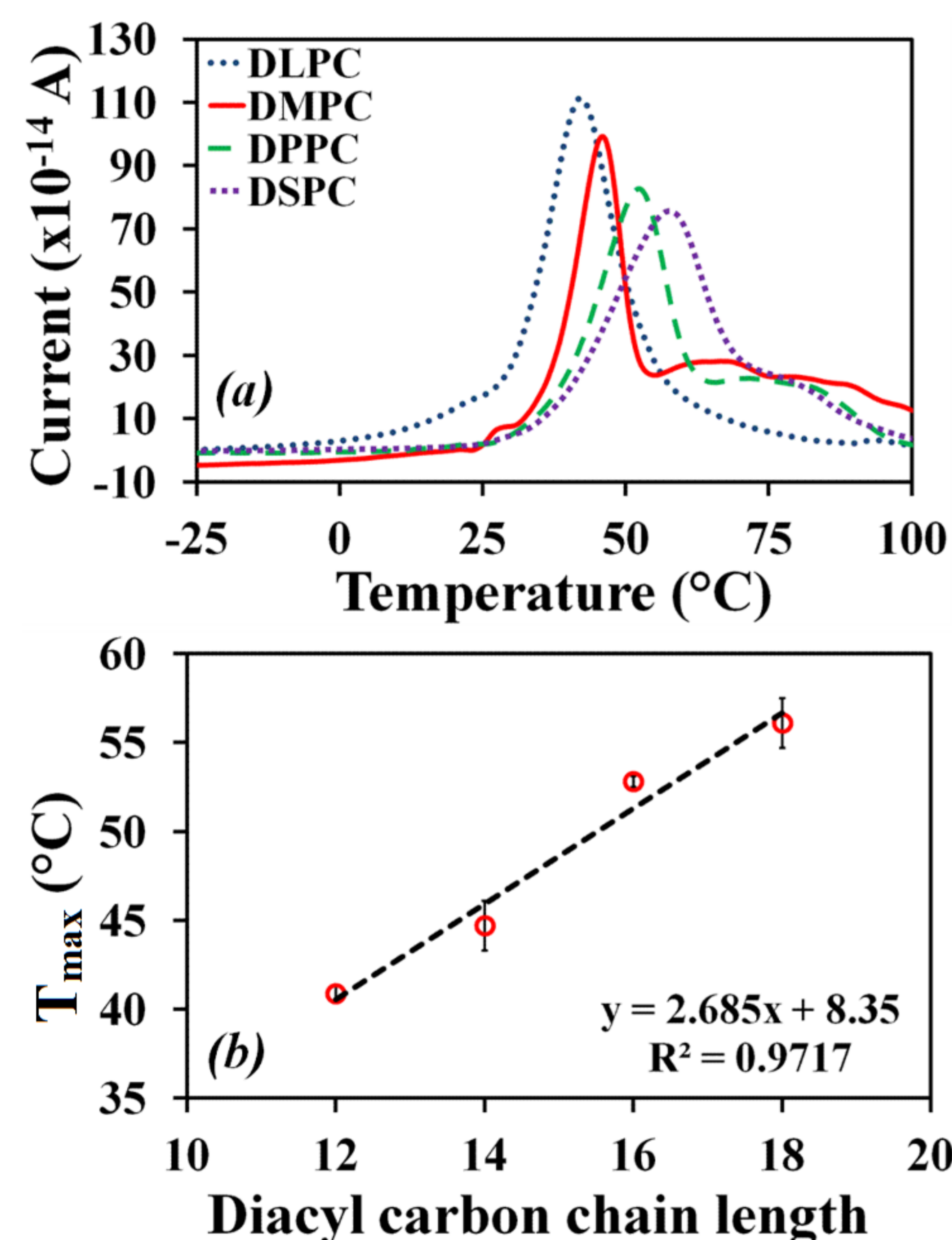

Fig. 2. Graphs showing (a) overlay of the global TSDC signal obtained for the PCs polarized at 90°C and (b) plot of relaxation current T_{max} against diacyl chain length.
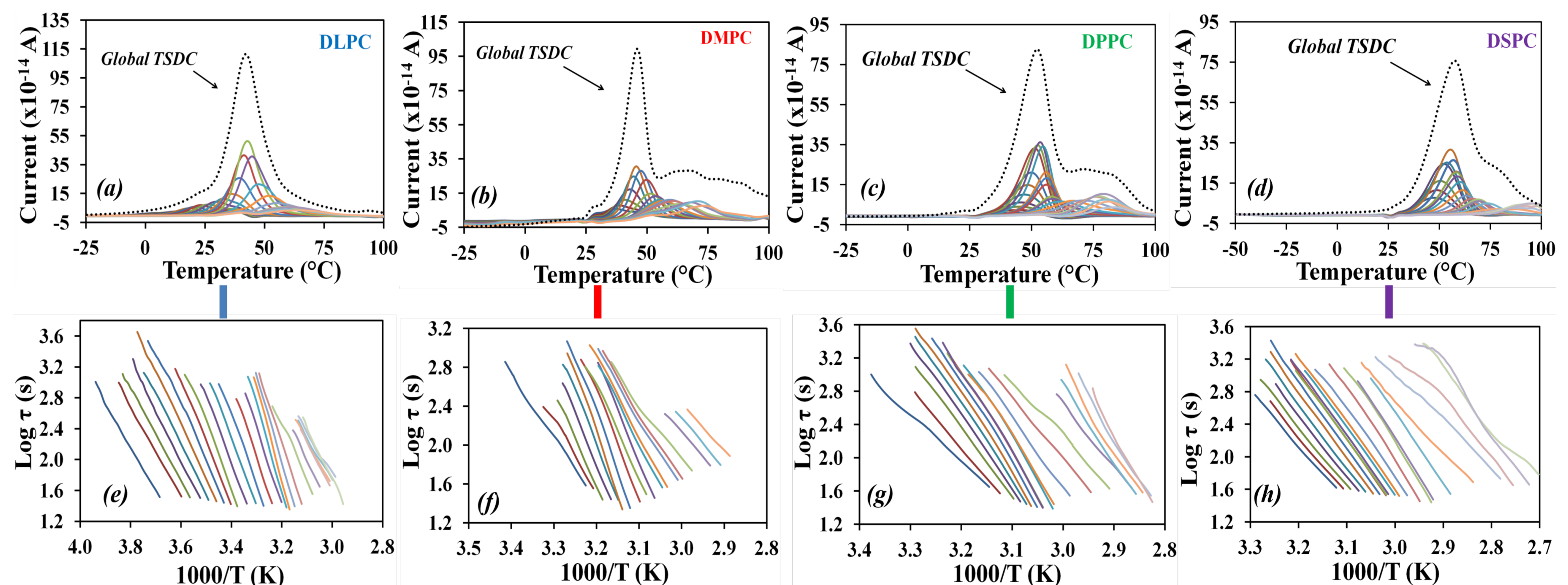

Fig. 3. Graphs of TW result showing discrete relaxation modes under the global TSDC signals for each PC (a-d) with their respective distribution of relaxation times (e-h).

The TW results (Fig. 3a-d) shows several discrete relaxation processes within the global TSDC curve. The respective distribution of relaxation times for the isolated processes (Fig. 3e-h) are generally straight and were fitted with the Arrhenius equation. The relaxation time (τ) and E_a of the isolated relaxation process with the highest current generated is used to compare the molecular mobility characteristics of the PCs (Table 1).

Relaxation time for the PCs in general increases with increasing carbon chain, which implies a more sluggish molecular mobility as you increase carbon chain length, that is due to increased hydrophobic interaction and viscosity. On the other hand, activation energy of molecular relaxation decreases with increasing carbon chain length. This observation is attributed to difference in the degree of ordering in the materials. PCs in the solid-state are semi-crystalline (confirmed by XRPD) and it is likely that PCs with longer carbon chain length will have greater state of mobility of their chains involved in formation of an amorphous fraction.

Table 1. Values obtained for Tm by DSC, T_{max}, relaxation times (τ) and activation energy (E_a) obtained from TSDC and TW experiments(n=3).

Compound	Diacyl carbon chain length	DSC Tm (°C)	T_{max} (°C)	τ (s)	E_a (kJ/mol)
DLPC	12	91	41 ± 2	21.1 ± 2.0	193 ± 38
DMPC	14	101	47 ± 2	19.3 ± 3.5	265 ± 33
DPPC	16	106	57 ± 2	25.0 ± 0.8	193 ± 8
DSPC	18	109	67 ± 3	32.8 ± 4.1	152 ± 8

CONCLUSIONS

The application of TSDC has provided novel information pertaining to anhydrous PCs below their Tm. This study has demonstrated that significant molecular mobility exist below the Tm and the temperature at which mobility occurs increases with carbon chain length. Relaxation rate is also more sluggish for PCs with longer carbon chain length. These offer some explanations as to why increasing carbon chain length of PCs increasing their physical stability. TSDC therefore enhances capabilities in understand physical stability of materials by analysing relaxation processes in materials that occur at temperatures well below any structural change.

REFERNCES

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