Molecular dynamics in pharmaceutical drug delivery systems — The potential of QENS and first experimental results

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ABSTRACT

In this contribution the potential of quasi–elastic neutron scattering (QENS) for studying molecular mobilities in complex colloidal pharmaceutical systems will be discussed. First experimental results from investigations on nanodispersions of supercooled melts and the dynamics of phospholipids used as stabilizer in lipid nanodispersions are presented. The measurements were performed at the time–of–flight spectrometer TOFTOF at FRM II. It could be demonstrated that it is possible to measure diffusive as well as internal motions of molecules inside nanoscaled droplets of aqueous pharmaceutical emulsions with only 5 weight% of the disperse phase. Furthermore it was possible to observe changes of the dynamics of phospholipid molecules, which form the stabilizer monolayer of a lipid emulsion, in dependence on temperature, phase transitions of the phospholipid and the addition of co–emulsifiers. This is remarkable in view of the fact that the total amount of phospholipid in the neutron beam could not be increased above 20 mg. The TOFTOF instrument seems to be the best suited spectrometer for such kind of QENS investigations due to its high neutron flux, good resolution and excellent signal to background ratio.

INTRODUCTION

Aqueous nanodispersions of liquid, liquid crystalline and solid lipids are under investigation as drug delivery systems for numerous therapeutic applications, such as intravenous administration of poorly water soluble drugs [1,2,3]. The reason for using a drug carrier is most often to reduce side effects of drugs, to modify the drug release kinetics or to deliver the drug to a specific body site (drug targeting). The mobility of the drug molecules in the drug carrier and their interface cross over rates are of particular interest in order to understand the drug release behaviour of such systems. The dynamics of the carrier and especially of the stabilizer (emulsifier) molecules should also influence drug release rates as well as physico–chemical properties of the pharmaceutical formulation such as recrystallization tendency (stability) of nanoscaled supercooled melts or the phase behaviour of lipid nanoparticles.

In contrast to a large number of publications on microscopic and mesoscopic structural investigations [4–9] only a few investigations on the molecular dynamics of lipid drug delivery systems have been published so far. This might be due to the lack of experimental methods to investigate the interesting molecular dynamics within the highly complex native systems. PFG–NMR–spectroscopy can not be used to determine e.g. the self–diffusion constant of molecules inside nanoscaled dispersed particles because of the spatially restricted diffusion inside a nanoparticle, whereas relaxation time measurements by ^{13}C–NMR should reveal information on the molecular dynamics. From PFG–NMR–spectroscopy the diffusion constant of the particles can, however, be obtained as discussed briefly below.

In this article we intend to demonstrate the high potential of QENS for the determination of dynamic properties of special molecules of interest in the mentioned colloidal pharmaceutical dispersions. According to first experimental investigations the discussion will be focused on two exemplary topics: The dynamics of coenzyme Q_{10} molecules in aqueous (D_2O) Q_{10} nanoemulsions and the behaviour of phospholipid molecules inside a monolayer stabilizing nanodroplets of hexadecane–d34 in D_2O. The first example has been selected because it is not understood so far why Q_{10}–nanodroplets exhibit such extremely high supercooling. In fact it is hard to crystallize the droplets at all. Phospholipids are used as stabilizers in many pharmaceutical dispersion formulations. But the stabilization mechanism especially during formation and crystallization of the particles, respectively, is not well understood. For both topics information about the molecular dynamics should lead to a better understanding of the observed effects.

EXPERIMENTAL DETAILS

The Q_{10}–dispersion (Q10D) was prepared by high–pressure homogenization of a pre–dispersion of the molten Q_{10} (10 weight%, Kyowa Hakko Kogyo) and D_2O (Euriso–top) containing phospholipid Lipoid S100 (1.6 %, Lipoid) and sodium glycocholate (0.4 %, Sigma) as emulsifiers. For the production of the pre–dispersion the S100 was allowed to swell in the aqueous phase for some hours and subsequently merged with the Q_{10} melt at 70 °C and stirred using an ultra turrax (IKA-Werke) at 10.000 rpm for 5 min. This pre–emulsion was successively homogenized at 2000 bar using a APV–2000 (APV Products, Denmark) homogenizer. The homogenizer was equipped with a special small volume sample inlet cylinder and outlet line such that a 35 ml sample could be cycled continuously without mixing the outcoming 'product' and the less often cycled educt in the inlet cylinder. The whole homogenizer valve including the inlet and outlet equipment was equilibrated at 60 °C before the homogenizing process by an external heating band. A cycle time of 2 min was applied which corresponds to cycling the whole product about ten times. The product was allowed to equilibrate in a sealed vial at room temperature. The corresponding reference sample (Q10Ref) was prepared by the same procedure but contained only the two emulsifiers and D_2O. The particle size was analyzed using photon correlation spectroscopy (PCS, ALV, laser wavelength: 632 nm). The average radii (z–averages) for all Q_{10}–dispersions were in the range from 60 nm to 120 nm. The dispersions were strongly diluted with purified water for the PCS measurements in order to operate the detecting photo multipliers in their linear range. Thus the measurements were not influenced by particle–particle interactions.

Three hexadecane–d34 (HD) nanoemulsions were prepared analogously to the Q10D sam-

ple. These dispersions contained only 0.5 % HD (Euriso–top) and either 0.2 % phospholipid or a mixture of 0.16 % phospholipid with 0.04 % sodium glycocholate (SGC, Sigma). As phospholipid either dimyristoylphosphatidylcholine (DMPC, Lipoid) or S100 (Lipoid) was used. The DMPC was allowed to swell over night in the aqueous phase at 40 °C before the preparation of the pre–emulsion. The homogenized emulsions labeled HD/DMPC/SGC (PCS z–average: 31 nm), HD/S100 (56 nm) and HD/S100/SGC (38 nm) were concentrated to 5 % of HD using a stirred cell (Millipore) with Isopore membrane filters (50 nm, VMPT, Millipore). The exact concentration was adjusted using the filtrate. The reference sample (HDRef, PCS z–average: 36 nm) was prepared by ultrasonic homogenization (Sonopuls HD 2070/MS73, Bandelin, 50 °C, 3 min, 10 % power) of a mixture of 5 % HD and 2 % sodium dodecylsulfate–d25 (SDS, Euriso–top) in D_2O. The HD/DMPC sample (5 % hexadecane, 2 % DMPC, PCS z–average: 61 nm) was prepared according to the HDRef sample but with an homogenization time of 18 min.

The QENS experiments were performed at the multichopper time-of-flight spectrometer TOFTOF at FRM II. The selected incident neutron wavelength of 6 Å and chopper speed of 16000 rpm lead to an instrument resolution of about 35 μeV. Typical measuring times were 8 h per spectrum for the HD–samples and 2 h per spectrum for the Q_{10} samples. Aluminium sample holders providing a hollow cylindrical sample volume with an outer diameter of 22.5 mm and a wall thickness of 0.2 mm were used. The wall thickness of the inner aluminium cylinder was 0.5 mm and the one of the outer cylinder was 0.25 mm. The sample holders had a height of about 60 mm. The width of the cylindrical sample was fully illuminated by the primary neutron beam but only about 50 mm of the sample height was exposed to the beam.

DSC measurements were carried out for the HD–samples using a VP-DSC MicroCalorimeter (MicroCal, Northampton, USA). For the measurement 0.2 ml of the respective dispersion were diluted with 0.45 ml D_2O. Pure D_2O was used as reference substance. After equilibration for 20 min at 10 °C, the cell was heated to 60 °C at a heating rate of 0.5 °C/min. Although the melting point of HD is about 18 °C no crystallization of the nanodispersed HD was observed at 10 °C.

NMR diffusion measurements of 0.5 ml of the Q10D sample were carried out with the pulse field gradient (PFG) double stimulated echo sequence with longitudinal eddy current delay (LED), compensating for possible convection effects [11]. Experiments were performed on a Bruker (Karlsruhe, Germany) DMX NMR Spectrometer operating at 600 MHz ^{1}H frequency, equipped with a TXI (^{1}H,^{13}C,^{15}N) probe with xyz gradients (maximum gradient strength $g \sim 55$ G/cm). Gradient length for the encoding/decoding gradients was 5 ms, with 32 steps for g. Diffusion time was set to 400 ms or 1 s for Q10D measurements, with a relaxation delay of 20 s between scans. Calibration of the gradient strength was performed on a H_2O sample with a diffusion time of 50 ms, 16 steps and a relaxation delay of 5 s. Evaluation was performed by a non-linear curve fitting of the experimental values to the Gaussian decay function, using the variable gradient function of the Topspin 1.3 software package (Bruker, Karlsruhe, Germany).

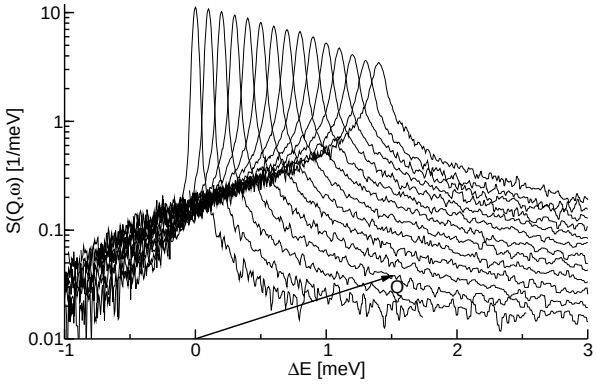

Figure 1: Scattering curves of sample Q10D at 80°C. The corresponding Q–values range from 0.4 Å^{-1} to 1.8 Å^{-1} (bottom to top) in steps of 0.1 Å^{-1}.

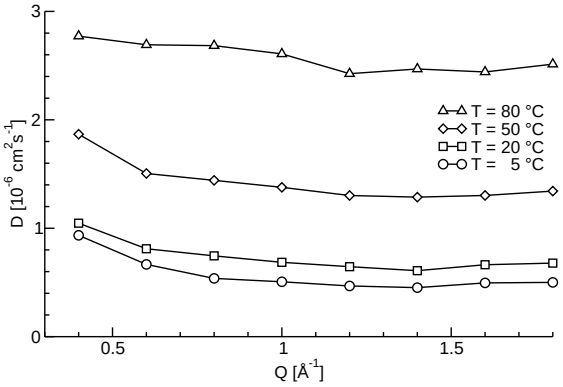

Figure 2: Diffusion constants of Q_{10}–molecules inside nanosized droplets at different temperatures. The data points were estimated from QENS measurements and are approximately Q–independent.

RESULTS AND DISCUSSION

Q_{10}–nanodispersions

QENS–spectra of a Q_{10}–nanodispersion were recorded for temperatures above and below the melting point of polycrystalline Q_{10} at about 47 °C. The Q_{10} nanoparticles do not crystallize at the lower temperatures but stay in a supercooled state. Fig. 1 shows the QENS–spectra of a Q_{10}–nanodispersion exemplarily at 80 °C, for different Q–values. The data was analyzed using a scattering function $S_{inc}(Q,\omega)$ with two Lorentzian functions, which represents a simplified model describing long range diffusion and a local motion of the Q_{10}–molecules inside the nanodroplets (e.g. molecular rotation):

$$S_{inc}(Q,\omega) = F(Q)\left(\frac{A_0(Q)}{\pi}\frac{DQ^2}{\omega^2+(DQ^2)^2} + \frac{1-A_0(Q)}{\pi}\frac{\Gamma(Q)}{\omega^2+\Gamma^2(Q)}\right). \quad (1)$$

The self diffusion constants D of the Q_{10}–molecules at different temperatures were derived from least squares fits of this function to the QENS data. The D–values are plotted as a function of Q in Fig. 2. Besides D the pre–factor $F(Q)$, the elastic incoherent structure factor $A_0(Q)$ and the half width of the second Lorentzian $\Gamma(Q)$ were allowed to be varied by the fit routine. Although the data could be quite well be approximated by the fit function a physically meaningful interpretation of the Q–dependence of the $\Gamma(Q)$–values could not be found.

It was found, however, while testing several other models that the evaluated D–values are almost independent of the particular model used for the local motion, which is described in the case of the simple model in eqn. 1 by the second Lorentzian. Therefore the determination of self diffusion constants of molecules inside colloidal particles by QENS is obviously possible even if the local molecular dynamics are not fully understood.

Nevertheless it should be possible to learn more about the molecular dynamics in nanodroplets besides the diffusion constant. With the introduction of a simple component to

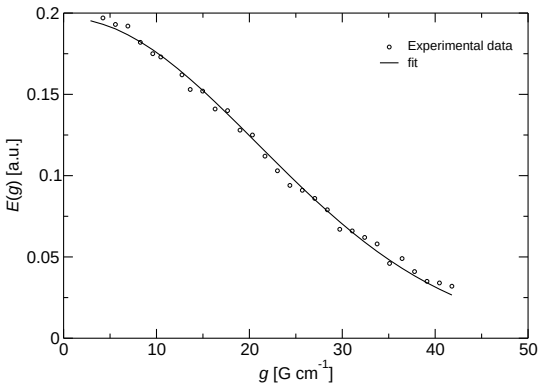

Figure 3: Spin echo intensity E as a function of the gradient strength g. The solid curve represents a least–squares fit of the Stejskal–Tanner equation $E(g) = E(0)\exp\left(-\gamma^2\delta^2 g^2 D(\Delta - \delta/3)\right)$ [12] to the data.

the model as e.g. molecular rotation, hydrocarbon chain motion or CH_3–group rotation it was not possible to come to convincing results. It thus seems that the scattering curves are influenced significantly by different dynamical processes simultaneously. In order to limit the number of free fit parameters it is necessary to obtain additional information about the dispersions from other measurements.

From a PFG–NMR investigation of a Q_{10}–nanodispersion the averaged diffusion constant of the nanodroplets inside the dispersion medium at 20 °C could be evaluated to be $4 \cdot 10^{-8}\,\mathrm{cm}^2/\mathrm{s}$ (cf. Fig. 3, [13]). The diffusion of the Q_{10}–molecules inside the droplets cannot be measured by this method because of the limited droplet size, which is much smaller than the spatial resolution of the spectrometer. The deduced diffusion constant is in perfect agreement with values estimated from the PCS measurements of $(4 \pm 1) \cdot 10^{-8}\,\mathrm{cm}^2/\mathrm{s}$. With respect to QENS measurements this diffusion would broaden the elastic line less than 2 μeV even for Q–values as large as $Q = 1.8$ Å^{-1}. Therefore it can be concluded that the diffusion of the nanoparticles is too slow to be detected by our measurements in which an elastic line width of about 35 μeV was used. That also means that this motion does not disturb the analysis of the QENS–data described above.

In order to get more information on the local motions, the rotation of the CH_3–groups of the Q_{10}–molecules in the nanodroplets was studied at low temperatures at which all other molecular motions should essentially be frozen. The corresponding data some of which are exemplarily displayed in Fig. 4 were analyzed using a model for uniaxial 3–fold jump rotation with logarithmic distributed relaxation times [14]:

$$S_{inc}(Q,\omega) = F(Q)[(1 - c_{fix})S_{meth}(Q,\omega) + c_{fix}\delta(\omega)] \tag{2}$$

$$S_{meth}(Q,\omega) = A_0(Q)\delta(\omega) + [1 - A_0(Q)]\frac{1}{\pi}\sum_{i=0}^{20} g_i \frac{\Gamma_i}{\Gamma_i^2 + \omega^2}$$

$$g_i = \frac{1}{\sqrt{2\pi}\sigma} e^{-\frac{1}{2\sigma^2}\ln^2\left(\frac{\Gamma_i}{\Gamma_0}\right)} \qquad \ln\frac{\Gamma_i}{\Gamma_0} = \frac{6\sigma}{20}i - 3\sigma$$

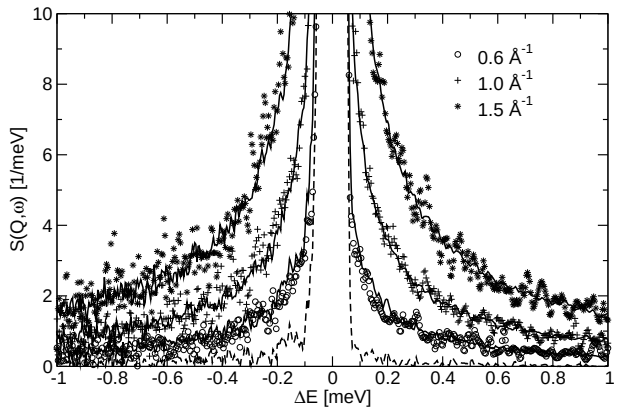

Figure 4: QENS spectra of sample Q10D at 150 K for 3 different momentum transfers Q. The dispersion was quenched in liquid nitrogen before inserting it into the pre–cooled closed cycle cryostat of the TOFTOF spectrometer. Therefore the Q_{10}–nanoparticles are in a glassy state, which is confirmed by the absence of Bragg reflections. The data were corrected for detector efficiency and absorption. The solid lines represent fits of function 2 to the data (symbols), which were numerically convoluted with the resolution function of the spectrometer determined by a 5 K measurement of the sample (dashed line). The maximum elastic intensity was normalized to a value of 1000 for all experimental curves.

$$A_0(Q) = \frac{1}{3}\left[1 + 2j_0\left(Qr\right)\right] \qquad \sum_{i=0}^{20} g_i = 1.$$

c_{fix} is the ratio of non–methyl group hydrogen atoms to the total amount of hydrogen atoms in the Q_{10}-molecule. The distribution of the CH_3–group rotation dynamics is discretized by 21 Lorentzians with the half widths Γ_i and the intensity weights g_i. For the jump rate a logarithmic Gaussian distribution with Γ_0 (most probable jump rate) and σ^2 (variance of the distribution) is used. Γ_0 is related to the correlation time τ, which is defined as the mean residence time between two consecutive infinite fast jumps, by $\Gamma_0 = 3/(2\tau)$. j_0 represents the zeroth–order Bessel function and r is the H–H distance in the CH_3–group.

QENS spectra of measurements at five temperatures between 50 K and 250 K exhibit quasi–elastic components which were assigned to methyl group dynamics. Even at 50 K the quasi–elastic component could clearly be separated from the resolution function. The estimated values of Γ_0, σ and τ are listed in Tab. 1. The small value of Γ_0 at 250 K might be due to a partial recrystallization of the dispersed Q_{10}. So far the number of measured temperatures is too low to allow an extrapolation of the τ–values to room temperature and above. It is intended to use such an extrapolation to estimate the contribution of the CH_3–group rotation to $S_{inc}(Q,\omega)$ at these temperatures.

Phospholipid dynamics in monolayers

In Fig. 5 the neutron scattering spectra of DMPC and S100 in the monolayer of n–hexadecane–d34 nanoemulsions are displayed. The scattering of the aqueous phase and of the interior of the HD–droplets were removed by the subtraction of the scattering curves of the

Table 1: Parameters estimated by least squares fits of function 2 to QENS spectra of sample Q10D at different temperatures.

T [K]	Γ_0 [meV]	τ [ps]	σ
50	0.015	66	4
100	0.09	11	3.7
150	0.54	1.8	2.6
200	0.78	1.3	1.3
250	0.61	1.6	1.4

reference sample. Therefore the observed effects can essentially be attributed to the dynamics of the emulsifier molecules, which are predominantly situated in the interface between the droplets and the continuous phase. The scattering contribution of the co–emulsifiers is small and should yield not more than 20 % compared to that of the phospholipids. It is thus assumed in the following discussion that the observed spectra are dominated by the dynamics of the phospholipid molecules.

The large elastic intensity of the spectra of the HD/DMPC sample indicates that the DMPC molecules are less mobile than the phospholipid molecules of S100. It must be mentioned here that this is a preliminary result because it can not be excluded that this effect is influenced by differences of the sample preparation (cf. section EXPERIMENTAL DETAILS). Interestingly the addition of SGC seems to increase the mobility of the DMPC molecules. An increased mobility of the surfactant molecules is held responsible for a better stabilization of the dispersion, which could in fact be observed for similar dispersions during and after the crystallization of their liquid nanodroplets [6].

Furthermore, the temperature dependence on the elastic scattering intensity is stronger for the samples containing DMPC compared to the corresponding samples containing S100 as the phospholipid component. This effect, which is particularly obvious for the temperature step from 20°C to 30°C can be attributed to the gel/liquid–crystalline transition of the DMPC monolayer. For S100 this transition is shifted to temperatures far below the investigated temperature range and the temperature effect in these samples is therefore limited to the temperature dependence of the Debye–Waller factor.

A detailed discussion of the phase behaviour of DMPC in the investigated dispersions is beyond the scope of this article. According to the DSC–curves presented in Fig. 6 it can be assumed that the peaks of the transitions of DMPC are sharpened and shifted to higher temperatures in the HD/DMPC–sample and strongly broadened and shifted to lower temperatures for the HD/DMPC/SGC sample. As expected all other samples exhibit no or only minor thermal effects.

Due to the poor counting statistics a quantitative analysis of the QENS–signal is not yet possible. It can, however, clearly be seen that the reduction of the elastic intensity due to the addition of SGC to the DMPC sample is accompanied with an increasing quasi–elastic intensity, which can be explained by the enhanced DMPC mobility. On the other hand the increase in molecular mobility with temperature is not reflected by higher quasi–elastic intensity for any of the samples. This effect is not yet understood but might be due to a quite fast molecular dynamics resulting in a very broad quasi-elastic component, which could

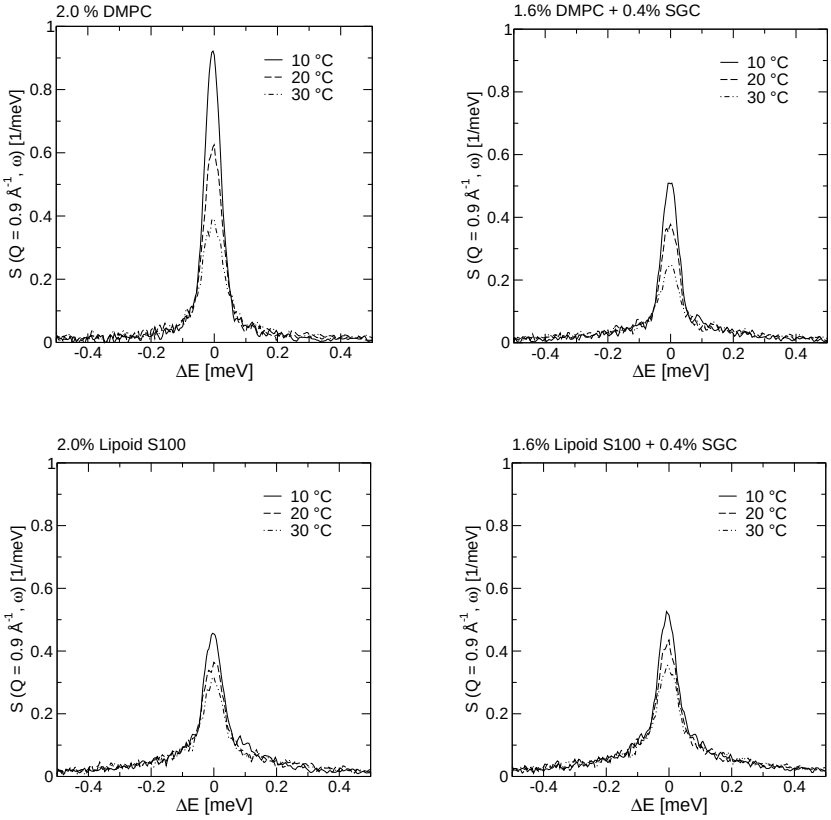

Figure 5: Neutron scattering spectra of DMPC and S100 in the monolayer of n–hexadecane–d34 nanoemulsions (samples HD/DMPC, HD/DMPC/SGC, HD/S100 and HD/S100/SGC). The displayed data represent spectra of the samples after subtraction of the spectra of the fully deuterated reference sample HDRef.

not be detected because of the poor counting statistics.

CONCLUSIONS

It could be demonstrated, that using quasi–elastic neutron spectroscopy it is possible to investigate the dynamics of special molecules of interest inside a complex pharmaceutical colloidal dispersion. By this method long range diffusional and local molecular motions can easily be distinguished, and the mechanism of the diffusion process or the type of intermolecular motion can be investigated.

The diffusion constants of the Q_{10}–molecules inside the nanosized droplets of a dispersion could be determined. Furthermore it was possible to observe internal molecular motions as e.g. CH_3–group rotation. From the methodical point of view this is remarkable, if one respects, that the Q_{10} concentration in the sample was only 5 % which produced about 50 % of the total scattering intensity.

An even worse intensity ratio of about 20 % is achieved from the phospholipid molecules in the stabilizing monolayer of hexadecane nanodroplets dispersed in D_2O where only 20 mg (2 weight%) of the phospholipid were in the neutron beam. Nevertheless it was possible to

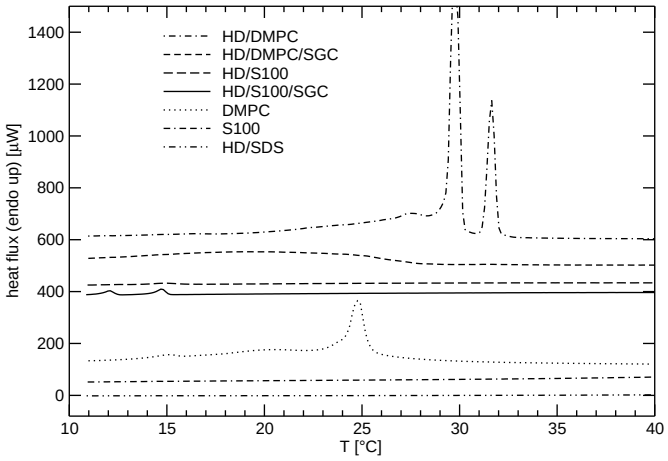

Figure 6: DSC curves of the investigated samples. The DMPC and S100 samples were prepared by ultrasonic homogenization of the swollen phospholipid (5 %) in D_2O for 5 min.

detect the changes of the phospholipid dynamics due to temperature change and temperature induced phase transitions. The reduced elastic line intensity of a dispersion stabilized with DMPC and SGC compared to a dispersion stabilized only with DMPC indicates that the addition of SGC increases the mobility of the DMPC molecules. This enhanced mobility of DMPC might be responsible for the increase of the stabilizing capability of DMPC for lipid nanodispersions when adding SGC.

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