6.3.2 Does PEGylation affect the osmotic activity of liposomes?

Typically, unilamellar liposomes present a very narrow size distribution and spherical shape, whose diameter ranges from 50 nm to some hundreds of nanometers, and emerge as suitable nanocarriers for drug delivery. The covalent attachment of biocompatible polymers can improve the liposome stability. For example, polyetyhlene glycol (PEG) shows very low toxicity Yamaoka *et al.* (1994) and is a widely used stabilizer Sou *et al.* (2000). PEGylated liposomal formulations, also called sterically stabilized liposomes (SSL), show longer blood circulation times *in vivo* Barenholz (2001) and exhibit a slow drug release rate. PEG-modified liposomes have become of importance lately due to their increased drug pharmakinetics, decreased plasma clearance and improved patient convenience Gabizon & Martin (1997); Harris & Chess (2003). Therefore, the self-assembly of lipid structures in the presence of PEG moieties has been studied for different lipids Lee & Pastor (2011).

The incorporation of biocompatible polymers increases the phospholipid bilayer strength and enhances the vesicle rigidity, which relates to the increase of the bending modulus Liang *et al.* (2005); Sou *et al.* (2000). The higher membrane stiffness of SSLs has been extensively characterized with methods such as Atomic Force Microscopy (AFM) Spyratou *et al.* (2009) though other techniques such as light scattering have found a higher osmotic activity in SSLs in comparison to their non-PEGylated counterparts when incubated in serum Wolfram *et al.* (2014). Further investigations about the relationship between PEGylation and the liposomal osmotic behavior in suspension are essential. In the following work, the different response of SSLs and plain liposomes to osmotic pressure is studied with SAXS. The creation of multilamellar domains in the phospholipid layer is evaluated and the role of the PEG moieties in the membrane resilience is also analyzed.

For this purpose, 5 PEGylated and 3 plain liposomes were extruded with different pore sizes, as explained in section 6.1.4. To simplify the following discussion, the liposomes are named after the hydrodynamic diameter measured by DLS. The SAXS measurements of the 8 liposomes are showed in figure 6.10, where the first minimum shifts from $\sim$0.1 nm^{-1} to smaller q-values for increasing size. For high polydispersities this scattering minimum gets smeared out, as it can be observed for the 274.1 nm SSL. It is cleary observed from these measurements and the DLS results that the polydispersity degree rises for increasing liposomal sizes. Besides, non-PEGylated liposomes show slightly broader size distributions than SSLs.

Focusing on the high q-region of the single-contrast SAXS curves as displayed in figure 6.11, the scattering feature related to the phospholipid bilayer structure can be

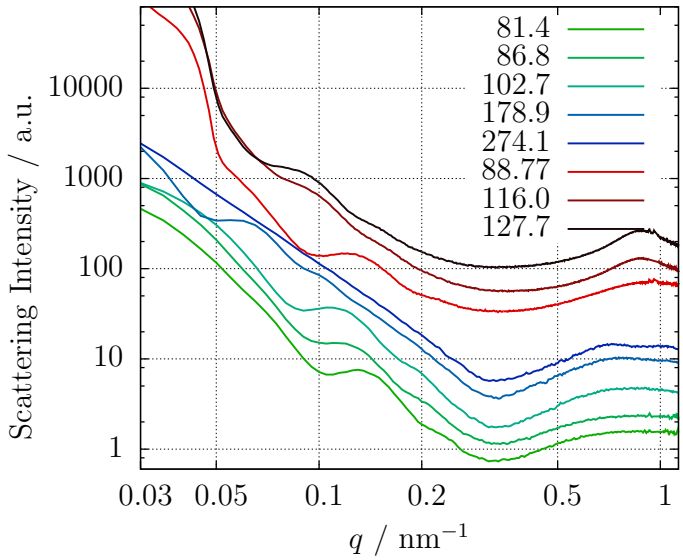

Figure 6.10: Scattering curves of the different liposomes in buffer. The curves are intensity shifted for clarity. The 5 SSLs are presented in the lower part of the plot. The sizes in the legend are extracted form DLS measurements.

analyzed. For Unilamellar Vesicles (ULV), the feature shape is typically round with a maximum around $q = 0.86$ nm^{-1} Varga *et al.* (2012), related to a distance of 7.3 nm, as it can be observed for small PEGylated liposomes. For SSLs extruded with larger pores, the bilayer shape shows incipient Bragg peaks which suggest the simultaneous presence of Multilamellar Vesicles (MLV) with unilamellar SSLs. Nevertheless, the MLV population cannot exceed 10 % of the total liposomes Sakuragi *et al.* (2011), as the scattering contribution from ULV is still clearly dominant.

However, the bilayer feature of the plain liposomes differs completely from the round shape visible in unilamellar vesicles. The diffraction peaks appearing at $q_1 = 0.88$ and $q_2 = 1.9 \simeq 2q_1$ nm^{-1} correspond to a lamellar repeat distance of 7.1 nm and the narrow shape of the bilayer feature indicates a variation of the phospholipid bilayer form factor. This change in the bilayer is emphasized for larger vesicles, as observed for the 127.7 plain liposome.

The effect of PEGylation induces a higher membrane stability due to the sterical stabilization of the phospholipid bilayer and the reduction of the electrostatic interactions within the membrane. The electrostatic repulsion is revealed in the plain liposomes by the appearance of the Bragg peaks. Nevertheless, secondary populations of MLVs coexisting with unilamellar liposomes can be observed for large

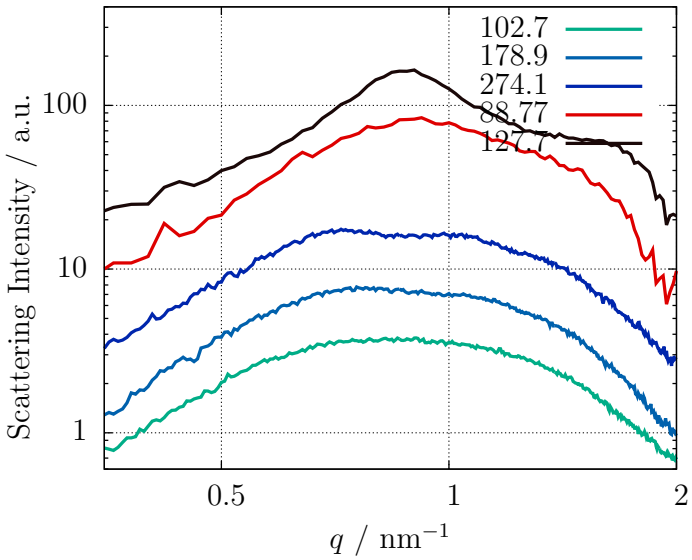

Figure 6.11: High q-region of the scattering curves of the 3 largest SSLs and 2 plain liposomes. The phospholipid bilayer feature is clearly observed. The SSLs are presented in the lower part of the plot.

extrusion pore sizes. In conclusion, the size and composition of the liposomes affects remarkably the formation of unilamellar vesicles and the shape of the phospholipid bilayer.