Lyotropic behaviour of cholesteric liquid-crystal polymers suspended in aqueous solution.

Mercedes Pérez Méndez, Javier Sanguino Otero, Daniel Rodríguez Martínez, Ana Labrador and Francois Fauth.

Biocompatible cholesteric liquid crystal polymers (CLCP), called PTOBEE and PTOBDME, were synthetized by enantioselective resolution through polycondensation reaction [1]. Their helical macromolecules behave both as thermotropic and lyotropic with mesophase in solution, which self-associates both by the effect of temperature and concentration, adopting different conformations depending on the solvent.

Besides they are capable of interacting with lipids, being inserted into the membranes without necessity of anchoring their molecules to those of the lipid by covalent bond [2].

Cationic liposomial/surfactant systems based on our CLCP were developed which entrapped DNA plasmids acting as non viral cationic vectors for gene therapy, which transfected successfully in several tumor cell lines [3]. Their structures were studied by SAXS [4].

Recently we have sinthetized new CLCP, funcionalized from the previous ones, called PNOBDME, PTOBEE-ammonium, PTOBDME-ammonium, PTOBEE-coline and PTOBDME-coline.

The new CLCP were dispersed in TAE (0,04M TRIS; 0,001M EDTA) at different concentrations: 10mg/ml, 7mg/ml, 5 mg/ml, 2,5 mg/ml and 1 mg/ml. Figure 1.

The CLC polymers were then complexed with commercial polynucleotides of increasing complexity [Poly-A]; [Poly-C], [Poly-G], [PolydT], [PolyC-PolyG], [PolyAC-PolydT], and commercial calf thymus DNA and plasmid. Three different proportions CLCP:DNA were studied respectively: (1:2), (1:1), and (2:1) by mixing and digesting for 12h in a swinging shaker.

The structure of the complexes has been studied by SAXS at the BM16 beamline at ESRF, at room temperature.

A monochromatized beam at λ= 0,9795 Å was used. Two-dimensional data recorded by an image-plate detector was placed at 5975 cm from the sample.

The program Fit2D was employed to evaluate the beam centre position and to generate a mask file. Binary data are normalized by the detector response and pixels are radially averaged into 1D. Silver behenate (d= 58.3 Å) was used to calibrate the angular axis.

The net scattering intensity I(n) was obtained through the standard equation.

$$I(n) = \frac{1}{c\,\mathrm{Det}(n)}\left[\frac{I_s(n)}{I_{0s}T_s} - \frac{I_m(n)}{I_{0m}T_m} - \frac{I_e(n)}{I_{0e}}\left(\frac{1}{T_s} - \frac{1}{T_m}\right)\right],$$

Where *s*, *m* and *e* correspond to sample, matrix and empty cell; *T* transmission and *c* sample concentration.

---

[1]. a) M. Pérez-Méndez and C. Marco Rocha, *Acta Polymerica*, 48, 502-506 1997; b) M. Pérez-Méndez and C. Marco Rocha, " Preparing cholesteric liquid crystals - by adding acid di:chloride and butanediol to chloro-naphthalene, heating in nitrogen, decanting into toluene, etc", Patent with nº EP1004650-A; WO9831771-A; WO9831771-A1; AU9854863-A; ES2125818-A1; ES2125818-B1; EP1004650-A1; US6165382-A; MX9906732-A1; JP2001513827-W; AU739076-B; EP1004650-B1; DE69824182-E.

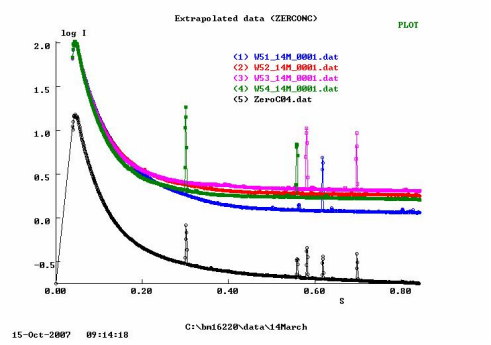
[2]. a) M. Pérez-Méndez, J. Fayos and C.R.Mateo, Advances in Biochirality, Elsevier Science S. A., Chapter 24 (1999). b) M. Pérez-Méndez, A. Alarcón Vaquero and M. H. J. Koch, Annual Report II. EMBL-HASYLAB- DESY, 116 (1997). c) M. Pérez-Méndez, S. Areso, A. Alarcón Vaquero, B. Elorza and M. Malfois, Annual Report EMBL, 372 (1998).

[3] M. Pérez-Méndez, R. Marsal Berenguel, A. Koenig, A. J. Vila-Coro, M. Timón, S. Moreno-López, ES200201317 patent, 6 de Junio 2002.

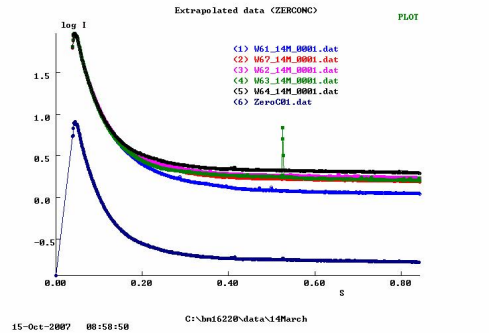
[4]. M. Pérez Méndez, R. Marsal Berenguel, S. S. Funari, "Structural Characterization of the Interaction Between Cholesteric Lquid-Crystal Polymers and Molecules of Biological Interest". HASYLAB Annual Report , 2003, 11150.

RESULTS.

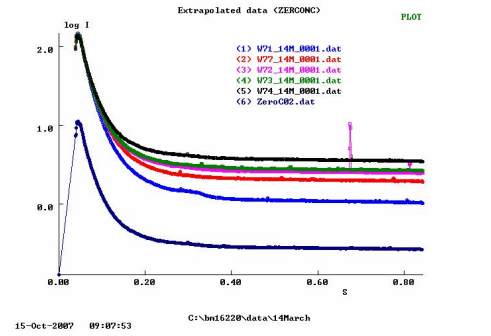
The technique proved to be sensitive to polymer concentration. Extrapolation to zero concentration was calculated for the new polymers. Radius of gyration Rg have been evaluated and the shape of the aggregates estimated by the slope in the Debye zone.



PTOBDME-NH2 Rg=37.52 nm, slope=2.5 (rod-like) (spheroidal)



PTOBEE-coline Rg= 39.06 nm, slope= 2,8 (spheroidal)



PTOBDME-coline Rg=42.3 nm slope=3,6 (spheres)

Figure 1. SAXS curves of the new functionalyzed polymers dispersed in TAE.

The SAXS spectra corresponding to complexes between PolyA and the new liquid-crystal polymers appear in Figure 2. Their size and shape obtained respective in the Guinier and Debye space, is shown in Table 1.

Similar results are obtained for PolyG complexes. Their structural parameters are given in Table 2.

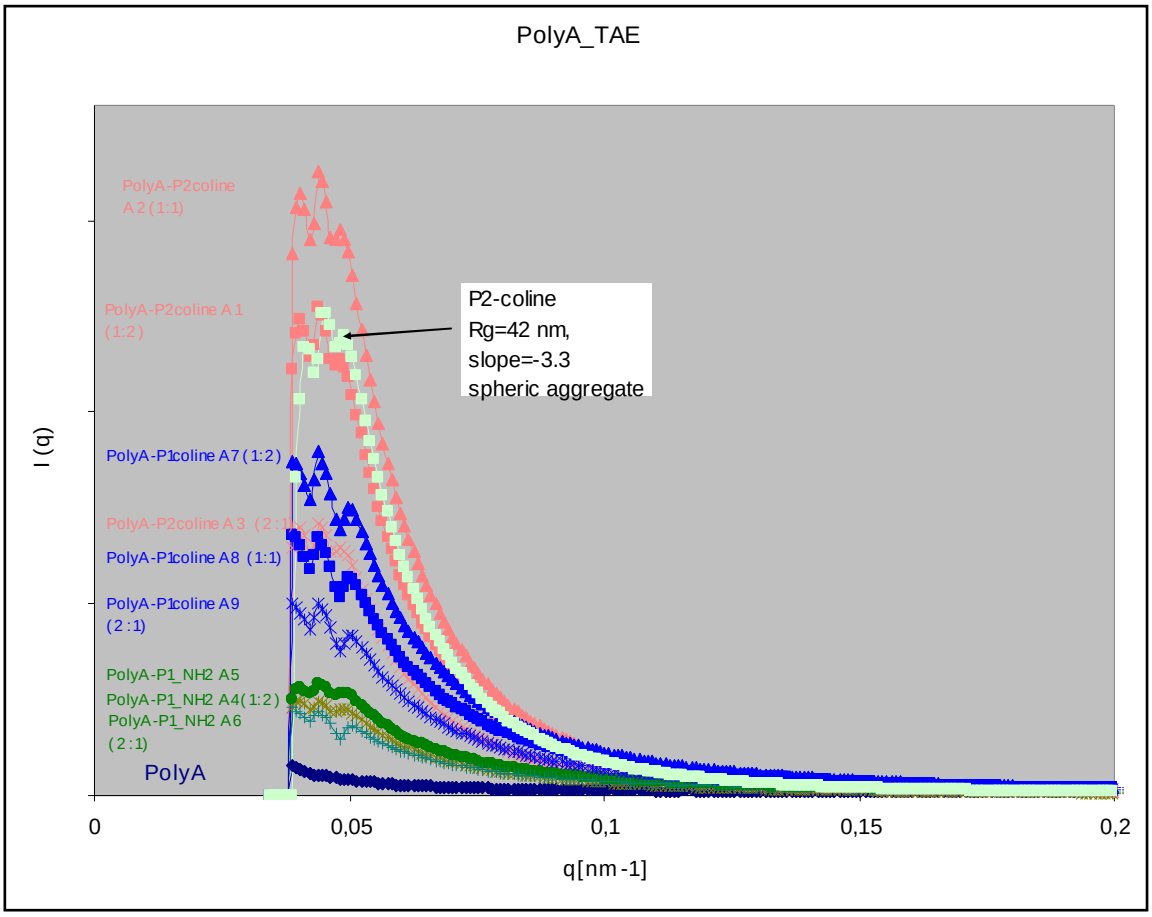


Figure 2.

Table 1. Complexes PolyA- Cholesteric LC Polymers

		Rg (Guinier) [nm]	Shape	Slope (Ln I(q) / Ln q)
PolyA		33,94	Rod	-1,6
PolyA-P2coline	(1:2)	40,37	sphere	-3,6
PolyA-P2coline	(1:1)	41,02	sphere	-3,7
PolyA-P2coline	(2:1)	39,8		-3,5
PolyA-P1_NH2	(1:2)	37,4	spheroidal	-2,4
PolyA-P1_NH2	(1:1)	37,4		-2,5
PolyA-P1_NH2	(2:1)	35,53		-2,26
PolyA-P1coline	(1:2)	37,33		-2,78
PolyA-P1coline	(1:1)	36,32		-2,63
PolyA-P1coline	(2:1)	36,38		-2,56

Table 2. Complexes PolyG- Cholesteric LC Polymers

		Rg (Guinier) [nm]	Shape	Slope Ln I(q) / Ln q
PolyG		21,043	Rod	-1,03
PolyG-PTOBDME_coline	(1:2)	38,86	sphere	-3,34
PolyG-PTOBDME_coline	(1:1)	37,95		-3,1
PolyG-PTOBDME_coline	(2:1)	36,05		-3,5
PolyG-PTOBEE_ammonium	(1:2)	34,86		-2,4
PolyG-PTOBEE_ammonium	(1:1)	32,21	rod-like	-1,9
PolyG-PTOBEE_ammonium	(2:1)	30,276		-1,67
PolyG-PTOBEE_coline	(1:2)	36,77	spheroidal	-2,62
PolyG-PTOBEE_coline	(1:1)	34,68		-2,27
PolyG-PTOBEE_coline	(2:1)	34,68		-2,27

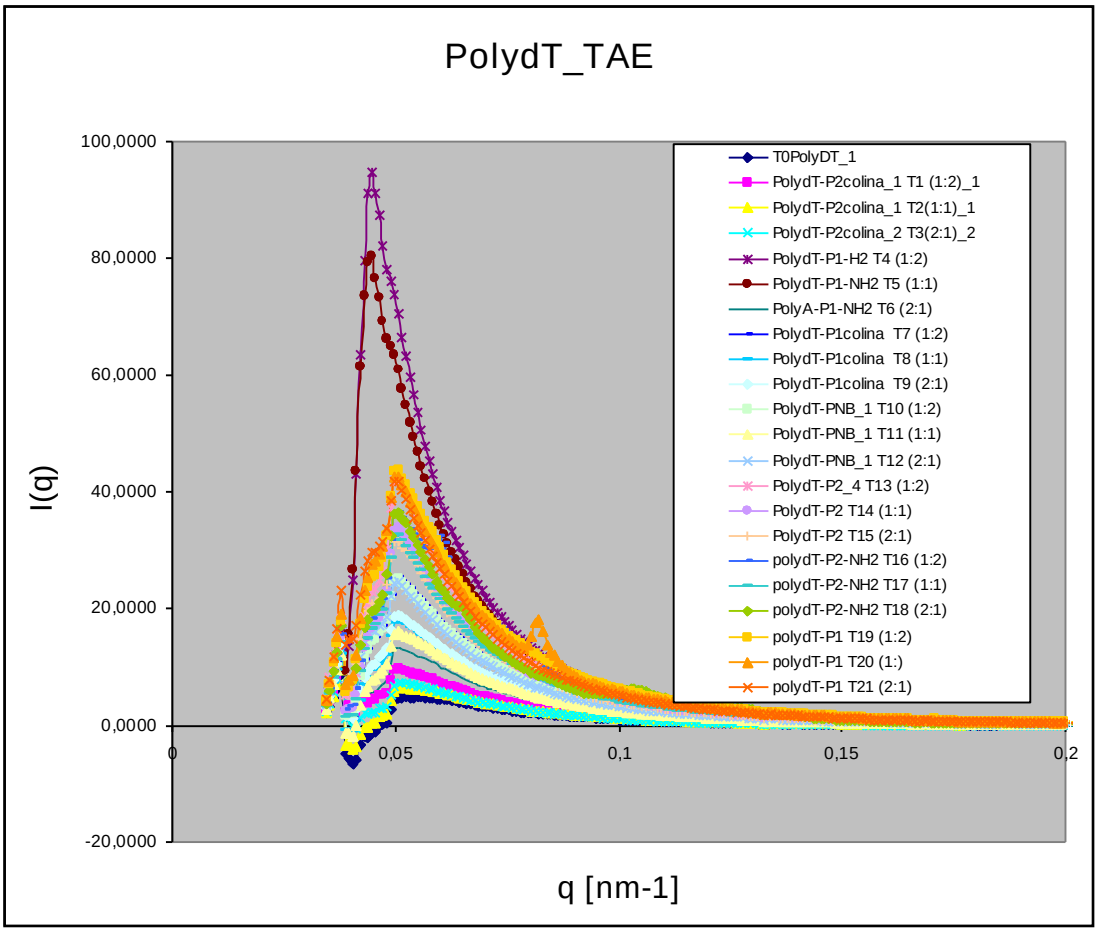


Figure 3

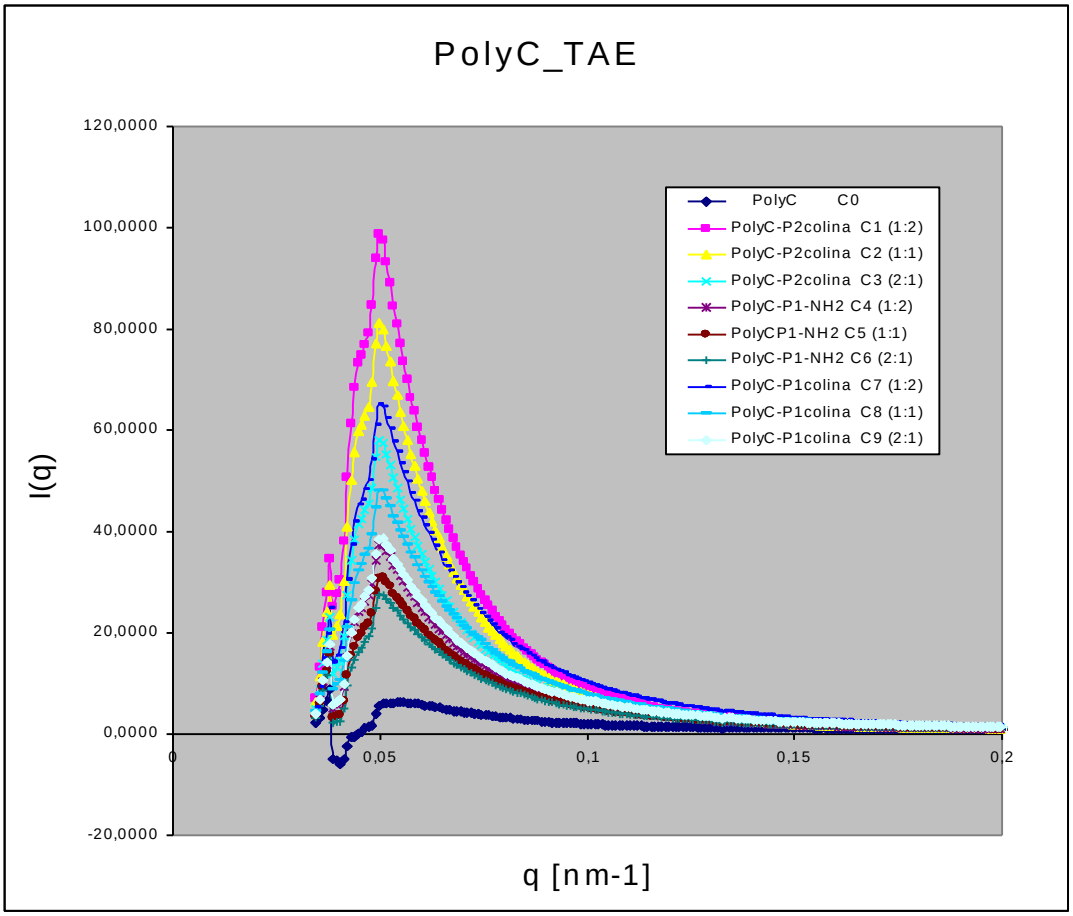


Figure 4

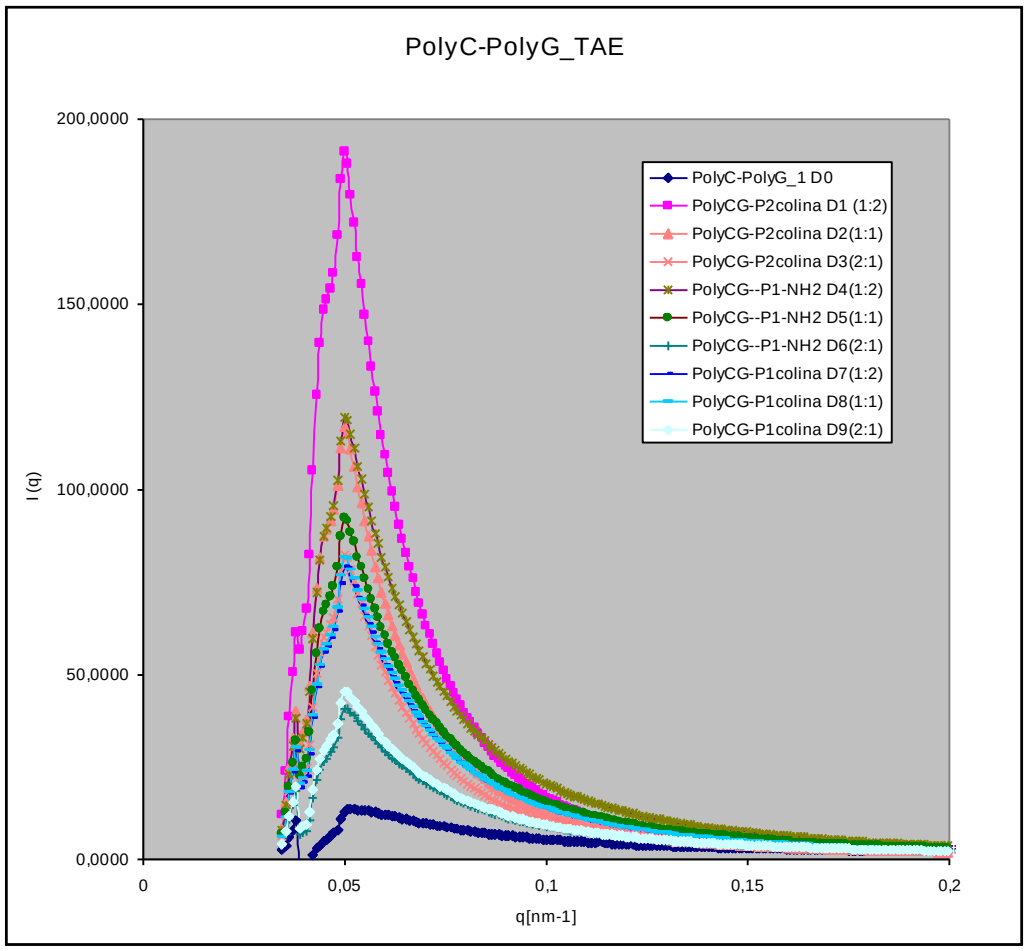


Figure 5

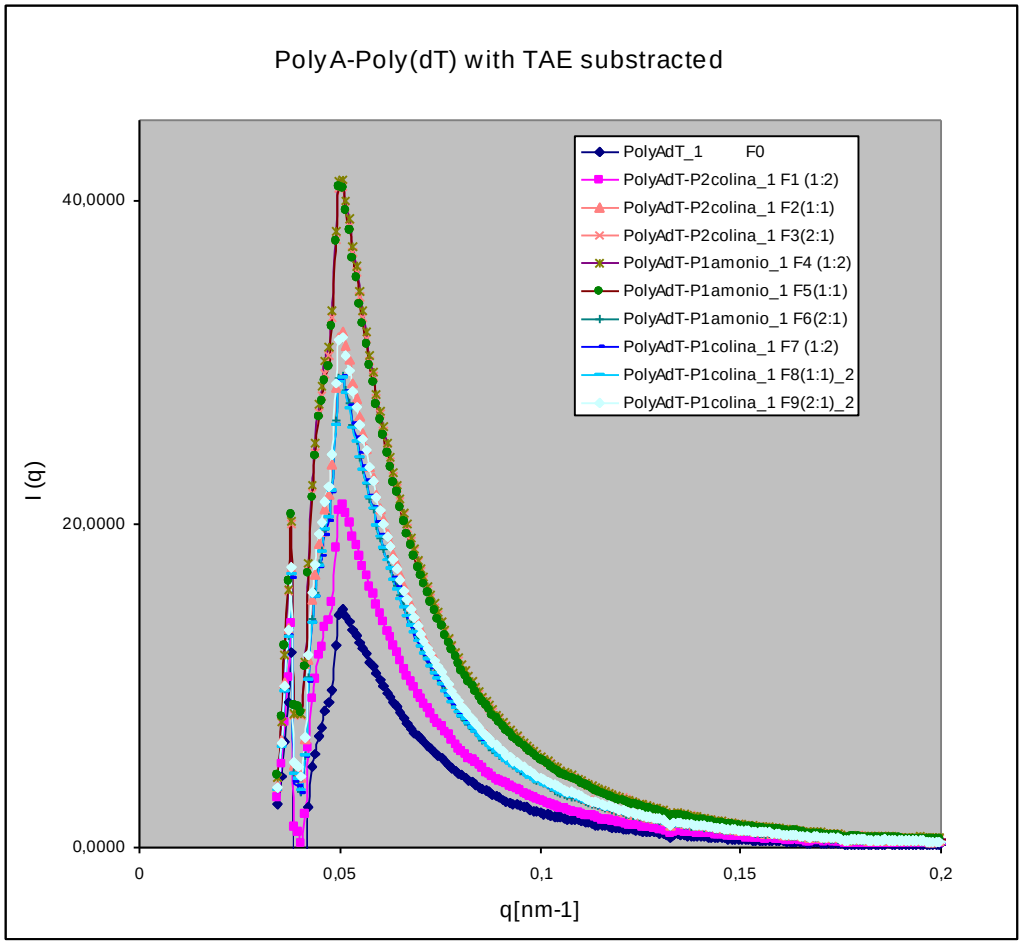


Figure 6

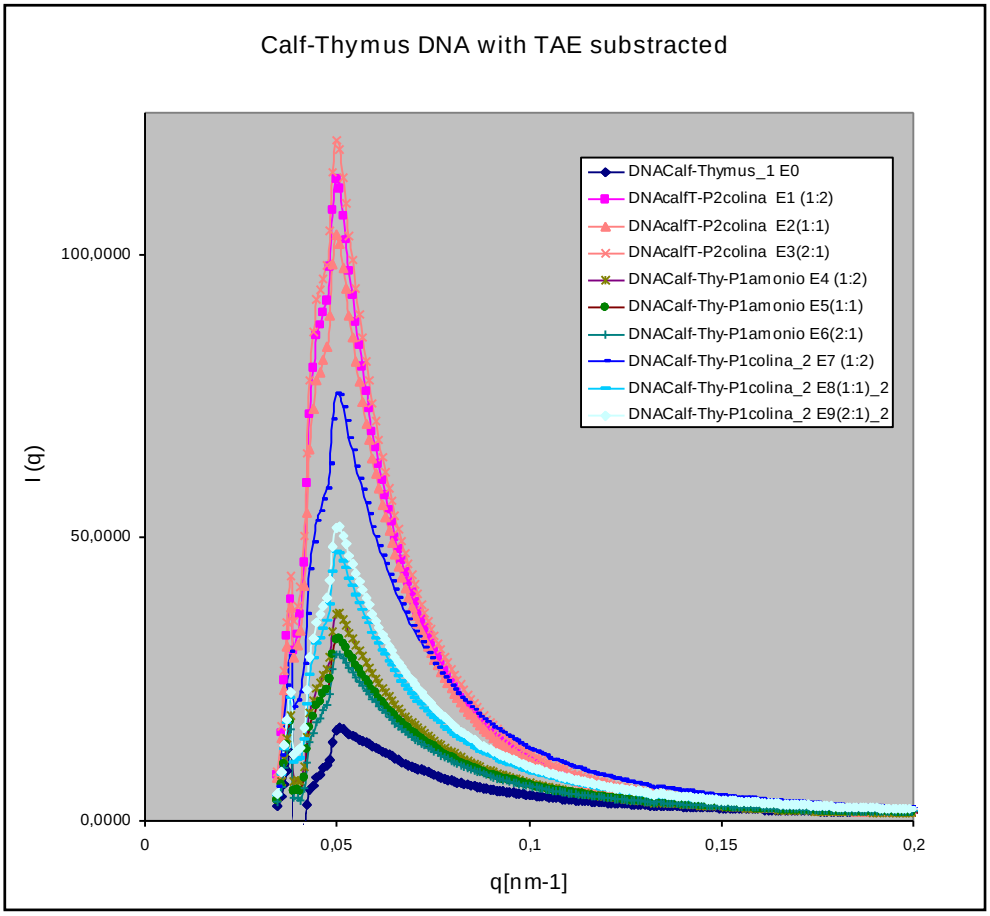


Figure 7

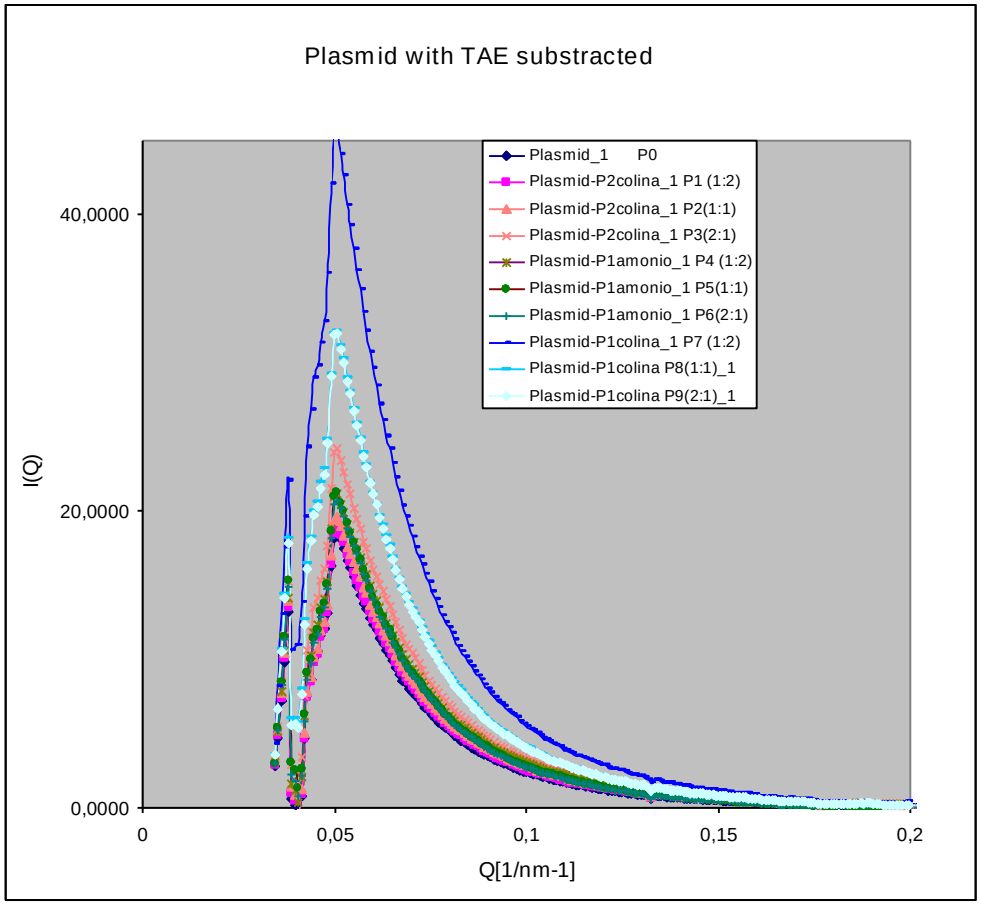


Figure 8

Table 3. Complexes PolyC- Cholesteric LC Polymers

		Rg (Guinier)	Slope
		[nm]	Ln I(q) / Ln q
PolyC		24,5	-2,16
PolyC-PTOBDMEcoline	(1:2)	38,02	-3,6
PolyC-PTOBDMEcoline	(1:1)	37,78	-3,56
PolyC-PTOBDMEcoline	(2:1)	36,77	-3,4
PolyC-PTOBEE_ammonium	(1:2)	34,015	-2,86
PolyC-PTOBEE_ammonium	(1:1)	33,20	-2,5
PolyC-PTOBEE_ammonium	(2:1)	32,36	-2,41
PolyC-PTOBEE_coline	(1:2)	34,58	-2,61
PolyC-PTOBEE_coline	(1:1)	33,94	-2,54
PolyC-PTOBEE_coline	(2:1)	33,14	-2,48

Table 4. Complexes PolyAdT (single strand)- Cholesteric LC Polymers

		Rg (Guinier	Slope
		[nm]	Ln I(q) versus Ln q
PolyAdT		31,95	-2,87
PolyAdT- PTOBDMEcoline	(1:2)	33,36	-3,0
PolyAdT- PTOBDMEcoline	(1:1)	34,75	-2,98
PolyAdT -PTOBDMEcoline	(2:1)	35,135	-2,95
PolyAdT -PTOBEE_ammonium	(1:2)	34,956	-2,96
PolyAdT -PTOBEE_ammonium	(1:1)	35,174	-3,03
PolyAdT -PTOBEE_ammonium	(2:1)	34,73	-3,00
PolyAdT -PTOBEE_coline	(1:2)	34,41	-3,03
PolyAdT -PTOBEE_coline	(1:1)	34,40	-2,99
PolyAdT -PTOBEE_coline	(2:1)	34,47	-3,02

Table 5. Complexes Calf-thymus DNA PolyC- Cholesteric LC Polymers

		Rg (Guinier)	Slope
		[nm]	Ln I(q) versus Ln q
Calf-thymus DNA		26,59	-1,55
Calf-thymus DNA -PTOBDMEcoline	(1:2)	38,90	-3,2
Calf-thymus DNA -PTOBDMEcoline	(1:1)	38,26	-3,11
Calf-thymus DNA -PTOBDMEcoline	(2:1)	38,63	-3,17
Calf-thymus DNA -PTOBEE_ammonium	(1:2)	32,96	-2,32
Calf-thymus DNA -PTOBEE_ammonium	(1:1)	32,16	-2,44
Calf-thymus DNA -PTOBEE_ammonium	(2:1)	31,43	-2,36
Calf-thymus DNA -PTOBEE_coline	(1:2)	34,12	-2,63
Calf-thymus DNA -PTOBEE_coline	(1:1)	33,23	-2,54
Calf-thymus DNA -PTOBEE_coline	(2:1)	33,25	-2,53

Table 6. Complexes: Plasmid DNA - Cholesteric LC Polymers

		Rg (Guinier)	Slope
		[nm]	Ln I(q) versus Ln q
Plasmid_DNA		34,22	-3,2
Plasmid_DNA -PTOBDMEcoline	(1:2)	33,38	-3,12
Plasmid_DNA -PTOBDMEcoline	(1:1)	33,17	-3,0
Plasmid_DNA -PTOBDMEcoline	(2:1)	33,66	-3,0
Plasmid_DNA -PTOBEE_ammonium	(1:2)	32,99	-3.06
Plasmid_DNA -PTOBEE_ammonium	(1:1)	33,66	-2,735
Plasmid_DNA -PTOBEE_ammonium	(2:1)	33,79	-2,738
Plasmid_DNA -PTOBEE_coline	(1:2)	35,22	-2,94
Plasmid_DNA -PTOBEE_coline	(1:1)	34,35	-2,82
Plasmid_DNA -PTOBEE_coline	(2:1)	34,37	-2,83

CONCLUSIONS:

1. Cholesteric LC polymers self associate in big spheroidal aggregates, with radius ~ 50 nm (about 3000 monomeric units). Some diffraction peaks indicates large space order at about 130 nm.
2. The polynucleotides particles are long isolated molecules with elongated forms between rods and spheroids. Plasmide DNA is in the supercoiled state. No diffraction peaks are observed.
3. PolyA and PolyG and PolyC-PolyG are more rodlike and PolydT and PolyC are more spheroids.
4. The complexes retain the structure of the CLC polymer aggregates, by modifying their internal order, increasing volume and loosing sphericity. Probably the DNA cover the polymer aggregates by charge balance.
4. Hence the Cholesteric Liquid Crystal, would act as carrier for the genetic material, with potencial application in Gene Therapy.