

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Solution Thermodynamics of highly fluorinated gemini bispyridinium surfactants for biomedical applications

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1593724> since 2016-10-13T22:32:35Z

Published version:

DOI:10.1016/j.colsurfa.2016.07.051

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in COLLOIDS AND SURFACES. A, PHYSICO-CHEMICAL AND ENGINEERING ASPECTS, 507, 2016, 10.1016/j.colsurfa.2016.07.051.

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>), 10.1016/j.colsurfa.2016.07.051

The publisher's version is available at:

<http://linkinghub.elsevier.com/retrieve/pii/S0927775716305726>

When citing, please refer to the published version.

Link to this full text:

<http://hdl.handle.net/2318/1593724>

*SOLUTION THERMODYNAMICS OF HIGHLY FLUORINATED GEMINI
BISPYRIDINIUM SURFACTANTS FOR BIOMEDICAL APPLICATIONS*

*Emilia Fiscaro^{*a}, Laura Contardi^a, Carlotta Compari^a, Franco Bacciottini^a, Erika Pongiluppi^a,
Guido Viscardi^b, Nadia Barbero^b, Pierluigi Quagliotto^b, Bożenna Różycka-Roszak^c*

^a University of Parma, Department of Pharmacy, Parco Area delle Scienze, 27/A - 43124 Parma –
Italy

^b University of Torino, Department of Chemistry, Interdepartmental “Nanostructured Surfaces and
Interfaces” NIS Centre, Via P. Giuria, 7 - 10125 Torino – Italy

^c Wrocław University of Environmental and Life Sciences, Department of Physics and Biophysics,
Norwida 25, 50-375 Wrocław – Poland

*Corresponding author e-mail: emilia.fiscaro@unipr.it

ABSTRACT

Highly fluorinated gemini surfactants are relevant in pharmaceutical field, owing their ability to deliver genes to the respiratory or to the biliar epithelium, where hydrogenated interfering surfactants are present. We report for the first time the thermodynamic properties of the solutions of 1,1'-Bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2 *n*-methylenebispyridinium dichloride ($n = 4, 8, 12$). The gene delivery ability of the dichlorides and the structure of the DNA nanoparticles are strictly related to their solution enthalpic properties, resulting in a very sensitive probe of the behaviour of the molecule in solution. Dilution enthalpies, density and sound velocities vs. molality for dichlorides and dimethanesulfonates with different spacer length, n , were measured. The evaluation of apparent and partial molar enthalpies as a function of concentration of the dimethanesulfonate suggests that the effect of the counterion is additive and the values obtained for the hydrogenated surfactants can be transferred to the fluorinated ones, allowing the prediction of enthalpic properties in solution. No evidence of the structure change in solution is found in the trends of volumetric properties vs. m . and the group contribution of the $-\text{CH}_2-$ for both the dichlorides and the dimethanesulfonates results additive as well as the effect of the counterion. The adiabatic molar compressibilities seem to be independent from the counterion and show in micellar region a plateau value much greater than in the case of the more traditional hydrogenated gemini surfactants, indicating a less compact structure of the micelles, probably due to the repulsion between hydrogenated and fluorinated moieties of the molecules.

KEYWORDS: highly fluorinated gemini surfactants; heterocyclic cationic gemini surfactants; micelle formation thermodynamics; fluoro-compounds solution thermodynamics; fluorinated synthetic vectors for gene delivery

INTRODUCTION

Gemini surfactants - i.e. surfactants consisting of at least two identical hydrophobic chains and two polar head groups covalently bound together by a spacer – have been attracting increased attention for more than 20 years, owing to their increased surface activity, low critical micelle concentration (cmc), and useful viscoelastic properties.¹⁻⁷ Among the *gemini* surfactants, the structures most widely investigated are those in which the hydrophobic tails and the spacer are made by hydrocarbon moieties. In particular, the bisquaternary ammonium salts (bisQUATS) are the best known with regard to the biological activity and the chemico-physical properties.³ More recently, gemini surfactants with heterocyclic polar heads have been proposed: their major synthetic access routes and the impact of structural elements on their physicochemical and aggregation properties are examined in a comprehensive review⁸. The interest in biomedical field of cationic *gemini* surfactants greatly increased when they were proposed as non viral vectors in gene therapy⁷⁻¹³, taking advantage of their multiple cationic character, necessary for binding and compacting DNA. Gene therapy is, in fact, one of the major goals pursued by the post-genomic research for treating diseases caused by a known defective gene. It is based on the idea of delivering to the cells a correct copy of the gene originating the genetic disease, by means of a specially designed viral or synthetic vector. Non-viral vectors have become in many cases a preferred means of gene delivery into eukaryotic cells, despite their still low transfection efficiency, because the use of viral vectors is not without the risk of adverse or immunogenic reaction, or replication. Recently, the use of gemini surfactants as candidates for the formation of nonviral vectors has been reviewed, emphasizing the effect on the efficiency of transfection of the chemical structure of the surfactant (variations in the alkyl tail length and spacer/head group) and of the resulting physicochemical properties of the lipoplexes¹¹. In this field we have obtained interesting results both by using very simple bisquaternary ammonium gemini surfactants, derivatives of N,N-bisdimethyl-1,2-ethanediamine (bis-C_nBEC)¹² and dipyridinium gemini surfactants^{13,20}, when formulated with DOPE [L- α -phosphatidylethanolamine dioleoyl (C18:1,[cis]-9)]. More recently, also fluorinated and partially fluorinated gemini surfactants have been proposed¹⁴⁻¹⁹, in particular to obtain an efficient gene expression in those biological fluids containing endogenous hydrogenated interfering surfactants, as pulmonary surfactants or bile salts, able to destroy lipoplexes, before the delivering to the diseased cells.¹⁴⁻¹⁶

This is an essential requirement, for instance, in the treatment of cystic fibrosis and cystic fibrosis-associated diseases.¹⁵

Partially fluorinated surfactants (otherwise called “hybrid surfactants”) are suited for biomedical applications, because, in general, they show low to moderate acute toxicity and low haemolytic activity compared to their hydrogenated analogues.^{19,21} We therefore started to synthesize and characterize from a chemico-physical and biological point of view a class of highly fluorinated gemini dipyridinium surfactants, differing for spacer length and counterion.^{20,22} As for their hydrogenated counterparts¹³, we were amazed at the tight relationship we have discovered between solution thermodynamics data and their biological performance, making “old” methods very useful for understanding “new” applications.

We are studying for many years the solution thermodynamics of surfactants by means of the direct methods typical of dilute solutions (generally, surfactant solutions are diluted), i.e. we are directly measuring the total property of the system, such as volume, compressibility, heat capacity, dilution enthalpy, vs. concentration. In fact, the advantages of measuring equilibrium properties are that they can be obtained very precisely²³. The experimental data are expressed in terms of apparent and partial molar quantities, affected by the solute-solvent and solute-solute interaction. It must be stressed that these thermodynamic properties are macroscopic and information at molecular level can be extracted only through models, as explained in the “results” section. The thermodynamics of solutions has been covered in a large number of excellent textbook²⁴.

For this reason and because the literature is lacking of solution thermodynamics data about fluorinated surfactants, we decided to study in depth the solution thermodynamics of the new compounds by means of direct methods, paying attention to partial molar volumes, compressibilities and to the effect of the counterion. The data here reported try to give an answer to the question if also for the gemini fluorinated surfactants the group contribution approach is valid. If it is so, their solution thermodynamic properties can be evaluated theoretically.

MATERIAL AND METHODS

Compounds

The synthesis of the compounds under study, prepared by us, is reported in detail in ref. 22. In the following the IUPAC names of the compounds studied and in brackets the names in short by which they will be referred in the paper, are shown: 1,1'-bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2'-tetramethylenepipyridinium dichloride (FGP4); 1,1'-bis(3,3,4,4,5,5,6,6,7,7,8,8,8-

tridecafluorooctyl)-2,2'-tetramethylenebispyridinium dimethanesulfonate (FGPS4); 1,1'-bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2'-octamethylenebispyridinium dichloride (FGP8); 1,1'-bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2'-octamethylenebispyridinium dimethanesulfonate (FGPS8); 1,1'-bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2'-dodecamethylenebispyridinium dichloride (FGP12); 1,1'-bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2'-dodecamethylenebispyridinium dimethanesulfonate (FGPS12). The compounds were obtained by ion exchange, after having synthesized the corresponding ditrifluoromethanesulfonate.²² Purity was checked by NMR, elemental analysis and TLC: eluent BAW (only the organic phase separated from butanol:acetic acid:water = 4:1:5 biphasic system phase) on silica gel plate. The general structure and the peculiarities of the compounds under study are shown in Figure 1.

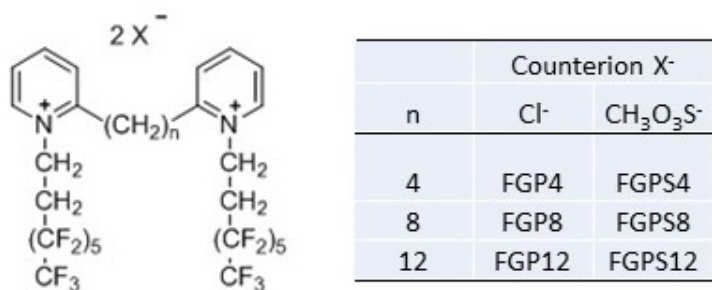


FIGURE 1. Compounds under study, n is the number of carbon atoms in the spacer.

Dilution enthalpies measurements

The enthalpies of dilution were measured by means of the TAM (Thermometric) microcalorimeter, equipped with 221 Nano Amplifier, at 298 K, by using the flow mixing cell. The freshly prepared surfactant solutions, kept before injection at the experimental temperature by means of a Heto cryothermostatic bath, were diluted into the "mixing" measuring cell of the microcalorimeter in a ratio 1:1 by using CO₂-free water. The solutions and the water were injected by means of a Gilson peristaltic pump, Minipuls 2, and their flows were determined by weight. For the dilution enthalpies measurements, the solutions were prepared by weight using freshly boiled bi-distilled water, stored under nitrogen and solution concentrations were expressed as molality, m (mol kg⁻¹).

Density and sound velocity measurements

Density and sound velocity of the solutions as a function of m were obtained by a Paar DSA 5000, an oscillating U-tube meter, able to measure density ($\pm 0.000001 \text{ g cm}^{-3}$) and sound velocity ($\pm 0.1 \text{ m sec}^{-1}$) to the highest accuracy in wide viscosity and temperature ranges. Based on an additional measuring cell made of stainless steel and high resolution electronics, the sound velocity of the filled in sample can be determined accurately. Both measuring cells are temperature controlled using a built-in solid state thermostat and two integrated Pt 100 platinum thermometers ($\pm 0.001^\circ\text{C}$). The instrument was calibrated by water and dry air.

RESULTS

The thermodynamics of the solutions of FGPS4 and FGPS8 is given in terms of apparent and partial molar quantities of the solute, assuming the infinite dilution as reference state. These quantities are derived from the experimental data using methods stated in detail elsewhere.²³⁻³³

For the sake of clarity, we recall that, with reference to the state of infinite dilution, the molar enthalpy of dilution, ΔH_d , is given by the difference between the apparent relative molar enthalpy in the final (after dilution) state, $L_{\Phi,f}$ and that in the initial (before dilution) state, $L_{\Phi,i}$:

$$\Delta H_d = L_{\Phi,f} - L_{\Phi,i} \quad (1)$$

For ionic surfactant in the premicellar region, the apparent relative molar enthalpy can be expressed by means of a polynomial of $m^{1/2}$, in which the coefficients B_L and C_L are obtained interpolating by a least squares curve fitting the experimental points in the premicellar region. A_L is the limiting Debye-Hückel slope for relative enthalpies accounting for the long range electrostatic solute-solute interactions:

$$L_{\Phi} = A_L m^{1/2} + B_L m + C_L m^{3/2} \quad (2)$$

In the micellar region, the apparent molar enthalpies are evaluated by means of eq. (1) and, when a

value of L_Φ vs. m not experimentally measured is needed, by graphical interpolation.

The partial molar enthalpies L_2 are determined by drawing the best curve for the apparent molar enthalpies vs. m and then by calculating the partial molar quantities as $\Delta(mL_\Phi)/\Delta m$ from points interpolated at regular intervals. The trends of the apparent and partial molar enthalpies for the compounds under investigation as a function of molality, m are shown in Figure 2 and 3, in comparison with dichlorides ones.

Dilution enthalpies, apparent and partial molar enthalpies vs. m for the compounds under investigation, are available as supporting information.

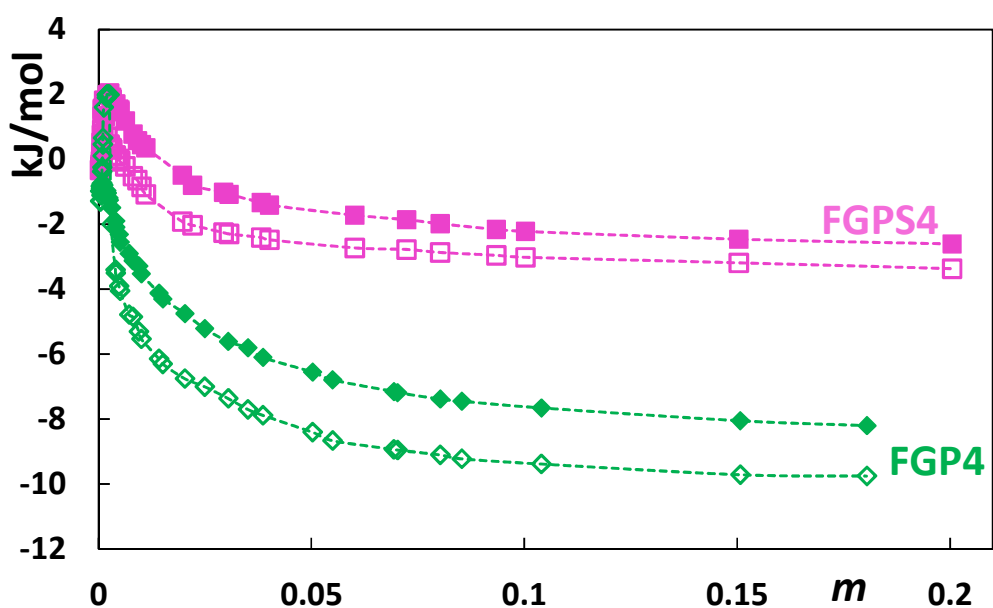


FIGURE 2. Apparent (full symbols) and partial (empty symbols) molar relative enthalpies of FGP4 (diamonds) and FGPS4 (full squares) as a function of surfactant molality, m .

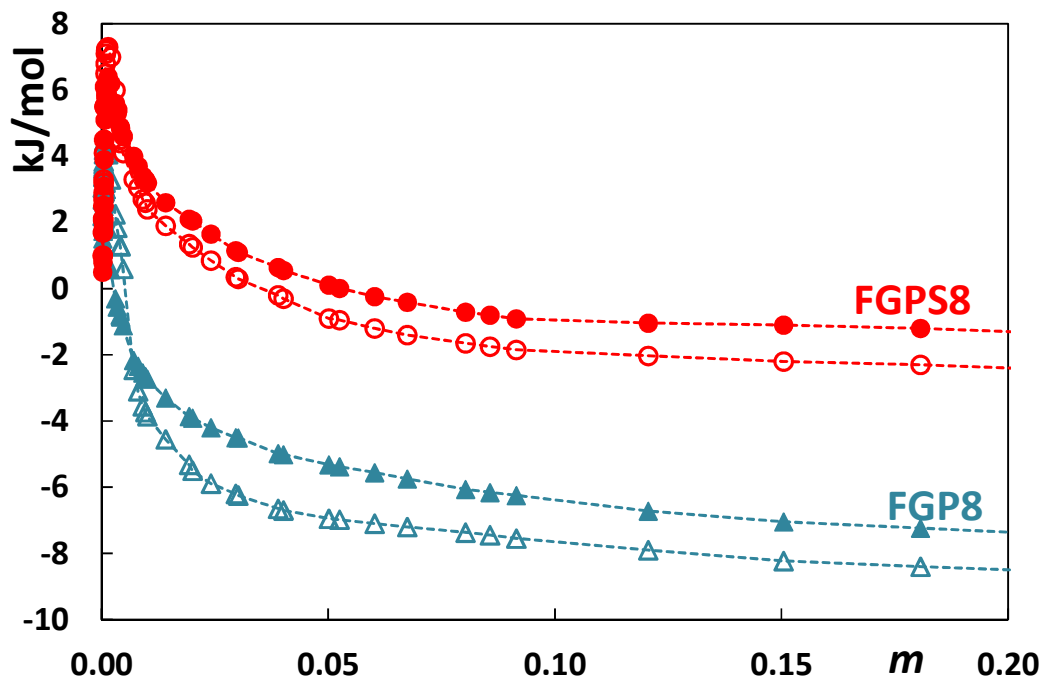


FIGURE 3. Apparent (full symbols) and partial (empty symbols) molar relative enthalpies of FGP8 (triangles) and FGPS8 (circles) as a function of surfactant molality, m .

Apparent volume and adiabatic compressibility

The apparent molar volumes, V_{Φ} are obtained by the equation:

$$V_{\Phi} = \frac{M}{d} - \frac{10^3 (d - d_0)}{m d d_0} \quad (3)$$

where d is the density of the solution of molality m , M is the molecular weight of the surfactants, and d_0 is the density of the solvent. The obtained trends of V_{Φ} vs. m are shown in Figure 4.

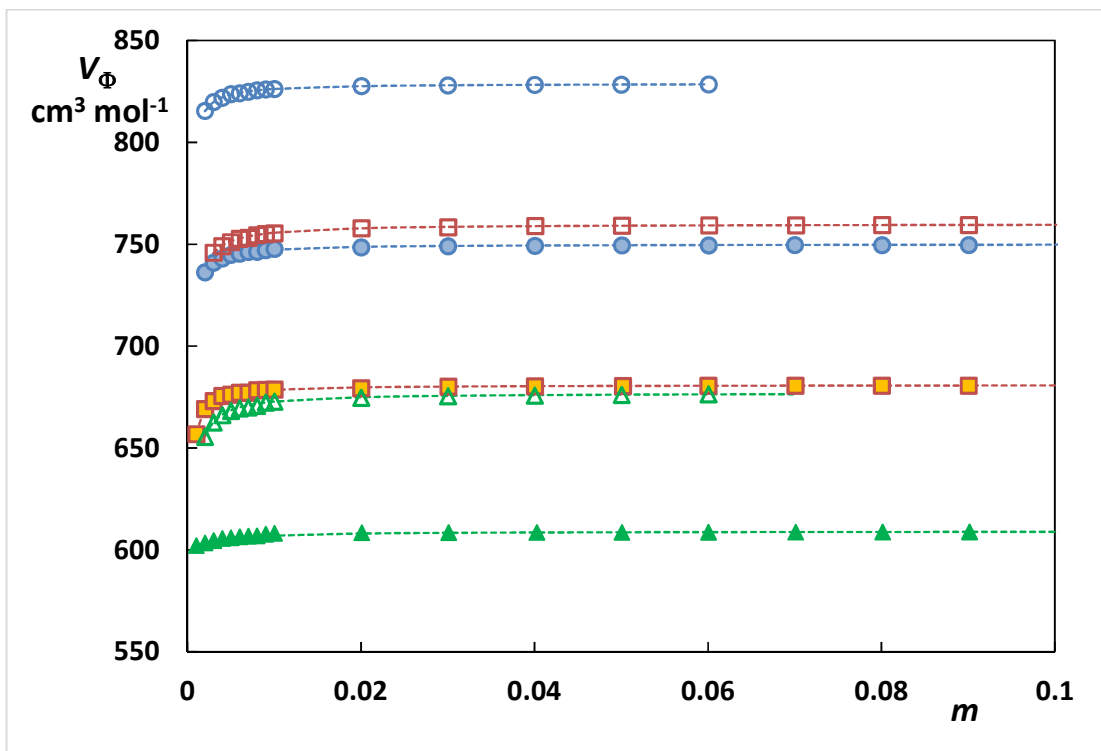


FIGURE 4. Apparent molar volumes of FGPn (full symbols) and FGPSn (empty symbols) with $n=4$ (triangles), $n=8$ (squares) and $n=12$ (circles) as a function of surfactant molality, m . Broken lines show the function computed by eq.6.

The apparent molar adiabatic compressibility, $K_{S,\Phi}$, is given by³⁰⁻³³

$$K_{S,\Phi} = \frac{M\beta_S}{d} - \frac{10^3(\beta_{S,0} d - \beta_S d_0)}{m d d_0} \quad (4)$$

where $\beta_{S,0}$ and β_S are the coefficient of adiabatic compressibility of the solvent and of the solute respectively. The latter is computed from sound velocity, u and density data as

$$\beta_S = 100/(u^2 d) \quad (5)$$

In Figures 5 and 6, the apparent molar isentropic compressibilities as a function of concentration

of sulfonates and chlorides, respectively are shown. Following a pseudo-phase transition model, the trends observed above the cmc can be described by the equation:

$$X_{\Phi} = X_{\Phi,M} - (\text{cmc} \cdot \Delta X_{\Phi}) \cdot (1/m) \quad (6)$$

where X stands for the property being investigated. The values of $X_{\Phi,M}$, the property in micellar phase, and ΔX_{Φ} , the change in property upon micellisation, can be obtained by a least square fit, if the values of cmc are known. Table 1 shows the values thus derived, together with the values of $X_{\Phi,S}$, the value at the cmc, from

$$X_{\Phi,S} = X_{\Phi,M} - \Delta X_{\Phi} \quad (7)$$

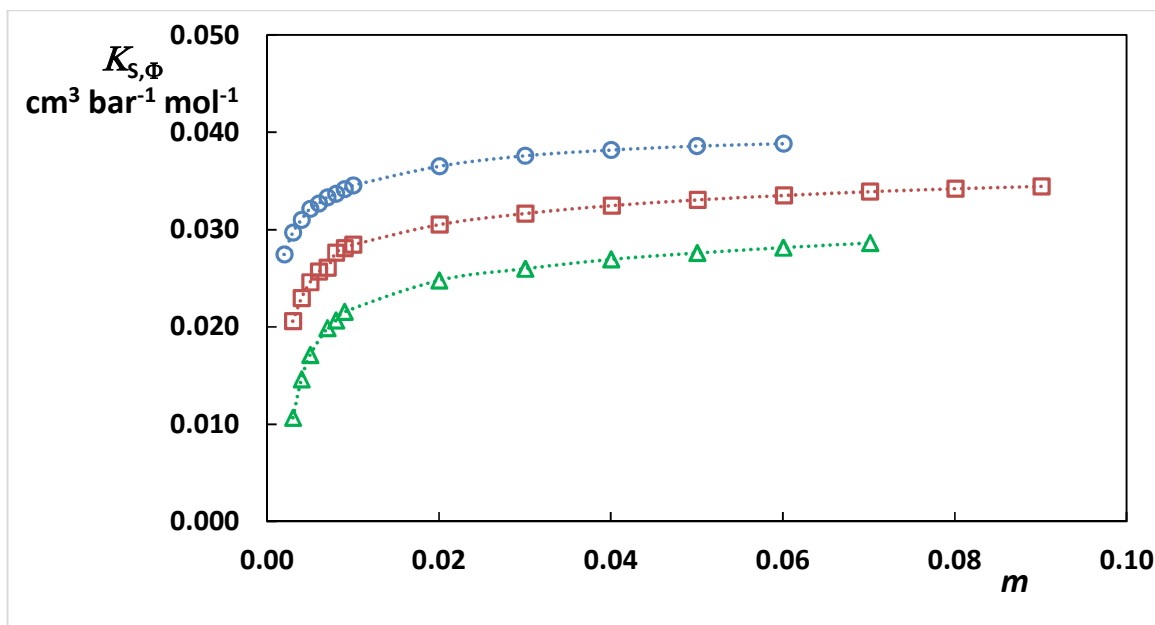


FIGURE 5. Apparent molar adiabatic compressibilities of FGPSn with $n=4$ (triangles), $n=8$ (squares) and $n=12$ (circles) as a function of surfactant molality, m . Broken lines show the function computed by eq.6.

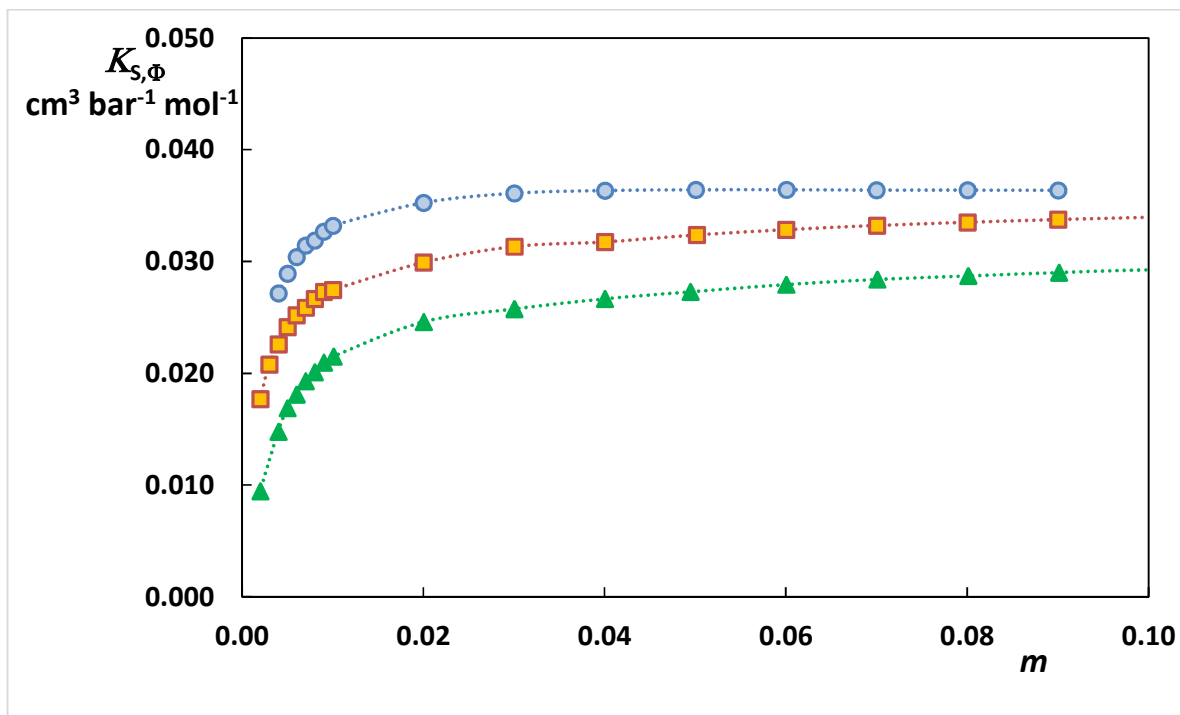


FIGURE 6. Apparent molar adiabatic compressibilities of FGPn with $n=4$ (triangles), $n=8$ (squares) and $n=12$ (circles) as a function of surfactant molality, m . Broken lines show the function computed by eq.6.

DISCUSSION

It is known, principally from the work of Shinoda³⁵, that the critical micelle concentrations (cmc) of fluorinated surfactants, determined by the balance of the hydrophobicity and hydrophilicity of the molecule, are close to those of ordinary surfactants whose hydrocarbon chain lengths are about 1.5 times longer. This is why we have synthesized the bis-tridecafluorooctyl compounds to be compared with the bis-dodecyl hydrogenated compounds. Moreover, we recall that thermodynamic properties in solution are dictated both by the difference in size between fluorine and hydrogen atoms (van der Waals radii, $F=1.35$ and $H=1.2$ Å) and by the difference in electronegativity (Pauling scale, $F=4.0$ and $H=2.1$).³⁴

In the field of solution thermodynamics, it is useful to express the properties of different molecules using a group contribution approach, i.e. to extract from the experimental data the effect of each brick constituting the molecule.^{24, 28} This approach has the advantage that the different properties of a new molecule can be evaluated theoretically with a good approximation by adding the contribution of each group. Moreover, if computed and calculated data are in strong disagreement, this could be

an indication that the behaviour of the molecule in solution has changed. We have obtained by direct methods the apparent and partial molar enthalpies at 298 K of the aqueous solutions of the homologous series of the protiated cationic gemini surfactants 1,1'-didodecyl-2,2'-alkylenebispyridinium dichloride²⁶ and dimethanesulfonate²⁵. They show a very peculiar behaviour as a function of the spacer length not allowing for the determination of a $-\text{CH}_2-$ group contribution when this group is added to the spacer. The deviation of the properties from those theoretically predicted, suggests that something new happens in solutions, such as a phase transition or a change in conformation of the molecule. We explained the unexpected behaviour of gemini pyridinium surfactants, independent on the counterion, by a conformation change of the molecule determined by stacking interactions between the two pyridinium rings, appearing at an optimum length of the spacer. The compound deviating from the predicted trend shows the greatest gene delivery ability probably because this conformation change in solution generates to a sort of molecular tongs able to grip basic DNA groups near each other.¹³ The study of thermodynamic and biological properties of the homologous series of partially fluorinated gemini pyridinium surfactants 1,1'-bis(3,3,4,4,5,5,6,6,7,7,8,8,8-tridecafluorooctyl)-2,2'-*n*-methylenebispyridinium dichloride (FGP n with $n = 3, 4, 8, 12$), confirms a behaviour similar to that of the hydrogenated analogues. The essential difference is that the most efficient structure in solution is obtained with the spacer eight carbon atoms long, instead as four, as for the hydrogenated ones.^{13, 20}

If the hydrophobic chain is modified by partial fluorination, a greater length of the spacer is needed for the molecule folding, due to the greater steric hindrance and rigidity of the fluorinated moiety. The comparison with the hydrogenated analogues of FGP3, FGP4 and FGP8, reveals a greater ability of the partially fluorinated compounds to compact DNA.

We were able to show, by comparing the trends of chlorides and of methanesulfonates, that, also in the case of dipyridinium gemini surfactants, the counterion plays a crucial role in determining the energetics of micellar solutions but the group contribution additivity for the counterion is respected, independently on the spacer length.²⁵ This means that the peculiar behaviour above described is independent on the counterion. Owing the lack of data in the literature for partially fluorinated gemini compounds, we have measured the dilution enthalpies of FGPS4 and FGPS8 and derived the apparent and partial molar enthalpies as a function of m (Figure 2 and 3).

The curves show the typical shape for ionic surfactants: after increasing in the pre-micellar region, they tend to level off at concentrations above the cmc, where they are almost parallel. The lowering of the curves in the micellar region is attributed to the electrostatic interactions in micellar solutions, strongly dependent on the counterion radius and polarizability. The substitution of methanesulfonate

by the chloride causes the lowering of the enthalpy curves of partially fluorinated compounds in micellar region of an amount of about 6.1 kJ mol^{-1} , the same value found for the protiated analogues²⁵, confirming the validity of the group contribution approach for the counterion of the gemini fluorinated surfactants, too.

The enthalpy change upon micellization, ΔH_{mic} , obtained by extrapolating at the cmc the trends of the partial molar properties before and after cmc, results -4.6 kJ mol^{-1} and -3.2 kJ mol^{-1} , for FGPS4 and FGPS8, respectively.

The value of micellization enthalpy is lower by about 5.7 kJ mol^{-1} if the methanesulfonate counterion is replaced by the chloride one. The same value was found in the case of protiated analogues²⁵, confirming the group contribution additivity for the counterion.

Volumes and compressibilities

To reach a complete view of their solution thermodynamics and to gain information about the structure of the hydration sphere of the molecules, we carried out the measurements of the apparent molar volumes and adiabatic compressibilities vs. m , never reported before in the literature for these systems. In fact, because volumetric properties such as volumes and compressibility are reflective of the solute-solvent interactions, a great change in these interactions occurs when micelle formation begins. In Figure 4, the apparent molar volumes of the compounds under study are shown. Volumes of fluorinated surfactants in solution have not been exhaustively studied in literature and, in particular, changes in volumes upon micellization are sometimes in disagreement.²⁷⁻²⁹ Tamaki *et al.* have studied some thermodynamic properties of sodium perfluoroalkanoates and lithium perfluoro-1-alkane sulfonates³⁶⁻³⁷. In particular, they have done an accurate study of volumetric properties of these compounds below the cmc³⁶. They obtained a group contribution of $22.63 \text{ cm}^3 \text{ mol}^{-1} -\text{CF}_2-$ for the infinite dilution value, in good agreement with the value obtained by us for a homologous series of monomeric partially fluorinated pyridinium chlorides, despite the difference in the charge on the polar head²⁸. Some time ago, we had the opportunity to study solution thermodynamics of highly fluorinated monomeric surfactants, with chloride as counterion, having a fluorinated alkyl chain (with 4, 6, and 8 fluorinated carbon atoms) bound to the positive nitrogen of the pyridinium ring through a hydrogenated methylene group, as the surfactants in this study.²⁸

For the term with six fluorinated carbon atoms, considered as the monomer from which our gemini surfactants were built up, we obtained a volume in micellar phase, $V_{\phi, \text{m}} = 281.6 \text{ cm}^3 \text{ mol}^{-1}$.

Using the generally accepted value of $16 \text{ cm}^3 \text{ mol}^{-1}$ for the $-\text{CH}_2-$ group²⁹, we tried to evaluate $V_{\Phi, \text{m}}$ for our dichloride gemini surfactants by the group contribution approach.

TABLE 1

	s^{a}	Cmc^{b} mM	$V_{\Phi, \text{M}}$ $\text{cm}^3 \text{ mol}^{-1}$	ΔV_{mic} $\text{cm}^3 \text{ mol}^{-1}$	$V_{\Phi, \text{cmc}}$ $\text{cm}^3 \text{ mol}^{-1}$	$K_{S_{\Phi, \text{M}}}$ $\text{bar}^{-1} \text{ cm}^3 \text{ mol}^{-1}$	$\Delta K_{S_{\text{mic}}}$ $\text{bar}^{-1} \text{ cm}^3 \text{ mol}^{-1}$	$K_{S_{\Phi, \text{cmc}}}$ $\text{bar}^{-1} \text{ cm}^3 \text{ mol}^{-1}$	ΔH_{mic} kJ mol^{-1}
FGP4	4	1.86	609	21	588	0.028688	0.031323	-0.002635	-10.5 ^c
FGP8	8	1.29	681	24	657	0.033062	0.034519	-0.001457	-8.9 ^c
FGP12	12	1.10	750	26	724	0.037146	0.037273	-0.000127	-23 ^c
FGPS4	4	1.93	677	22	655	0.028582	0.031290	-0.002708	-4.6
FGPS8	8	1.40	760	24	736	0.033991	0.034286	-0.0002947	-3.2
FGPS12	12	1.05	829	26	803	0.038908	0.037020	0.0018880	--

^a s , number of carbon atoms in the spacer.

^b cmc , from conductivity measurements (Ref. 22).

^c Ref. 20.

They resulted 625 , 689 and $753 \text{ cm}^3 \text{ mol}^{-1}$ for $n = 4, 8, 12$, respectively. These values are greater than the experimental ones (see Table 1) and the difference decreases with the increasing of the spacer length. This means that the presence of the hydrogenated spacer changes the interactions inside the micelles. We have to outline that, because of the very low values of the cmc s, we were not able to measure volumetric properties near and below the cmc . Therefore, the values of $V_{\Phi, \text{s}}$, the volume at the cmc , and the change in volume upon micellization, ΔV_{m} have been obtained by a least square fitting, using eq. 6. Results are reported in Table 1. These values are strictly dependent on the values of the cmc of the gemini surfactants under investigation. The cmc values of the gemini surfactants is still an open question in the literature. We have shown how the cmc determined by surface tension measurement is even lower by one order of magnitude than that obtained via conductometric measurements, probably owing the formation of small premicellar aggregates, not surface active²².

If the volumetric parameters obtained from the conductometric value of the cmc are plotted vs. the number of carbon atoms of the spacer (Figure 7), a nice linear relationships can be obtained in the limit of the experimental error. The group contribution of the $-\text{CH}_2-$ group in the spacer can be derived from the slope. For the dichlorides it results $17 \text{ cm}^3 \text{ mol}^{-1} -\text{CH}_2-$ and $17.5 \text{ cm}^3 \text{ mol}^{-1} -\text{CH}_2-$ for $V_{\Phi, \text{s}}$ and $V_{\Phi, \text{M}}$, respectively, and a little greater for the dimethanesulfonates. In both

cases, the group contribution to the change in volume upon micellization results $0.5 \text{ cm}^3 \text{ mol}^{-1} - \text{CH}_2^-$, a value lower of that generally accepted when the $-\text{CH}_2-$ group is added to the hydrophobic tail of the traditional hydrogenated surfactants²⁹, confirming the data proposed in the literature³⁸.

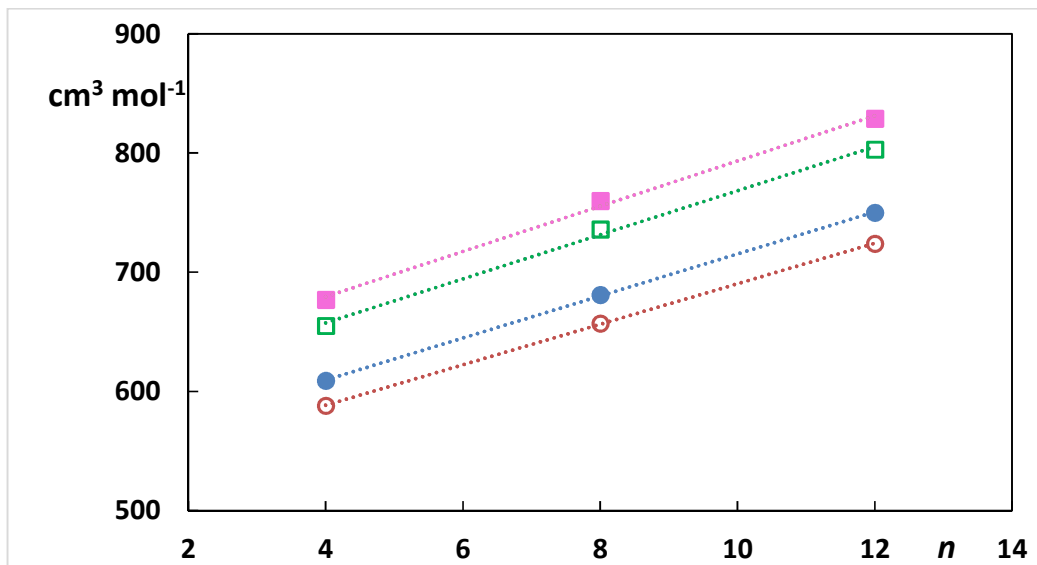


FIGURE 7. $V_{\phi,M}$ (full symbols) and $V_{\phi,S}$ (empty symbols) vs. the number, n of carbon atoms in the spacer for FGPN (circles) and FGPSn (squares).

In the same way, using eq. 6 and 7, we have processed the apparent molar adiabatic compressibility data, shown in Figures 5 and 6. They show trends vs. m similar to those of volumes with a sharp increase at the cmc till a plateau value in the micellar region. The values of the apparent molar adiabatic compressibility in the micellar state, $K_{S,\phi,M}$, are high and positive, because when micelles are formed, the stiff cavity formed by water molecules and surrounding the hydrophobic moiety of the molecule is destroyed and the electrostriction is reduced by the counterion binding to the micelle. The molar compressibility in micellar phase for the highly fluorinated surfactants under investigation is much higher than for gemini hydrogenated surfactants and increases with the spacer length in a linear way. A group contribution for the methylene group in the spacer equal to $+1.2 \cdot 10^{-3} \text{ cm}^3 \text{ bar}^{-1} \text{ mol}^{-1}$, independent from the counterion is obtained. We have found, for a homologous series of gemini surfactants, double amphiphilic betaine ester derivatives,¹² a group contribution for the $-\text{CH}_2-$ added to the hydrophobic tails, equal to $+0.94 \cdot 10^{-3} \text{ cm}^3 \text{ bar}^{-1} \text{ mol}^{-1}$. This value is very close to that reported in Ref. 37 ($0.97 \cdot 10^{-3} \text{ cm}^3 \text{ bar}^{-1} \text{ mol}^{-1}$), and to that obtained for some propanediyl- α ,- ω -bis (dimethylalkylammonium bromide) *gemini* surfactants ($1.15 \cdot 10^{-3} \text{ cm}^3 \text{ bar}^{-1} \text{ mol}^{-1}$)⁴⁰. This means that the effect of the methylene group on the isoentropic molar compressibility is the same, no matter if it is added to the tail or to the spacer of the gemini surfactant.

As said before, the values of $K_{s,\phi,S}$ were obtained from Eq. 7, because the cmcs of the fluorinated compounds are too low to allow accurate measurements below the cmc. The group contribution for each $-\text{CH}_2-$ group of $+5 \cdot 10^{-4} \text{ cm}^3 \text{ mol}^{-1} \text{ bar}^{-1}$ is obtained, regardless of the counterion. It is generally accepted that the group contribution to the molar compressibility of the $-\text{CH}_2-$, when added to the hydrophobic tail, is negative and quite small at room temperature, due to the balance between the negative contribution of the increased density of water molecules around the cavity and the positive contribution of the cavity itself^{12, 39-41}. The $-\text{CH}_2-$ group, when added to spacer, plays a minor role in the total hydrophobicity of the molecule, as suggested from the trend of the cmc with the lengthening of the spacer. For the change in molar isoentropic compressibility upon micellization a $-\text{CH}_2-$ group contribution of $+8 \cdot 10^{-4} \text{ cm}^3 \text{ mol}^{-1} \text{ bar}^{-1}$ is obtained, which is not affected by the counterion.

CONCLUSIONS

The evaluation of apparent and partial molar enthalpies as a function of concentration at 298 K of the aqueous solutions of the highly fluorinated dipyridinium gemini surfactants FGPS4 and FGPS8, differing from the previously studied FGP4 and FGP8 for having two methanesulfonates as counterions, instead of two chlorides, suggests that the effect of the counterion is additive.

The values obtained for the hydrogenated surfactants can be transferred to the fluorinated ones, allowing the prediction of enthalpic properties in solution. We have previously shown²⁵ that the gene delivery ability of the highly fluorinated dipyridinium gemini surfactants and the structure of the DNA nanoparticles resulted strictly related, particularly, to their enthalpic properties in solution, resulting in a very sensitive probe of the behaviour of the molecule in solution.

The additivity of the group contribution of the counterion confirms that this peculiar behaviour is distinctive of the dication, particularly of the structure of the polar head, and independent on the counterion and fluorination.

On the other hand, no evidence of the structure change in solution is found in the trends of volumetric properties *vs.* m , in the limits of the accuracy of the experimental methods used. If it is not possible to define a group contribution of the $-\text{CH}_2-$ group in the spacer as far as apparent and partial molar enthalpies are considered, it is not the case for apparent molar volume and isoentropic compressibilities.

From the nice linear relationships obtained by plotting the volumetric parameters *vs.* the number of the carbon atoms of the spacer, the group contribution of the $-\text{CH}_2-$ group in the spacer results $17 \text{ cm}^3 \text{ mol}^{-1} -\text{CH}_2^{-1}$ and $17.5 \text{ cm}^3 \text{ mol}^{-1} -\text{CH}_2^{-1}$ for $V_{\phi,S}$ and $V_{\phi,M}$, respectively, and a bit greater for

the dimethanesulfonates. In both cases, the group contribution to the change in volume upon micellization results $0.5 \text{ cm}^3 \text{ mol}^{-1} -\text{CH}_2^{-1}$ and the effect of the counterion is additive, as expected. The adiabatic molar compressibilities seem to be independent from the counterion. They show in micellar region a plateau value much greater than in the case of the more traditional hydrogenated gemini surfactants, indicating a less compact structure of the micelles, probably due to the repulsion between hydrogenated and fluorinated moieties of the molecules. The tight relationship we have discovered between solution thermodynamics data and their biological performance, makes “old” methods very useful for understanding at molecular level “new” applications.

ACKNOWLEDGEMENTS

This work was supported by the Italian Ministry of Education, University and Research (MIUR), PRIN2011 “NANO Molecular tEchnologies for Drug delivery – NANOMED” and by the University of Torino (Ricerca Locale ex-60% Bando 2014).

Supporting Information: Dilution enthalpies, apparent and partial molar enthalpies, densities, sound velocities, apparent molar volumes and isoentropic compressibilities *vs. m* for the compounds under investigation.

REFERENCES

1. Rosen, M. J.; Tracy, D. J. Gemini surfactants. *Surfactants Deterg.* **1998**, *1*, 547-554 and references therein.
2. Menger, F. M.; Keiper, J. S. Gemini surfactants. *Angew. Chem. Int. Ed.* **2000**, *39*, 1906-1920.
3. Zana, R. Novel Surfactants-Preparation, Applications, and Biodegradability. Dimeric (Gemini) Surfactants, in: K Holmberg (Eds.), *Surfactant Science Series*, Volume 74, Marcel Dekker, Inc., New York, 1998, p. 241.
4. Fisicaro, E. Gemini surfactants: chemico-physical and biological properties. *Cellular & Molecular Biology Letters* **1997**, *2*, 45-63 and references therein.
5. R. Zana and J. Xia, Gemini Surfactants, Interfacial and Solution Phase Behavior, and Applications, Marcel Dekker, Inc., New York, **2004**
6. Rosen, M. J. Geminis: a new generation of surfactants. *Chemtech* **1993**, *23*, 30-3.
7. Elsabahy, M.; Badea, I.; Verrall, R.; Donkuru, McD.; Foldvari, M.; Dicationic gemini nanoparticle design for gene therapy. Edited by Torres, T.; Bottari, G., *From Organic Nanomaterials* **2013**, 509-528,, 1 plate.
8. Sharma, V.D.; Ilies, M. A. Heterocyclic Cationic Gemini Surfactants: A Comparative Overview of Their Synthesis, Self-assembling, Physicochemical, and Biological Properties. *Med. Res. Rev.* **2014**, *34*, 1-44.
9. Bell, P. C.; Bergsma, M.; Dolbnya, I. P.; Bras, W.; Stuart, M. C. A.; Rowan, A. E.; Feiters, M. C.; Engberts, J. B. F. N. Transfection mediated by gemini surfactants: engineered escape from the endosomal compartment. *J. Am. Chem. Soc.* **2003**, *125*, 1551-1558.
10. Kirby, A. J.; Camilleri, P.; Engberts, J. B. F. N.; Feiters, M. C.; Nolte, R. J. M.; Soderman, O.; Bergsma, M.; Bell, P. C.; Fielden, M. L.; Garcia Rodriguez, C. L.; Guedat, P.; Kremer, A.; McGregor, C.; Perrin, C.; Ronsin, G.; van Eijk, M. C. P. Gemini surfactants: New synthetic vectors for gene transfection. *Angew. Chem. Int. Ed.* **2003**, *42*, 1448-1457.
11. Ahmed, T.; Kamel, A. O.; Wettig, S. D. Interactions between DNA and Gemini surfactant: impact on gene therapy: part I. *Nanomedicine (London, United Kingdom)* (2016), *11*(3), 289-306.
12. Fisicaro, E.; Compari, C; Duce, E.; Donofrio, G.; Różycka-Roszak B.; Woźniak E., Biologically Active Bisquaternary Ammonium Chlorides: Physico-Chemical Properties of Long Chain Amphiphiles and Their Evaluation as Non-Viral Vectors for Gene Delivery, *Biochimica et Biophysica Acta-General Subjects* **2005**, *1722*, 224-233.
13. Fisicaro, E.; Compari, C.; Bacciottini, F.; Contardi, L.; Barbero, N.; Viscardi, G.; Quagliotto, P.; Donofrio, G.; Różycka-Roszak, B.; Misiak, P.; Woźniak, E.; Sansone, F. Nonviral Gene-Delivery: Gemini Bispyridinium Surfactant-Based DNA Nanoparticles, *J. Phys. Chem. B* **2014**, *118*, 13183-13191.
14. Raczka, E.; Kukowska-Latallo, J. F.; Rymaszewski, M.; Chen, C.; Baker, J. R. Jr. The effect of synthetic surfactant Exosurf on gene transfer in mouse lung in vivo. *Gene Therapy* **1998**, *5*, 1333-1339.

15. Boulanger, C.; Di Giorgio, C.; Gaucheron, J.; Vierling, P. Transfection with fluorinated lipoplexes based on new fluorinated cationic lipids and in the presence of a bile salt surfactant. *Bioconjugate Chemistry* **2004**, *15*, 901-908.
16. Gaucheron, J.; Santaella, C.; Vierling, P. Improved in Vitro Gene Transfer Mediated by Fluorinated Lipoplexes in the Presence of a Bile Salt Surfactants. *J. Gen. Med.* **2001**, *3*, 338-344.
17. Gaucheron, J.; Santaella, C.; Vierling, P. Highly Fluorinated Lipospermines for Gene Transfer: Synthesis and Evaluation of Their in Vitro Transfection Efficiency. *Bioconjugate Chemistry* **2001**, *12*, 114-128.
18. Courier, H. M.; Pons, F.; Lessinger, J. M.; Frossard, N.; Krafft, M. P.; Vandamme, Th. F. In Vivo Evaluation of a Reverse Water-in-fluorocarbon Emulsion Stabilized with a Semifluorinated Amphiphile as a Drug Delivery System through the Pulmonary Route. *International Journal of Pharmaceutics* **2004**, *282*, 131-140.
19. Li, X.; Turanek, J.; Knoetigova, P.; Kudlackova, H.; Masek, J.; Pennington, D. B.; Rankin, S. E.; Knutson, B. L.; Lehmler, H. J. Synthesis and Biocompatibility Evaluation of Fluorinated, Single-tailed Glucopyranoside Surfactants. *New Journal of Chemistry* **2008**, *32*, 2169-2179. Erratum in *New Journal of Chemistry* **2009**, *33*, 2491-2493.
20. Fiscaro, E.; Compari, C.; Bacciottini, F.; Contardi, L.; Pongiluppi, E.; Barbero, N.; Viscardi, G.; Quagliotto, P.; Donofrio, G.; Krafft, M. P. Nonviral Gene-Delivery by Highly Fluorinated Gemini Bispyridinium Surfactant-based DNA nanoparticles, submitted.
21. Abe, M. Synthesis and applications of surfactants containing fluorine. *Current Opinion in Colloid & Interface Science* **1999**, *4*, 354-356.
22. Quagliotto, P.; Barolo, C.; Barbero, N.; Barni, E.; Compari, C.; Fiscaro, E.; Viscardi, G. Synthesis and Characterization of Highly Fluorinated Gemini Pyridinium Surfactants. *Eur. J. Org. Chem.* **2009**, *19*, 3167-3177.
23. Desnoyers, J. E.; Perron, G. Surfactant Solutions - New Methods of Investigation, Ed. R. Zana, Marcel Dekker, Inc., New York, **1987**, p.1 and references therein.
24. Lewis, G.N. and Randall, M., Thermodynamics, Mc Graw-Hill, New York (1961).
25. Fiscaro, E.; Compari, C.; Biemmi, M.; Duce, E.; Peroni, M.; Barbero, N.; Viscardi, G.; Quagliotto, P. L. The Unusual Behaviour of the Aqueous Solutions of Gemini Bispyridinium Surfactants: Apparent and Partial Molar Enthalpies of the Dimethanesulfonates, *J. Phys. Chem. B*, **2008**, *112*, 12312-12317.
26. E. Fiscaro, C. Compari, F. Bacciottini, N. Barbero, G. Viscardi and P.L. Quagliotto: Is the Counterion Responsible for the Unusual Thermodynamic Behavior of the Aqueous Solutions of Gemini Bispyridinium Surfactants? *Colloids and Surfaces, A: Physicochemical and Engineering Aspects* **2014**, *443*, 249-254.
27. Fiscaro, E.; Pelizzetti, E.; Bongiovanni, R.; Borgarello, E. Perfluorinated Surfactants: Properties and Applications. I. Thermodynamic Properties. *Colloids Surf.* **1990**, *48*, 259.
28. Fiscaro, E.; Pelizzetti, E.; Viscardi, G.; Quagliotto, P. L.; Trossarelli, L. Thermodynamic Properties of Aqueous Micellar Solutions of N-1H,1H,2H,2H Perfluoro-Octyl and Decyl

Pyridinium Chlorides. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **1994**, *84*, 59-70.

29. Fiscaro, E.; Ghiozzi, A.; Pelizzetti, E.; Viscardi, G.; Quagliotto, P. L. Effect of the Counterion on Thermodynamic Properties of Aqueous Micellar Solutions of 1-(3, 3, 4, 4, 5, 5, 6, 6, 6-nonafluorohexyl) Pyridinium Halides. Part I: Apparent and Partial Molar Enthalpies and Volumes at 298 K. *J. Colloid Interface Sci.* **1996**, *182*, 549-557.
30. Wetting, S.D.; Verrall, R.E. Thermodynamic Studies of Aqueous m-s-m Gemini Surfactant Systems. *Journal of Colloid and Interface Science* **2001**, *235*, 310-316.
31. De Lisi, R.; Milioto, S.; Verrall, R.E. Partial molar volumes and compressibilities of alkyltrimethylammonium bromides. *J. Solution Chem.* **1990**, *19*, 665-692.
32. Iqbal, M.; Verrall, R.E. Partial molar volumes and adiabatic compressibilities of glyceryl peptides at 25°C. *J. Phys. Chem.* **1987**, *91*, 967-971.
33. Chalikian, T.V.; Sarvazyan, A.P.; Breslauer, K. Partial molar volumes, expansibilities, and compressibilities of α,ω -aminocarboxylic acids in aqueous solutions between 18 and 55°C. *J. Phys. Chem.* **1993**, *97*, 13017-13026.
34. Krafft, M. P.; Riess, J. G. Selected Physicochemical Aspects of Poly- and Perfluoroalkylated Substances Relevant to Performance, Environment and Sustainability- Part One. *Chemosphere* 2015, *129*, 4-19.
35. Shinoda, K.; Hato, M.; Hayashi, T. The Physicochemical Properties of Aqueous Solutions of Fluorinated Surfactants. *J. Phys Chem* **1972**, *76*, 909.
36. Tamaki, K.; Ohara, Y.; and Watanabe, S.; Solution Properties of Sodium Perfluoroalkanoates. Heats of Solution, Viscosity *B* Coefficients, and Surface Tensions. *Bull. Chem. Soc. Jpn* **1989**, *62*, 2497-2501.
37. Tamaki, K.; Watanabe, S.; and Daikyoji, Y. Partial Molar Volumes of Sodium Perfluoroalkanoates and Lithium Perfluoro-1-alkanesulfonates in Aqueous Solutions. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 3681-3682.
38. De Lisi, R.; Ostiguy, C.; Perron, G.; Desnoyers, J. E. Complete Thermodynamics Properties of Nonyl- and Decyltrimethylammonium Bromides in Water. *J. Colloid Interface Sci.* **1979**, *71*, 147-166.
39. Kudryashov, E.; Kapustina, T.; Morrissey, S.; Buckin, V.; Dawson, K. The Compressibility of Alkyltrimethylammonium Bromide Micelles. *Journal of Colloid and Interface Science*, **1998**, *203*, 59-68.
40. Fiscaro, E.; Compari, C.; Duce, E.; Contestabili, C.; Viscardi, G.; Quagliotto, P. First Evaluation of the Thermodynamic Properties for Spheres to Elongated Micelles Transition of Some Propanediyl- α , ω -bis(dimethylalkylammonium bromide) Surfactants in Aqueous Solution. *J. Phys. Chem. B* **2005**, *109*, 1744-1749.
41. Fukada, K.; Li, J.; Fujii, M.; Kato, T.; Seimiya, T. Adiabatic Compressibility of Aqueous solutions of Amphiphiles with an Ammonium Group as the Hydrophilic Domain. *J. Oleo Sci.* , **2002**, *51*, 103-111.