BABEŞ-BOLYAI UNIVERSITY OF CLUJ-NAPOCA
FACULTY OF CHEMISTRY AND CHEMICAL ENGINEERING
CHEMICAL ENGINEERING DEPARTAMENT

PhD THESIS- ABSTRACT

LIPID NANOSTRUCTURES IN THE PRESENCE OF SOME BIOMOLECULES SOLUBLE IN WATER OR VARIOUS METAL NANOPARTICLES OF GOLD OR SILVER

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2013

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Defense: **20th of September 2013**

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INTRODUCTION

This PhD thesis has a multidisciplinary character and it contains studies from the following fields: Colloids and Interfaces, Biophysics, Chemical Thermodynamics and Structure, having applications in Nanotechnology and Nanomedicine.

The PhD thesis is devised in two parts. The first part (Cap. 1 and 2) contains literature data: different ways of studying the lipid nanostructures using Langmuir-Blodgett and other methods of studying nanostructured systems: UV-VIS, IR and Raman spectroscopy, zeta potential measurements, AFM and TEM microscopy.

The second part (Cap. 3-8) describes the lipid nanostructures formed in the absence and in the presence of proteins (collagen) and drug (propranolol) using microscopy AFM, as well as the methods used in the preparation of the gold and silver nanoparticles (including green chemistry), nanoparticles studied in the presence of lipid nanostructures, of anesthetics (procaine, dibucaine and tetracaine), and of propranolol.

The PhD thesis contains 113 figures, 25 schemes, 10 tables, 336 references, 265 pages: 30 pages describe the literature study in the field of thesis, 170 pages present the original experimental results obtained and 65 pages show some annexes (general conclusions, general bibliography-alphabetically, list of figures, list of schemes, list of abbreviations).

Some elements of originality are the following: obtaining lipid nanostructures in the presence of a collagen type I dispersion or in the presence of propranolol using Langmuir-Blodgett technique; the synthesis of gold nanoparticles using plant extracts; the synthesis of silver nanoparticles stabilized with silica and asparagine; the study of the interaction of gold nanoparticles with anesthetics for biomedical applications. Those original results have been published in 8 articles (6 ISI articles), have been presented at 4 scientific manifestations abroad and 15 national conferences. Some of the results have been obtained in collaboration with foreign universities: Newcastle

University, UK (cap 3) and Budapest University of Technology and Economics (cap 5), during 2 research stages.

KEY WORD

1. lipids
2. proteins
3. Langmuir-Blodgett technique
4. biomolecules
5. metal nanoparticles

1. MONOLAYERS AND LANGMUIR-BLODGETT FILMS

Biological membranes are lipo-proteic structures, which are separating the cells form the external medium [1-3]. Langmuir monolayers (films) can offer important models for investigation of the existing molecular forces in oriented lipid arrangements [4] at the air water interface and of the interactions between the water soluble proteins and insoluble lipids [5], therefore are considered membrane models.

Lipids can be divised in 3 groups: simple lipids (such as cholesterol), complex lipids (such as glycerophospholipids) and other lipids [3]. Due to the presence in their structure of some hydrophilic groups, as well as of some hydrophobic parts, the lipids have the ability to orient at the air-water interface.

Proteins can be classified into 2 great groups: globular proteins and fibrilar proteins, such as collagen [3].

The technique of depositing monolayers from the air water interface on solid supports is named Langmuir-Blodgett Technique. A KSV 5000 was used in studying the monolayer, namely: obtaining the insoluble Langmuir monolayers and the transfer from the air water interface on solid support, using Langmuir-Blodgett technique. There are some different types of experiments that can be done by this:

- Obtaining the surface pressure isotherms (π-A);
- Measuring the surface potential and representing the surface potential isotherms (ΔV-A);
- Deposition of the monolayers and multilayers on different solid supports, hydrophilic or hydrophobic, at different constant surface pressures

Langmuir–Blodgett Technique enables precise control of the monolayer thickness and a homogeneous deposition of the monolayer over large areas, so it can be used in studying the properties of the natural biological membranes and in building of biosensors with a high stability and good

response [6]. Solid supports can be further studied using various methods of investigation, presented in Cap. 2.

Surface pressure isotherms, plots of surface pressure versus area per molecule, offer valuable information regarding the size and the shape of the molecules from the monolayer, as well as about the interactions that appear between those molecules [7]. To prepare an insoluble Langmuir film, the substance studied must be soluble in an organic volatile solvent (such as benzene, hexane, toluene, chloroform), then it will be spread at the interface. A time for solvent evaporation is needed (Fig. 1.4). After 10-15 min., the experiment commences, by compressing the film with the help of the movable barriers and the values of the surface pressure are automatically registered [8]. So, the compression isotherm is obtained. KSV 5000 system is using the vibrating plate method to determine the surface tension and the surface pressure is simultaneously calculated.

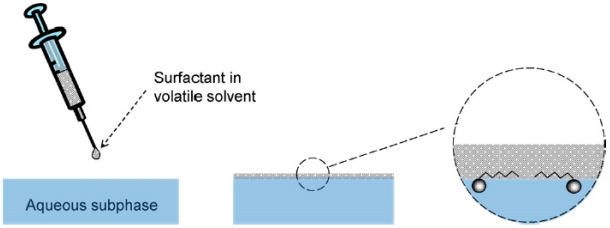


Figura 1.4 Obtaining insoluble Langmuir monolayers [8]

With increasing concentration of the molecules the monolayer is passing form a very diluted "gaseous" state (G), when the molecules are situated at great distances from one another and the surface pressure is quite low, to a "liquid" state. Generally, there are 2 types of liquid state: a liquid expanded state (LE) and a liquid condensed state (LC), depending on the orientation of the molecules (Fig. 1.5. B). The next state is the solid one, where the molecules are close packed and the surface pressure is quite high; for

further compression the collapse of the monolayer occurs and now the molecules are forming different supramolecular structures such as micelles or multilayers. As seen from the Fig. 1.5A , the transition between those is not always well defined.

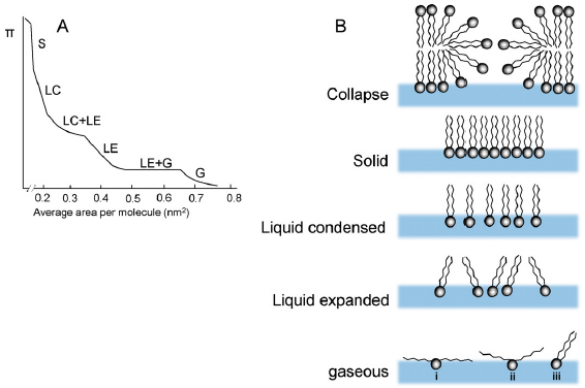


Figura 1.5 A Compression isotherm Π-A representation G-gas; LE- liquid expanded; LC- liquid condensed; S-solid. B. Lipid packing at the air water interface, adapted from literature data [8].

Surface potential ΔV at the air water interface is measured using the vibrating plate method in KSV 5000 system.

There are many ways to transfer the film from the air water interface on solid support at a constant surface pressure, but Langmuir-Blodgett (vertical transfer, Fig. 1.7.) and Langmuir-Schafer (horizontal transfer) are the most common ones [9].

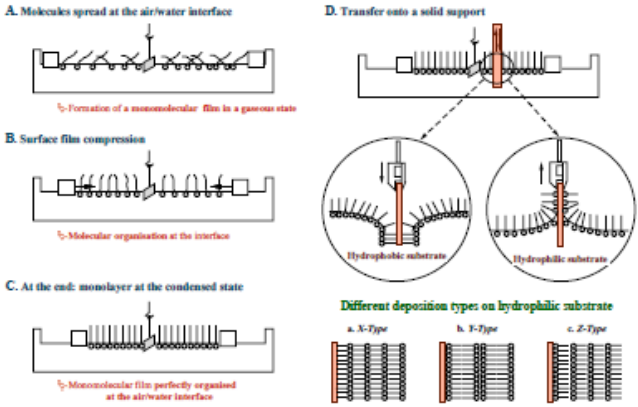


Figura 1.7 Langmuir-Blodgett Technique, literature data [10]

Further, some characteristics of the monolayer can be determined:

- The surface compressibility modulus (C_s), which shows the state of the monolayer
- The excess Gibbs free energy for mixing (ΔG_m^E), which describes the thermodynamic stability of the mixed monolayers

2. METHODS TO INVESTIGATE THE NONOSTRUCTURED SYSTEMES

The methods presented in this chapter are: spectroscopic methods (UV-VIS, IR and Raman spectroscopy), zeta potential measurements and microscopic methods (AFM and TEM microscopy).

To determine the absorption spectrum a Jasco UV/VIS V-650 spectrophotometer that measures in the wave length range 190-900 nm was used. The solutions containing metal nanoparticles present characteristic absorption peak in this domain, due to their surface plasmon resonance.

Zeta potential characterizes the stability of the colloidal systems; such a system (including metal nanoparticles) is stable if the potential is situated in the range -30 mV- +30 mV [11]. Zeta potential was measured using a Zetasizer Nano ZS90, Malvern apparatus.

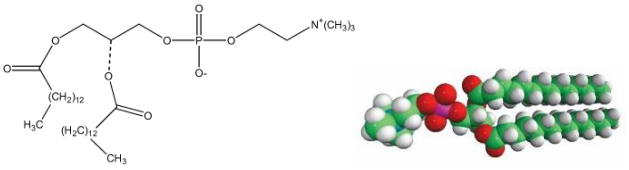
For atomic force microscopy measurements (AFM) a AFM JEOL 4210 was used [12].

Transmission Electron Microscopy (TEM) can be successfully used in studying the metal nanoparticles (gold or silver), to establish the shape and the size of the particles [13]. A JEOL – JEM 1010 apparatus was used for all measurements.

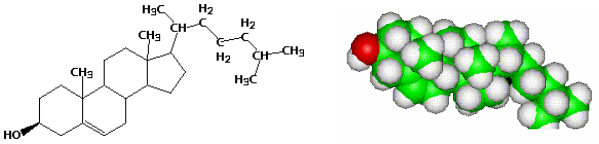
3. MIXED OR MONOCOMPONENT LIPID NANOSTRUCTURES

In the present study, we have chosen dimyristoyl phosphatidyl choline (DMPC) and cholesterol (CHO) to be investigated at the air-water interface; as monolayers, considered as membrane models (Scheme 3.1), for a better understanding of their surface behavior in mixed films or in presence of some biomolecules soluble in water.

These films are important as membrane models, and their investigation is a step towards the understanding of membrane structure and properties.



a.



b.

Scheme 3.1. Chemical structures of DMPC (a) and cholesterol (b) [14]

So, pure cholesterol (CHO) and pure DMPC, as well as their mixture were investigated in monolayers at the air water interface and on solid supports, using Langmuir-Blodgett technique [15].

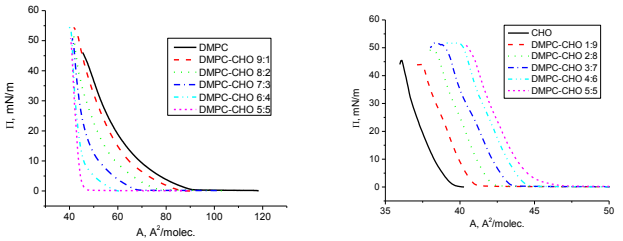
The main aim of this work is to determine the role of the polar headgroup conformation of DMPC molecules on the behavior of the mixed

DMPC and CHO monolayers at the air/water interface using surface potential measurements coupled with those of lateral surface pressures.

The influence of some proteins (collagen) or drugs (propranolol) on the DMPC pure or mixed with cholesterol films was also studied by means of surface pressure and AFM imaging. The influence of stearic acid on collagen sub phase was also investigated.

The *compression isotherms* measured for DMPC, cholesterol and their mixtures at different ratios are represented in Fig. 3.1.

All compression isotherms are shifted towards left (smaller areas per molecule) with increasing x_{CHO} values, *i.e.* a condensing effect of cholesterol is manifested.



a b

Figure 3.1 Representative compression isotherms for DMPC and DMPC-CHO mixed monolayers with $x_{CHO} \leq 0.5$ (a), and for CHO and DMPC-CHO mixed monolayers with $x_{CHO} \geq 0.5$ (b)

The differences in the collapse pressures for the various monolayers are also visible in these isotherms. While for the pure DMPC and CHO the values are lowest (about 46 mN/m for DMPC, respectively 42 mN/m for CHO), the mixed monolayers present higher collapse pressures.

A proof for the condensing effect of cholesterol in the mixed monolayers is given by the representation of mean areas per molecule, A, against the mixture composition, x_{CHO}, at constant values of the surface

pressure, π. Such *isobars* are given in Fig. 3.2 for π-values from 5 up to 40 mN/m. This condensing effect, i.e. negative deviations from the mixing rule, represented by a straight line connecting the points for pure components is most pronounced for small values of the surface pressure and it is maintained even with highest lateral surface pressures.

This condensing effect of cholesterol, and therefore the higher packing density of mixed DMPC-CHO monolayers, can be ascribed to the attraction van der Waals forces and the hydrogen bonding between the phospholipids and cholesterol, stabilizing these mixed structures [16-19].

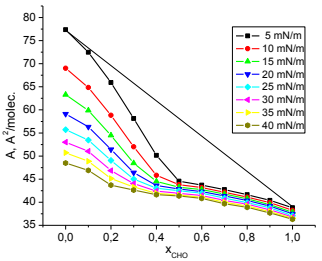


Figure 3.2. Mean molecular areas *vs.* cholesterol mole fractions in DMPC-CHO mixed monolayers at the surface pressures given in the inset

The isothermal compressibility modulus was calculated from the compression isotherms $\pi = f(A)_T$ in Fig. 3.1, by graphical derivation. The values of C_s^{-1} are roughly growing with increasing surface pressure, and reach a maximum for a surface pressure corresponding to the high packing in the monolayers before collapse. DMPC and mixed DMPC-CHO monolayers for $x_{CHO} \leq 0.2$ present the lowest and cholesterol (at least for high π-values) the highest C_s^{-1} values for the same surface pressure, while mixed monolayers show intermediate values. For cholesterol mole fractions above 0.5 the differences between mixed layers and pure cholesterol are diminished.

This surface compressibility modulus C_s^{-1}, is considered to be an indicator for the physical state of the monomolecular film [20]. When these

values pass beyond 100 mN/m, the layer should attain the liquid-condensed (LC) state, whereas values above 250 mN/m suggest the presence of the solid state, implying a close packing of the hydrocarbon chains [14]. Applying this criterion, all the mixed DMPC-CHO monolayers can pass by compression in the LC state, and even in the solid state, for various compositions with higher cholesterol content ($x_{CHO} \geq 0.4$).

A representation of the surface pressures at which film collapse occurs, against the cholesterol content of the DMPC-CHO mixtures (Fig. 3.5) shows a maximum for cholesterol mole fractions between 0.4 and 0.5, thus the most stable monolayers are obtained for these compositions [21-23].

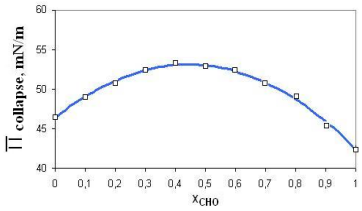


Figure 3.5. Surface pressures at film collapse *versus* cholesterol content in binary DMPC-CHO mixed monolayers

The values of the *surface potential*, ΔV, measured at different surface pressures, π, for monolayers of pure DMPC and pure CHO and of their mixed monolayers at different cholesterol mole fractions, x_{CHO}, were represented against the mean area per molecule, A, in Fig. 3.6. At the same chosen A value, pure DMPC monolayer presents the highest surface potential and pure cholesterol the lowest value (Fig. 3.6). Clearly, the surface potential is increasing with diminishing average molecular area, i.e. with increasing lateral surface pressures. For mixed DMPC and CHO monolayers, higher surface potentials are reached with an increase of x_{DMPC} content.

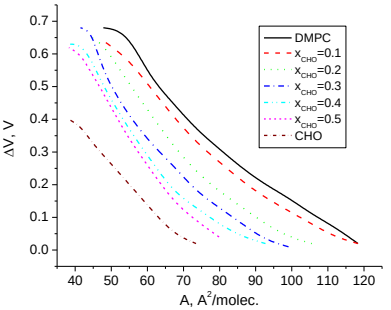


Figure 3.6. Representative isotherms of surface potentials, ΔV, versus mean area per molecule, A, for DMPC and CHO and for their mixed monolayers, at different cholesterol mole fractions, x_{CHO}, given in the insert.

These trends are more visible in the plots of the surface potentials against the cholesterol mole fraction, x_{CHO}, at constant lateral surface pressures (isobars), presented in Fig. 3.7., for pure DMPC and CHO monolayers and for their mixed monolayers.

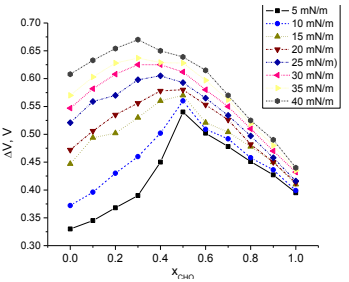


Figure 3.7. Surface potentials, ΔV, for DMPC, cholesterol and mixed DMPC-CHO monolayers against cholesterol mole fractions (x_{CHO}), at constant lateral surface pressures (given in the insert).

Surface potential rises with increasing x_{CHO} values, but after attaining a maximum value it diminishes toward the values for pure cholesterol monolayer. The maximum is reached at a molar ratio DMPC:CHO of about 1:1

at lower surface pressures, and it is shifted towards mixtures with lower CHO amounts at higher surface pressures of these monolayers.

The lack of linearity in the variation of surface potentials with cholesterol content in the mixed DMPC and CHO monolayers could be a consequence of the formation of localized domains, as suggested for the similar behavior of mixed phospholipids and usnic acid monolayers [24].

The component of the *molecular dipole moment* in vertical direction to the monolayer plane, denoted μ_v, was estimated from the plots of the surface potentials, ΔV versus 1/A, for each of the monolayers of DMPC and CHO and of the DMPC-CHO mixtures up to 0.5 in cholesterol mole fraction [25].

The μ_v value initially increases for all monolayers with decreasing A values (Fig. 3.8), reflecting the change from the liquid expanded (LE) to the liquid condensed (LC) state [15] within the monolayers. For the DMPC monolayer, this corresponds to a modification in the orientation of the polar headgroup of the DMPC molecules at the water/air interface from a horizontal arrangement (polar headgroup parallel oriented to the air/water interface) to a vertical one (polar headgroup oriented perpendicular to the interface).

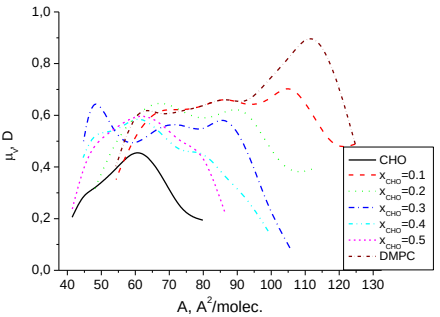
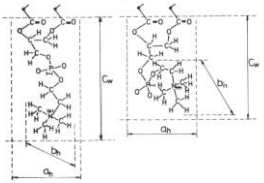


Figure 3.8. Dipole moment, μ_v, perpendicular to the monolayer plane against mean molecular area, for pure DMPC and CHO monolayers and for their mixtures. Symbols are given in the insert.

The μ_v values can be discussed using two models proposed previously [16] for the phosphatidylcholine moiety (Fig. 3.9a and Fig. 3.9b), both conformations perpendicular to the air/water interface. The notations a_h and b_h represent respectively the length and the width of the horizontal cross section of the polar headgroup, while c_w is the height of the polar part of the DMPC molecule, presumed to be anchored in the water phase.

For the pure DMPC monolayer, a maximum in the μ_v versus A curve (Fig. 3.8) is observed and it might correspond to an extended predominant conformation of the DMPC polar headgroup, as given in Fig. 3.9, within monolayer. After the maximum the observed values of μ_v decrease to an apparent plateau. The smaller μ_v values suggest that an internal salt conformation (Fig. 3.9b) would be preferred within the monolayer. This fact is in substantial agreement with the dipole moment μ_v value of the extended *a* conformation (Fig. 3.9a) which is bigger than its corresponding value for the *b* conformation of the internal salt (Fig. 3.9b).

For the DMPC and CHO mixed monolayers, the aspect of the μ_v versus A curves is rather similar, but the curves are shifted toward lower A values (Fig. 3.8), suggesting that the internal salt conformation of phosphatidylcholine moiety (b) might also be favored in mixed DMPC and CHO monolayers.



a b

Figura 3.9. Models of the polar headgroup of DMPC molecule, for two conformations of the phosphatidylcholine moiety [26], namely in its extended form (a) and in its internal salt form (b), both oriented perpendicular to the air/water interface. $(\mu_v)_a > (\mu_v)_b$. For symbols see the text.

In order to analyze the interactions of the two components in the mixed films, it is useful to investigate the variation of the mean molecular area and of the excess free surface energy with the system composition. The condensing effect of cholesterol in the mixed monolayers was mentioned (Fig. 3.2) .An even clearer picture of this effect is given by the representation of *partial molecular surface areas* $\overline{A}_i$ for DMPC and cholesterol in the mixed films, for different lateral surface pressures [27, 28].The effects, both of pressure and composition on the partial molar areas is pronounced in mixtures with lower cholesterol content (up to $x_{CHO} = 0.4$).

Another indicator of the interactions between the components of the system, and also of the thermodynamic stability of the mixed film is the *surface excess Gibbs energy of mixing*, ΔG_m^E [29, 30].

From the plots of the experimental area per molecule minus the ideal area *versus* pressure, we obtained by integration the excess Gibbs free energy for mixing per molecule; from these values we calculated the corresponding molar quantities [31, 32].

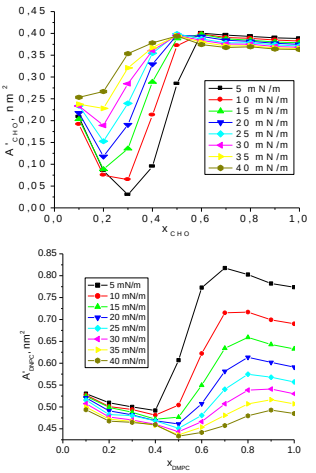


Figure 3.10. Partial molecular areas for cholesterol (a) and DMPC (b) against the their mole fraction for the lateral surface pressures given in the insets

They were represented as a function of π values in Fig. 3.11 and molar fraction in cholesterol, at a constant pressure (isobars), in Fig. 3.12.

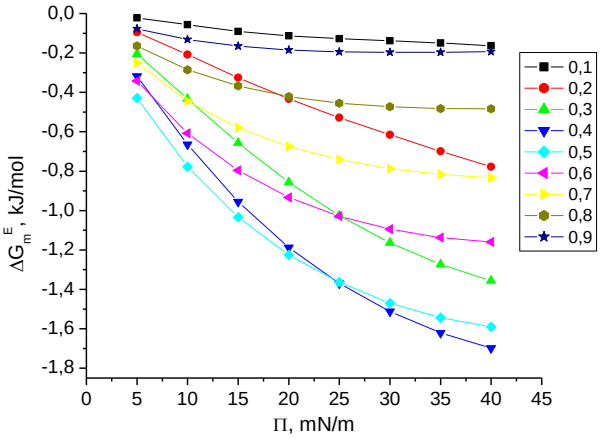


Figure. 3.11 Surface excess Gibbs energy of mixing, ΔG^{E}_{m}, against lateral surface pressures, π, for different compositions of the DMPC-cholesterol mixed films

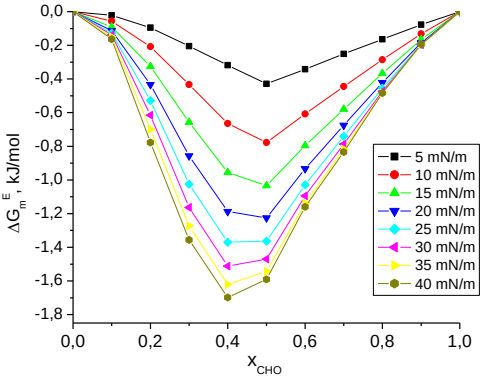


Figura 3.12 The excess surface Gibbs energy for mixing, $\Delta G_m{}^{E}$ against cholesterol mole fraction at the surface pressures given in the inset.

All the calculated ΔG_m^E values (and thus the enthalpy for mixing, ΔH_m, values) are negative, indicating that there are intermolecular attraction forces between the components of the mixed films, and therefore the mixing of DMPC and cholesterol is favored.

The minima of the plots, i.e. the maximum stability of the mixed films, occur for a nearby equimolar ration of DMPC and cholesterol (x_{CHO} = 0.5 for lower surface pressures and 0.4 for the highest lateral pressures).

AFM observations were used to complete the picture of DMPC, CHO and mixed DMPC-CHO layers near collapse pressure, transferred on glass surface [33-35]. In Fig. 3.18, some AFM images for a mixed film DMPC-COL (x_{CHO} =0,5) are presented, as an exemplification.

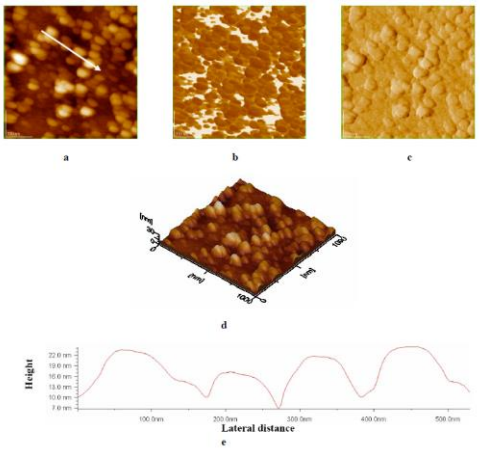


Figure 3.18. AFM images of a mixed DMPC-cholesterol film (x_{CHO} = 0.5) transferred on optical polished glass at $\pi_{collapse}$ = 10 mN/m. Scanned area 1 μm x 1 μm. a) 2D- topography; b) amplitude image; c) 3D-topography; d) profile of the cross section along the arrow in panel a.

Interaction between lipids and drugs was intensively studied in the last years [36-54], but it is for the first time when the interaction between DMPC, CHO and their mixture (1:1, 7:3, 3:7) with *propranolol*, is studied using LBT and AFM, and compared with the behavior of mixed lipid films on water subphase in absence of this drug. The compression isotherms on different concentrations of propranolol in subphase were registered. They show the interaction of lipids with propranolol. Also AFM imaging reveals the influence of propranolol, leading to some spherical structures.

Interaction between lipids and proteins is often present in literature [55-67], but it is for the first time when the interaction was investigated between DMPC, CHO and stearic acid, AS (as comparison) with *collagen* type I dispersion, using LBT and AFM, in order to simulate the interactions that appear between lipids and proteins within biological membranes [68-70]. The type I collagen was dissolved in 1% (0.167 M) acetic acid solution at 4^{o}C and an aqueous acidic solution of collagen of the desired concentration (1 g/L) was obtained ($pH \approx 3$). Collagen fiber can be seen using AFM, in Fig. 3.28.

The compression isotherms for DMPC, COL, and AS films, spread on ultrapure water, were compared with the ones obtained on collagen aqueous dispersion. In all cases an expansion of the films in presence of collagen was observed. Collapse surface pressure has quite the same values as on aqueous subphase. The lipid nanostructures in presence of collagen were analyzed using AFM imaging.

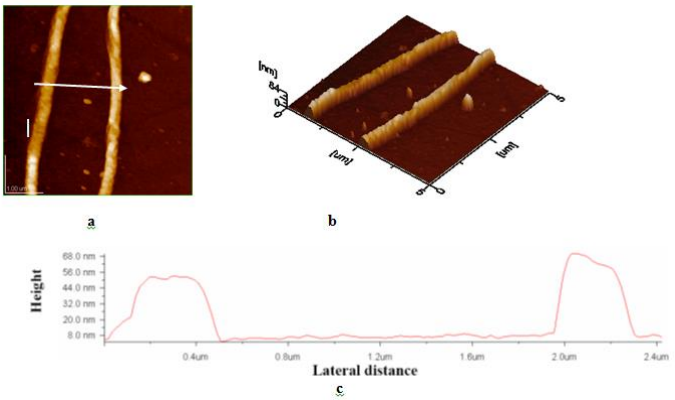


Figure 3.28. AFM images on collagen type I on NaCl 2N subphase, deposited on glass support at π = 30 mN/m. Scanned area 5 µm x 5 µm. a) 2D-topography; b) 3D-topography; c) profile of the cross section along the arrow in panel a.

During a research stage at Newcastle University, UK, some liposomal lipid nanostructures between DPPC and DOPC were studied [71-77]. The AFM images of the structures show the presence of unilamellar vesicles

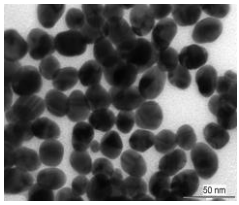
4. THE PREPARATION OF METAL NANOPARTICLES OF GOLD OR SILVER

Gold nanoparticles were obtained using two different methods of preparation: reduction with citrate in aqueous medium [78] and reduction with tetrahidroborate in organic medium (toluene), using cetyltrimethyl ammonium bromide as stabilizer [79]. Gold nanoparticles obtained by reduction with citrate had 4 different sizes: 30 nm, 20 nm, 10 nm and 5 nm. For this purpose $Na_3Au(SO_3)_2$ (30 mm) and $HAuCl_4$ (for the other sizes) were used. For the smallest particles (10 and 5 mm) tannic acid was added [80].

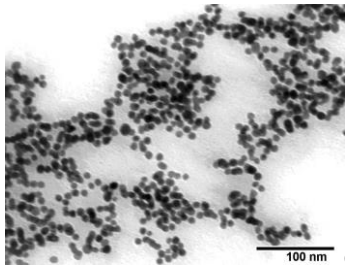
AgNPs were prepared by reduction of silver nitrate, $AgNO_3$, with glucose in aqueous solution in presence of tetraethyl orthosilicate TEOS and asparagine, as stabilizing agent.

The metal nanoparticles have been characterized using UV-Vis spectroscopy. AuNP dispersions present a maximum of absorption at 515-529 nm, depending on the particle size, while for silver nanoparticles the absorption occurs at 414 nm.

TEM images present the shape of the gold and silver nanoparticles (Fig. 4.4) so their average diameter and the size distribution can be evaluated (e.g. histogram Fig. 4.5).



a). b).



c).

Figure 4.4. TEM image of gold nanoparticles; average size: 30 nm (a), 20 nm (b) and 10 nm (c)

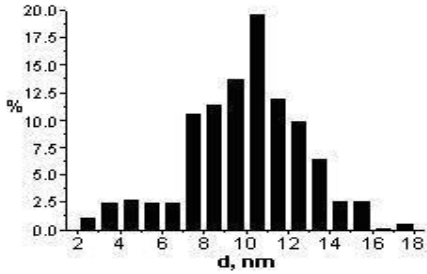


Figure 4.5. Histogram of size distribution for 10 nm GNPs

5. LIPID NANOSTRUCTURES CONTAINING METAL NANOPARTICLES

The Langmuir-Blodgett Technique (LBT) proved to be suitable in obtaining gold or silver nanoparticles networks by self-assembly of the nanoparticles on water surface [81-85]. The goal of this study was to gain insights into the assembly formation of gold nanoparticles of different sizes at air/water interfaces by LB technique [86-87]

The initial dispersion of GNPs was centrifuged using a PJ 180 rotor at 10,000 rpm for 30 min., and GNPs were washed with ultra pure water and centrifuged again. This process was repeated for five times, until GNPs without surfactants were obtained.

Then, the GNPs precipitate was dispersed in organic solvents, such as chloroform, hexane or toluene, and spread out at the air-water interface.

For example, in the case of chloroform, the compression isotherms (pressure, Π– area, A) of GNPs layers were obtained for the three particle sizes: 30 nm, 20 nm and 10 nm, as presented in Fig. 5.6.

At a constant lateral surface pressure (about 10 mN/m, 15 mN/m or 18 mN/m), the GNPs layer was transferred from air-water interface on different solid supports, such as glass optically polished, silanized glass, positively charged glass, conductive glass, silicon, mica and TEM grids in horizontal transfer configuration. Subsequently the GNPs layers were investigated by AFM, in tapping mode AFM observations revealed a spontaneous ordered arrangement of the particles, closed packed by self-organization of GNPs at the air/water interface.

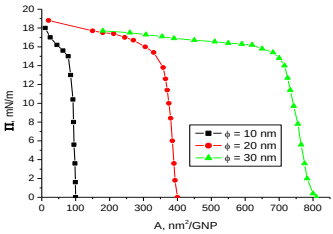


Figure 5.6. Compression isotherms for GNPs (10, 20 and 30 nm) spread at the air/water interface

A new approach was the investigation of the arrangement and behavior of GNPs at the air/water interface, by the LB technique, using stearic acid as surfactant.

Stearic acid was spread over the GNP film spread at the air/water interface; compression at a specific surface pressure; resulted in the interaction of stearic acid with the GNPs and the partial functionalization of the latter at the air/water interface. The compression isotherms evidenced the influence of GNPs upon the stearic acid surface film. The transfer of the composite film (stearic acid with GNPs) at selected constant surface pressures on solid supports, made possible the AFM investigation of the films.

On this GNPs film, compressed at lateral surface pressure of about 10 mN/m, stearic acid was spread from two different solvents: benzene or hexane, as well as their mixture.

The compression isotherms (Fig. 5.7.) of composite films show an expanding of the stearic acid film in the presence of GNPs. This expanding is greater the greater the GNP size is. The interactions between stearic acid and the GNPs are also reflected in the higher values for the collapse pressure: about 55 mN/m, against 44 … 50 mN/m for pure stearic acid.

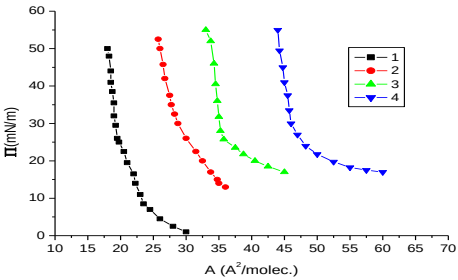


Figure 5.7. Compression isotherms for stearic acid film and hybrid films stearic acid + GNPs spread at the air/water interface.
Stearic acid (Curve (1); Stearic acid and GNP 10nm (Curve 2);
20nm (Curve 3) and 30 nm (Curve 4)

The AFM observations proved that the GNPs layers stabilized with stearic acid build ordered arrangements – in compactly packed domains - by self assembly at fluid interfaces (Fig. 5.20). The AFM images evidence an advanced structuration both in the hybrid monolayers and in the multilayers of composite film, consisting of GNPs half surfactant covered at the air/water interface. The roughness and the morphology of the composite film show the formation of large compactly packed domains [86-87].

The influence of the nature of the solid support was also evidenced by the AFM investigations. For instance the hybrid film on silicon support presents a very low roughness, showing that the composite film is nearly planar.

The AFM images show a nearly compact packing of the nanoparticles within the stearic acid film. The composite film consists of two LB layers and has a very low roughness (about 5 – 6 Å), implying a nearly plane hybrid film.

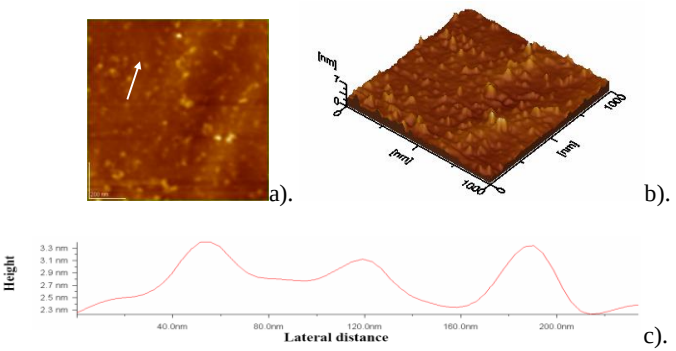


Figure 5.20. AFM images of GNPs (size 20 nm) in stearic acid, deposited on silicon (2 layers). Scanned area: 1 x 1 μm^2. a) 2D- and b) 3D-topographic image; c) cross section along the arrow in Fig. 5.20a. LBT horizontal transfer. Push method of transfer, π = 15 mN/m.

The assembly of GNPs at the air water interface by their partial functionalization with stearic acid and the transfer on solid supports has potential applications as building blocks for elaborating new materials with a controlled architecture at nano level.

6. METAL NANOPARTICLES FUNCTIONALIZED WITH BIOMOLECULES

Metal nanoparticles, particularly noble metal nanoparticles, are in the focus of interdisciplinary research on nanomaterials, due to their specific properties and potential applications in the field of nanotechnology, catalysis, molecular electronics, but also in biomedicine, with applications for biosensors, medical imaging, molecular diagnostics and therapeutics or controlled drug delivery [88-94].

The tendency of nanoparticles self assembly in solution or on various substrates, with the formation of agglomerates is also a much investigated problem, and the clusters size and arrangement of particles play an important role in the behavior of the materials [95]. Noble metal nanoparticles (gold, silver, platinum, palladium, or hybrid nanoparticles) are the most used in this context [96-98]. Among these nanoparticles, for this work, we select gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs).

Here the interactions between AuNPs and AgNPs with three local anesthetics are investigated. The chosen molecules are: two amino esters, namely procaine, 2-(diethylamino)ethyl 4-aminobenzoate, and tetracaine, 2-(dimethylamino)ethyl 4-(butylamino)benzoate, and an amino amide, dibucaine (cinchocaine), 2-butoxy-*N*-[2-(diethylamino)ethyl]quinoline-4-carboxamide. Their pharmacological action in producing the local anesthesia is mediated by membranes, and modeling such phenomena was one of our major research interests [99-103]. The acid-basic properties and the protolytic equilibria of these anesthetics were also investigated by us [104, 105] since they play an important role in the interaction of the molecular species present in solution at a given pH value with charged metal nanoparticles [99-101].

Also, the interaction between AuNPs and propranolol: (*RS*)-1-(1-methilethilamino)-3-(1-naftiloxi) propan-2-ol, a drug used in treatment of hypertension [106] was investigated.

Gold nanoparticles (AuNPs) were obtained in our laboratory by reduction of gold (III) chloride, $AuCl_3$ with sodium citrate in aqueous solution, containing tannic acid in order to control the particles size.

AgNPs were prepared by reduction of silver nitrate, $AgNO_3$, with glucose in aqueous solution in presence of TEOS and asparagine, as stabilizing agents.

The metal nanoparticles with/without biomolecules have been characterized using UV-Vis spectroscopy, TEM and AFM imaging and zeta potential measurements.

AuNP dispersion [107] presents a maximum of absorption at 515-517 nm and high stability, having a zeta potential value of -40 mV. Their diameter is 5 ± 1.3 nm, from TEM images.

AgNP dispersion is very stable (zeta potential: -44.2 mV); maximum of absorption at 406 nm, the diameter (from TEM data): 24.5 ± 5.3 nm [108-111].

The addition of anesthetic solution in increasing amounts to the AuNPs solution produces a clear progressive red shift of the absorption maximum and its broadening, a characteristic feature for the assembly of the AuNPs, mediated in our case by the biologically active molecules adsorbed on their surface. Although the aggregates appear practically immediately after the mixing of solutions, the self-assembly process continues progressing in time. The absorbance maximum decreases for longer times, due to coagulation and sedimentation of larger aggregates. After 1 or 2 weeks the deposition of the assembled AuNPs was complete, and the color of the solution disappeared.

The TEM images confirm the building of GNPs assemblies mediated by the anesthetic molecules. For dibucaine and tetracaine spherical aggregates are formed, whereupon for dibucaine larger and more compact aggregates are found, including hundreds of particles.

We could conclude that the interactions between AuNPs and anesthetic molecules are the strongest in case of dibucaine and the least intense for procaine.

In the case of silver nanoparticles, the situation is very similar, as it can be seen from UV-Vis spectra (Fig. 6.9), TEM (6.10) and AFM imaging (Fig. 6.11). The same order is maintained for the three anesthetics.

In our experimental conditions (pH = 5.5) the three anesthetic should be almost exclusively in the protonated monocationic form AH^+. Since the citrate capped AuNPs and the silica capped AgNPs are both negatively charged, the adsorption of the anesthetic molecules should be favored by electrostatic attractions. Their adsorption results in a decrease in the zeta potential values of the NPs, promoting the coagulation of the colloidal system. Nevertheless the marked differences in the behavior of the NPs (especially AuNPs) against the three anesthetics suggest that specific interactions between nanoparticles and the anesthetic molecules should also play a role in linking NPs to build assemblies.

For instance the organic molecules can mediate the self assembly by the formation of hydrogen bonds to oxygen atoms or hydroxylic H atoms in the coatings of the AuNPs. Dibucaine molecules present the highest disposability for bridging MPs by forming such bonds – by the O and H atoms of the amide group, by the O in the ether group, and even by the quinoline N atom.

Undoubtedly, the hydrophobic interactions can also not be ruled out. Anyway, a combination of these interactions will lead to these self assemblies

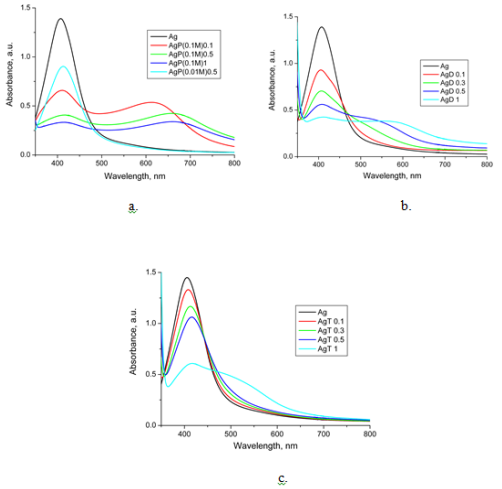


Figure 6.9. Optical absorption spectra of AgNPs with 10^{-2}M and 10^{-1}M procaine, P (a), 10^{-2}M dibucaine, D (b), and 10^{-2}M tetracaine, T (c) solutions; volumes (ml) of anesthetic solutions added to 2 ml AgNPs solution are indicated in the inserted frame.

The significant interactions revealed by our investigation between local anesthetic molecules and both gold and silver nanoparticles, resulted in UV-VIS spectra modifications and in TEM images of the assembles, show their potential for building molecularly well defined surfaces with controlled surface characteristics with possible industrial, biological and medical applications, such as selective sensing of anesthetics in various biological fluids

On the other hand, colloidal systems containing AgNPs could find medical applications, based on the well known bactericidal properties of silver [44].

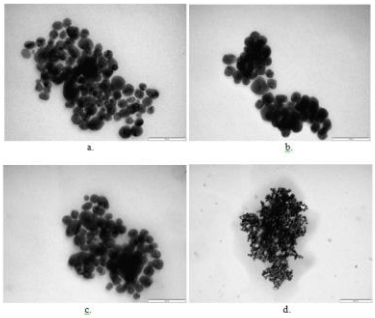


Figure 6.10. TEM images of the AgNPs with 10^{-2} M procaine (a), 10^{-3} M dibucaine (b), and 10^{-2} M tetracaine (c, d) solutions in the 1:1 volume ratio, bars in the images correspond to 100 nm (a, b, c), respectively to 200 nm (d)

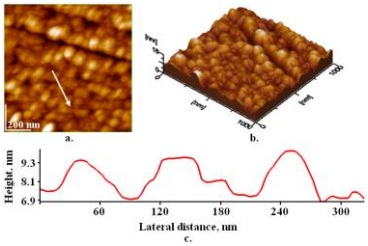


Figure 6.11. AFM images AgNPs on glass in presence of dibucaine; scanned area 1 μm x 1 μm: (a) 2D- topography; (b) 3D – topography; (c) profile of the cross section along the arrow in panel a

The interaction between propranolol and AuNP is less intense and it was observed using UV-VIS spectroscopy and TEM imaging.

7. BIOSYSTEMS CONTAINING GOLD NANOPARTICLES AND COLLAGEN

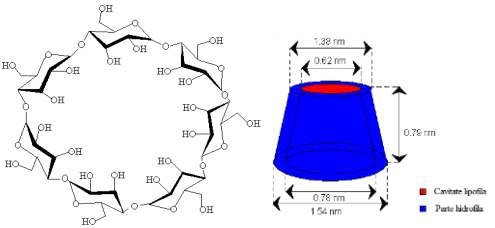
In our experiments we employ β-cyclodextrine (β-CD) both as reducing and protecting agent for obtaining GNPs to be used in biological applications. As a first test molecule for the functionalization of the GNPs we selected collagen.

Gold nanoparticles (GNPs) were prepared by reduction of $HAuCl_4$ solution with β-CD (Scheme 7.1) at basic pH without addition of any stabilizer, at room temperature. The process was monitored by measuring the UV-Vis spectra.

The GNPs (average diameter from TEM measurements: 7.7 ± 1.3 nm) are slightly negatively charged (zeta potential -23.4 mV), but very stable .

Therefore the high stability observed for the Au nanoparticles obtained by reduction with βCD can not be primarily assigned to electrostatic repulsions between particles as preventing the coagulation of the system, but rather to the effect of the βCD coating on the surface of nanoparticles.

The FT Raman spectra of the β-CD solution and of the same solution after the reaction with $HAuCl_4$ and the formation of the GNPs are quite similar, no major differences could be revealed. Therefore direct Au-O interactions between the gold atoms and the 21 hydroxyl groups of β-CD are not probable.



Schema 7.1. Structure and geometric sizes of β-cyclodextrin [112]

Adding increasing volumes of acidic (pH 3) collagen solution (concentration 1 g/L) to the colloidal gold solution, the absorption peak is

initially red shifted, then remains unchanged for higher amounts of collagen, while the decrease of the absorption in time is slow. The color of the solution remains red-violet even after 2 days. Here there is only a limited aggregation of the GNPs.

The effect of an acetic acid solution of the same concentration as that used in preparing the collagen solution is different: the sedimentation of the GNPs aggregates begins immediately. Thus collagen prevents the advanced aggregation of GNPs and their separation from the solution. The self assembly of GNPs in presence of the acidic collagen solution is not due exclusively to the acidity of the solution, but the collagen molecules have a specific mediating effect on the assembly of nanoparticles.

The zeta potential measured for the acidic collagen solutions was positive: + 29.7 mV, and the average particles size was given by DLS as 740 nm, with a size distribution between 525 and 950 nm.

In the mixture of the acidic collagen solutions (positively charged particles) with the GNPs solution (slightly negatively charged), the measured zeta potential value was positive: + 23.1 mV (3 g/L collagen). By DLS two very distinct populations were observed: particles with an average size of 70-90 nm and 530 nm. We can assume that the lower sizes correspond to associations of GNPs, while the large size fraction corresponds to collagen aggregates, possibly also containing GNPs. The conjugation of GNPs with collagen particles is favored by the electrostatic attraction between the differently charged particles, but specific interactions should play a more important role.

In some of the AFM images obtained by drying the mixed collagen-GNP films obtained by vertical adsorption for 10 s on glass and drying in air, collagen fibrils appear. But also GNPs are observed, both on the fibril surface and outside it (Fig. 7.8). Probably GNPs were also absorbed within the fibrils, during the self association of collagen molecules and aggregates into fibrils. Thus collagen fibrils obtained in presence of GNPs solution are mineralized with metal nanoparticles.

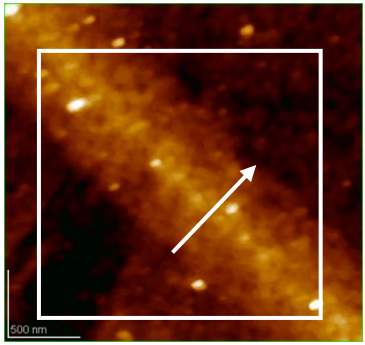


Figure 7.8. 2D topographies of collagen fibers with GNPs on glass, assembled from aqueous solution of collagen in presence of GNPs.
Scanned area: 5 μm x 5 μm

8. THE SYNTHESES OF GOLD NANOPARTICLES USING PLANT EXTRACTS

The introduction of biogenic methods in the obtaining the metal nanoparticles has become an important field in chemistry, biology and materials science [113-115]. The nanoparticles prepared using biological materials have the same shape and size like the ones obtained using chemical methods [116]. The stabilizing agents are, in general, the same as the reduction ones. Most of the syntheses take place at room temperature or very close to it.

In the present work we investigate the biogenic synthesis of gold nanoparticles by the reduction of $HAuCl_4$ with extracts obtained from different parts of three medicinal plants: garden angelica (*Angelica archangelica*) roots, St. John's wort (*Hypericum perforatum*) *herba*, and witch-hazel (*Hamamelis virginiana*) bark [117, 118].

We explore the possibility of using these plant extracts as reducing agents for Au (III), and simultaneously as stabilizing agents for the obtained GNPs, as well as the self assembly of the NPs, mediated by active substances also contained in these extracts. To this end, the working conditions (concentrations of reagents, succession of their adding, temperature, pH) were varied.

The three plants are well known medicinal plants.

Angelica roots extracts contain angelic acid [trans-CH_3-CH=C(CH_3)-COOH], resins, a fitosterol, a volatile oil containing terpenes (phellandrene) and sesquiterpenes [119], polysaccharides and tannins. *Hypericum* plant and blooms contain tannin, essential oil [120], choline, anthracene derivatives, flavonoids, caffeic and chlorogenic acid, *Hamamelis* [121] contains in the bark tannins, flavonoids, volatile oil, resins, fitosterols etc.

By photometric dosing of the plant extracts used, the concentrations of polyphenolic compounds were determined; they are given in equivalent

caffeic acid concentrations. Their amount in the extracts is important, since they are reducing agents for the obtaining of Au (0) from Au (III).

The AFM imaging shows a different behavior of the gold nanoparticles synthetized using plant extracts, as it can be seen for Hypericum in Fig. 8.13 and for Angelica in Fig. 8.14.

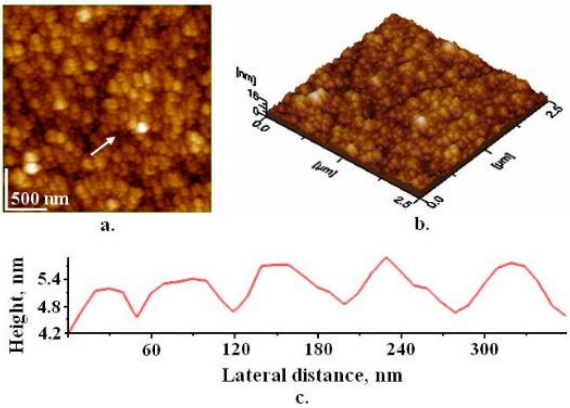


Figure 8.13. AFM images GNPs on glass, obtained with *Hypericum* extract (dilution 1:100); scanned area 2.5 µm x 2.5 µm: (a) 2D- topography; (b) 3D – topography; (c) profile of the cross section along the arrow in Fig. 8.13 a

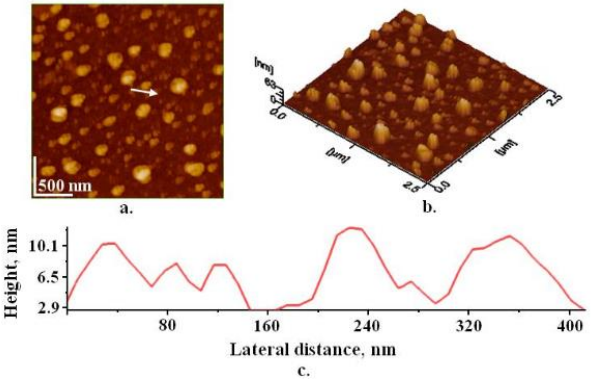


Figure 8.14. AFM images GNPs on glass, obtained with *Angelica* extract (dilution 1:6); scanned area 2.5 μm x 2.5 μm: (a) 2D- topography; (b) 3D – topography; (c) profile of the cross section along the arrow in Fig. 8.14 a

From FTIR spectrum it can be noticed that in presence of Angelica, the intensities of the OH (3370 cm^{-1}) and C-OH (1055 cm^{-1}) bands decrease while they are increased for COOH bands (1410 cm^{-1} and 1655 cm^{-1}).

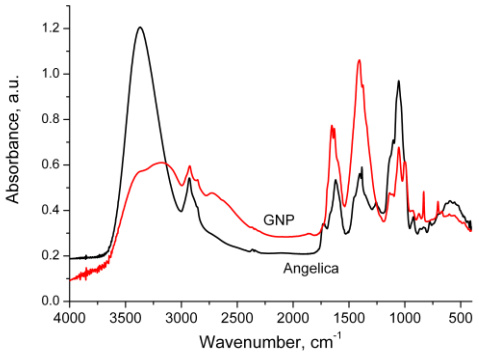


Figure 8.15 FTIR spectra for *Angelica* extract (solid line) and GNPs obtained by reduction with this extract (dotted line).

9. GENERAL CONCLUSIONS

The behavior of dimyristoyl phosphatidylcholine (DMPC) and cholesterol (CHO), as well as of their mixed monolayers of various compositions (ratios from 9:1 to 1:9) has been investigated by surface pressure and surface potential (ΔV) measurements at the air/water interface, using Langmuir technique coupled with the vibrating plate method.

Langmuir-Blodgett films were transferred on solid glass support (optical polished glass). These films are important as membrane models, and their investigation is a step towards the understanding of membrane structure and properties.

The interaction between DMPC, CHO and their mixture (1:1, 7:3, 3:7) with propranolol was also studied and compared with the compression isotherms obtained on pure water subphase and their nanostructures have been visualized using AFM.

The interaction of DMPC, CHO and stearic acid (chosen for comparison) with collagen type I is presented. In all cases, an expansion of the films in the presence of protein can be noticed and it is higher for higher quantities of protein dispersed in subphase. The collapse pressure of the films is quite the same in all cases. Lipid nanostructures obtained in the presence of collagen were also investigated using AFM imaging.

Along with the lipid nanostructures obtained using Langmuir-Blodgett technique, we present also liposomal structures formed by DPPC and DOPC, obtained using the specific techniques for vesicles.

Gold nanoparticles having different particle size (5 nm, 10 nm, 20 nm, 30 nm) were prepared by adapting some methods given in the literature. Silver nanoparticles (12 nm) have been prepared using a new method of preparation.

They were characterized using UV-VIS spectroscopy (to determine the absorption maxima) and TEM (to determine the diameter of the particles). Using TEM data, the histograms of the size distribution of the particles were constructed.

Surface films containing gold nanoparticles (different size of the particles) were produced at the air water interface using Langmuir-Blodgett technique.

These films were further functionalized with stearic acid and their compression isotherms were studied. After their deposition on solid supports they were investigated by AFM.

The GNPs films spread from dispersions in organic solvents (chloroform) gave ordered arrangements at the air/water interface. Applying a lateral surface pressure, close packed structures of particles were obtained. These structures were also maintained after LB transfer on the interface solid support / air.

The spreading of a stearic acid film on the GNPs film, previously compressed at a given lateral pressure resulted in the interaction of stearic acid with the GNPs and the partial functionalization of the latter at the air/water interface.

The compression isotherms evidenced the influence of GNPs upon the stearic acid surface film. The transfer of the composite film (stearic acid with GNPs) at selected constant surface pressures on solid supports, made possible the AFM investigation of the films.

The AFM observations proved that the GNPs layers stabilized with stearic acid build ordered arrangements – in compactly packed domains - by self assembly at fluid interfaces. The influence of the nature of the solid support was also evidenced by the AFM investigations.

The LB technique proved to be important and useful, providing thin LB films, monolayers or multilayers, well organized and containing gold nanoparticles.

Silver nanoparticles (AgNPs) were prepared in aqueous colloid dispersions by the reduction of Ag^+ with glucose in alkaline medium. Tetraethyl orthosilicate (TEOS) and L-asparagine were added as stabilizers of nanoparticles.

The silver nanoparticles obtained by the method proposed by us proved to be quite stable in time, due to their mixed hydrate silica and asparagine coating. Nevertheless, they show a high sensitivity toward the investigated anesthetic molecules.

The interaction of gold and silver nanoparticles with three local anesthetics (procaine, dibucaine or tetracaine) was investigated.

The interaction of gold nanoparticles with propranolol was also investigated and it was observed that it appears after 3 days after the preparation of the mixtures.

Optical spectra reveal the modifications in the absorption band of nanoparticles related to their self assembly mediated by anesthetic molecules and depending on the progress in time of the aggregation process and reveal marked differences in the behavior of the nanoparticles against the three anesthetics.

Zeta potential measurements have been applied to characterize the electrostatic stability of NPs.

AFM images show the characteristics of AgNPs films deposited on glass support.

The main effect of various anesthetics can be described in terms of electrostatic forces between the negatively charged metal nanoparticles and anesthetic molecules, existing in their cationic form at the working pH.

The significant interactions revealed by our investigation between local anesthetic molecules and gold and silver nanoparticles show their potential for building molecularly well defined surfaces with controlled surface characteristics with possible industrial, biological and medical applications, such as selective sensing of anesthetics in various biological fluids.

On the other side, colloidal systems containing silver nanoparticles could have medical applications, based on their well known antimicrobial properties of silver.

Gold nanoparticles (GNPs) were prepared by reduction of $HAuCl_4$ solution with β-cyclodextrine (β-CD) at pH 11 without addition of any stabilizer, at room temperature. The obtained gold colloidal solution presents a high stability.

UV-Vis spectra, AFM images and FT Raman spectra were also used to characterize the obtained GNPs.

Their interaction with a solution of type I collagen from bovine Achilles tendon dissolved in acid medium was investigated by UV-Vis and zeta potential measurements and by TEM and AFM imaging.

The assembly of GNPs nanoparticles in presence of collagen solution was observed. In mixed collagen-GNPs films on glass support the formation of collagen fibrils charged with gold particles was revealed by AFM images.

The introduction of biogenic methods in the obtaining of metal nanoparticles is a consequence of the trend to apply approaches of green chemistry in nanotechnology.

The advantages of using the biogenic methods for obtaining gold nanoparticles are: low cost of preparation, avoiding contamination with organic solvents and their biocompatibility, having possible biomedical applications.

We prepared gold nanoparticles starting with hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), using three reduction agents extracts from medicinal plants, (from Angelica, Hypericum and Hamamelis).

The content of reducing compounds (polyphenols) in each plant extract was determined by photometric dosing.

The resulting colloidal gold solutions were characterized by UV-Vis and FT-IR spectroscopy and transmission electron microscopy (TEM) imaging.

The gold nanoparticles had an average diameter of about 4-8 nm, were obtained at room temperature, and pH value about 8. They present various shapes from spherical, to ovals, heart or polyhedral forms.

Some of the colloidal solutions obtained are rather stable in time, for other their self assembly is observed. At lower concentration of the plant extract the tendency to self aggregation of the GNPs increased.

The plant extracts contain reducing agents, compounds with stabilizing effect on the GNPs, but also components which mediate their self assembly.

The GNPs obtained by these biogenic syntheses have potential biological and medical applications, due to the medical properties of the vegetal extracts adsorbed and to the antibacterial characteristics of gold nanoparticles.

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11. LIST OF PUBLICATIONS

1. PUBLISHED/ ACCEPATED ARTICLES
1.1. ISI PAPERS (IF- impact factor);

1. Roxana-Diana Paşca, Aurora Mocanu, Simona Cobzac, Ioan Petean, Ossi Horovitz, Maria Tomoaia-Cotişel, "Biogenic syntheses of gold nanoparticles using plant extracts", *Particulate Science and Technology*, **2013**, accepted August 27 th (IF=0,545).
2. Aurora Mocanu, **Roxana-Diana Paşca**, Gheoghe Tomoaia, Corina Gabro, Petre Frangopol, Ossi Horovitz, Maria Tomoaia-Cotisel, "New procedure to synthesize silver nanoparticles and their interaction with local anesthetics", *International Journal of Nanomedicine*, **2013**, accepted August 9 th (IF=4,027).
3. Aurora Mocanu, **Roxana-Diana Paşca**, Gheorghe Tomoaia, Antonis Avranas, Ossi Horovitz, Maria Tomoaia-Cotisel, "Selective effect of procaine, tetracaine and dibucaine on gold nanoparticles", *Journal of Nanoscience and Nanotechnology*, **2012**, 12 (12) 8935-8939 (IF=1,563).
4. Maria Tomoaia-Cotişel, **Roxana-Diana Paşca**, Ossi Horovitz, Aurora Mocanu, "Surface Potentials of Cholesterol and Dimyristoyl Phosphatidylcholine Monolayers at the Air-Water Interface", *Revue Roumaine de Chimie*, **2011**, 56 (10-11), 1047-1053 (IF=0,418).
5. Csaba Pal Racz, **Roxana-Diana Paşca**, Szabolcs Santa, Irina Kacso, Aurora Mocanu, Ossi Horovitz, Maria Tomoaia-Cotisel, „Inclusion Complex of β-Cyclodextrin and Quercetin. Thermodynamic Approach", *Revista de Chimie (Bucharest)*, **2011**, 62 (10), 992-997 (IF=0,599).
6. Aurora Mocanu, **Roxana-Diana Paşca**, Ossi Horovitz, Maria Tomoaia-Cotişel, "Behavior of Mixed DMPC-Cholesterol Monolayers at the Air-Water Interface", *Studia Universitatis Babeş-Bolyai- Chemia*, **2010,** 2 (II), 303-312 (IF=0,231).

1.2. PROCEEDINGS PAPERS:
7. **Roxana-Diana Paşca**, Maria Tomoaia-Cotişel, Ossi Horovitz, Aurora Mocanu, "Cholesterol and Dimyristoyl Phosphatidylcholine Monolayers at the Air-Water Interface. Structural and Thermodynamic Characterization", *in Proceedings of the Humboldt-Kolleg "Knowledge, Culture, Science. The Fundament of Quality of Life in Society*", Editura Politehnică Timişoara, Colecţia Conferinţe, **2011**, p. 196-202, ISBN 978-606-554-314-0.
8. **Roxana-Diana Paşca**, Ossi Horovitz, Aurora Mocanu, Maria Tomoaia-Cotişel, „Self Assemblies of Mixed Lipids at the Air-Water Interface", *Annals of „Dunarea de Jos" University of Galati, Mathematics, Physics, Theoretical Mechanics,* Fascicle II, Year III (XXXIV), **2011**, pp. 141-147 (BDI).
9. **Roxana-Diana Paşca**, Oana-Alexandra Rusu, "Studies on the Behavior of Gold Nanoparticles at the Air-Water Interface", *in Proceedings of the 9th National Symposium on Colloids and Surfaced Chemistry,* Galaţi University Press, **2008**, pp. 129-132, ISSN 2065-3603.

2. CONFERENCES
2.1. Abroad
1. Emőke Volentiru, Réka Csutak, **Roxana-Diana Paşca**, Norbert Nagy, Zoltán Hórvölgyi, "Silica coatings with controlled morphology", *10th Conference on Colloid Chemistry (10CCC)*, **Budapest, Hungary**, 29-31 August, **2012** (oral presentation).

2. Aurora Mocanu, **Roxana-Diana Paşca**, Gheorghe Tomoaia, Ossi Horovitz, Maria Tomoaia-Cotişel, "Structural and Thermodynamic Characterization of Cholesterol and Dimyristoyl Phosphatidyl Choline Monolayers at the Air/Water Interface", *14th International Conference on Organized Molecular Films (ICOMF 14-LB 14)*, **Paris, France,** 10-13 July, **2012** (poster).
3. Roxana-Diana Paşca, "The interaction of cholesterol-DMPC mixed monolayers and type I collagen. Langmuir-Blodgett technique and AFM approach", *2nd EBSA BIOPHYSICS COURSE ON: Membrane Biophysics and Lipid-Protein Interaction*, **Bordeaux-Lacanau, France**, 24-29 June, **2012**, (oral presentation).
4. Roxana-Diana Paşca, Réka Csutak, Norbert Nagy**,** Emőke Volentiru, Zoltán Hórvölgyi, "SiO_2 thin films containing Stöber silica particles: structural, optical and wettability properties", *Conference of Chemical Engineering 2012*, **Veszprem**, **Hungary**, 24-26 April, **2012** (oral presentation).

2.2. in Romania
5. Roxana-Diana Paşca, Aurora Mocanu, Gheorghe Tomoaia, Ossi Horovitz, Maria Tomoaia-Cotişel, "The interaction between lipids and collagen type I", *11th Conference on Colloid and Surface Chemistry – 11 CCSC*, **Iasi**, **Romania**, 09-11 May, **2013** (oral presentation).
6. Roxana-Diana Paşca, Daniel Frankel, "Interaction of lipids with lectins", *COST Action TD0906 Biological Adhesives: from biology to biomimetics "WG3 & WG4 Scientific Workshop"*, **Cluj-Napoca**, **Romania,** 09-11 April, **2013** (poster).
7. **Roxana-Diana Paşca**, Aurora Mocanu, Gheorghe Tomoaia, Ossi Horovitz, Maria Tomoaia-Cotişel, "Membrane Models. The interaction between lipids and proteins", *A XXXII Conferinţă Naţională de Chimie*, **Călimăneşti-Căciulata, Romania**, 03-05 October, **2012** (poster).
8. Gheorghe Tomoaia, Lacrimioara-Bianca Pop, Gabriel Furtos, Cristina Prejmerean, Ioan Petean, **Roxana-Diana Pasca**, Alexandra-Gertrud Hosu-Prack, Aurora Mocanu, Maria Tomoaia-Cotisel. "The effect of various calcium phosphate particles on collagen mineralization", *The 3rd Workshop and 4th Management meeting of the COST TD0903, "Understanding and Manipulating Enzymatic and Proteomic Processes in Biomineralization*", **Cluj Napoca**, **Romania,** 11-13 October, **2011** (poster).
9. Roxana-Diana Paşca, Ossi Horovitz, Aurora Mocanu and Maria Tomoaia-Cotişel, "Interaction of Silver Nanoparticles with some Biomolecules", *Processes in Isotopes and Molecules (PIM)*, **Cluj-Napoca, Romania**, 29 September-01 October **2011** (poster).
10. Aurora Mocanu, **Roxana-Diana Paşca**, Ossi Horovitz and Maria Tomoaia-Cotişel, "Thermodynamic Characterization of Mixed Lipids at the Air-Water Interface", *Processes in Isotopes and Molecules (PIM)*, **Cluj-Napoca, Romania**, 29 September-01 October **2011** (poster).
11. Ioana Bozga, Codruţa Cobzac, **Roxana-Diana Paşca**, Aurora Mocanu, Maria Tomoaia-Cotişel, Ossi Horovitz, „Sinteza biogenică a unor nanoparticule", *A VI-a conferinţă naţională cu participare internaţională „Coroziune şi protecţie anticorozivă, CPA"*, **Cluj-Napoca, Romania**, 22-24 September **2011** (oral presentation).
12. Roxana-Diana Paşca, Ossi Horovitz, Aurora Mocanu, Maria Tomoaia-Cotişel, „Interaction of Silver Nanoparticles with some Molecules of Biological Interest" *The 10th International conference on colloids and surfaces chemistry*, **Galaţi**, **Romania**, 9-11 June, **2011**, (poster).
13. Roxana-Diana Paşca, Ossi Horovitz, Aurora Mocanu, Maria Tomoaia-Cotişel, „Self Assemblies of Mixed Lipids at the Air-Water Interface", *10th International conference on colloids and surfaces chemistry*, **Galaţi**, **Romania**, 9-11 June, **2011**, (oral presentation)

14. Roxana-Diana Paşca, Maria Tomoaia-Cotişel, Ossi Horovitz, Aurora Mocanu, "Cholesterol and Dimyristoyl Phosphatidylcholine Monolayers at the Air-Water Interface. Structural and Thermodynamic Characterization", *Humboldt-Kolleg "Knowledge, Culture, Science. The Fundament of Quality of Life in Society"*, **Timişoara**, **Romania**, 23-28 November, **2010**, (oral presentation).
15. Roxana-Diana Paşca, Aurora Mocanu, Ossi Horovitz, Maria Tomoaia-Cotisel, "Phase Behavior of Cholesterol and Dimyristoyl Phosphatidylcholine Monolayers at the air- water interface", *14th Interntional Conference of Physical Chemistry, ROMPHYSCHEM*, **Bucureşti, Romania**, 2-4 June, **2010**, (poster).
16. Roxana-Diana Paşca, "The Interfacial Behavior and Nanostructures of Cholesterol and Dimyristoyl Phosphatidylcholine Monolayers", *7th Interntional Conference "Students for Students",* **Cluj-Napoca**, **Romania**, 23-25 April **2010**, (poster).
17. Roxana-Diana Paşca, "Studiu comparativ privind relaţia dintre modul de preparare al nanoparticulelor de aur şi comportamentul la interfaţa aer-apă", *6th Interntional Conference "Students for Students",* **Cluj-Napoca**, **Romania**, 10-12 April **2009**, (oral presentation).
18. Roxana-Diana Paşca, Oana-Alexandra Rusu, "Studies on the Behavior of Gold Nanoparticles at the Air-Water Interface", *9th National Symposium on Colloids and Surfaced Chemistry* – **Galaţi**, **Romania**, 29-30 May **2008**, (poster).
19. Roxana-Diana Paşca, Oana-Alexandra Rusu, "Studies on the Behavior of Gold Nanoparticles at the Air-Water Interface", *5th Interntional Conference "Students for Students",* **Cluj-Napoca**, **Romania**, 18-20 April **2008**, (oral presentation).

2.3. PRISIS
1. "The best poster" **Roxana-Diana Paşca,** "Thermodynamic Characterization of Mixed Lipids at the Air-Water Interface", *Processes in Isotopes and Molecules (PIM)*, **Cluj-Napoca, Romania**, 29 September-01 October 2011 (poster).
2. "The best poster for young scientists" **Roxana-Diana Paşca**, „Interaction of Silver Nanoparticles with some Molecules of Biological Interest", *10th International conference on colloids and surfaces chemistry*, **Galaţi, Romania**, 9-11 June, **2011** (poster).
3. "First place" for **Roxana-Diana Paşca**, "The Interfacial Behavior and Nanostructures of Cholesterol and Dimyristoyl Phosphatidylcholine Monolayers", *7th Interntional Conference "Students for Students",* **Cluj-Napoca**, **Romania**, 23-25 April **2010**, (poster)
4. "The best poster" for **Roxana-Diana Paşca**, "Studies on the Behavior of Gold Nanoparticles at the Air-Water Interface", *9th National Symposium on Colloids and Surfaced Chemistry* – **Galaţi**, **România**, 29-30 May **2008** (poster)

3. RESEARCH GRANTS
Roxana-Diana Paşca, as a Ph. D. student (2010-2013) was a member in the research team in the following research projects:
1. Name of the project: "Multifunctional nanostructures formed of gold or silver nanoparticles and different biomolecules with medical applications" (NANOMED), period: 2011-2014.
Director: Univ. Prof. Dr. Maria Tomoaia-Cotisel
2. Name of the project: "Methods and technologies based on molecular and cellular medicine with applications in surgery and treatment of bone cancer, bone metastases and osteo-articular lesions" (OSMOCEL), period: **2007-2010**.
Director: Univ. Prof. Dr. Maria Tomoaia-Cotisel

4. MEMBER OF SOME PROFESSIONAL SOCIETIES:
2010-Associatin for Thermodynamics and Surface Chemistry, **TECHIS**, **Romania**
2010- Romanian Chemical Society, **SChR**, **Romania**

5. SCHOLARSHIPS AS PhD STUDENT
Octomber 2010- September 2013, Sectoral Operational Programme for Human Resources Development 2007-2013, co-financed by the European Social Fund, under the project number POSDRU/107/1.5/S/76841 with the title „Modern Doctoral Studies: Internationalization and Interdisciplinarity".