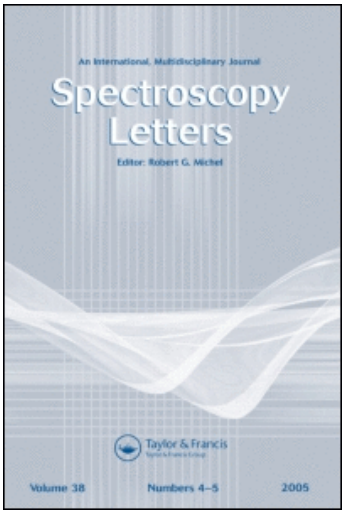

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Interaction of Scopolamine and Cholesterol with Sphingomyelin Bilayers by FT-Raman Spectroscopy

Bing Zhao[a]; Xinmin Li[b]; Daqing Zhao[b]; Jiazuan Ni[b]; Jianwen Chen[c]; Fen Hwang[c]
[a] Key Laboratory for Supramolecular Structure and Spectroscopy, Jilin University, Changchun, P. R. China [b] Laboratory of Rare Earth Chemistry and Physics, Changchun Institute of Applied Chemistry, the Chinese Academy of Sciences, Changchun, P.R. China [c] National Laboratory of Biomacromolecules, Institute of Biophysics, the Chinese Academy of Sciences, Beijing, P. R. China

To cite this Article Zhao, Bing , Li, Xinmin , Zhao, Daqing , Ni, Jiazuan , Chen, Jianwen and Hwang, Fen(1998) 'Interaction of Scopolamine and Cholesterol with Sphingomyelin Bilayers by FT-Raman Spectroscopy', Spectroscopy Letters, 31: 8, 1825 — 1837
To link to this Article: DOI: 10.1080/00387019808007457
URL: http://dx.doi.org/10.1080/00387019808007457

SPECTROSCOPY LETTERS, 31(8), 1825-1837 (1998)

INTERACTION OF SCOPOLAMINE AND CHOLESTEROL WITH SPHINGOMYELIN BILAYERS BY FT-RAMAN SPECTROSCOPY

Key Words: Scopolamine, Cholesterol, Sphingomyelin, FT-Raman

Bing Zhao[1*], Xinmin Li[2*], Daqing Zhao[2], Jiazuan Ni[2], Jianwen Chen[3] and Fen Hwang[3]

[1] Key Laboratory for Supramolecular Structure and Spectroscopy, Jilin University, Changchun 130023, P. R. China

[2] Laboratory of Rare Earth Chemistry and Physics, Changchun Institute of Applied Chemistry, the Chinese Academy of Sciences, Changchun 130022 P.R. China

[3] National Laboratory of Biomacromolecules, Institute of Biophysics, the Chinese Academy of Sciences, Beijing 100101, P. R. China

ABSTRACT

The interaction of scopolamine and cholesterol with sphingomyelin bilayers has been investigated by FT-Raman spectroscopy in head-group region (600-1000 cm^{-1}), the C-C stretching (1000-1200 cm^{-1}), CH_2 deformation (1400-

* To whom corresponding should be addressed.

1500 cm^{-1}) and the C-H stretching (2800-3000 cm^{-1}) mode regions. The results indicate that scopolamine and cholesterol do not change the conformation of O-C-C-N^+ backbone in the choline group of sphingomyelin bilayers, the polar headgroup is still extending parallel to the bilayer surface and O-C-C-N^+ group is still in its gauche conformer. Scopolamine and cholesterol lower the order of the interface, the interchain, CH_2 crystal lattices and the lateral chain-chain packing, and increase their fluidity.

INTRODUCTION

Phospholipids in excess water have been extensively studied as a model for understanding the structure and properties of the complex lipid matrices of biological membranes. The physical properties of phospholipids provide information of fundamental significance to the determination of the structure and functional of biomembranes. It is known that the molecular mechanism of drugs with biomembranes is either involved in membrane lipids or membrane-associated proteins such as channels, receptors and pumps to perform their physiological functions(1). *Hyoscyamus niger* L., medical herbs recorded in the famous ancient Chinese medical book, Compendium of Materia Medica, are widely dispersed throughout China. Drugs belongs to this group are anisodamine, scopolamine and atropine. These drugs, showing an inhibitory effect on the cholinergic nerve function, as well as the improvement of the microcirculation, are extensively used in clinic, especially in the case of toxic shock and organophosphorus intoxication. Anisodamine and scopolamine, if they are in combination with chlorpromazine, can be used as combined intravenous anesthetics. The interaction of anisodame with membrane has been extensively studied by Fen Hwang et al.. The results showed that anisodamine increases the fluidity of membranes(2), causes phase separation of dipalmitoylphosphatidic acid (DPPA) (3), and induces the hexagonal

phase of cardiolipin and dioleoylphosphatidylcholine liposomes(4). The drug induces acyl chain interdigition in phosphatidylglycerol(5,6).

Cholesterol appears to have several different functions in cells, one of its primary roles is a modulator of the physical properties of the plasma membrane phospholipid bilayers. Cholesterol, glycerol-based phospholipids and sphingomyelin make up the bulk of lipid components in mammalian plasma membranes, and as a results, a great deal of effects has been devoted to understanding their interaction in model membrane assemblies(7). Renewed interest in cholesterol-lipid interaction has been fueled by recent findings in the viral fusion and lipid signal transduction fields. For instance, human immunodeficiency virus envelops reportedly contain high cholesterol-to-phospholipid ratios and are selectively enriched in sphingomyelin relative to host cell membranes (8). With respect to lipid signal transduction events, certain phospholipids, sphingolipids, and their metabolic derivatives are known to act as second messengers. Sphingomyelin, in particular, has been the focus of recent study (9). Because biological evidence suggests coordinate regulation of sphingomyelin and cholesterol content in cellular membranes and certain biophysical evidence indicates that cholesterol has a great "affinity" for sphingomyelin compared to phosphatidylcholine, the possibility exists that GM1-rich and cholesterol-rich distinct domains may be involved in regulating lipid signal transduction (10). Hence understanding cholesterol's interaction with simple sphingolipids in important from the biological as well as physical perspective. In this paper, the interaction of scopolamine and cholesterol with sphingomyelin bilayers have been investigated by FT-Raman spectroscopy. All the results show that scopolamine and cholesterol increase the membrane fluidity. The results enable us to elucidate further the molecular mechanism of the interaction of the drugs with biomembranes and to understand the interaction scopolamine/ sphingomyelin/cholesterol model membrane systems.

EXPERIMENTAL

Porcine brain sphingomyelin(SM) and cholesterol were purchased from Sigma Chemical Co. (St. Louis, MO) and used without further purification. Scopolamine as hydrobromides was obtained from Chengdu First Pharmaceutical Factory and purified before use. The chemical structure of the drug is shown in Fig. 1.

SM and cholesterol-SM bilayers were prepared according to previous studies(11) by first dissolving the lipids in chloroform and evaporating the solvent first under a nitrogen flow and finally under high vacuum. Buffer (10 mmol/L Hepes, 100 mmol/L NaCl pH 7.0) was added to disperse the lipid and the samples were incubated, accompanied by gentle mixing for an hour at 50 °C in the liquid-crystalline phase. Adding the appropriate volume of scopolamine to SM and cholesterol-SM bilayers and shaking mechanically at 50 °C for 30 min, the SM concentration of all samples was 100 mg/ml. Samples were sealed in glass capillary tubes and were stored in a refrigerator at 4°C for 24 hr before recording the spectra.

Raman spectra were recorded on a Bruker RFS-66v FT-Raman spectrometer equipped with a liquid nitrogen cooled Ge detector. Excitation radiation was provided at the sample of ~100 mw by a 1064 μm CW Nd:YAG laser. Samples were maintained at ambient temperature (~18°C) during spectral scans.

RESULTS AND DISCUSSION

FT-Raman spectroscopy holds great promise for providing vibrational data on biological systems(12). In the present studies, the perturbations to the lipid

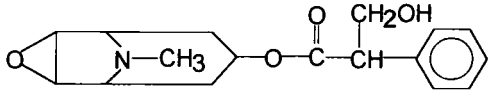

FIG. 1 The chemical structure of scopolamine

matrix resulting from adding of scopolamine and cholesterol were examined separately, and the experiments were designed in order to assess simultaneous bilayer effects of adding both scopolamine and cholesterol. For the purpose of comparing the order/disorder properties of scopolamine-SM and scopolamine-SM-cholesterol multilamellar assembles, the first present the Raman spectra results of anhydrous SM and SM bilayers. Vibrational frequencies and intensities characteristics of the lipid interface, head group and hydrocarbon chain regions provide structural probes for the bilayer system.

1. Effects of Scopolamine and Cholesterol on the Conformation of O-C-C-N^+ Backbone and the Lipid Interface Region

The Raman spectra of sphingomyelin bilayers in 600-1000 cm^{-1} regions with or without the presence of scopolamine and cholesterol were shown in Fig. 2. The characteristic bands observed in this region are 717, 770, 873 and 890 cm^{-1}. The Raman band at 717 cm^{-1} has been assigned to C_4N^+ totally symmetric stretching mode(13), while 873 and 890 cm^{-1} have been assigned to carbon-carbon stretch in flid-like and crystallinity state of interfacial region (14). The band at 717 cm^{-1} that originated from the totally symmetric C-N stretching mode of C_4N^+ group, characteristic of O-C-C-N^+ backbone in its gauche conformation (13) remains invariant after adding scopolamine, cholesterol separately, and both scopolamine and cholesterol. No new band observed at 770 cm^{-1} that originated from the totally symmetric C-N stretching mode of C_4N^+ group when the

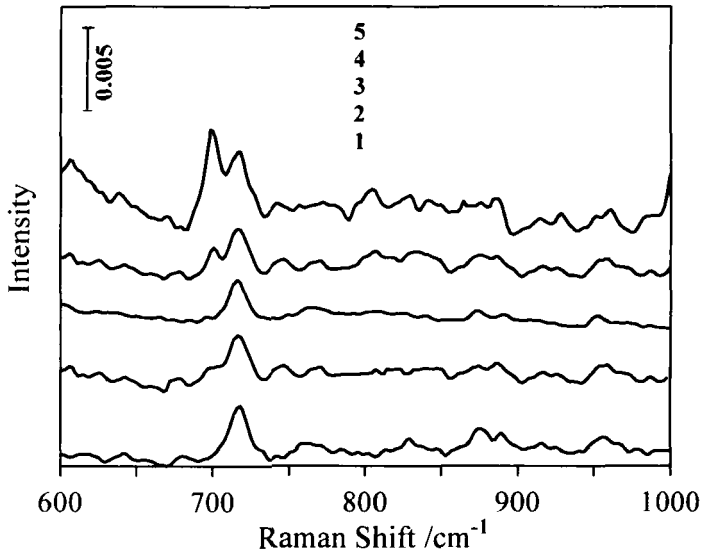

FIG.2 Raman spectra of head-group region (600-1000 cm^{-1}) for (1) anhydrous SM, (2) SM bilayers (100 mg/ml), (3) scopolamine-SM at 30 weight % scopolamine, (4) cholesterol-SM at 30 % weight cholesterol, (5) Scopolamine-SM-cholesterol at 30 weight % scopolamine and cholesterol

backbone of O-C-C-N^+ in its trans conformation(15). The constancy of both the intensity and the frequency of 717 cm^{-1} band and no 770 cm^{-1} band occurred indicate that no conformational change occurred in polar headgroup of sphingomyelin bilayers in the presence of scopolamine and cholesterol. The conformation of choline group is still in its gauche form which allows the polar headgroup extend parallel to the surface of sphingomyelin bilayers.

The characteristic Raman-active doublet bands around 873 and 890 cm^{-1} have been identified as choline deformation and C_1-C_2 stretch with little contribution from methyl terminal C-C stretch (16). The intensity ratio, I_{873}/I_{890}, of these bands has been shown to be sensitive to the geometry of the interface

region and lateral interaction with the choline head. An apparent change observed in this region is the increase of the intensity ratios of I_{873}/I_{890} in the presence of scopolamine and cholesterol (Table 1). The peak intensity ratio of I_{873}/I_{890} was used to monitor the physical state changes in the interfacial region (14). The increase of the intensity ratio of I_{873}/I_{890} suggest that the interfacial region of sphingomyelin bilayers is in a disordered state. Intensity ratio for the three component scopolamine-SM-cholesterol system compares closely to the two component scopolamin-SM or cholesterol-SM system.

2. Effects of Scopolamine and Cholesterol on C-C Stretching and CH_2 Deformation Modes

Information regarding trans-gauche isomerism can be obtained from the Raman spectra of the optical skeletal mode. These modes occur over a wide frequency range, However, in-phase and out-of-phase C-C stretching modes of the rigid hydrocarbon chain fall in a narrow frequency range, 1000-1200 cm^{-1}. The Raman spectrum displays a cluster of three bands at 1064, 1086 and 1127 cm^{-1} and a fairly isolated strong band at 1296 cm^{-1} (Fig. 3). The bands at 1064 and 1127 cm^{-1} are the B_{1g} and A_g modes of all-trans chain segments while the band at 1086 cm^{-1} results from structures containing gauche conformers. The 1296 cm^{-1} mode, which is also one of the strongest bands in the Raman spectrum, is the in-plane methylene twist (16). Intensity ratio of 1086 cm^{-1} for sphingomyelin bilayers to the all-trans conformer peaks are currently used as a probes of the gauche/trans population. As shown in Table 1, an increase in the I_{1086}/I_{1064} and I_{1086}/I_{1127} ratios occurs with addition of 30 weight % scopolamine to sphingomyelin bilayers, this indicates that there was a increase of the gauche/trans population compared with pure sphingomyelin bilayers, the degree of the interchain order decreased and the membranes fluidity increased.

TABLE 1 Effects of scopolamine and cholesterol on the peak height intensity (I_a/I_b) of sphingomyelin bilayers

	I_{873}/I_{890}	I_{1086}/I_{1064}	I_{1086}/I_{1127}	I_{1457}/I_{1437}	I_{2847}/I_{2882}	I_{2932}/I_{2882}
Anhydrous SM	0.932	0.310	0.433	0.891	0.920	0.412
SM bilayers	0.793	0.749	1.029	0.909	0.823	0.598
Scopolamine+SM	1.019	0.812	1.059	0.875	0.835	0.770
Cholesterol+SM	1.003	0.960	1.033	0.830	0.909	0.792
Scopolamin+SM+ cholesterol	0.986	0.936	1.035	0.824	0.914	0.877

30% weight scopolamine and cholesterol to sphingomyelin

An increase in the I_{1086}/I_{1064} and I_{1086}/I_{1127} ratios was also observed when 30 weight % cholesterol incorporated sphingomyelin bilayers (Table 1), this reflects an increase of the gauche/trans population compared with sphingomyelin bilayers, an increased molecular mobility of sphingomyelin bilayers was caused by cholesterol. Simultaneous effects of adding both scopolamine and cholesterol on sphingomyelin bilayers was shown in Table 1, no additive effect of scopolamine and cholesterol on gauche/trans population was observed.

The methylene deformation band is observed as a strong doublet at 1437 cm^{-1} and 1457 cm^{-1} in the Raman spectrum of the aqueous gel (Fig.3). Multiple components associated with the deformation mode in the Raman spectra are sensitive to the type of packing of the hydrocarbon chains in the unit cell(16). The CH_2 deformation (1400-1500 cm^{-1}) spectral region in Fig. 3 has been used to monitor changes in the lipid-chain lateral packing characteristic. In general, as a pure gel, sphingomyelin for example, approaches its gel to the liquid-crystalline

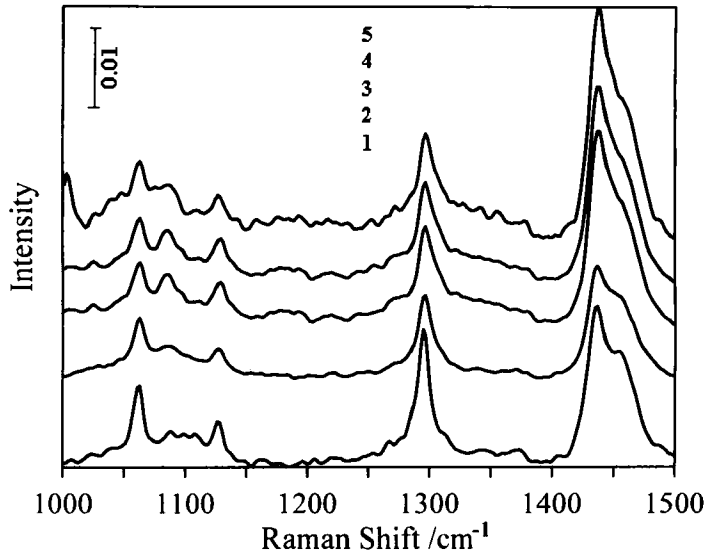

FIG. 3 Raman spectra of C-C stretching and CH_2 deformation modes (1000-1500 cm^{-1}) for (1) anhydrous SM, (2) SM bilayers (100 mg/ml), (3) scopolamine-SM at 30 weight % scopolamine, (4) cholesterol-SM at 30 % weight cholesterol, (5) Scopolamine-SM-cholesterol at 30 weight % scopolamine and cholesterol

phase transition temperature and undergoes lattice disorder. The 1457 cm^{-1} feature, reflecting the high-frequency component of the CH_2 deformation doublet, decreases in intensity and broadens into a shoulder of 1437 cm^{-1} as sphingomyelin undergoes lattice disorder. The I_{1457}/I_{1437} peak height ratios in Table 1 demonstrated cholesterol's participation in decreasing lipid interchain interactions, the addition of scopolamine also decreasing the lipid interchain interactions. No additive lipid interchain interactions was observed in scopolamine-SM-cholesterol system.

3. Effects of Scopolamine and Cholesterol on Lipid Fluidity of Sphingomyelin Bilayers

The C-H stretching region in Fig. 4 extends from 2800-3000 cm^{-1} , the 2847 and 2882 cm^{-1} modes refer to the acyl chain methylene CH_2 symmetric and asymmetric stretching vibrations respectively, I_{2847}/I_{2882} reflects the lateral chain-chain interactions defining the bilayer dynamics. Order/disorder measures also arise from intrachain trans/gauche isomerizations, are determined from the I_{2932}/I_{2882} intensity parameter (11). The 2932 cm^{-1} feature represents a methylene moieties and, separately, the C-H symmetric stretching modes of the chain methyl termini of the lipids, comprising the liposomes(11,16). The peak height intensity ratios I_{2847}/I_{2882} and I_{2932}/I_{2882} are used to characterize the lateral chain-chain, order-disorder rearrangements(16). The comparison of I_{2847}/I_{2882} and I_{2932}/I_{2882} order parameters for various sphingomyelin bilayers in Table 1 demonstrates that scopolamine and cholesterol decreased the intermolecular ordering of the lipid lattice, and increased the membrane lipid fluidity. Interestingly, scopolamine-sphingomyelin-cholesterol complex is more disordered than either scopolamine-SM or cholesterol-SM complex, and further change on the membrane fluidity was observed.

It was proved by Raman spectroscopy that an electrostatic interaction exists between the trialkylamino group of anisodamine and headgroup of acidic lipids, While the benzene ring of anisodamine inserts into the phospholipid bilayers (17). Scopolamine has a structure very similar to anisodamine. The hydrophilic and hydrophobic interactions of scopolamine with sphingomyelin bilayers indicate that there was little influence of scopolamine on the conformation of O-C-C-N^+ backbone in the choline group of sphingomyelin bilayers. Scopolamine induces the disorder of the interface, the acyl chain, lattice packing and intrachain conformation. Upon the addition of cholesterol, modifications in lateral chain interactions were observed by monitoring spectral

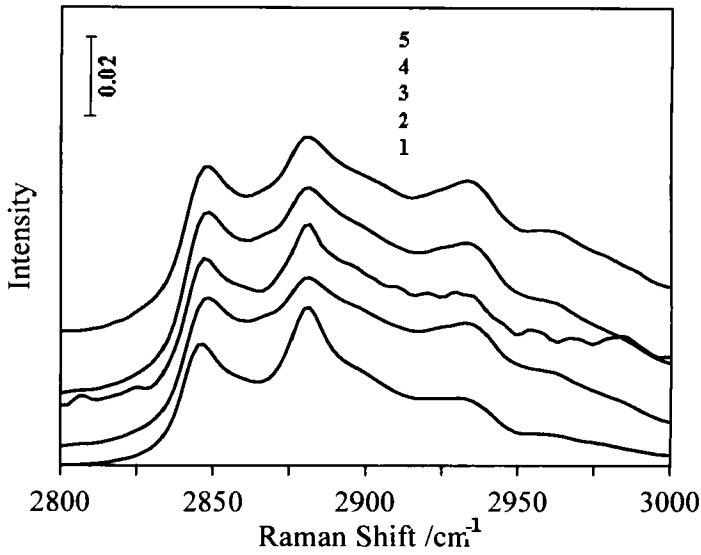

Fig.4 Raman spectra of C-H stretching modes (2800-3000 cm^{-1}) for (1) anhydrous SM, (2) SM bilayers (100 mg/ml), (3) scopolamine-SM at 30 weight % scopolamine, (4) cholesterol-SM at 30 % weight cholesterol, (5) Scopolamine-SM-cholesterol at 30 weight % scopolamine and cholesterol

change in the methylene C-H stretching and the CH_2 deformation regions, trans/gauche isomerization is developed along the hydrocarbon chains. However, adding both scopolamine and cholesterol, no further headgroup changes nor increases in either acyl chain trans/gauche or lattice disorder were observed.

ACKNOWLEDGEMENT

The Project Was Supported by the National Natural Science Foundation of China and the President Foundation of the Chinese Academy of Sciences.

REFERENCES

1. Franks N.P.,Lieb W.R. What is the Molecular Nature of General Anestetic Target Sites. Trends. Pharmacol. Sci. 1987, 8:169.

2. Hwang F., Wang S.M., Hu, C.Q. The Disordering Effect of Hyoscyamine Drugs on Phospholipid Membranes. Biochim.Biophys.Acta. 1983,736:220.

3. Wang S.M.,Hwang F., Fu Y.Z., Zhang Z.L., Dong, W.J., Hu C.Q. The Interaction of Anisodamine with Phospholipid Membranes. Chinese Sci. Bull. 1984, 29:529.

4. Hwang F., Wu Y.W., Wen D.C. Anisodamine Induce the Hexagonal Phase in Phospholipid Liposomes. Biochim. Biophys. Acta, 1986,860:713.

5. Wang P.Y., Chen J.W. ,Hwang F. Anisodamine Causes Acyl Chain Interdigition in Phosphatidylglycerol. FEBS, 1993,332:193.

6. Hao Y.H., Chen J.w., Hwang F. Atropine Induces An Interdigitated Structure in DPPG Liposomes. Chinese Sci. Bull. 1996, 41:1788.

7. Finegold, L. Ed. Cholesterol in Membrane Models. Boca Raton, FL:CRC Press, 1993.

8. Smaby J.M., Brockman H.L., Brown R.E. Cholesterol's Interfacial Interactions with Sphingomyelins and Phosphatidylcholine: Hydrocarbon Chain Structure Determines the Magnitude of Condensation. Biochemistry, 1994, 33:9135.

9. Ahmed S.N., Brown D.A., London E.L. on the Orgin of Sphinolipid/Cholesterol-rich Detergent-insoluble Cell Membranes: Physiological Concentrations of Cholesterol and Sphingolipid Induce Formation of a Detergent-insoluble, Lipid-ordered Lipid Phase in Model Membranes. Biochemistry, 1997, 36:10944.

10. Ferraretto A., Pitto M., Palestini P., Masserini M., Lipid Domain in the Membrane: Thermotropic Properties of Sphingomyelin Vesicles Containing GM1 Ganglioside and Cholesterol. Biochemistry, 1997, 36:9232.

11. Li X.M., Zhao B., Zhao D.Q., Ni J.Z., Wu Y., Xu W.Q., Interaction of La^{3+} and Cholesterol with Dipalmitoylphosphatidylglycerol Bilayers by FT-Raman Spectroscopy, Thin Solid Films, 1996, 284-285:762.

12. Levin I.W., Lewis E.N. Fourier Tansform Raman Spectroscopy of Biological Materials. Anan. Chem. 1990, 62:1101A.

13. Yuan C.B., Zhao D.Q., Zhao B., Ni J.Z. NMR and FT-Raman Studies on the Interaction of Lanthanide Ions with Sphingomyelin Bilayers. Spectro. Lett. 1996, 29:841.

14. Brown E.B., Brown K.G., Ladjadj M. Raman-active Bands Sensitive to Motion and Conformation of the Chain Termini and Backbones of Alkanes and Lipids. J. Phys.Chem. 1987, 91:3436.

15. Akutsu H. Direct Determination by Raman Scattering of the Conformation of the Choline Group in Phospholipid Bilayers. Biochemistry, 1981, 20:7359.

16. Lamba O.P., Borchman D., Sinha S.K., Lal S., Yappert M.C., Lou M.F. structure and Molecular Confoamation of Anhydrous and of Aqueous Sphingomyelin Bilayers Determined by Infrared and Raman Spectroscopy. J. Mol. Struct. 1991, 248:1.

17. He J.P., Wang S.M. The hydrophilic and hydrophobic interactions of anisodamine with acidic phospholipid liposomes. Acta Biophys. Sinica. 1988, 4:7.

Date Received: July 6, 1998
Date Accepted: August 4, 1998