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FEATURES OF LASER DESORPTION/IONIZATION MASS SPECTROMETRY FRAGMENTATION OF VITAMIN B_{12}

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A comparative analysis of the laser desorption/ionization of vitamin B_{12} by matrix-assisted laser desorption/ionization (MALDI) and desorption/ionization on porous silicon (DIOS) was carried out. The mass spectra obtained were interpreted and the pathways for ion formation and decomposition were established. The MALDI fragmentation of the positive vitamin B_{12} ions is more extensive than the DIOS fragmentation. The most extensive fragmentation was found using the MALDI method for negative vitamin B_{12} ions, which are lacking when using the DIOS method.

Key words: *MALDI mass spectrometry, DIOS mass spectrometry, vitamin B_{12}, porous silicon, 2,5-dihydroxybenzoic acid.*

The development of matrix-assisted laser desorption/ionization has provided renewed interest in the mass spectrometry of biological and synthetic macromolecules [1]. The major advantage of this method relative to the known ionization methods such as electron impact, chemical ionization, and fast atom bombardment [2] is the more reliable generation of nonfragmented or in tact ions or of partially fragmented ions of the molecules studied. This advance is a consequence of the use of a matrix (as a rule, an organic acid) as a buffer between the laser radiation and the molecules of the samples studied [3].

However, there are also disadvantages along with the significant advantages of the MALDI generation of in tact molecular ions. One such disadvantage is the need to select special matrices for each new type of compound studied. Furthermore, the use of a matrix leads to the appearance of a background mass spectrum of the matrix in the range up to 1000 Da. Thus, alternative non-matrix variants of this method are now used. One such variant is the recently proposed desorption/ionization on porous silicon (DIOS) [4]. The DIOS method permits the generation of high-quality mass spectrometric information in the mass range to 5000 Da, which is useful for the quality control of food products, pharmaceuticals, and chemical polymers.

The development of non-matrix laser desorption/ionization methods has led to increasing interest in the effect of the surface of nanostructurized bases on the desorption and ionization of the compounds studied. Furthermore, the pathways for the mass spectrometric fragmentation of some compounds are extremely sensitive to variation of the experimental parameters. Vitamin B_{12} is one of these compounds. The fragmentation of vitamin B_{12} strongly depends on the experimental conditions [5]. Vitamin B_{12} was previously studied by plasma desorption mass spectrometry [6], secondary ion mass spectrometry, and field desorption [8]. However, in the overwhelming majority of cases, in tact vitamin B_{12} molecular ions were not obtained or detected in small amounts, while the strongest signal in the mass spectra corresponded to the $[M\text{-}CN]^+$ fragment.

In the present work, we evaluated the use of the DIOS method for obtaining nonfragmented molecular ions of the title compound in a comparative analysis of the ionization and fragmentation pathways of vitamin B_{12} ions in the MALDI and DIOS methods.

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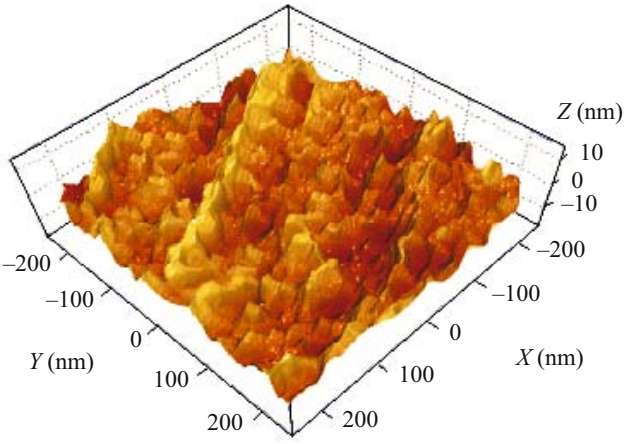


Fig. 1. Atomic force microscopic image of a porous silicon sample.

EXPERIMENTAL

The reagents were supplied by Sigma Aldrich (USA) and a sample of vitamin B_{12} (cyanocobalamine) was obtained from Darnitsa (Ukraine). Epitaxial plates of *p*-type monocrystalline silicon (7.5 Ω·cm) were used to prepare porous silicon.

2,5-Dihydroxybenzoic acid (DHB) was used as the matrix. The ratio of the compound studied to the matrix was 1 : 1.

Porous silicon was prepared by the electrochemical etching of *p*-type monocrystalline silicon (7.5 Ω·cm) according to the procedure described by Wei et al. [4]. The morphology of the mesoporous silicon with pore size from 20 to 50 nm was established by atomic force microscopy (AFM) (see Fig. 1).

The experiments were carried out on a Bruker Daltonics Autoflex II mass spectrometer (Germany) in positive and negative ion modes using reflectronic analysis. The samples were irradiated with a pulse nitrogen UV laser at 337 nm (3 ns pulse). The overall result was obtained by the accumulation of 100 individual mass spectra.

The MALDI study of samples of vitamin B_{12} in a DHB matrix began with determination of the laser radiation intensity, at which the peaks corresponding to the molecular ions, exceed the noise level. We found that the threshold values both for positive and negative ions were at a level of 40% of the maximum laser intensity. After accumulation, the mass spectra of the positive ions (Fig. 2a) showed peaks with *m/z* = 1351.6, 1329.6, 1209.5, 1183.5, and 1067.5. For negative ions (Fig. 2b), we found peaks with *m/z* = 1481.6, 1328.6, 386.2, 356.2 as well as a set of peaks with *m/z* from 490 to 680.

The image of the porous silicon surface used in this work obtained using an atomic force microscope is given in Fig. 1, which shows that the pore size of the porous silicon obtained ranges from 20 to 50 nm.

In a study of the laser desorption/ionization of vitamin B_{12} from the porous silicon surface, the mass spectra were obtained at 40% laser radiation intensity as in the case of using the DHB matrix. The positive ion mass spectra given in Fig. 2c show peaks with *m/z* = 1354.6, 1054.5, and 972.5. DIOS spectra were not obtained for negative ions.

Table 1 gives the structural formula of vitamin B_{12} as well as characteristics for the ions obtained by the MALDI and DIOS methods in positive and negative ion modes.

RESULTS AND DISCUSSION

The interpretation of the positive ion MALDI mass spectra showed that the peak with *m/z* = 1329.6 is the fragment of the vitamin B_{12} molecule corresponding to the $[M\text{-}CN+H]^+$ ion, the peak with *m/z* = 1351.6 corresponds to $[M\text{-}CN+Na]^+$, and the peak with *m/z* = 1183.5 corresponds to $[M\text{-}CN\text{-}base]^+$ (Fig. 2a), which is in good accord with the literature data on MALDI measurements of vitamin B_{12} using porphyrin matrices [9] and the HCCA matrix (α-cyano-4-hydroxycinnamic acid) [5]. The mass spectrum also shows a peak with *m/z* = 1209.5 corresponding to the $[M\text{-}base]^+$ fragment and a peak with *m/z* = 1067.5

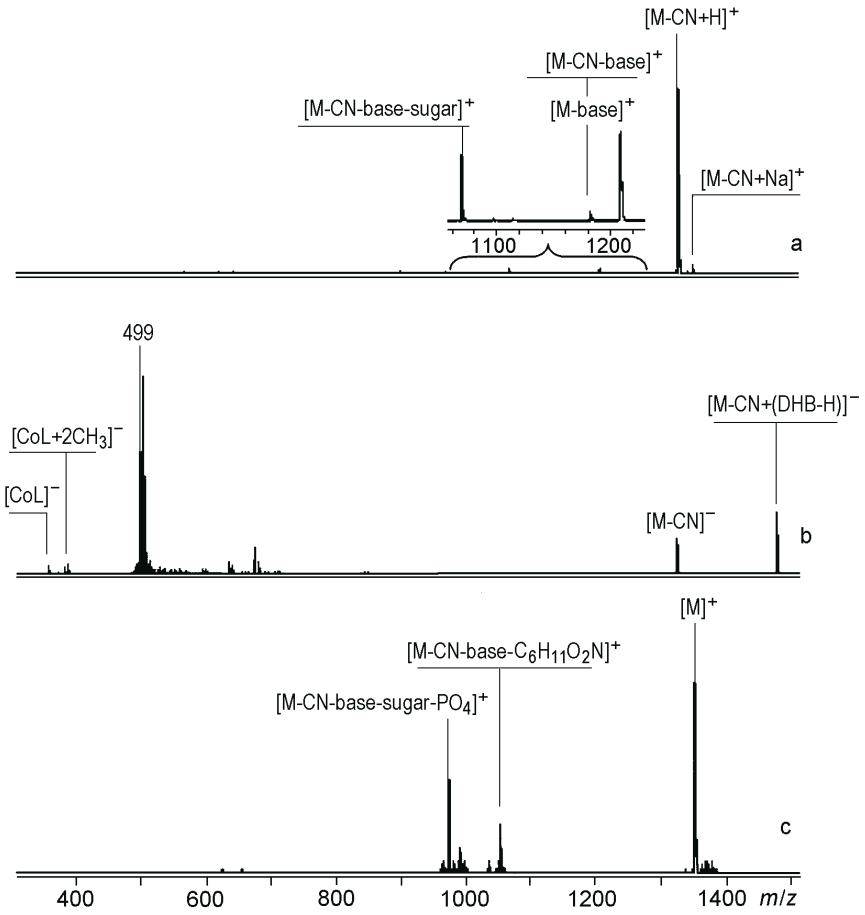
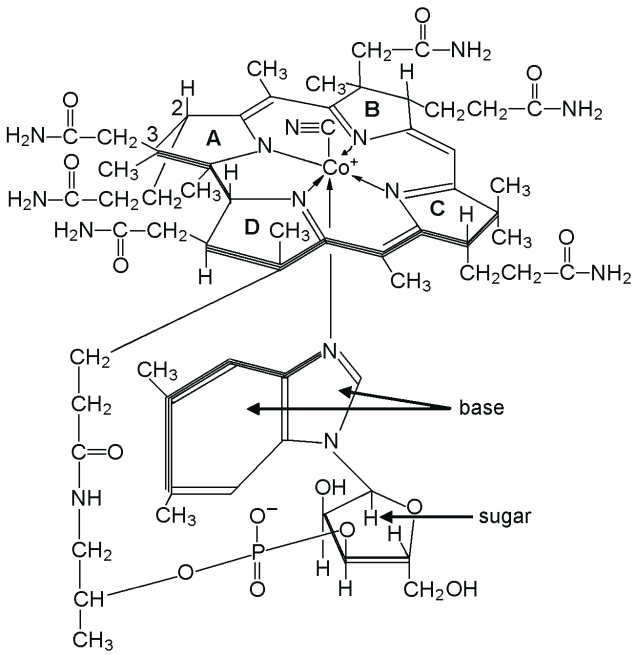


Fig. 2. Mass spectra of vitamin B_{12}: a) positive ion MALDI, b) negative ion MALDI, c) desorption ionization from the porous silicon surface for positive ions; $[\ldots]^+$ is a positive ion, $[\ldots]^-$ is a negative ion, $[M]^+$ is the molecular ion. The minus sign in the brackets indicates bond breakage, while a positive sign indicates addition of the corresponding fragment.

corresponding to $[M\text{-CN-base-sugar}]^+$ (the sugar is ribofuranose). Virtually all the fragments, with the exception of $[M\text{-base}]^+$, are formed in the positive MALDI experiment by loss of the CN group. The loss of this group probably is through hemolytic cleavage. The formation of the $[M\text{-CN+H}]^+$ ion occurs, as usually observed in MALDI mass spectra, through the addition of a proton to the $[M\text{-CN}]^{\cdot}$ radical. Since doubly-charged ions are not seen in the mass spectra of vitamin B_{12}, we might well assume that the initial step in the fragmentation process is the hemolytic cleavage of the Co—CN bond with subsequent protonation of the radical formed. The protonation presumably occurs through addition of a proton to the negatively-charged 5,6-dimethylbenzimidazolyl nucleotide phosphate.

The ionization of vitamin B_{12} with detection of the negative ions occurs upon the addition of an electron to the $[M\text{-CN}]^{\cdot}$ fragment (m/z = 1328.6). The peak with m/z = 1481.6 is interpreted as an associated species formed by fragments of vitamin B_{12} and the matrix molecule and corresponds to the formula $[M\text{-CN+(DHB-H)}]^-$. Peaks in the mass range m/z = 490-680 correspond to the corrin ligand with various extents of fragmentation of the side propionamide and acetamide groups. These groups provide for the solubility of vitamin B_{12} in polar solvents, are weakly bound to the corrin ring, and are readily lost [10]. For example, the peak with m/z = 499 may be interpreted as the corrin ligand with retention of the bond with side-groups at positions 2 and 3 in ring A (Table 1). The consecutive loss of side-groups leads to the formation of the unsubstituted corrin

TABLE 1. Characteristic Ions and Their Molecular Masses Obtained in the MALDI and DIOS Analyses of Vitamin B_{12}

Method and mode	Structural formula	Characteristic ion	Molecular mass
MALDI			
Positive ions		$[M\text{-}CN+Na]^+$	1351.6
		$[M\text{-}CN+H]^+$	1329.6
		$[M\text{-}base]^+$	1209.5
		$[M\text{-}CN\text{-}base]^+$	1183.5
		$[M\text{-}CN\text{-}base\text{-}sugar]^+$	1067.5
Negative ions		$[M\text{-}CN+(DHB\text{-}H)]^-$	1481.6
		$[M\text{-}CN]^-$	1328.6
		—	499
		$[CoL+2CH_3]^-$	386.2
		$[CoL]^-$	356.2
DIOS			
Positive ions		$[M]^+$	1354.6
		$[M\text{-}CN\text{-}base\text{-}C_6H_{11}O_2N]^+$	1054.6
		$[M\text{-}CN\text{-}base\text{-}sugar\text{-}PO_4]^+$	972.5

ligand with a central cobalt ion $[CoL]^-$ (*m/z* = 356.2), whose ionization in the negative ion mode occurs through addition of an electron.

The interpretation of the DIOS mass spectra showed that the peak with *m/z* = 1354.6 corresponds to the molecular ion of vitamin B_{12} $[M]^+$, while the peaks with *m/z* = 1054.5 and 972.5 correspond to fragments of the vitamin B_{12} molecule, $[M\text{-}CN\text{-}base\text{-}C_6H_{11}O_2N]^+$ and $[M\text{-}CN\text{-}base\text{-}sugar\text{-}PO_4]^+$, respectively (Fig. 2c). The fragment with *m/z* = 972.5 is presumably formed by the loss of 5,6-dimethylbenzimidazolyl nucleotide.

This interpretation suggests that the ionization of vitamin B_{12} on the porous silicon surface proceeds through loss of an electron by the molecule, in contrast to ionization involving the matrix, which occurs due to loss of the CN group and protonation of the fragment formed. Since the absorption maximum of vitamin B_{12} λ_{max} = 361 nm, which is close to the wavelength of the laser used as the ionizing factor (337 nm), vitamin B_{12} can efficiently absorb the laser radiation and is capable of photodissociation, such that it is natural that vitamin B_{12} molecule fragments are also found in the mass spectra obtained using porous silicon. We should note that fragments of the starting molecule are also observed in the mass spectra taken using an organic matrix but these fragments are different from those found in the mass spectra obtained using the porous silicon surface. This discrepancy indicates different pathways for the fragmentation of vitamin B_{12} in both cases. In matrix-activated laser desorption/ionization, the extent of fragmentation of vitamin B_{12} depends mainly on the properties of the matrix chosen, specifically, on the amount of energy transferred from the matrix to the compound analyzed [5], while the decisive factor in ionization on the porous silicon surface may be the tendency of the molecule to absorb laser radiation and to undergo photodissociation. We assume that the nanostructural porous surface moderates the ionization process, which leads to the formation of in tact ions. Furthermore, the surface layer of porous silicon, specifically in light of its porosity, acquires high UV absorption capacity. When using a nitrogen laser with wavelength 337 nm, we observed an absorption coefficient of 10^5 cm^{-1}

[11]. Thus, rapid heating of the surface occurs due to absorption of the laser emission. This heating may be evaluated using the equation given by Alimpiev et al. [12]:

$$\Delta T_{sur} = (2I/K)(kt/\pi)^{1/2},$$

where I is the laser emission intensity, K is the thermal conductivity, k is the thermal conductivity coefficient, and t is the laser pulse duration.

In accord with this evaluation under the experimental conditions, the heated surface temperature may reach 600 K and, thus, significantly affect the desorption of molecules from the surface. Local energy fields (10^5 V/cm) existing near the edges on the porous silicon surface may also play an important role in the ionization of molecules of the compound studied [12].

The most striking finding obtained in comparing the mass spectra taken using porous silicon and the MALDI mass spectra is the complete absence of negatively-charged ions in the former. The existence of strong local electric fields near structural features on the surface of nanoporous silicon presumably facilitates the loss of an electron from the vitamin B_{12} molecule in the surface region of the porous base through a tunneling transition with subsequent transfer of the electron to the silicon conductance band. Thus, the formation of positive molecular ions is stimulated. The reverse process of loss of an electron from silicon proceeds through autoelectron emission and the addition of the electron to an atom or ion in the vast majority of cases is unlikely as shown in our previous work [13]. The lack of negative ions in the mass spectra obtained using the porous silicon surface is a weighty argument for the decisive role played by strong electric fields in this case in the mechanism of laser desorption/ionization from the porous silicon surface.

In comparing the MALDI and DIOS mass spectra of vitamin B_{12}, we note the advantages of porous silicon bases for obtaining peaks of unfragmented molecular ions and simple mass spectra. When using matrices, the molecular ion peak is lacking in the mass spectra and extensive fragmentation of the compound analyzed is observed when recording both positive and negative ions.

Thus, we have shown that the ionization of vitamin B_{12} using the DHB matrix for positive ions proceeds through loss of a CN group with subsequent protonation or addition of a sodium ion. The negative ions are formed by addition of an electron to the $[M\text{-}CN]^{\cdot}$ fragment or association with a deprotonated matrix molecule. Extensive fragmentation of negative ions of vitamin B_{12} was found when using the MALDI method, indicating the excitation of vibrational degrees of freedom upon negative ion formation.

When porous silicon is used, ionization occurs through loss of an electron by the molecule to give positive ions. Negative ions are not observed in these mass spectra. The mass spectra taken using 2,5-dihydroxybenzoic acid as the matrix show more extensive fragmentation of the vitamin B_{12} in comparison with the mass spectra taken using porous silicon.

The reason for the observed differences in the fragmentation of vitamin B_{12} in the MALDI and DIOS methods may arise since the ionization of vitamin B_{12} from the porous silicon surface occurs on sites characterized by strong electric fields enhanced by coherent laser radiation without significant excitation of the vibrational degrees of freedom of the free vitamin B_{12} molecule such that the extent of its fragmentation is much less than with the MALDI method.

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