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UV-VIS AND FTIR SPECTROSCOPIC INVESTIGATIONS OF GAMMA-RAY IRRADIATED ANTIBIOTICS

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Abstract. The effect of gamma irradiation on three antibiotics (Nalidixic acid, Spectinomycin, and Rifampicin) were studied. The drugs were exposed to different doses of radiation emitted by ^{60}Co source, up to 205 kGy. The ionizing induced degradation on the above antibiotics was discussed based on their UV-VIS and FTIR absorption spectra.

Key words: antibiotics, FTIR, gamma irradiation, UV-VIS absorption.

1. INTRODUCTION

Radiation sterilization has been successfully used in the pharmaceutical industry [1,2] . Apart from its bactericidal effect, gamma-ray exposure can change the drugs' active ingredient properties. This may lead to the loss of drugs pharmacological potency or even worse, to have a harmful effect [3]. The most often found changes induced by ionizing radiation in drugs include the generation of free radicals [4] as well as degradation by-products, discoloration, changes in crystal lattice and/or crystal structure [5]. For the above-mentioned reasons, it appears necessary to determine the radiochemical stability of therapeutic drugs and to check the possibility of their sterilization by irradiation.

Apart from radiosterilization purposes, gamma-ray irradiation may successfully be used to the hospital wastes treatment and removal of harmful pharmaceuticals from environment [6].

The aim of this study was to determine the impact of ionizing radiation emitted by a ^{60}Co source on three antibiotics exposed in solid phase – Nalidixic acid, Spectinomycin, and Rifampicin. Each drug was irradiated with doses of 6, 24,

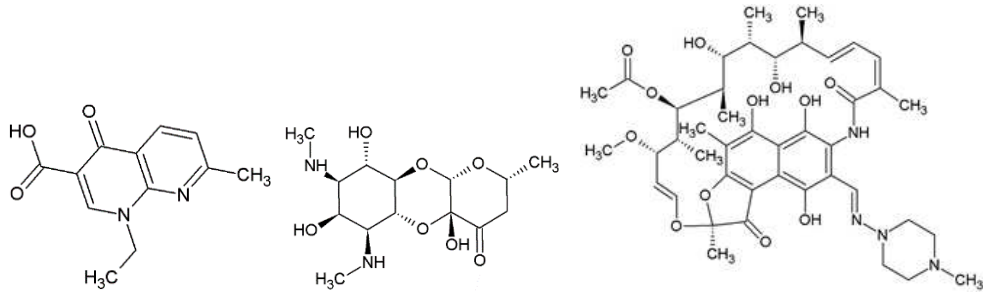
48, 102, and 204 kGy, and then subjected to analytical examination by spectrophotometric methods (UV-VIS and FTIR). According to the European Norm EN ISO 11137-1:2015 [7], the dose recommended for medical sterilization is 25 kGy, but use of higher doses in the reported experiments explores the possibility to easier detect the molecular changes induced by gamma irradiation, if any.

2. MATERIALS AND METHODS

Nalidixic acid (NAL, $C_{12}H_{12}N_2O_3$, M = 232.239 g/mol), whose molecular structure is presented in Fig. 1(a), was purchased from Sigma Aldrich. It is a synthetic antimicrobial agent with a limited bactericidal spectrum which is active against most gram-negative organisms that cause urinary tract infections [8].

Spectinomycin (SPEC, $C_{14}H_{24}N_2O_7$, M = 332.35 g/mol), bought also from Sigma Aldrich is an aminocyclitol aminoglycoside antibiotic derived from *Streptomyces spectabilis* with bacteriostatic activity of gynecological infections [9]. Its molecular structure is depicted in Fig 1(b).

Rifampicin (RIF, Fluka-BioChemika, $C_{43}H_{58}N_4O_{12}$, M = 822.94 g/mol) is an anti-infectious drug used along with other antibiotics to treat several types of bacterial infections, including tuberculosis, leprosy, and Legionnaire's disease [10]. The molecular structure of Rifampicin is shown in Fig. 1(c).



a) b) c)

Fig. 1 – The 2D molecular structure of Nalidixic acid (a), Spectinomycin (b), and Rifampicin (c)

All the three drugs were exposed in powder form to gamma-ray at BGS Beta-Gamma-Service GmbH (Wiehl, Germany) facility. Ionizing radiation doses between (6 - 204) kGy emitted by a 5 MCi Cobalt-60 (^{60}Co) gamma source were used [11].

Control (unirradiated) samples were kept at room temperature and protected from environment radiation in the microbiology laboratory (DLR) at German

Aerospace Center in Köln (noted with D), at the BGS irradiation facility (noted with B) in the same environment conditions and in the fridge (noted with T) at 4-8°C and protected from environment light at DLR.

Comparative UV-VIS and FTIR absorption spectra analysis of control and gamma-ray exposed NAL, SPEC, and RIF were performed in order to determine their stability. These measurements were made four weeks after gamma irradiation in order to avoid the presence of transient free radicals in the reconstituted solutions. Pellets of the three drugs in KBr were investigated using the Nicolet IS50 FTIR spectrophotometer (Thermo Scientific, USA), with spectral resolution of 4 cm^{-1}. The spectral data were processed with Omnic 9 standard software.

For UV-VIS absorption measurements, 10^{-3}M stock solutions of the three drugs in different solvents were prepared as follows:

- NAL in ultrapure water delivered *via* a sterile filter (TKA Pacific UP/UPW6, Thermo Electron LED GmbH, Germany) with addition of 1M NaOH;
- SPEC in ultrapure water;
- RIF in a mixture 1:1 of ultrapure water and ethanol (Merck Millipore, Germany).

The UV-VIS spectra were acquired using the Lambda 950 (Perkin Elmer, USA) spectrophotometer with the experimental spectral resolution of 0.05 nm. The measuring errors limit was ±1.045%, which cumulates the measuring error limit of the spectrophotometer, and the cells positioning error as measured in [12].

3. RESULTS AND DISCUSSIONS

Due to their accuracy and high sensitivity, UV-VIS and FTIR measurements are powerful spectrophotometric methods for qualitative and quantitative detection of the molecular changes of sensitive materials such as medicines [13-15].

FTIR spectroscopy was employed to establish the intensity, location, and type of characteristic vibrations changes, whereas by UV-VIS spectroscopy the location and the intensity of the absorption maxima were determined. The study of the spectral characteristics was performed separately for FTIR and respectively for UV-VIS registered spectra of the individual antibiotics: NAL, SPEC, and RIF. As a reference, the sample kept in dark at 4-8°C at DLR, noted with T, was considered for all the discussed results.

3.1. FTIR SPECTRA ANALYSIS

The FTIR spectra of control and respectively gamma irradiated NAL samples are exhibited in Fig. 2, where the molecule's chemical bonds that undergo vibrational changes are also represented. In the region of characteristic bands related to intramolecular vibrations of the molecules, modification in the position and shape of the band arising between (1705 – 1720) cm^{-1}, noted with 1 in Fig. 2, registered for 6 KGy and 24 KGy irradiated samples but for B control also, may be due to carboxyl stretching vibration. The inset of modified band labeled 1 in Fig.2 shows more detailed the peak structure around 1700 cm^{-1}. The sample preserved at the BGS irradiation facility shows also changes of the broad band centered at 3440 cm^{-1} (2 in Fig.2), which correspond to O-H stretching vibrations [16]. All these vibrational changes observed in NAL FTIR spectra are specified in Table 1.

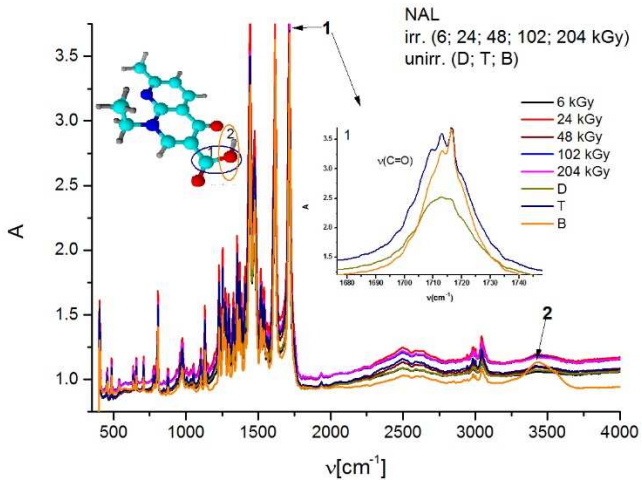


Fig. 2 – The FTIR spectra of NAL: D, T, B control samples as well as of samples exposed to gamma-ray up to 204 kGy; 1 and 2 are the bands that undergo changes.

Table 1

Vibrational changes of the RIF molecules

Nr.crt.	ν[cm^{-1}]	Assignments	Comments
1	1705-1720	ν(C=O)	- Molecular changes of samples irradiated at 6/24 kGy - Molecular changes of the sample kept at BGS irradiation facility
2	3440	ν(O-H)	Molecular changes of the sample kept at BGS irradiation facility

As for FTIR spectra of SPEC samples (Fig. 3), the O-H torsion vibrations of molecules may cause shifts of the weak maximum centered at 418 cm^{-1} for the D control and its disappearance from all FTIR spectra of irradiated samples, while the O-H stretching vibrations could determine the shift of the second maximum of the doublet 431/439 cm^{-1} to higher frequencies. In the molecular fingerprint region, changes of the peak centered at 645 cm^{-1} are observed. These correspond to C-H bending vibrations. The doublet at 665/672 cm^{-1} undergoes a shift and allure alteration, which may be due to O-H torsion in nitro- area of the analyzed molecule. Spectral changes observed for the bands between (3000 – 3440) cm^{-1} may be due to O-H stretching vibrations [16,17].

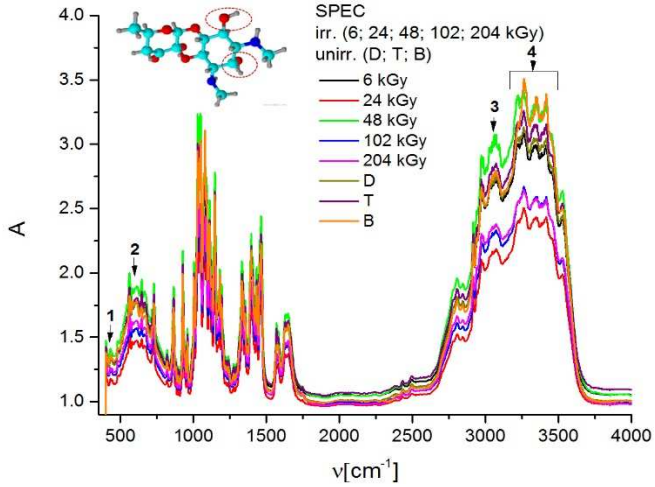


Fig. 3 – The FTIR spectra of SPEC: D, T, B control samples as well as of samples exposed to gamma-ray up to 204 kGy; from 1 to 4 are indicated the bands that undergo changes.

Table 2 synthesizes the vibrational changes registered for control and, respectively, for SPEC samples exposed to ionizing radiations.

Table 2

Vibrational changes of the SPEC molecules

Nr.crt.	ν[cm^{-1}]	Assignments	Comments
1	418-440	τ(O-H) + ν(O-H)	Vibrational changes to all irradiated samples
2	645-670	ν_{bend}(C-H) + τ (O-H)	Vibrational changes to all irradiated samples
3	3020-3100	ν (O-H)	Vibrational changes of the 48 kGy irradiated sample

4	3200-3450	ν (O-H)	Molecular changes of the sample kept at BGS irradiaton facility

The FTIR spectra of RIF that are displayed in Fig. 4 suggest also the poor stability of gamma-ray irradiated antibiotic, especially when 6 kGy and 24 kGy irradiation doses are applied. The radioinduced vibrational changes are briefly listed in Table 3.

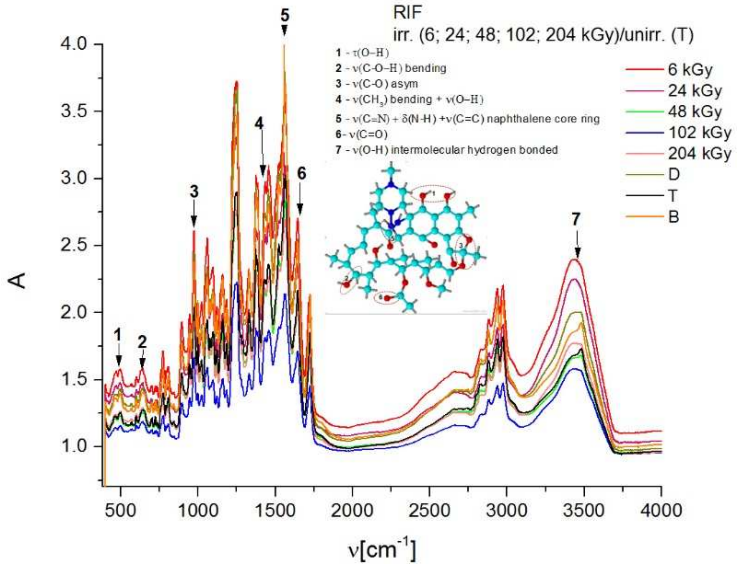


Fig. 4 – The FTIR spectra of RIF: D, T, B control samples as well as of samples exposed to gamma-ray up to 204 kGy; from 1 to 7 are indicated the bands that undergo changes.

They refer to torsional mode vibrations of O-H bonds suggested by modifications observed for maxima at 492 cm^{-1}. Also, FTIR spectra allure disturbance for the band between (626-660) cm^{-1} suggests modifications of molecular C-O-H bending vibrations. The band corresponding to the C-O absorption at 1078 cm^{-1} is accompanied by the O-H band which usually overlaps with the C-H bending vibration for the methyl group at 1330 cm^{-1}. The disappearance of the band centered at 1559 cm^{-1} for the 102 kGy irradiated sample could be due to C=N stretching, N-H deformation and C=C (naphthalene core ring) stretching vibrations [15]. The shift to lower frequencies of the peak centered at 1726 cm^{-1} for the samples exposed to doses of 6 kGy and 24 kGy may be assigned to C=O stretching vibrations, while complete vanishing of the band at 3480 cm^{-1} of the same irradiated samples may be caused by O-H hydrogen bonded (intermolecular) stretching vibrations [16].

Table 3

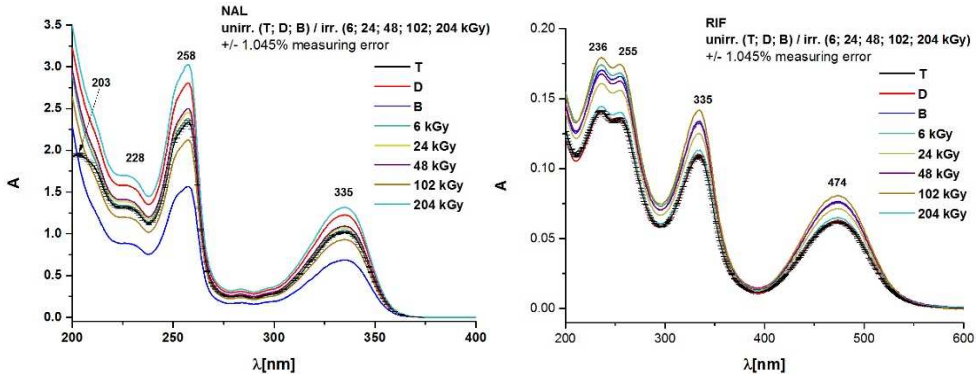
Vibrational changes of the RIF molecules

Nr.crt	ν[cm^{-1}]	Assignments	Comments
1	492	τ(O-H)	Vibrational changes of 6/24 kGy irradiated samples
2	626-660	ν_{bend}(C-O-H)	Vibrational changes of 6/24 kGy irradiated samples
3	1078	ν_{asym}(C-O)	Vibrational changes of 24 kGy irradiated sample
4	1330-1340	$\nu_{bend}(CH_3)$ + ν(O-H)	Vibrational changes of 6/24 kGy irradiated samples
5	1559	ν(C=N)+ δ(N-H) +ν(C=C) of naphthalene core ring	Vibrational changes of 102 kGy irradiated sample
6	1726	ν(C=O)	Vibrational changes of 6/24 kGy irradiated samples
7	3480	Intermolecular ν(O-H)	Vibrational changes of 6/24 kGy irradiated samples

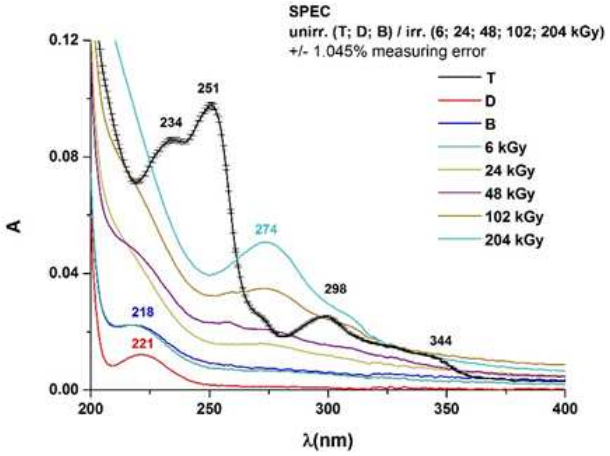
3.2. UV-VIS SPECTRA ANALYSIS

The absorption measurement in UV-VIS spectral range reveals peaks as follows:

- NAL: at 203, 228, 258, and 335 nm (Fig. 5a);
- RIF: at 236, 255, 355, and 474 nm (Fig. 5b);
- SPEC: at 234, 251, 298 nm, and a week shoulder at 344 nm (Fig. 5c).



a) b)



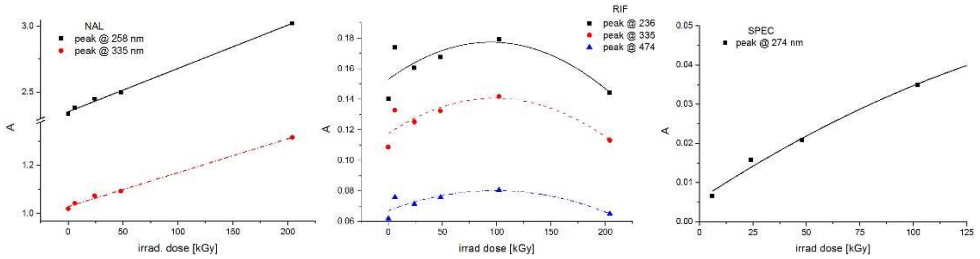
c)

Fig. 5 – The UV-VIS spectra of NAL (a), RIF (b) and SPEC (c): D, T, B control and ionizing radiation exposed samples up to 204 kGy .

As first observation, in Fig. 5 the location of these peaks did not modify for NAL and RIF gamma-ray exposed drugs samples, but they suffer important changes for SPEC in, all control and irradiated samples.

In the UV-VIS spectra of SPEC, the blue shift of 236 nm absorption peak was registered, as well as the vanishing of all the other absorption maxima for control samples D and B. This is observed also for the sample exposed to 6 kGy irradiation dose. Furthermore, with increasing of ionizing radiation dose a new absorption peak arising at 274 nm is observed as shown in Fig. 5c.

The evolution of absorption maxima for NAL and RIF function of ionizing radiation doses used for drugs exposure is depicted in Fig. 6a and 6b.



a) b) c

Fig. 6 – Linear fit of the absorption maxima at 258 nm and 335 nm of NAL (a); polynomial fit of the absorption maxima at 236 nm, 335 nm, and 474 nm of RIF (b); polynomial fit of the SPEC absorption maxima arising at 274 nm as function of gamma-ray irradiation dose (c).

This evolution may be approximated by linear fit which shows an ascendant trend in Fig.6a for NAL or polynomial fit showing a maximal modification of RIF sample at exposure to 100 kGy in Fig. 6b. Likewise the progress of the peak arising at 274 nm which may belong to a side-product of irradiated SPEC is approximated by polynomial fit in Fig. 6c. Its behavior suggests the increasing concentration of this product in the total mass of SPEC along with irradiation dose increment.

4. CONCLUSIONS

Ionizing radiation initiates in drugs not only the oxidation, but also some other reactions, including radiolytic dissociation leading to the breaking of different types of bonds, hydrolysis, deamination, deacetylation, decarboxylation, polymerization and isomerization [18].

Following the spectrophotometric investigation we found that all three antibiotics show variations in absorption intensity in UV-VIS spectral range as a result of ionizing radiation exposure. Hypsochromic shifts and absorption maxima extinction of Spectinomycin, all control and 6 kGy irradiated samples were observed. This may suggest very high sensitivity of drugs to environment conditions, especially to temperature. More, the new Spectinomycin absorption peak arising at 274 nm, whose intensity increases with the irradiation dose, proves the drug instability as a consequence of gamma-ray exposure.

As a general remark, molecular modifications of control samples for Nalidixic acid and Spectinomycin stored in the BGS irradiation facility laboratory were observed, and all the three antibiotics are particularly sensitive to irradiation doses up to 24 kGy.

The analysis of spectrophotometric data allows to conclude that sterilization by exposure to gamma radiation is not recommended for the studied antibiotics. It is rather suggested the use of ionizing radiation exposure for the treatment of medical wastes containing this kind of antibiotics [19].

Further experiments may extend optical spectroscopy studies using, supplementary, techniques such as Laser Induced Fluorescence and Raman measurements [20] along with chromatographic methods [21,22] to identify the degradation by-products. Also, microbiological tests are needed to determine the activity of gamma irradiated Nalidixic acid, Rifampicin and Spectinomycin on selected sensitive microorganisms.

The obtained results show that modifications induced at intra-atomic levels by exposure to ionizing radiation in the case of Nalidixic acid, Rifampicin and Spectinomycin may be reflected in the modifications of their optical spectra.

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