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ESR INVESTIGATION OF X-RAY EXPOSURE ON SOME ANTI-DIABETICS AND PROTON PUMP INHIBITORS

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Abstract. *X-ray is ionizing radiation used in several areas such as analytical sciences, medicine and security areas. X-ray machines are used in the entrance of important places (airports, shopping centers, etc.) for security purposes. The aim of this study was the investigation of the potential effects of X-ray irradiation on anti-diabetics (metformin HCl, pioglitazone HCl) and proton pump inhibitors (PPI) (lansoprazole, pantoprazole sesquihydrate) pharmaceuticals which are used in chronic diseases by Electron Spin Resonance (ESR). ESR analysis was done before and after different X-ray irradiation doses. Afterwards, ESR spectra and resonance peaks were evaluated. As a result, no significant free radicals were detected by ESR resonance peaks and also, their ESR intensities did not change significantly by increasing X-ray doses.*

Keywords: *Electron Spin Resonance, X-ray, ionizing radiation, X-ray irradiation on pharmaceuticals*

1. Introduction

X-ray irradiation having short wavelength and high penetration capability is used in several areas nowadays. Especially, it is used for security in several control points such as passenger bags screening at airports, shopping centers, etc., with evolving technologies [1].

X-ray exposure can cause the formation of reactive molecular fragments and induced radical types which can be determined previously by Electron Spin Resonance (ESR) spectroscopy [2,3]. ESR is a non-destructive, highly sensitive spectroscopic technique allowing direct observation of free radicals and enabling the differences between radical types. Thus, this method is accepted to be one of the most reliable methodologies for identifying free radicals and can provide valuable information about structural and chemical properties for especially stable radical species formed in drugs after X-ray exposure [4].

In this study, physicochemical properties and formed radicals of X-ray exposed anti-diabetics (metformin HCl, pioglitazone HCl) and proton pump inhibitors (PPI) (lansoprazole, pantoprazole sesquihydrate) were investigated before and after X-ray irradiation.

2. Materials and methods

X-ray irradiation doses were selected based on the doses used in X-ray machines for security purposes (0.12 mGy from one pass) [1]. Doses were determined from the reference dose for the investigation of X-ray effects on pharmaceuticals. X-ray irradiation doses are 0.24 mGy and 1.2 mGy which were equivalent to 2 and 10 passes, respectively and 58 mGy which was chosen as an extremely high dose.

Organoleptic properties (color, odor, and appearance) were investigated before and after X-ray exposure. The spectroscopic features of radiolytic intermediates produced in X-ray irradiated (0.24; 1.2; and 58 mGy) and non-irradiated drug samples were investigated after 1 hour at room temperature by using ESR spectroscopy. The utilization of quantitative electron paramagnetic resonance spectrometry has been increasing with increasing rate in the chemical, physical, biological, and medical sciences. This is because of its high sensitivity and broad dynamic range property, and its ability to be calibrated with any convenient paramagnetic materials [5]. This technique is also accepted to be high sensitivite when it is compared with other conventional techniques [6]. ESR studies were performed by using Bruker EMX 113X-Band ESR spectrometer

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which is around the 10^{10} sensitivity. The optimum experimental conditions of ESR measurements for anti-diabetics and PPI's were adjusted (Center Field: 355.0 mT; Sweep Width:100 mT; Microwave Frequency: 9.75 GHz; Microwave Power: 5 mW; Modulation Frequency:100 kHz; Modulation Amplitude:0.1 mT; Receiver Gain: $6.32x10^4$; Sweep Time: 167.77 s; Time Constant:81.92 ms; Conversion Time:163.84 ms; at Room Temperature). For determination of spin concentration, two measuring methods are possible where in the first method the standart sample (In this study DDPH is used as standard material) and investigated samples can be located in the cavity at the same time and in the second method these samples can be insert into the cavity after each other. For the second method the two samples do not influence each other and will be much more accurate [7]. During the experiments we have also use the second method mentioned.

3. Results and Discussion

Organoleptic properties of anti-diabetics did not show any differences as in PPI's. Color of pioglitazone HCl and metformin HCl containing samples were white before and after X-ray irradiation. Pantoprazole sesquihydrate and lansoprazole containing samples were yellow and orange before and after 0.24, 1.2 and 58 mGy doses of X-ray irradiation, respectively.

Non-irradiated metformin HCl and pioglitazone HCl containing samples designated ESR spectra with relatively low-intensity ESR resonance lines. Seven resonance peaks were recorded for metformin HCl and pioglitazone HCl containing samples, respectively. ESR intensities of these resonance peaks were not changed much by the increasing rate of absorbed X-ray dose and spectrum pattern remained nearly the same at higher absorbed dose for both anti-diabetics (Table 1).

The ESR spectra of metformin HCl-containing samples were spread over 40 mT of the magnetic field region. The spectroscopic splitting factor obtained for g_{mid} value of the ESR spectra of metformin HCl was 1.9809. The spectroscopic splitting factors of the recorded 7 resonance signals of metformin HCl were obtained as g_1: 2.0639; g_2: 2.0078; g_3:1.9953; g_4: 1.9825; g_5: 1.9759; g_6: 1.9429; g_7:1.9002. Peak-to-peak line width of $\Delta H_{pp,2\text{-}3}$: 3.0 mT; $\Delta H_{pp,4\text{-}5}$: 1.2 mT; $\Delta H_{pp,6\text{-}7}$: 8.4 mT (Figure 1A).

The ESR spectra of pioglitazone HCl containing samples were spread over 40 mT of the magnetic field region. The spectroscopic splitting factor obtained for g_{mid} value of the ESR spectra of pioglitazone HCl was 1,9798. The spectroscopic splitting factors of the recorded 7 resonance signals of pioglitazone HCl were obtained as g_1: 2.0627; g_2: 2.0123; g_3:1.9965; g_4: 1.9842; g_5: 1.9759; g_6: 1.9429; g_7:1.8961. Peak-to-peak line width of $\Delta H_{pp,2\text{-}3}$: 3.1 mT; $\Delta H_{pp,4\text{-}5}$: 2.0 mT; $\Delta H_{pp,6\text{-}7}$: 8.5 mT (Figure 1B).

Non-irradiated lansoprazole and pantoprazole sesquihydrate samples were indicated ESR spectra with very low-intensity ESR resonance lines (Table 1).

Table 1. Dose-response results of anti-diabetics

	Absorbed X-ray Dose (mGy)	Resonance Signal (a.u.) I_{pp}: I_6+I_7	Area (a.u.)
Metformin HCl containing samples	0	1758	1544800.4
	0.24	1928	1614889.4
	1.2	2013	1903825.2
	58	2000	1707939.9
Pioglitazone HCL containing samples	0	2094	1473166.2
	0.24	2298	1406243.7
	1.2	1814	1468202.7
	58	2089	1489399.8
Lansoprazole	0	1723	1683482.8
	0.24	1802	1775484.8
	1.2	1993	1725458.9
	58	1688	1617471.3
Pantoprazole Sesquihydrate	0	2366	2257094.4
	0.24	2294	2064821.7
	1.2	2201	2043992.1
	58	2205	2189404.6

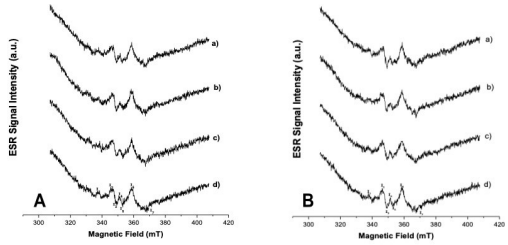

Figure 1. ESR Spectra of X-ray irradiated Metformin HCl (A); Pioglitazone HCl (B); a)non-irradiated b)0.24 mGy c)1.2 mGy d)58 mGy at room temperature.

14 resonance peaks of lansoprazole samples were recorded and ESR intensities of these peaks were not much increased by the increasing rate of absorbed X-ray dose and the spectrum pattern remained same at higher absorbed doses (Table 1) and resonance signals of lansoprazole were obtained as g_1: 2.1500; g_2: 2.1389; g_3:2.0917; g_4: 2.0855; g_5: 2.0358; g_6: 2.0317; g_7:2.0156; g_8: 1.9974; g_9: 1.9839; g_{10}:1.9773; g_{11}: 1.9443; g_{12}: 1.9237; g_{13}: 1.8853; g_{14}:1.8748. Peak-to-peak line width of $\Delta H_{pp,1\text{-}2}$: 1.7 mT; $\Delta H_{pp,3\text{-}4}$: 1.0 mT; $\Delta H_{pp,5\text{-}6}$: 0.5 mT; $\Delta H_{pp,7\text{-}8}$: 2.8 mT; $\Delta H_{pp,9\text{-}10}$: 1.2 mT; $\Delta H_{pp,11\text{-}12}$: 3.5 mT; $\Delta H_{pp,13\text{-}14}$: 1.9 mT. The ESR spectra of lansoprazole have spread over 60 mT of the magnetic field region. The spectroscopic splitting factor obtained for g_{mid} value of the ESR spectra of lansoprazole is 1.9812 (Figure 2A).

10 resonance peaks were recorded for pantoprazole sesquihydrate samples and the spectrum pattern remained nearly the same at higher absorbed doses (Table 1). The spectroscopic splitting factors of the recorded signals were obtained as g_1: 2.0609; g_2: 2.0497; g_3:2.0315; g_4: 2.0315; g_5: 2.0090; g_6: 1.9977; g_7:1.9849; g_8: 1.9788; g_9: 1.9435; g_{10}:1.8968. The ESR spectra of

pantoprazole sesquihydrate were spread over 50 mT of the magnetic field region. Peaks to peak line width of $\Delta H_{pp,1\text{-}2}$: 1.8 mT; $\Delta H_{pp,3\text{-}4}$: 0.8 mT; $\Delta H_{pp,5\text{-}6}$: 1.8 mT; $\Delta H_{pp,7\text{-}8}$: 1.2 mT; $\Delta H_{pp,9\text{-}10}$: 8.2 mT (Figure 2B).

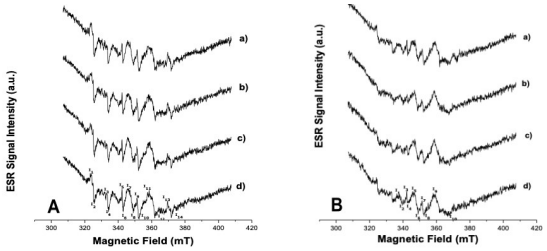

Figure 2. ESR Spectra of X-irradiated Lansoprazole (A); Pantoprazole sesquihydrate (B); a)non-irradiated b)0.24 mGy c)1.2 mGy d)58 mGy at room temperature.

In principle, the area under the absorption curve obtained by ESR is proportional to the number of unpaired spins in the sample [8]. To obtain accurate analytical ESR data and calculating the number of spins contributing to the recorded ESR spectra, either double integration or measurement of the peak-to-peak amplitudes can be preferred. The double integration offers the greatest generality because it permits any paramagnetic material to be used as a standard, but it is less accurate than measurement of the amplitude (3). For improving the accuracy of double integration, the baseline corrections were also used by using WinEPR program. We had used both of the mentioned methods together and have got similar results confirming each other and share the results in *Table 1*.

As certain conditions were fulfilled in our experiments, accuracy of EXM Bruker X-band spectrometer is at about ±2%**.** This value can be taken to be less for the high dose irradiated sample as the spin concentration are expected to be higher for these samples (the error degrees can be less).

5. Conclusion

X-ray irradiation has been used in many areas in daily life, especially for the purpose of security. Thus, pharmaceuticals used for the therapy of chronic diseases, are exposed to X-ray irradiation in different doses and different periods. This study was a part of the investigation of the effect of X-ray irradiation on pharmaceuticals. ESR studies are an important part of the investigation of free radicals that are formed after exposure. Free radicals show different resonance peaks with different intensities during different periods and those peaks can be detected by ESR.

In this study, organoleptic properties and ESR data were evaluated for anti-diabetics and PPI's. Organoleptic analyses showed no significant differences in color, odor, and appearance meaning that X-ray did not penetrate so deeply and did not make any significant changes chemically.

ESR spectra did not show any differences before and after X-ray irradiation for anti-diabetics and PPI's. Resonance peaks detected before and after X-ray irradiation however, their ESR intensities did not increase with increasing X-ray doses. It might be the reason for doing ESR analysis 1 hour after X-ray irradiation. Although it can be concluded that free radicals could be decreased or disappeared after 1 hour, it can be assumed that X-ray did not affect the pharmaceuticals and form any free radicals due to the detection of so low ESR intensities.

Therefore, X-ray is safe for solid form pharmaceuticals as a result of our study, but, other analyses should also be done and investigated to comment more thoroughly.

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