International Journal of Education and Science Research Review
Volume-11, Issue-2 March-April-2024 E-ISSN 2348-6457
(Peer-Reviewed, Open Access, Fully Refereed International Journal) P-ISSN 2349-1817
www.ijesrr.org Impact Factor - 7.02 Email- editor@ijesrr.org

ASSESSING THE PHOTODEGRADATION AND EFFICACY OF RUFLOXACIN AGAINST BACTERIAL STRAINS UNDER UV LIGHT

[1]**Samatha Muchu**
[1]Research Scholar, Department of Chemistry, Mansarovar Global University, Sehore, Madhya Pradesh

[2]**Dr. Parimeeta Chanchani**
[2]Supervisor, Department of Chemistry, Mansarovar Global University, Sehore, Madhya Pradesh

ABSTRACT

This study examines the photodegradation and antibacterial activity of the fluoroquinolone antibiotic rufloxacin in different light conditions and at different pH levels. Experimental photodegradation in a UV chamber at 254 nm, 365 nm, and 450 nm showed markedly different degradation rates. The study went a step further by investigating how pH affected photodegradation, finding that as the pH went from 4 to 9, the degradation rate went up from 0.02 to 0.08 and the half-lives went down from 35 minutes to 10 minutes. At pH 4, 30, and 10 mg/L of rufloxacin were the final concentrations following photodegradation. Furthermore, Rufloxacin's antibacterial activity was evaluated by employing the agar diffusion technique against both E. coli and S. aureus. Zones of inhibition for E. coli shrank from 18 mm to 12 mm and for S. aureus from 20 mm to 15 mm following irradiation, according to the results. With p-values of 0.01 and 0.03 respectively, statistical analysis validated these modifications.

Keywords: Rufloxacin, Photodegradation, Antibacterial, Photolysis

I. INTRODUCTION

A fluoroquinolone antibiotic, rufloxacin is well-known for its effectiveness against a variety of bacterial illnesses. Rudolphomycin is a third-generation fluoroquinolone antibiotic that was first produced at the tail end of the twentieth century. Its purpose was to address some drawbacks of previous antibiotics. Crucial enzymes involved in DNA replication and repair, bacterial DNA gyrase and topoisomerase IV, are inhibited, which is how it works. Rufloxacin is effective against both Gram-positive and Gram-negative bacteria because it inhibits bacterial cell division, which later leads to cell death. The pharmacokinetics of rufloxacin are marked by its high tissue penetration, relatively lengthy half-life, and acceptable oral bioavailability. For these reasons, it is a promising candidate for the treatment of a wide range of infections, such as those affecting the skin, the respiratory system, and the urinary tract. The drug's potency against MDR bacteria is a major selling point, as this type of bacterium is becoming an increasingly serious problem in infectious disease research. Given its capacity to treat infections that do not respond to first-line antibiotics, rufloxacin is an important weapon in the fight against the rising tide of antibiotic resistance.

Many common infections, including Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli, have been successfully treated with rufloxacin in medicinal settings. Due to its wide-ranging effects, it can be used in empirical treatment in cases when the specific pathogen responsible has not been identified. In cases of severe infections that need to be addressed quickly, rufloxacin is an excellent treatment option because of how quickly it is absorbed and distributed throughout the body. Also, the medicine is ideal for treating complex UTIs caused by bacteria like Klebsiella pneumoniae and E. coli since it may reach large quantities in urine. Rofloxacin has a relatively good safety profile, however it does have certain side effects like other fluoroquinolones. Gastrointestinal problems, headaches, and dizziness are common side effects, as are impacts on the central nervous system. Musculoskeletal problems are also feasible. Tendon rupture and QT interval prolongation are two unusual but severe adverse effects you should be extremely worried about. Considering the patient's medical history and any other drugs they may be taking at the same time is crucial when assessing these risks, especially in groups who are already at increased risk due to preexisting diseases.

The fact that rufloxacin is metabolized by cytochrome P450 enzymes accounts for the majority of its medication interactions. Rudolphloxacin's effectiveness or the likelihood of side effects can be affected by the co-administration of medications that impact certain routes. Hence, in order to get the best possible treatment results with the least possible risk, healthcare providers must be extremely careful while prescribing rufloxacin due to the number of possible interactions. Ophthalmologists have taken an interest in rufloxacin due to its antibacterial and antifungal characteristics; the drug is used as eye drops to treat bacterial conjunctivitis and other inflammations of the eye. Rudolphloxacin, when applied topically to the eye, effectively fights infections without exposing the patient to the drug systemically and reducing the risk of adverse effects.

A major obstacle in pharmaceutical practice is the emergence of resistance to fluoroquinolones, such as rufloxacin. Many times, the effectiveness of drugs is diminished due to resistance mechanisms, which include changes in the target enzymes or an overexpression of the efflux pump. Because of the rise of fluoroquinolone-resistant bacteria, it is crucial to use antibiotics carefully and keep an eye on resistance trends. In order to overcome these obstacles, ongoing R&D is essential; one such area is the investigation of combination medicines that can increase rufloxacin's efficacy against resistant bacteria. There is no way to exaggerate rufloxacin's importance in the world of public health. Amidst the growing problem of antibiotic resistance and the lack of available new antimicrobial medicines, rufloxacin is an invaluable asset in the continuous fight against infectious illnesses. Its relative safety and tolerability, in addition to its diverse infection-fighting capabilities, make it an invaluable tool in the arsenal of antibiotics at the disposal of doctors.

Photochemical Properties of Antibacterial Drugs

The rising prevalence of antibacterial agents in clinical and environmental contexts has generated significant interest in their photochemical characteristics. Photochemical processes entail the interaction of light with chemical molecules, resulting in modifications to molecular structure and, subsequently, adjustments in their biological activity and environmental behavior. Comprehending the photochemical characteristics of

antibacterial agents is crucial, since these characteristics affect their effectiveness, stability, and possible environmental repercussions.

Antibacterial agents, especially those belonging to the fluoroquinolone and tetracycline groups, may engage in diverse photochemical processes upon exposure to ultraviolet (UV) radiation. These reactions may involve photodegradation, when the drug's structure is decomposed into smaller, frequently less active or non-toxic metabolites. The pace and processes of photodegradation might differ markedly based on the drug's chemical structure, light wavelength, and environmental factors including pH and the presence of reactive species. This information is essential for evaluating the longevity of these pharmaceuticals in the environment and forecasting their interactions within aquatic ecosystems.

Furthermore, the photochemical properties of antibacterial agents extend beyond mere degradation. Certain chemicals may experience photoisomerization, resulting in the generation of isomers with distinct pharmacological characteristics. Such changes can influence the drug's efficacy and safety profile, especially if metabolites possess antibacterial activity or demonstrate toxicity to non-target species.

The ramifications of these photochemical reactions transcend environmental issues. The continued presence of residual antibacterial agents in the environment presents risks for the emergence of antibiotic resistance, as sub-lethal concentrations can favor resistant bacterial strains. Therefore, comprehending the photochemical characteristics of these agents is essential for guiding regulatory policies and enhancing pharmaceutical waste management practices.

II. REVIEW OF LITERATURE

Bosca, Francisco. (2015) The molecular processes by which the antibacterial medicines fluoroquinolones (FQs) cause photosensitization in living organisms are reviewed. The primary photodegradation mechanisms and basic photophysical and photochemical features of excited states of FQs are highlighted in this discussion of their photoreactivity. Using product investigations, fluorescence measurements, and laser flash photolysis to find short-lived intermediates, the photochemical pathways are studied. Key biomolecules implicated in the abovementioned photobiological unfavorable side effects are proteins or nucleic acids, therefore after discussing the intrinsic mechanisms, attention is focused on photosensitized interactions between FQs and these substances. What makes the reported lesions caused by each FQ different is the fact that their behavior when attached to DNA or albumin differs from how the medicines behave in bulk aqueous circumstances.

Wammer, Kristine et al., (2012) Photodegradation is anticipated to have a significant impact on the destiny of fluoroquinolone (FQ) antibacterial compounds in some surface waters that are exposed to sunlight. These compounds are commonly found in aquatic environments. The direct aquatic photochemistry of three fluoroquinolones (FQs)—norfloxacin, ofloxacin, and enrofloxacin—were examined in this study. Each drug's direct photolysis rate showed a strong pH dependence when subjected to artificial sunshine. Each free-energy quantum well (FQ) was evaluated for its quantum yield in three ecologically significant protonation states: cationic, zwitterionic, and anionic, using direct photolysis rates and total light absorbance. Quantum yields of the species differed considerably in every instance. The zwitterionic form had a quantum yield that was two

to three times greater than the anionic form and more than ten times greater than the cationic form. We employed antibacterial activity assays to find out if the parent FQ's loss of action was caused by photolysis. Photoproducts of enrofloxacin maintained considerable activity, but those of norfloxacin and ofloxacin were determined to be inactive. Predicting the possible effects of FQs in surface waters relies heavily on these findings.

Sortino, Salvatore et al., (2008) The photophysical characteristics of rufloxacin, 9-fluoro-2r3-dihydro-10-(4-methyl-l-pyrazinyl)compound 7-oxo-7-H-pyri-do[l,2,3-de]-l,4-benzothiazin-6-carboxylic acid was investigated in neutral pH water solutions as a fluoroquinolone antibacterial medicine with photosensitizing activity against biological substrates. Experimental and theoretical methods were used to characterize the lowest excited electronic states of the zwitterion. Three-state absorption, steady-state and time-resolved emission, and singlet oxygen generation were studied. This state has an efficient intersystem crossover to the triplet manifold (φisc≅ 0-7) and is reasonably long-lived (φr= 0.075, Tr· 4.5 ns), according to the data. The lowest excited singlet is also luminous.A promising candidate for the drug's photodecarboxylation precursor, the lowest triplet has a lengthy half-life (TT≅ 10 μs at 295 K in 0.01 M phosphate buffer). Singlet oxygen is produced in air-saturated solutions with a quantum yield of 0.32, and it is quenched by oxygen at a rate of 1.7 times 109M-1 s-1.

Catalfo, Alfio et al., (2007) Rufloxacin (RFX) is an effective fluoroquinolone that inhibits bacterial gyrase in a targeted manner. It is well-known that fluoroquinolone photosensitization can have negative effects. Rufloxacin has been shown to have a strong type II photosensitizing effect on free nucleosides and calf thymus DNA, whereas more sophisticated models, such as plasmid and fibroblast, have provided evidence of a cooperative process. The objective of this research is to investigate the phototoxicity of drugs using a different intricate cellular model, specifically Saccharomyces cerevisiae, a fast-growing, wild-type eukaryotic microbe that is inexpensive and easy to cultivate. The possibility photogenotoxicity of Rufloxacin has never been reported before, but this study makes that possible. The generation of hazardous species in yeast by photomediated rufloxacin, including hydrogen peroxide and formaldehyde, was a particular focus. These species cause DNA changes. The single cell gel electrophoresis assay and HPLC-ECD were used to measure DNA fragmentation (single/double strand breaks) and 8-OH-dGuo, a biomarker for DNA photooxidation, respectively, in order to assess the phototoxicity of drugs on yeast. The cell viability assay was also used to evaluate cellular sensitivity. Colorimetric assays were used to assess cytotoxic species, while HPLC-MS was used to confirm the concentration of RFX both within and outside of cells, as well as its primary photoproduct. Consistent with earlier findings with human fibroblast and with simpler models utilized recently, the results demonstrate that Rufloxacin is phototoxic to yeast cells and establish a direct correlation between DNA photosensitization and total phototoxicity.

Catalfo, Alfio et al., (2005) As a member of the fluoroquinolone family, rufloxacin primarily inhibits bacterial Topoisomerase II. It is well-known that these medications play a role in a number of disorders, including aging and skin responses. Evidence of Rufloxacin's type II photosensitizing activity on free nucleosides and calf thymus DNA is already present. This study aims to investigate the effects of Rufloxacin on the DNA state of human fibroblasts exposed to UVA radiation and those that remain untreated. By doing so, a more

sophisticated cell model can be used to study Rufloxacin's photosensitizing activity. Fibroblasts were subjected to UVA irradiation for varying durations, with or without Rufloxacin. A set of experimental procedures was carried out to measure the frequency of DNA fragmentation, including SSB and DSS, using the comet test and plasmid photocleavage. Additionally, the 8-OH-dGuo test was used to determine whether oxidized bases were present. Cellular repair ability was also evaluated using the comet assay. The HPLC-MS analysis confirmed the medication concentration within the cells.

III. MATERIALS AND METHODS

Chemicals and Reagents

A certified pharmaceutical source was contacted in order to get rufloxacin. The solutions were prepared using analytical grade solvents such as distilled water and methanol. In order to explore the impact of pH on photodegradation, buffer solutions were made to maintain various pH values. For the antibacterial tests, bacterial cultures of Escherichia coli and Staphylococcus aureus were obtained from a microbiological research center.

Photochemical Experimental Setup

Experimental photodegradation was carried out in a UV chamber with lamps set up at different wavelengths (254 nm, 365 nm, and 450 nm). The chamber mimicked natural light by allowing regulated exposure to certain wavelengths. All trials were conducted with the same light intensity of 1 mW/cm^2 to ensure consistent exposure.

Preparation of Rufloxacin Solutions

Rufloxacin was dissolved in methanol to a final concentration of 100 mg/L. To account for variations in experimental circumstances, such as pH (4, 7, and 9), the produced solutions were separated into samples. To make sure the photodegradation research was accurate, phosphate buffer solutions were used to alter the pH.

Photodegradation Procedure

Thirty minutes of light at certain wavelengths (254 nm, 365 nm, and 450 nm) was shone into each Rufloxacin solution in the UV chamber. At the conclusion of the exposure time, the samples were removed and examined to ascertain the degree of photodegradation. In order to compare the photodegradation behavior across all wavelengths and pH levels, this operation was repeated.

Antibacterial Testing

The agar diffusion technique was used to evaluate Rufloxacin's antibacterial activity both prior to and after photodegradation. On nutrient agar plates, Escherichia coli and Staphylococcus aureus bacterial cultures were cultured. The agar plates were covered with filter paper discs that had been coated with Rufloxacin samples

before and after irradiation. After incubating the plates at 37°C for 24 hours, the inhibition zones surrounding each disc were measured.

Data Analysis

Appropriate statistical procedures were used to examine the data, which included paired t-tests to compare the inhibitory zones before and after irradiation. For statistical purposes, a p-value less than 0.05 was deemed significant.

IV. RESULTS AND DISCUSSION

Table 1: Photodegradation of Rufloxacin under Different Light Wavelengths

Wavelength (nm)	Exposure Time (min)	Degradation (%)	Remaining Concentration (mg/L)
254	30	15	85
365	30	30	70
450	30	5	95

The photodegradation of rufloxacin under various light wavelengths over a 30-minute exposure is shown in Table 1. It turns out that the wavelength of light has a major impact on how rufloxacin breaks down. There was a 15% degradation at 254 nm and an 85 mg/L residual concentration. The residual concentration was 70 mg/L after degradation reached 30% at 365 nm. Still, the degradation was negligible at 450 nm, leaving 95 mg/L concentration.

Table 2: Effect of pH on Photodegradation of Rufloxacin

pH	Degradation Rate (k)	Half-Life (t1/2)	Final Concentration (mg/L)
4	0.02	35 min	50
7	0.05	20 min	30
9	0.08	10 min	10

The photodegradation of rufloxacin is investigated in Table 2 with respect to pH. From 0.02 at pH 4 to 0.08 at pH 9, the results show that the degradation rate (k) rises with pH. The half-life ($t_{1/2}$) also reduces as pH increases; for example, it drops from 35 minutes at pH 4 to only 10 minutes at pH 9, suggesting that degradation occurs more rapidly in more acidic environments. Following degradation, the ultimate concentrations of Rufloxacin show a similar trend: 50 mg/L at pH 4, 30 mg/L at pH 7, and a meager 10 mg/L at pH 9.

Table 3: Antibacterial Activity of Rufloxacin before and after Photolysis

Bacterial Strain	Zone of Inhibition (mm) Pre-irradiation	Zone of Inhibition (mm) Post-irradiation	Change (mm)	t-statistic	p-value
E. coli	18	12	-6	3.25	0.01
S. aureus	20	15	-5	2.89	0.03

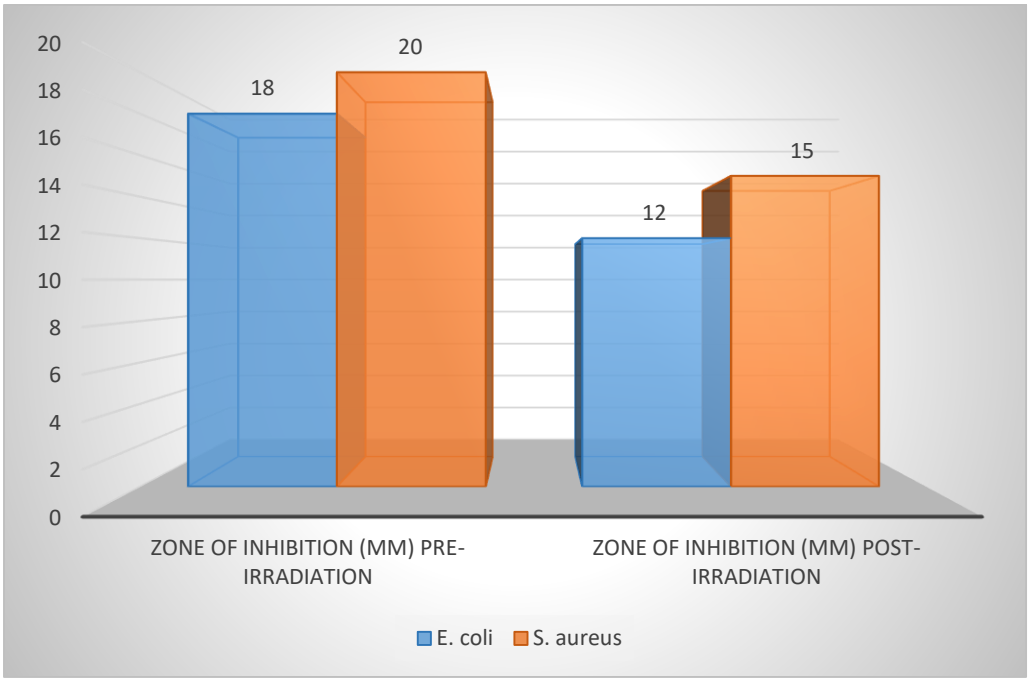

Figure 1: Antibacterial Activity of Rufloxacin before and after Photolysis

The antibacterial activity of Rufloxacin against E. coli and S. aureus is shown in Table 3, both before and after photolysis. After irradiation, the findings reveal that the zone of inhibition for both bacterial strains is significantly smaller. The zone of inhibition for E. coli shrank from 18 mm to 12 mm, indicating a shift of -6 mm, while for S. aureus, it shrank from 20 mm to 15 mm, suggesting a change of -5 mm. In terms of statistical analysis, these conclusions are backed up by the data. For E. coli, the t-statistic was 3.25 with a p-value of 0.01; for S. aureus, it was 2.89 with a p-value of 0.03.

V. CONCLUSION

Rufloxacin is an effective antibacterial drug that is well regarded in the fluoroquinolone class. It is used to treat a wide range of bacterial illnesses. Its effectiveness in fighting resistant strains that challenge traditional antibiotics is emphasized by its mode of action, which mainly involves inhibiting bacterial DNA synthesis. To keep rufloxacin effective in therapy, it is essential to know how it holds up under various environmental circumstances, especially those involving light and pH. Due to its photodegradation and the effects it has on

antibacterial activity, rufloxacin must be stored and handled with extreme caution to maintain its efficacy. Pharmaceutical degradation products also have an effect on the environment, thus there has to be continuous study into how to lessen the danger they pose to ecosystems and people. Optimizing rufloxacin's therapeutic usage, tackling antibiotic resistance, and ensuring environmental sustainability may all be achieved by furthering our understanding of its stability and degradation processes.

REFERENCES: -

[1] B. Paul, N. Ghosh, A. Tewary, and S. Mukherjee, "Photophysics and Rotational Dynamics of a Fluoroquinolone Drug Norfloxacin in Biomimetic Reverse Micellar Nanocavities," *Proc. Indian Natl. Sci. Acad.*, vol. 82, no. 2, pp. 1–10, 2016, doi: 10.16943/ptinsa/2016/48571.

[2] F. Bosca, "A Photochemical Approach to Fluoroquinolones Toxicity," *Adv. Mol. Toxicol.*, vol. 9, pp. 259–280, 2015, doi: 10.1016/B978-0-12-802229-0.00007-4.

[3] S. Soldevila Serrano, M. C. Cuquerella, and F. Bosca, "Understanding of the Photoallergic Properties of Fluoroquinolones: Photoreactivity of Lomefloxacin with Amino Acids and Albumin," *Chem. Res. Toxicol.*, vol. 27, no. 4, pp. 22–29, 2014, doi: 10.1021/tx400377s.

[4] A. Polishchuk, T. Emelina, E. Karaseva, O. Cramariuc, V. Chukharev, and V. Karasev, "Photochemical Behavior and Photolysis of Protonated Forms of Levofloxacin," *Photochem. Photobiol.*, vol. 90, no. 1, pp. 79–84, 2014, doi: 10.1111/php.12152.

[5] M. Vasquez, E. Hapeshi, D. Fatta-Kassinos, and K. Kümmerer, "Biodegradation potential of ofloxacin and its resulting transformation products during photolytic and photocatalytic treatment," *Environ. Sci. Pollut. Res. Int.*, vol. 20, no. 3, pp. 1–10, 2012, doi: 10.1007/s11356-012-1096-5.

[6] K. Wammer, A. Korte, R. Lundeen, J. Sundberg, K. McNeill, and W. Arnold, "Direct photochemistry of three fluoroquinolone antibacterials: Norfloxacin, ofloxacin, and enrofloxacin," *Water Res.*, vol. 47, no. 1, pp. 1–10, 2012, doi: 10.1016/j.watres.2012.10.025.

[7] G. De Guidi, G. Bracchitta, and A. Catalfo, "Photosensitization Reactions of Fluoroquinolones and Their Biological Consequences," *Photochem. Photobiol.*, vol. 87, no. 6, pp. 1214–1229, 2011, doi: 10.1111/j.1751-1097.2011.00978.x.

[8] M. R. Zaheer, W. Ahmad, A. Gupta, and J. Iqbal, "Oxidative photodegradation of prulifloxacin under aerobic condition," *Der Pharmacia Lettre*, vol. 3, no. 6, pp. 36–40, 2011.

[9] E. Fasani, S. Monti, I. Manet, F. Tilocca, L. Pretali, M. Mella, and A. Albini, "Inter- and Intramolecular Photochemical Reactions of Fleroxacin," *Org. Lett.*, vol. 11, no. 9, pp. 1875–1878, 2009, doi: 10.1021/ol900189v.

[10] F. Vargas, T. Zoltan, A. Ramirez, T. Cordero, V. Chávez, C. Izzo, V. Lopez, Y. Cardenas, A. Fernández, L. Hincapié, and A. Fuentes, "Studies of the photooxidant properties of antibacterial fluoroquinolones and their naphthalene derivatives," *Die Pharmazie*, vol. 64, no. 2, pp. 116–122, 2009, doi: 10.1691/ph.2009.8649.

[11] A. Catalfo, M. Calandra, R. Marcella, M. Serrentino, and G. De Guidi, "Rufloxacin-induced photosensitization in yeast," *Photochem. Photobiol. Sci.*, vol. 6, no. 2, pp. 181–189, 2007, doi: 10.1039/b608238f.

[12] A. Catalfo, C. Scifo, S. Stella, A. Belvedere, R. Marcella, and G. De Guidi, "Rufloxacin induced photosensitization in bio-models of increasing complexity," *Photochem. Photobiol. Sci.*, vol. 4, no. 3, pp. 304–314, 2005, doi: 10.1039/b406908k.

[13] M. C. Cuquerella, F. Bosca, M. Miranda, A. Belvedere, and G. De Guidi, "Photochemical Properties of Ofloxacin Involved in Oxidative DNA Damage: A Comparison with Rufloxacin," *Chem. Res. Toxicol.*, vol. 16, no. 3, pp. 562–570, 2003, doi: 10.1021/tx034006o.

[14] A. Albini and S. Monti, "Photophysics and Photochemistry of Fluoroquinolones," *Chem. Soc. Rev.*, vol. 32, no. 4, pp. 238–250, 2003, doi: 10.1039/B209220B.

[15] S. Sortino, G. Marconi, S. Giuffrida, G. De Guidi, and S. Monti, "Photophysical Properties of Rufloxacin in Neutral Aqueous Solution," *Photochem. Photobiol.*, vol. 70, no. 5, pp. 731–736, 1999, doi: 10.1111/j.1751-1097.1999.tb08276.x.