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THE STERILIZATION OF FIVE NEW ANTI-PSEUDOMONAL SEMI-SYNTHETIC PENICILLINS BY GAMMA RAYS

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ABSTRACT

The effect of γ-irradiation on the potency of five semi-synthetic penicillins possessing anti-pseudomonal action has been examined with a view to their radiation-sterilization. Whilst carfecillin sodium and piperacillin sodium may be safely irradiated at the commonly employed sterilization dose of 2.5 Mrads, the other three antibiotics examined, namely, azlocillin sodium, mezlozillin sodium and ticarcillin disodium, display reduced potency at this dose level. The use of a lower radiation dose for these three compounds is not excluded.

INTRODUCTION

The susceptibility of most semi-synthetic penicillins to hydrolysis particularly at elevated temperatures eliminates sterilization of parenteral products by conventional methods

such as autoclaving and 'heating with a bactericide'. The alternative and necessary practice of sterilizing powders for injections by techniques involving costly and highly demanding aseptic processes makes sterilization by γ-irradiation most desirable. The high penetratibility of γ-rays, combined with only a very small temperature rise of the irradiated material during treatment, makes radiation processing applicable to the product in its final pack, and often without removal from its transport container.

Since the introduction of carbenicillin in 1967, one of the principal directions in the development of new semi-synthetic penicillins, has been in the search for other compounds with increased anti-pseudomonal activity. This has been achieved to some extent by the use of either α- carboxy esters of carbenicillin, principally for improved absorption from the gastrointestinal tract, or, by the synthesis of compounds structurally related to carbenicillin, or, by the preparation of N-acyl derivatives of ampicillin (1).

Although quite a number of investigations on the effects of high energy radiation on semi-synthetic penicillins have been reported (2-8), the only reported antipseudomonal compounds to have been so tested are carbenicillin (6) and carindacillin (8). The present investigation is aimed at studying the effect of γ-radiation on five newer antipseudomonal semi-synthetic penicillins.

Because of the destructive nature of ionizing radiation and the difficulty in predicting its radiolytic effect, particularly in more complex molecules, it is necessary to analyse each compound individually for radiation damage in order to determine the feasibility of its radiation sterilization. The compounds have been irradiated in the dry-state, and after pertinent chemical and microbiological tests, our findings have been related to the feasibility of their radiation sterilization.

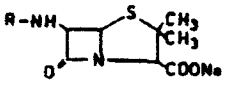
MATERIALS and METHODS

Penicillins

The semi-synthetic penicillins tested were azlocillin sodium and mezlocillin sodium (Bayer, West Germany), carfecillin sodium and ticarcillin disodium (Beecham Research Laboratories, U.K.) and piperacillin sodium (Lederle Laboratories Division U.S.A.). All the above compounds, whose chemical structures are depicted in Figure 1, were tested without any further purification.

Irradiations

γ-irradiation was carried out using a model M-38-3 Gammator 2400 Ci ^{137}Cs radiation source (Radiation Machinery Corp., U.S.A.) with an average dose rate of 1.64 krads min^{-1}. Irradiation vessels were as previously described (9). Routinely 5 g samples of the drugs were γ-irradiated at ambient temperature with 2.5 and 5 Mrad doses (that is, 1.56×10^{20} and 3.12×10^{20} eV g^{-1}, respectively) checked by periodic dosimetric determinations using



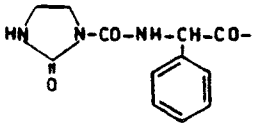
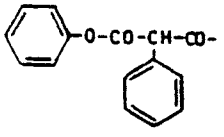
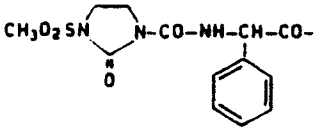
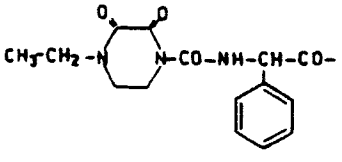
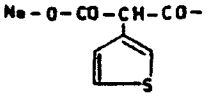
	R
AZLOCILLIN SODIUM	
CARFECILLIN SODIUM	
MEZLOCILLIN SODIUM	
PIPERACILLIN SODIUM	
TICARCILLIN DISODIUM	

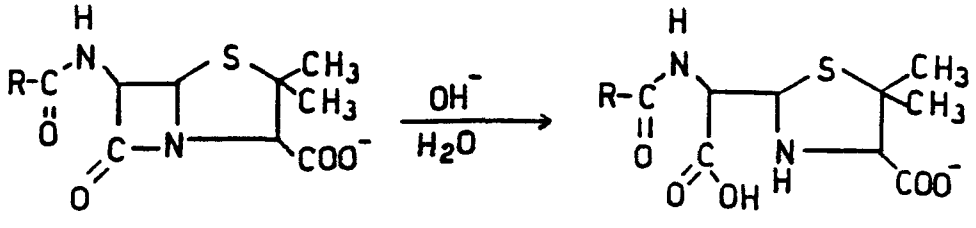
FIGURE 1

Chemical structures of the semi-synthetic penicillins used in the present study.

a ferrous sulphate dosimeter ($G_{Fe}3+ = 15.3$ (10)) and routinely confirmed by means of a clear Perspex HX dosimeter (11). A 2.5 Mrad dose is that recommended by several pharmacopoeias for sterilization purposes (12). Whilst the 5 Mrad dose is in excess of that generally used for this purpose, the testing of drugs having received this exaggerated dose is useful for indicating the type of radiolytic decomposition that the penicillin might be expected to undergo at lower radiation levels.

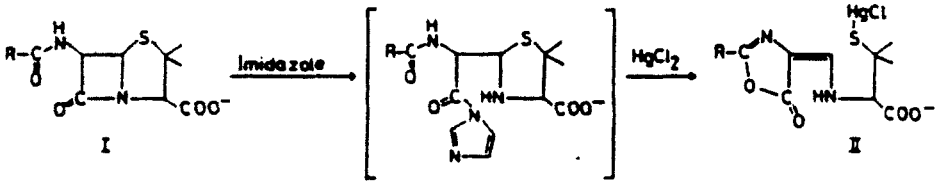
Chemical Analyses

Melting point determinations were made with a Thomas Unimelt apparatus. UV spectrophotometric determinations, using a Pye Unicam SP 1800 spectrophotometer with 10 mm matched quartz cells, were carried out on aqueous solutions (pH 6.5) of the irradiated drugs at the appropriate λ_{max} and concentration (Table 2). Chemical assays were undertaken using the methodology of the British Pharmacopoeia 1973 (13) based on the alkaline hydrolysis of the penicillin to penicilloic acid followed by back titration of the excess alkali,



and that of Bundgaard and Ilver (14), based on the spectrophotometric

measurement of penicillenic acid mercuric mercaptide of the penicillins.



I II

Specific optical rotation was determined on 0.5% $^{w}/v$ aqueous solutions of the irradiated penicillins using a 100 mm microcell in a Perkin Elmer 141 polarimeter.

TLC examination was carried out on 1% solutions of the penicillins in aqueous methanol (70% $^{v}/v$) using pre-coated silica-gel plates (Polygram Sil N-HR/UV_{254}, Mackery Nagel & Co.), with the mobile phase being either an equal part mixture of acetone and methanol, or a mixture of isopropanol and methanol (30:70 parts by volume respectively), or a 1.5% $^{v}/v$ solution of strong ammonia in methanol, or chloroform. Each solvent (of analytical grade)was tested separately with each antibiotic. Detection was under UV light at 254 nm followed by spraying with either a 1% aqueous solution of potassium permanganate or 0.1% ninhydrin spray reagent (Merck, W. Germany). The rationale for the use of chloroform, a non-polar solvent, with the polar antibiotics was essentially to detect any radiolysis products that may be masked by the large excess of unchanged antibiotic.

pH of 5% w/v aqueous solutions of the irradiated semi-synthetic penicillins was determined using a PHM 64 Research pH Meter (Radiometer, Denmark).

Microbiological Assay

The microbiological assay of the irradiated penicillins was carried out by a two-dose cylinder plate method (15) using Difco Antibiotic Medium 1 seeded, whilst molten, with 0.1 ml of an overnight culture of Staphylococcus aureus (Teva 29) (16). The choice of antibiotic concentrations of 10 and 100 $\mu g\ ml^{-1}$ was based on the determination of a linear relationship between concentration and diameter of zone of inhibitition over this range of concentrations. Following eighteen-hour incubation at 37°C diameters of zones of inhibition of bacterial growth were measured.

Sterility Testing

Sterility testing was by a membrane filtration technique in which 20 ml aliquots of 1% aqueous solution of the drug followed by four similar aliquots of saline (0.9% w/v) were passed through a membrane filter (25 mm diameter) having a mean pore diameter of 0.22 µm (Millipore type GSWP), using a Millipore Swinnex apparatus (code SX0002500) attached to a 20 ml disposable syringe. Immediately following filtration each membrane was cut into two, with one half aseptically introduced into 50 ml of Difco Brewer Thioglycollate Medium (for detection of aerobic and anaerobic

bacteria) and the other half onto Difco Sabouraud Dextrose Agar (for detection of fungi and moulds). All manipulations were undertaken in a laminar airflow cabinet. Incubation of the media was at 32°C for the thioglycollate and 25°C for the sabouraud, both for fourteen days. The usual media controls as stipulated by the United States Pharmacopoeia (17) were used. The rinsing of the filter with saline solution ensured no antibiotic residue that might otherwise interfere with microbial growth. This was ascertained by deliberately contaminating penicillin solutions with small inocula of Staphylococcus aureus Teva 29 (100 organisms per ml) prior to filtration. In the absence of rinsing no growth was apparent, whereas membranes which had been through the rinsing process, showed bacterial contamination. Sterility testing for each set of conditions was carried out in duplicate.

The efficacy of the radiation sterilization process was assessed by the sterility testing, as described above, of 1 g aliquots of the semi-synthetic penicillin powders deliberately contaminated with 10^6 spores of the radiation resistant Bacillus pumilus E601 (ATCC 27142), prior to irradiation.

RESULTS

The preliminary screening of the semi-synthetic penicillins for radiation-induced damage was carried out by determination of melting points. Our findings, summarised in Table 1, show no significant change in azlocillin Na, pipercillin Na and ticarcillin

TABLE 1

Melting Point* Determinations (^{o}C) of Irradiated Semi-Synthetic Penicillin Powders.

	Dose (Mrads)		
	0	2.5	5.0
Azlocillin Na	220	220	218
Carfecillin Na	219	221	214
Mezlocillin Na	215	211	211
Piperacillin Na	189	188	189
Ticarcillin di-Na	212	212	213

* These values are mean values with a maximum s.d. of $\pm$ 1^{o}C.

di-Na even after a 5 Mrad radiation dose. Carfecillin Na shows change at the 5 Mrad dose level but not at the 2.5 Mrad level. Mezlocillin Na, however, is affected even following a 2.5 Mrad radiation dose.

The effect of a 5 Mrad radiation dose on the antibiotics as depicted by UV absorbance of aqeuous solutions is summarised in Table 2. Our results show that on the basis of this determination

TABLE 2

UV Absorbance* of Aqueous Solutions of Irradiated Semi-Synthetic Penicillins.

	Conc. ($\mu g\ ml^{-1}$)	λ_{max} (nm)	Absorbance 0 Mrads	Absorbance 5 Mrads
Azlocillin Na	10	210	0.51	0.51
Carfecillin Na	15	210	0.51	0.51
Mezlocillin Na	10	215	0.34	0.34
Piperacillin Na	15	200	0.49	0.49
Ticarcillin di-Na	25	205	0.52	0.52

* Mean of 2 determinations within $\pm$ 0.5%

the compounds remain quite unaffected at this dose level. Unfortunately, the penam moiety of penicillins is devoid of characteristic absorption, the compounds showing only end absorption in the 200-215 nm range.

The above UV spectrophtometric results are supported by the chemical assay values which indicate little change in the semi-synthetic penicillins tested. There is possibly slight decomposition in azlocillin Na ($\sim$1%) following a 2.5 Mrad treatment, and in mezlocillin Na following a 5 Mrad treatment. The chemical assay values obtained by the methodology of Bundgaard and Ilver,

TABLE 3

Chemical Assay Values (± s.d) for Irradiated Semi-Synthetic Penicillins carried out by the Methodologies of (I) the British Pharmacopoeia (13) and (II) Bundgaard and Ilver (14).

		B.P. Method		Bundgaard & Ilver Method	
	0 Mrads	2.5 Mrads	5.0 Mrads	2.5 Mrads	5.0 Mrads
Azlocillin Na	(100)	99.1 ± 0.1	98.6 ± 0.5	100.1 ± 3.5	97.6 ± 1.7
Carfecillin Na	(100)	99.5 ± 0.3	99.8 ± 1.6	101.3 ± 1.8	96.6 ± 1.6
Mezlocillin Na	(100)	99.7 ± 0.4	98.5 ± 1.3	100.0 ± 0.0	99.3 ± 1.1
Piperacillin Na	(100)	99.4 ± 0.5	99.7 ± 0.0	98.7 ± 0.0	97.8 ± 1.1
Ticarcillin di-Na	(100)	99.9 ± 0.1	99.4 ± 0.0	100.7 ± 0.9	97.9 ± 0.4

however, show that only piperacillin Na is affected at the 2.5 Mrad dose level and that all the compounds except mezlocillin Na are decomposed slightly (not more than 3%) at the 5 Mrad level.

No products of radiolysis could successfully be detected by any of the TLC examinations adopted even following a 5 Mrad dose.

Specific optical rotation measurements (Table 4) show there to be no change in the stereochemistry of carfecillin Na

TABLE 4

Specific Optical Rotation of Aqueous Solutions (0.5%) of Irradiated Penicillins.†

	Dose (Mrads)		
	0	2.5	5
Azlocillin Na	(+)165°	(+)157°	(+)150°
Carfecillin Na	(+)206°	(+)206°	(+)207°
Mezlocillin Na*	(+)170°	(+)168°	(+)165°
Piperacillin Na	(+)174°	(+)173°	(+)172°
Ticarcillin di-Na	(+)148°	(+)124°	(+)129°

* A 0.1% solution used.

† Mean of ∓ 2 determinations ± 1%

and piperacillin Na following a 5 Mrad radiation dose. Changes in optical activity, even following a 2.5 Mrad dose, however, are observed for the other three antibiotics.

pH values of aqueous solutions of the γ-irradiated penicillins are summarised in Table 5. Considering the 5 Mrad results, change in pH of carfecillin Na and ticarcillin di-Na are not thought to be significant (a change of 0.1 pH units). Conversely, there does appear to be a real change in the pH of solutions of the other three irradiated antibiotics. The significance of these results are discussed below.

Table 6 summarises our microbiological assay values. With the large experimental error inherent in microbiological assaying,

TABLE 5

pH Values for Aqueous Solutions (5% $^{w}/_{v}$) of γ-Irradiated Semi Synthetic Penicillins. The numbers in parenthesis represent pH change. Values are means of ∤ 2 determinations ± 1%

	0 Mrads	2.5 Mrads	5 Mrads
Azlocillin Na	6.398	6.146 (-0.252)	5.469 (-0.929)
Carfecillin Na	5.729	-	5.626 (-0.103)
Mezlocillin Na	5.213	5.031 (-0.182)	4.744 (-0.469)
Piperacillin Na	5.456	5.486 (+0.030)	6.091 (+0.635)
Ticarcillin di-Na	6.245	-	6.140 (-0.105)

TABLE 6

Percentage Microbiological Assay Values (± s.d.) for Irradiated Semi-Synthetic Penicillins Using a Two-Dose Cylinder Method with Staphylococcus aureus (Teva 29) as the Test Organism.

	Dose (Mrads)		
	0	2.5	5
Azlocillin Na	(100)	-	100.4 ± 2.9
Carfecillin Na	(100)	100.6 ± 0.8	94.5 ± 0.4
Mezlocillin Na	(100)	98.4 ± 2.3	97.7 ± 1.9
Piperacillin Na	(100)	97.9 ± 0.1	98.4 ± 0.8
Ticarcillin di-Na	(100)	-	101.1 ± 1.5

only carfecillin Na (5 Mrads) appears to display a real change in potency following γ-irradiation. No significance can be attached to the 1-2% change noted for several of the other compounds.

Sterility testing indicated that both irradiated and unirradiated samples of antibiotics were free of bacterial or fungal contaminants. Whilst bacterial growth did occur in unirradiated samples deliberately contaminated with 10^6 B. pumilus spores,

no growth was apparent in similarly contaminated samples which subsequently received even the minimal irradiation dose (i.e. 2.5 Mrads).

DISCUSSION

To facilitate the discussion of the above results, the mean of the percentage changes in UV absorbance, chemical and microbiological assays and specific optical rotation measurements of the irradiated penicillins (Tables 2-4 and 6) have been presented in Table 7. The values thus obtained for each compound at each dose level have been used to estimate G (-penicillin) values, where G(-) is defined as the number of molecules changed for each 100 eV of radiation energy absorbed (see Appendix for sample calculation). The use of this parameter, which is effectively independent of radiation dose, is especially useful for the inter-laboratory comparison of results, particularly when different irradiation conditions have been used.

Azlocillin Sodium

Our results show that slight decomposition of azlocillin Na occurs following a 2.5 Mrad radiation dose, and that this is accentuated at the 5 Mrad dose level. The fact that changes are not detected by melting point determinations, microbiological assaying, UV absorbance of TLC suggests that these changes are small. Such a conclusion is borne out by the slight change noticed in the chemical assays carried out by the two quite distinct

TABLE 7

Mean Percentage Change in Irradiated Penicillins (Δ%) at Each Dose Level (calculated from values of UV absorbance, chemical and microbiological assays and specific optical rotation measurements) and the Corresponding G (-penicillin) value.

	Dose (Mrads)				Mean G(-)
	2.5		5.0		
	Δ%	G(-)	Δ%	G(-)	
Azlocillin Na	-1.1	8.8	-1.9	7.6	8.2
Carfecillin Na	+0.3	2.4	-1.8	7.3	4.8
Mezlocillin Na	-0.6	4.1	-1.5	5.2	4.7
Piperacillin Na	-0.9	6.4	-1.1	3.9	5.2
Ticarcillin di-Na	-1.2	10.8	-2.4	10.8	10.8

methods adopted. The large pH change, which interestingly is present following irradiation of all three N-acyl derivatives tested, that is, azlocillin, mezlocillin and piperacillin, is suggestive of rupture of the N-acyl bond with the release of D-α-amino benzilpenicillin (ampicillin) (see Figure 1). With azlocillin and mezlocillin, the formation in aqueous solution of a free carboxyl group could account for the fall in pH. The formation of

ampicillin in this reaction could possibly explain the lack of change noticed in either antimicrobial efficiency, or chemical assaying. Both these tests would only detect alteration of the B-lactam ring or the intact penicillin molecule.

G(-azlocillin Na) at both dose levels is estimated at around 8.

Carfecillin Sodium

This compound is apparently unaffected following a 2.5 Mrad radiation dose as demonstrated by a G(-carfecillin sodium) value of 2.4. The only significant change at the 5 Mrad dose level is in its melting point and microbiological assay, suggesting that radiolysis is slight and not detected by the other techniques adopted. With the large errors inherent in microbiological assaying, care must be exercised in overestimating the significance of the results of this particular analysis. Comparison of the radiation stability of carfecillin sodium with carindacillin sodium (8), the indanyl ester of 6-[D-2-carboxy-2-phenylacetamido] penicillanic acid (which like carfecillin is an α carboxy ester of carbenicillin) showed that carindacillin Na was more susceptible to radiolysis (G (-carindacillin Na) of 16) (8).

Mezlocillin Sodium

The results with this compound appear a little confusing. At the 5 Mrad dose level, there is a change in melting point, pH and specific optical rotation and yet the other tests, namely, microbiological and chemical assays, UV absorbance and TLC reveal no degradation (hence the low G(-mezlocillin Na) value of 4.7).

As suggested above, this could be as a result of differences in sensitivities of analyses used. The pH change could be accounted for in a similar fashion to that described for azlocillin (to which mezlocillin is very closely related). Furthermore, breakdown of mezlocillin to (say) ampicillin could well explain the fact that no change is observed in the microbiological or chemical assays (vide supra). On the other hand, specific optical rotation and melting point determinations which would detect a change even in the presence of an intact β-lactam ring structure reveal an alteration in the parent molecule.

Piperacillin Sodium

Our results show that the 2.5 Mrad irradiated piperacillin Na is virtually uneffected, and that a 5 Mrad radiation dose is only effective at raising the pH of an aqueous solution of this compound. This may be due to rupture of one of the C-N bonds (see Figure 1) with the formation in an aqueous solution of an amino moiety.

Ticarcillin Disodium

Only changes in the specific optical rotation of ticarcillin disodium are observed following γ-irradiation. All our other results show this compound to be unaffected even following a 5 Mrad dose. No satisfactory explanation can be offered at this stage for this somewhat unusual finding. The fact that all the analytical techniques adopted (other than the specific optical rotation determination) show this compound to be unchanged by γ-rays, is no doubt linked to its unique structure; of all the antibiotics

examined in the present study it is the only compound in which the phenyl group on the α-carbon atom of the N-acyl side chain is replaced by a 3-thienyl ring.

The absence of contaminants in samples deliberately inoculated with 10^6 spores of Bacillus pumilus is somewhat anticipated from the known D_{10} value (18) for this organism when irradiated in air-dried conditions in air. Using a D_{10} value of 0.17 Mrads (19), a 2.5 Mrad dose should give an inactivation factor of approximately 10^{15}.

In conclusion, the results of our tests indicate that carfecillin sodium and piperacillin sodium may be safely irradiated at the commonly employed sterilization dose of 2.5 Mrads. The other three antibiotics examined, azlocillin sodium, mezlocillin sodium and ticarcillin di-sodium, display reduced potency at this dose level. Nevertheless, since radiolysis products are within an acceptable pharmaceutical level it might be possible to safely subject samples of these three antibiotics with low initial contamination (say < 10 microbes g^{-1}) to a radiation dose of 1 Mrad, once the chemical nature of the radiolysis products has been conclusively established and the products shown to be non-toxic. The use of such low radiation doses is not uncommon for other antibiotics (20, 21).

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REFERENCES

1. G.N. Rolinson, J. Antimicrob. Chemother., 5, 7 (1979).
2. G. Sekules, S. Fantelli and E. Zannini, Bull. Chim. Farm., 114, 217 (1975).
3. J. Fleurette, S. Madier and M.J. Transy, in " Radiosterilization of Medical Products 1974 ", I.A.E.A., Vienna, 1975, p.239.
4. J. Dziegielewski, B. Jezowska-Trzebiatowska, E. Kalecinska, I.Z. Siemion, J. Kalecinski and J. Nawojska, Nukleonika, 18, 513 (1973).
5. G.P. Jacobs, Trans. Israel Nucl. Soc., 5, 165 (1977).
6. G.P.Jacobs, Int. J. appl. Radiat. Isotopes, 30, 417 (1979).
7. G.P. Jacobs, Trans. Israel Nucl. Soc., 7, IV 20 (1979).
8. G.P. Jacobs, Int. J. Pharmaceut., in the press.
9. G.P. Jacobs and D. Melumad, Pharm. Acta Helv., 51, 313 (1976).

10. J. W. Spinks and R.S. Woods, " An Introduction to Radiation Chemistry ", 2nd ed., Wiley, London, 1976.

11. R.J. Berry and C.H. Marshall, Physics Med. Biol., 14, 585 (1969).

12. " Radiosterilization of Medical Products 1974 ", I.A.E.A., Vienna, 1975, p.513.

13. " British Pharmacopoeia 1973 ", H.M.S.O., London, 1973.

14. H. Bundgaard and K. Ilver, J. Pharm. Pharmacol., 24, 790 (1972).

15. F. Kavanagh, " Analytical Microbiology ", Academic Press, New York, 1963.

16. Kindly supplied by Teva Pharmaceutical Industries, Ltd., Israel.

17. " United States Pharmacopoeia XIX ", Mack Publishing Co., Penn., 1975.

18. D_{10} is defined as the radiation dose that will reduce a given microbial population by a factor of 10.

19. F.J. Ley and A. Tallentire, Pharm. J., 193, 59 (1964).

20. G.P. Jacobs, Pharm. Acta Helv., 52, 302 (1977).

21. R.A. Nash, Bull. parent. Drug Ass., 28, 181 (1974).

APPENDIX

calculation of G(-penicillin)value

The following calculation of G(-penicillin) value is for azlocillin Na (MW 484) which shows a Δ% of 1.9% following a 5 Mrad radiation dose.

Moles azlocillin Na changed = $\frac{1.9}{100 \times 484}$ moles g^{-1} (1)

Using an Avogadro's Number of 6.023×10^{23} molecules $mole^{-1}$,

Molecules changed = $\frac{1.9}{100 \times 484} \times 6.023 \times 10^{23}$ molecules g^{-1} (2)

Since 1 rad = an energy absorption of 100 ergs g^{-1} =

6.24×10^{13} eV g^{-1} then a 5 Mrad dose = $5 \times 10^{6} \times 6.24 \times 10^{13}$ eV g^{-1}

..... (3)

Combining (2) and (3), the number of molecules changed per eV

= $\frac{1.9}{100 \times 484} \times 6.023 \times 10^{23} \times \frac{1}{5 \times 10^{6} \times 6.24 \times 10^{13}}$

molecules eV^{-1} (4)

By definition, a G value is the number of molecules changed for each 100 eV of energy absorbed, then, from equation (4)

G(-azlocillin Na) =

$\frac{1.9}{100 \times 484} \times \frac{6.023 \times 10^{23}}{5 \times 10^{6} \times 6.24 \times 10^{13}} \times 100$ molecules/100 eV (5)

= 7.58 molecules /100 eV