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Notes

Correlation of degradation in metronidazole infusion with F_0 applied during steam sterilization at 122°C

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Summary

Metronidazole infusion was sterilized in an autoclave at 122°C for different values of F_0 up to 65.3 min. Both metronidazole degradation and the Production of nitrite ion correlated with F_0. A typical sterilization cycle of approx. 121°C for 15 min. is likely to lead to 1.5–2% loss of metronidazole and the production of 1.5 ppm nitrite. The yield of nitrite was only 3–9% of the metronidazole destroyed.

The F_0 value of a steam-sterilization process is an expression of the equivalent time in minutes required by the process to produce the same degree of lethality to a micro-organism with a standardized degree of heat resistance, compared to that occurring at 121°C (Kirk et al., 1982; British Pharmacopoeia, 1988).

In solution, metronidazole degrades with the formation of nitrite ion on autoclaving or storage under light (Theuer, 1983) or by addition of cefuroxime sodium (Barnes, 1990). The purpose of this paper is to report the effect of F_0 applied during sterilization of metronidazole infusion at 122°C on the degree of degradation of metronidazole and production of nitrite ion.

Metronidazole infusion (500 mg metronidazole in 100 ml polyvinyl chloride bags) was Metrozol brand, from Parkfields Sterile Supply Unit, Wolverhampton, U.K. Metronidazole powder was from Becpharm Ltd, South Woodford, London, U.K. *N*-1-Naphthylethylenediamine dihydrochloride was general purpose reagent grade (Merck, Poole, Dorset, U.K.). Other reagents were of analytical or high-performance liquid chromatography (HPLC) grade.

Bags of metronidazole infusion were sterilized for various values of F_0 at a maximum temperature of 122°C in an SAL Dataclave autoclave (Southtrim Autoclaves, Bradford, U.K.). The appearance, pH, metronidazole assay and nitrite levels were then determined.

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The pH of samples was determined with an Orion 811 pH meter (MSE Scientific Instruments, Crawley, West Sussex, U.K.) with a glass combination electrode.

Metronidazole analysis was performed by HPLC with a Cecil CE1100 pump (Cecil Instruments, Cambridge, U.K.), Pye LC-3 variable wavelength detector with an 8 μl flow-cell (Pye Unicam, Cambridge, U.K.), a Talbot ASI-4 autosampler equipped with a Rheodyne 7010 injection valve (Talbot Scientific, Alderley Edge, Cheshire, U.K.) and a Shimadzu C-R3A integrator (Dyson Instruments, Houghton-le-spring, Tyne and Wear, U.K.).

The method was closely based on that of the United States Pharmacopeia XXII (1990) monograph for metronidazole injection. The column was a 100 × 4.5 mm reversed-phase Spherisorb 5 μm ODS column (Hichrom, Reading, U.K.). The mobile phase was methanol: 0.005 M potassium dihydrogen orthophosphate buffer pH 4.0 ± 0.5 (9:91). The flow-rate was 1 ml/min (approx. 6 MPa pressure), the detector wavelength was 320 nm (0.16 absorbance units full scale) and the injection volume was 20 μl.

A 5 ml sample was diluted to 25 ml with water. A 2 ml volume of this dilution was mixed with 2 ml methanol and diluted to 10 ml with mobile phase. The standard was prepared by dissolving 50 mg metronidazole in methanol and diluting to 50 ml. A 2 ml volume of this was mixed with 2 ml water and diluted to 10 ml with mobile phase. Triplicate injections were made of the standard and each sample solution. Quantification was by measurement of peak area.

Nitrite ion was determined with a method closely based on that of Theuer (1983). Equal volumes of a 0.6% w/v solution of sulphanilic acid in 25% v/v hydrochloric acid and a freshly prepared aqueous solution of *N*-1-naphthylethylenediamine dihydrochloride (0.1% w/v) were mixed immediately before use. To a 10 ml sample was added 5 ml mixed reagent and the solution diluted to 25 ml with water. This solution was incubated at ambient temperature for 30 min and the absorbance measured at 540 nm with a Cecil CE 594 spectrophotometer (Cecil Instruments, Cambridge, U.K.) in a 1 cm cell against a reagent blank. Nitrite ion levels were obtained by comparison with a freshly prepared aqueous solution

TABLE 1

Effect of autoclaving at 122°C for various values of F_0 on pH, metronidazole degradation and NO_2^- formation

F_0 (min)	pH [a]	Metronidazole [b]				NO_2^- [a] (ppm) (mean ± SD)	Yield of NO_2^- relative to metronidazole, destroyed (%)	
		Assay value (mg/ml)		% lost on autoclaving				
		Replicates	Mean	Replicates	Mean		Replicates	Mean
0	4.92 [c]	4.99	4.98	–	–	0.05 ± 0.004	–	
		4.98						
4.4	4.95	4.92	4.91	1.30	1.46	0.62 ± 0.03	3.3	3.0
		4.90		1.61			2.6	
9.4	4.88	4.93	4.90	1.04	1.66	1.03 ± 0.01	7.0	5.1
		4.87		2.29			3.2	
17.7	4.93	4.91	4.91	1.49	1.49	1.57 ± 0.08	7.6	7.6
		4.60 [d]		7.73 [d]			–	
41.2	4.93	4.79	4.82	3.85	3.18	3.19 ± 0.05	6.1	7.7
		4.86		2.51			9.3	
65.3	4.88 [c]	4.81	4.81	3.39	3.49	4.09 ± 0.08	9.0	8.8
		4.80		3.59			8.5	

[a] Mean of single determination on five bags unless otherwise indicated below.
[b] Mean of triplicate determination on two randomly selected bags.
[c] Mean of single determination on two randomly selected bags.
[d] Outlier – value rejected.

of sodium nitrite, equivalent to 4 ppm NO_2^-, treated concurrently with the samples.

The method for the determination of NO_2^- was validated for use with metronidazole infusion from the source used. The calibration curve was linear for sodium nitrite standards equivalent to 0–10 ppm NO_2^- ($r = 0.9995$). Six replicate determinations of a 4 ppm NO_2^- standard gave a precision of 1.0% relative standard deviation. The recovery of sodium nitrite equivalent to 4 ppm NO_2^-, spiked into metronidazole infusion from a routine production batch, was 99.1% (mean of duplicate determinations).

The only change in appearance was that the samples subject to an F_0 of 41.2 and 65.3 developed a slight yellow colour. However, these high values are beyond those which would be employed for sterilization at 122°C.

The F_0 value applied did not affect the pH (Table 1) of the infusion, which is formulated with a buffer. An unbuffered formulation may, however, display different properties in this respect.

Both the production of NO_2^- and loss of metronidazole showed a correlation with F_0.

In the U.K. the minimum F_0 value normally employed for the steam sterilization of pharmaceuticals is 8 min (British Pharmacopoeia, 1988). Metrozol injection is normally sterilized at F_0 values between 12 and 20 min to minimise degradation whilst still achieving a good level of sterility assurance. This would correspond to a typical cycle of approx. 121°C for approx. 15 min. These conditions would lead to 1.5–2% loss of metronidazole and the production of approx. 1.5 ppm nitrite.

Kraus and Vermeij (1981) reported that metronidazole infusions, buffered at pH 5.0, lost approx. 2.0% metronidazole when autoclaved at 120°C for 20 min, in reasonable agreement with the findings of the present work.

Other authors (Bannert et al., 1983; Theuer, 1983) have found no evidence of degradation in metronidazole infusions at similar pH during sterilization.

Nitrite ion levels in metronidazole infusion were found to be in the range 0.5–1.5 ppm, when sterilized in a spray-cooled autoclave (Theuer, 1983). With an autoclave lacking spray cooling, metronidazole infusion at a similar pH to that in the present study, had NO_2^- levels of 2.0–4.0 ppm. NO_2^- levels established in the present work agreed with those found with the spray-cooled autoclave in the former study. This is because although the autoclave lacked spray cooling, it employed jacket cooling and an internal fan to increase heat distribution.

A level of 20 ppm NO_2^- has been quoted as the maximum acceptable for metronidazole infusion (Messerschmidt, 1984; Theuer, 1984). In the present work this was not approached even at high values of F_0.

Aztreonam, and potentially other antibiotics containing the 2-aminothiazole moiety, reacts with NO_2^- to cause a pink discoloration (Thakur et al., 1991). The intensity is related to the nitrite concentration so it may be desirable to minimise it in this particular case.

The mechanism for the production of nitrite during autoclaving is likely to be nucleophilic displacement of the nitro group by water (Beck, 1978). Attack of the nucleophile 2-aminoethanethiol at both the 5- and 4-positions of metronidazole leads to NO_2^- elimination from the 5-position (Goldman and Wuest, 1981). Nucleophilic attack of water at either of these positions may similarly be capable of causing NO_2^- formation.

The yield of NO_2^- accounted for only 3–9% of the metronidazole destroyed. This compares with a similarly low yield during the photolysis of metronidazole (Gattavecchia, 1982).

On the basis of appearance, pH, metronidazole degradation and the formation of nitrite, the F_0 value applied to metronidazole infusion during sterilization at 121–124°C affects the amount of degradation occurring. Consequently, excessively high F_0 values obtained with older, less efficient autoclaves will give rise to increased levels of degradation products. These results would not necessarily apply to F_0 values obtained at a different maximum temperature or under situations where the heating and cooling phases of the sterilizing cycle were markedly different. This would require knowledge of the kinetics of degradation at all temperatures encountered.

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