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Stability of ampicillin infusions in unbuffered and buffered saline

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Summary

The stability of ampicillin sodium injections, 250 mg in 50 ml and 1 g in 100 ml 0.9% sodium chloride at 5°C, was investigated. The degradation of ampicillin was rapid, resulting in greater than 10% loss in 24 h. Degradation was most rapid in the more dilute solution. The addition of small volumes of potassium acid phosphate 13.6% as a buffer to the infusion resulted in a significant reduction in the degradation rate of ampicillin. Results indicate that the shelf life of ampicillin injection diluted in buffered saline can be extended to 6–12 days, depending on the ampicillin concentration.

Ampicillin is unstable in aqueous solutions and the rate of degradation is influenced by many factors. The most important of these are temperature, concentration, diluent and pH, the last three being interrelated. Degradation is highly dependent of temperature. It has been reported, for example, that degradation rates more than double if the storage temperature is increased from refrigeration (4–6°C) to ambient (Warren et al., 1972). Drug concentration is also an important influence on degradation rate for a number of reasons. At high concentrations (above around 5% w/v ampicillin) both molecular polymerisation and self-catalysis have been observed (Savello and Shengraw, 1971), while at lower concentrations, degradation can be effected by the brand of ampicillin used, the nature, source and batch of diluting infusion and the type of container (Trissel, 1990). In particular, ampicillin is less stable in dextrose infusions compared with solutions in saline. One variable factor causing this difference appears to be pH, but the catalytic effect of dextrose on ampicillin degradation is probably more significant (Savello and Shengren, 1971). Ampicillin sodium injection in solution has an alkaline pH, usually in the range 8–9, depending on the brand of drug, concentration and vehicle. The optimum pH for maximising ampicillin stability after dilution appears to be between 5 and 6 (Trissel, 1990) although one report suggests that infusions with a pH of 7.5 are optimum (Raffanti

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and King, 1974). In addition, any buffering capacity of the diluent must be considered, since the products resulting from ampicillin degradation are acids and will reduce pH as the reaction proceeds (Savello and Shengren, 1971).

The assay method used to measure degradation of ampicillin in infusion fluids and aspects of the experimental design can substantially distort the true situation. Various analytical methods have been employed in studies, some of which have been found, on closer examination, to be inappropriate and have, therefore, resulted in incorrect conclusions (Savello and Shengraw, 1971; Warren et al., 1972). Many of the early studies relied on microbiological assay methods. Gallelli et al. (1969) employed a tube dilution method and was later discredited (Savello and Shengraw, 1971). Microbiological disc plate methods give some measure of total antibiotic activity but are not sufficiently specific and inherently more variable than a method suitable for determining degradation rates with any degree of accuracy. Certain colorimetric methods employed (Hianaka et al., 1972) may not be stability-indicating. Perhaps the best method available in the 1970s was the specific iodometric titration technique (Ashwin and Lynn, 1975; Stjernstrom et al., 1978). This method still lacks the specificity and precision necessary to obtain accurate and reproducible results as would be expected from a chromatographic assay. Great care is also required with respect to experimental design. Because of the rapid degradation of ampicillin in aqueous solutions, all samples for analysis should be cooled in ice water and assayed immediately after sampling to avoid further degradation which could significantly distort the results obtained (James and Riley, 1985).

Recommendations summarised by Trissel (1990) from various sources tentatively suggest that ampicillin sodium injection, if diluted in 0.9% sodium chloride infusion to a solution containing between 1 and 2% ampicillin may be stored for 3–5 days at 4–5 °C, based on $t_{10\%}$ calculations. However, the more reliable studies already highlighted provide data indicating that ampicillin may not be sufficiently stable in 0.9% sodium chloride to support this recommendation (Warren et al., 1972; James and Riley, 1985). It was therefore the aim of the present investigation examine, using a chromatographic method of analysis, the degradation of ampicillin in Minibags and assess the use of buffering, both to improve stability and provide greater consistency in pH between batches of vehicle. Studies were conducted at two drug concentrations representing those doses most commonly used in clinical practice.

Ampicillin sodium injection B.P., 250 and 500 mg vials, Penbritin, were obtained from Beecham Research Laboratories (Ltd, Brentford, U.K. Sodium chloride 0.9% infusions, 50 and 100 ml Minibags (Viaflex), were obtained from Baxter Healthcare Ltd, Thetford, U.K. Potassium acid phosphate injection 13.6%, 10 ml ampoules, was obtained from Pharmaceutical Manufacturing Unit, Torbay Hospital, Torquay, U.K.

The contents of each vial were reconstituted in a small volume of 0.9% sodium chloride transferred from the appropriate Minibag using a transfer needle. As much as possible of the resulting solution was returned to the Minibag and the contents shaken thoroughly. Three infusions were prepared for each experiment. Bags were packed in polyethylene pouches, placed in a light-protective overwrap and transferred to the refrigerator at 5°C (±1°C). A sample (approx. 1.5 ml) was removed at appropriate time intervals, including the commencement of each experiment, and cooled in an ice bath. 1 ml, accurately measured, was diluted to 20 ml in cold (5°C) water and maintained in an iced water bath until analysis. A new standard was prepared at each test interval, using an accurately weighed amount from the pooled contents of two 500 mg vials. All standards for one experiment were prepared from the same pooled powder. The weighed powder was diluted accurately in distilled water to approximately the same concentration as the test solution. This solution was maintained in an ice bath.

Analysis was by a stability-indicating HPLC method (Vree et al., 1978). A stainless-steel column (10 cm in length, i.d. 4.6 mm) packed with Spherisorb 5 μm ODS was used. The solvent was methanol : potassium dihydrogen phosphate 0.067

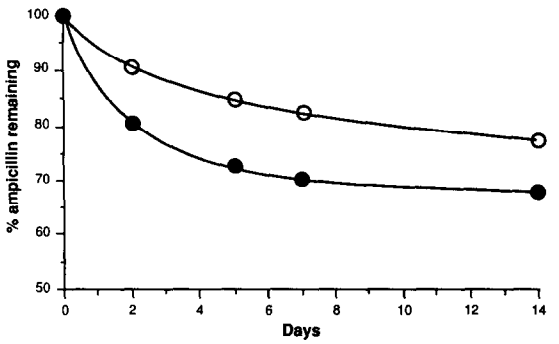

Fig. 1.

M, at a flow rate of 1.5 ml min^{-1}. Detection was at 225 nm and quantitation was by electronic integration using PC software (Chromate, Philips Scientific Ltd, Cambridge, U.K.). The method was validated for linearity of response with concentration ($r = 0.9999$, four concentrations over the range 62.5–250 μg/ml) and precision (coefficient of variation of repeated injections, 0.54%; 125 μg/ml, $n = 5$). The method was also confirmed as stability-indicating by comparing chromatograms of heat-stressed (60°C, 2 h) solutions of ampicillin with untreated solutions. Significant falls in the parent compound peak areas were observed and new peaks were evident in stressed solutions.

The pH was measured using a standard combination pH electrode and meter (Jenway Model 3020, Jencon's Scientific Ltd, Leighton Buzzard, U.K.).

The degradation of ampicillin sodium reconstituted and diluted in 0.9% sodium chloride infusion in Minibags (Fig. 1). The rate of degradation was not linear over the 14 day period. A rapid initial fall was followed by a progressive diminution in degradation rate at both concentrations studied. The degradation rate was less rapid at the higher concentration examined. The initial pH values of solutions containing 250 mg ampicillin in 50 ml were 8.4–8.5, while solutions containing 2 g in 100 ml were slightly higher at pH 8.7–8.8. A fall of approx. 0.5 units was observed over the storage period examined. There was no evidence of visible changes in colour or clarity of solutions. Approx. $t_{90\%}$ values derived from Fig. 1 were 1 and 2 days for solutions containing 250 mg in 50 ml and 1 g in 100 ml Minibags, respectively.

The stability of reconstituted ampicillin should be enhanced if the pH is reduced and the solution buffered. The addition of a small volume (1–2 ml) of 13.6% potassium acid phosphate (chosen because of its availability in injectable form) was found to reduce the pH of ampicillin infusions by approx. 1.5 pH units. Therefore, to test the effect of buffering on stability, potassium acid phosphate 13.6% injection was added to each bag in the following quantities: 50 ml 0.9% sodium chloride + 1 ml potassium acid phosphate + 250 mg ampicillin B.P. and 100 ml 0.9% sodium chloride + 2 ml potassium acid phosphate + 1 g ampicillin B.P. Larger quantities of the buffer caused precipitation when stored at 5°C. Samples were withdrawn at 2–3 day intervals over 14 days for analysis and pH measurement. Each test was repeated and the pooled analytical data subject to regression analysis. Degradation was approximately linear over the 7 day investigation ($r =$ 0.970–0.972; intercepts between 99 and 100%). From the slopes of the graphs, the $t_{90\%}$ values were calculated in bags containing 250 mg in 50 ml 0.9% sodium chloride and 1 g in 100 ml 0.9% sodium chloride to be 12.2 and 6.8 days, respectively. The pH values for these solutions were approx. 6.82–6.88 at the commencement of the tests, falling to 6.76–6.78 in bags containing 250 mg in 50 ml, and 7.29–7.36 falling to 7.22–7.25 in bags containing 1 g in 100 ml 0.9% sodium chloride.

This study has shown that ampicillin is too unstable to recommend storage after reconstitution or dilution in infusions, including 0.9% sodium chloride. The present study indicates that such infusions must be used within 12–24 h after preparation unless buffered. The following recommendations are indicated by these studies: 50 ml Minibag 0.9% sodium chloride + 1 ml potassium acid phosphate injection 13.6% + 250 mg ampicillin sodium – maximum shelf life of 12 days; 100 ml Minibag 0.9% sodium chloride + 2 ml potassium acid phosphate injection 13.6% + 1 g ampicillin sodium – maximum shelf life of 6 days.

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