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The chemical and physical stability of three intravenous infusions subjected to frozen storage and microwave thawing

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Summary

This study is concerned with the physical and chemical stability of erythromycin, fusidic acid and methylprednisolone infusions when subjected to frozen storage and microwave thawing. Stability-indicating HPLC assays were developed for the active principle of each infusion. In each case, storage of infusions for 12 months at −20 °C, followed by microwave thawing, resulted in no drug loss. The chemical stability of erythromycin and fusidic acid infusions thawed after 6 months storage at −20 °C and subjected to three further freeze/thaw cycles was also demonstrated. Physical stability of the infusions was unaffected by frozen storage and microwave thawing, there being only minor changes in pH, infusion weight and sub-visual particulate levels. Prolonged frozen storage and rapid microwave thawing under the conditions described may be used to facilitate the batch-scale preparation of the three infusions detailed in this report.

Introduction

The benefits of batch-scale production of intravenous (i.v.) infusions by hospital pharmacy departments as part of a centralised i.v. additive service have been well documented (Kleinberg et al., 1980; Brown et al., 1986). A key issue in batch-scale production is that of drug stability. Attempts to improve drug stability have focussed on the storage of i.v. infusions in the frozen state. Studies on drug stability under frozen storage conditions have been reported for a number of i.v. admixtures including antibiotic infusions (Stiles, 1981; Sanburg et al., 1987). This approach has been combined with the use of microwave ovens to enable the rapid thawing of frozen infusions.

The freezing of some i.v. infusions may actually increase the rate of drug degradation. The degradation rate of amoxycillin sodium in aqueous solution was 25 times more rapid at −7.5 °C than at room temperature (Concannon et al., 1986). This effect was attributed to the concentration-dependent degradation kinetics of amoxycillin coupled with increased concentrations of the drug in liquid vesicles as the temperature was lowered

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from the freezing temperature of the solvent to the eutectic temperature of the infusion.

Microwave thawing of frozen infusions may also promote drug degradation. Variable drug stability has been reported for infusions subjected to freeze-thaw procedures (Lynn, 1982) and concern has been expressed that infusions in PVC minibags could be overheated (Williamson and Luce, 1987). Although satisfactory microwave thawing has been reported for a variety of i.v. infusions (Elliott et al., 1983; Awang and Graham, 1987; Sanburg et al., 1987) rigorous drug stability studies under 'in-use' freezing and thawing conditions have been advocated (Tabor and Norton, 1985).

We have previously described a freeze-thaw system with a novel microwave oven tumbling drum mechanism designed to promote mixing of infusions during thawing to prevent localised overheating (Sewell et al., 1991). The objective of this study was to determine the stability of three commonly used i.v. infusions under the frozen storage and microwave thawing conditions defined in our previous report. Two antibiotic infusions (erythromycin and fusidic acid) and one corticosteroid infusion (methylprednisolone sodium succinate) were selected for study. In each case the manufacturers recommend a shelf-life of only 24 h under refrigerated conditions when these drugs are incorporated into infusions (Data Sheet Compendium, 1990). Stability-indicating high-performance liquid chromatography (HPLC) assays were developed and validated for each infusion. Physical parameters including pH, sub-visual particulate levels and the weight change of infusions subjected to frozen storage and microwave thawing were also determined.

Materials and Methods

PVC 'Viaflex' bags containing 100 and 500 ml volumes of sodium chloride infusion (0.9% w/v) were obtained from Baxter Laboratories Ltd, Thetford, U.K. Ampoules of Water for Injections BP (20 ml volume) were obtained from Antigen Ltd, Roscrea, Ireland, Erythromycin 1 g (erythromycin as the lactobionate) vials were obtained from Abbott Laboratories Ltd, Queensborough, U.K.), fucidin (fusidic acid 0.5 g + diluent) vials were obtained from Leo Laboratories, Aylesbury, U.K. Solu-Medrone (methylprednisolone as the sodium succinate 0.5 g + diluent) vials were obtained from Upjohn Ltd, Crawley, U.K.

Infusions were prepared under aseptic conditions by reconstituting vials with Water for Injections BP or the diluent provided. Bulk solutions of each drug were prepared in sterile sodium chloride infusion (0.9% w/v) which had previously been withdrawn from PVC Viaflex bags. Measured volumes of each drug solution were then dispensed via a 0.2 μm Minisart filter (Sartorius Ltd, Surrey, U.K.) into empty PVC Viaflex containers to produce the following infusions: erythromycin 500 mg in 110 ml, fusidic acid 500 mg in 550 ml and methylprednisolone 500 mg in 108 ml. In each case the final concentration and volume of the infusion was representative of that used in clinical practice.

Reference samples of erythromycin lactobionate, fusidic acid and methylprednisolone sodium succinate were obtained from Sigma, Poole, U.K. All other chemicals and reagents were of high-performance liquid chromatography (HPLC) grade or analytical reagent grade, as appropriate, and were obtained from BDH Ltd, Poole, U.K.

Determination of chemical stability

Determination of drug concentration in all three infusion types was by HPLC using a standard system comprising a stainless-steel column (125 × 4.9 mm) packed with 5 μm particle size ODS-2 (Phase Separations Ltd, Clwyd, U.K.), Merk-Hitachi 655A-12 pump, Rheodyne 7125 injection valve (20 μl loop) and Merk-Hitachi 655A variable wavelength UV detector. Peak heights were computed using a CI-10 integrator/printer-plotter (LDC Milton-Roy, Stone, U.K.). The HPLC operating conditions used for each drug are given below, together with the linearity of response and precision of the method (10 separate dilutions and assays of each infusion type). Peak purity was determined with a model LC-235 Diode Array Detector (Perkin Elmer Ltd, Beaconsfield, U.K.).

Erythromycin Mobile phase comprised acetonitrile : methanol : 0.1 M KH_2PO_4 (41.2 : 12.6 :

46.2). The flow rate was 1.0 ml min^{-1} and the detection wavelength was 215 nm.

Linearity of response over the range 0.1–0.7 mg ml^{-1}:

$y = 5.7x + 0.14$, $r = 0.9996$, $n = 7$.

Precision of method (infusion diluted to 0.4 mg ml^{-1}):

CV = 0.61%, $n = 10$.

Fusidic acid Mobile phase comprised acetonitrile: 0.01 M pentanesulphonic acid (70 : 30) adjusted to pH 3 with 1 N sulphuric acid. The flow rate was 1.0 ml min^{-1} and the detection wavelength was 215 nm.

Linearity of response over the range 0.001–0.025 mg ml^{-1}:

$y = 4.5x - 0.0041$, $r = 0.9997$, $n = 7$.

Precision of method (infusion diluted to 0.006 mg ml^{-1}):

CV = 1.16%, $n = 10$.

Methylprednisolone Mobile phase comprised methanol : water (65 : 35) adjusted to pH 3.5 with glacial acetic acid. The flow rate was 0.7 ml min^{-1} and the detection wavelength was 243 nm.

Linearity of response over the range 0.04–1.25 mg ml^{-1};

$y = 7.1x + 0.03$, $r = 0.9993$; $n = 7$

Precision of method (infusion diluted to 0.1 mg ml^{-1});

CV = 0.91%, $n = 10$.

To confirm that HPLC methods were stability-indicating, samples of each infusion were subjected to chemical stress prior to assay. Sealed glass ampoules containing each infusion and equal volumes of either 1 N HCl, 1 N NaOH or 20 vols H_2O_2 were heated at 60 °C for 1 h. The solutions were then cooled to ambient temperature, neutralised if appropriate and diluted to within the analytical range. Control ampoules containing the test infusion and an equal volume of water were stored at 4 °C prior to dilution and assay. Chromatograms of control and stressed samples were obtained using the relevant HPLC assay method as described previously. In each case, HPLC chromatograms of the stressed infusion samples exhibited either a decrease in the analyte peak height or elimination of the analyte peak. With the exception of erythromycin, chromatography of the control samples produced only the analyte peak in addition to the solvent front. HPLC chromatograms of the erythromycin control sample and standard solution exhibited three minor peaks (too small for quantification) which eluted after the principal peak. A detailed study (Tsuji and Kane, 1982) on the reversed-phase HPLC of erythromycin formulations also confirmed the presence of three minor peaks in addition to the principal peak (erythromycin A). The minor peaks were assigned to: erythromycin B, erythromycin C and 8,9-anhydro-6,9-hemiketal erythromycin. In this study only the principal peak (erythromycin A) was determined. Peak homogeneity of analyte peaks (erythromycin A, fusidic acid, methylprednisolone sodium succinate) was confirmed by simultaneous monitoring of UV absorbance in the region 190–350 nm using diode array detection. Each assay was considered to be stability-indicating with respect to non-specific degradation of the analyte.

Diluted samples of infusions from freeze-thaw studies were injected in duplicate and bracketed by injections of the external standard solution. The content of sodium chloride in the infusions was determined by the BP assay for sodium chloride infusion.

Determination of physical stability

Weights of infusions before and after the freeze-thaw process were obtained with a Model L2200P electronic top-pan balance (Sartorius Instruments Ltd, Surrey, U.K.). Subvisual particulate levels at $\geqslant 2$ and $\geqslant 5$ μm were determined with an electrical zone sensing method using an Industrial Model D Coulter Counter (Coulter Electronics, Luton, U.K.). Infusion pH was determined with an analogue pH meter and glass electrode (Pye Model 291 pH meter). The

thawed temperature of the infusions was determined by an Infratrace 800 infrared temperature monitor (Kane and May Ltd, Welwyn Garden City, U.K.).

Freeze-thaw studies

PVC containers of each infusion were tared immediately after preparation and samples were taken for initial ($t = 0$) analysis. Batches of 20 containers of each of the test infusions were then frozen to $-20\,^{\circ}C$ under the conditions previously described (Sewell et al., 1991). After various time intervals, duplicate infusions were removed from frozen storage and immediately subjected to microwave thawing (Sewell et al., 1991). The erythromycin and methylprednisolone infusions were thawed as batches of two containers in the microwave oven, with thawing times of 4.0 min at the maximum energy setting. Fusidic acid infusions were thawed singly for 8.5 min at the maximum energy setting. The temperature of each infusion was determined immediately after thawing, and each container was then re-weighed and subjected to visible inspection. Samples of the infusion were taken for drug assay, assay of sodium chloride excipient, determination of sub-visual particulate levels and measurement of pH.

In a further experiment the erythromycin and fusidic acid infusions thawed after 6 months storage at $-20\,^{\circ}C$ were subjected to further cycles of freeze-thaw. 1 h after the initial thawing, during which time infusions were maintained at $20\,^{\circ}C$ and samples were withdrawn for analysis, the infusions were returned to the freezer and then re-thawed 24 h later. This cycle was repeated twice so that infusions were subjected to 6 months stor-

TABLE 1

Effect of frozen storage and microwave thawing on the chemical stability of (A) erythromycin, (B) fusidic acid and (C) methylprednisolone infusions

Storage time at $-20\,^{\circ}C$	Thawed temp. ($^{\circ}C$)	Drug assay		NaCl assay	
		(% w/v)	(% of initial)	(% w/v)	(% of initial)
(a) Erythromycin infusion (500 mg in 110 ml)					
0	–	0.497	100	0.83	100
48 h	23.5	0.501	100.8	0.83	100
7 days	25.0	0.507	102.0	0.82	98.8
14 days	23.0	0.509	102.4	0.83	100
1 month	26.0	0.504	101.4	0.83	100
3 months	22.5	0.506	101.8	0.82	98.8
6 months	24.5	0.505	101.6	0.82	98.8
12 months	26.0	0.495	99.6	0.83	100
(b) Fusidic acid infusion (500 mg in 550 ml)					
0	–	0.090	100	0.83	100
48 h	24.0	0.091	101.1	0.82	98.8
7 days	21.0	0.089	98.8	0.82	98.8
14 days	20.0	0.091	101.1	0.83	100
1 month	19.0	0.090	100	0.84	101.2
3 months	23.0	0.089	98.8	0.84	101.2
6 month	24.0	0.091	101.1	0.84	101.2
12 months	21.0	0.090	100	0.84	101.2
(c) Methylprednisolone infusion (500 mg in 108 ml)					
0	–	0.483	100	0.89	100
24 h	21.0	0.475	98.15	0.89	100
1 month	22.0	0.483	100	0.88	98.9
6 months	23.0	0.494	102.34	0.89	100
12 months	25.0	0.492	101.86	0.89	100

Data presented are mean values obtained for two containers.xxxxx

age at −20 °C, microwave thawing and then three further 24 h freeze-thaw cycles.

The infusion samples were subjected to HPLC analysis after the thawing stage of each cycle.

Results and Discussion

Chemical stability of infusions subjected to freeze-thaw

Data relating to the chemical stability of erythromycin infusion, fusidic acid infusion and methylprednisolone infusion after frozen storage and microwave thawing are presented in Table 1. The infusion temperature (mean of two infusions) immediately after microwave thawing ranged from 22.5 to 26 °C and 21 to 25 °C for infusions of erythromycin and methylprednisolone, respectively (both of approx. 110 ml volume) and from 19 to 24 °C for fusidic acid infusion (550 ml volume). These thawed temperature ranges should be incorporated into quality assurance programmes when the freeze-thaw techniques described in this report are applied to pharmaceutical practice.

There was no loss of active principal in erythromycin, fusidic acid and methylprednisolone infusions subjected to up to 12 months frozen storage at −20 °C and microwave thawing. HPLC chromatograms of the three test infusions were not affected by the period of frozen storage and no additional peaks were present in infusion samples thawed after storage at −20 °C for up to 12 months. In the case of erythromycin three minor peaks (previously assigned, see Materials and Methods) were observed in all HPLC chromatograms of this infusion irrespective of the storage period ($t = 0$–$t = 12$ months).

The technique of preparing bulk solutions of each infusion under study and aseptically filling these solutions into empty PVC containers ensured identical initial concentrations for each infusion type. This avoided the variable results reported in previous studies which were attributed to errors in the preparation of infusions (Sanburg et al., 1987).

Chemical stability data for erythromycin and fusidic acid infusions subjected to 6 months frozen storage at −20 °C, microwave thawing and then three further freeze-thaw cycles are presented in Table 2. Both infusions remained clear and colourless after each thawing cycle. Visual inspection of the PVC containers revealed no evidence of mechanical damage.

TABLE 2

Effect of repeated freezing and microwave thawing cycles, at 24 h intervals, on the chemical stability of (A) erythromycin and (B) fusidic acid infusions previously subjected to 6 months frozen storage and microwave thawing

Additional freeze-thaw cycles	Thawed temp. (°C)	Drug assay (% w/v)	Drug assay (% of initial)
(a) Erythromycin infusion (500 mg in 110 ml)			
0	24.5	0.505	101.6
1	24.0	0.504	101.4
2	25.0	0.496	99.8
3	26.0	0.511	102.8
(b) Fusidic acid infusion (500 mg in 550 ml)			
0	24.0	0.091	101.1
1	22.0	0.091	101.1
2	24.0	0.091	100.1
3	24.0	0.090	100.1

Data presented are mean values for two infusions.

The drug assay of erythromycin and fusidic acid infusions, thawed after 6 months storage at −20 °C, was unaffected by three repeat freeze-thaw cycles (Table 2). If erythromycin and fusidic acid infusions were thawed erroneously it would be possible to freeze the infusions for subsequent re-issue providing that re-freezing was commenced within 1 h of thawing.

Physical stability of infusions subjected to freeze-thaw

The corresponding physical stability data for the three infusions is shown in Table 3 (a–c). All infusions remained clear and colourless after microwave thawing from the frozen state (−20 °C). There was no evidence of damage to the infusion containers after the freeze-thaw process and both the pliability and transparency of the PVC material from which the containers were constructed was unchanged.

No significant weight change occurred in any of the test infusions as a result of the freeze-thaw

TABLE 3

Effect of frozen storage and microwave thawing on the physical stability of (a) erythromycin, (b) fusidic acid and (c) methylprednisolone infusions

Storage time at −20 °C	pH	Sub-visual particles (per ml)		Weight change (%)
		⩾ 2 μm	⩾ 5 μm	
(a) Erythromycin infusion (500 mg in 110 ml)				
0	7.15	642	78	–
48 h	7.10	568	104	+0.004
7 days	7.10	464	62	+0.012
14 days	7.10	1376	85	0
1 month	7.10	684	101	+0.029
3 months	7.08	318	13	+0.060
6 months	6.90	604	30	+0.060
12 months	6.90	884	60	+0.006
(b) Fusidic acid infusion (500 mg in 550 ml)				
0	7.55	1571	133	–
48 h	7.45	2030	122	+0.005
7 days	7.45	1790	133	+0.018
14 days	7.35	1992	120	+0.016
1 month	7.40	1430	147	+0.041
3 months	7.20	1893	113	+0.064
6 months	7.50	1240	97	+0.072
12 months	7.45	1771	152	+0.088
(c) Methylprednisolone infusion (500 mg in 108 ml)				
0	7.40	1620	207	–
24 h	7.43	1020	171	+0.004
1 month	7.60	1356	215	−0.008
6 months	7.43	1878	158	+0.040
12 months	7.50	1803	177	−0.019

Data presented are mean values obtained for two containers.

process. There was therefore no possibility that loss of water from infusions during storage could have masked a decrease in drug assay occuring as a result of degradation. There was also no significant change in the pH of the three test infusions further supporting the absence of either drug degradation or extensive gaseous exchange through the container wall during the freeze-thaw process.

Sub-visual particulate levels of all three infusions were high (Table 3) and, in the case of fusidic acid and methylprednisolone infusions, almost all counts at ⩾ 2 and ⩾ 5 μm levels exceeded the BP limits (British Pharmacopoeia, 1988). It may be erroneous to compare sub-visual particulate levels of antibiotic and corticosteroid infusions with Pharmacopeial limits for sodium chloride and glucose infusions since the presence of additional electrolyte has been shown to produce a significant increase in particulate counts where electrical-zone sensing measuring techniques are used (Haines-Nutt and Munton, 1983).

With only one exception, the infusions subjected to frozen storage and microwave thawing exhibited no apparent increase in sub-visual particulate levels with respect to the initial ($t = 0$) samples. The notable exception was the value of 1376 particles/ml obtained for particles of ⩾ 2 μm in the erythromycin infusion thawed after 14 days. This mean value comprised one 'normal' result (732 particles/ml) and one high result (2020 particles/ml). There was no obvious explanation for this observation which was not reflected in the corresponding particulate count at ⩾ 5 μm. In the absence of any other major deviation from the initial ($t = 0$) particulate counts it was concluded that sub-visual particulate levels of the test infu-

sions were not affected by either the freeze-thaw process or the storage time at −20 °C. The issue of sub-visual particulate matter in hospital prepared i.v. infusions, whether subjected to freeze-thaw processes or not, is worthy of detailed investigation.

This study has demonstrated the physical and chemical stability of three hospital-prepared i.v. infusions subjected to frozen storage at −20 °C for up to 12 months followed by microwave thawing. The extended shelf-life obtained under frozen conditions will enable the batch-scale preparation of the three infusions described in this report. In addition to improving efficiency and reducing costs, this approach could enhance patient safety by enabling samples of each batch to be taken for quality control. Although we have previously reported low microbial survival rates in frozen infusions subjected to microwave thawing (Tidy et al., 1988) it is essential that batch-scale production of i.v. infusions is carried out under validated aseptic conditions.

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