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Stability of methotrexate and vinblastine in burette administration sets

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Summary

The stability of methotrexate and vinblastine when stored for up to 48 h in a range of burette administration sets (A200, A2000 and A2001; Avon Medicals, U.K.) under differing lighting conditions has been examined. Photodegradation was the main problem with methotrexate which showed a maximal fall in concentration of 79.13% when stored in polybutadiene administration tubing of an A2001 burette administration set. The photodegradation did not require direct sunlight but occurred more rapidly in the presence of direct sunlight. The use of A2000 burette administration sets, or wrapping the standard administration sets in tinfoil, largely prevented the degradation. Adsorption was the main problem with vinblastine which was bound chiefly by components constructed from polyvinyl chloride or cellulose propionate. The maximum loss recorded was 44.12% when stored in polyvinyl chloride administration tubing of a A2000 burette administration set. The use of A2001 sets, constructed from methacrylate butadiene styrene (burette) and polybutadiene (tubing) overcame the problem of sorption.

Introduction

Although there are many advantages of plastics as a packaging material for pharmaceuticals, important problems do arise particularly with parenteral products. These problems include sorption, leaching, permeation, photodegradation and polymer modification. With the exception of leaching and polymer modification the problems are exacerbated during intravenous infusion of drugs, since the drugs are present in solution at low concentration and the surface area of contact with the plastic materials is large.

A range of intravenous burette administration sets and accessories designed to avoid the two main problems, namely sorption and photodegradation, have recently been introduced in the United Kingdom. The sets which are designed to resist drug adsorption are prepared from the novel plastics, methacrylate butadiene styrene (burette) and polybutadiene (tubing), while the sets designed to prevent photodegradation are amber in colour and filter out light between 220 and 470 nm. To prevent the amber-coloured plastic from coming into direct contact with the fluid, with the possibility of leaching into the fluid, the burette is

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constructed of cellulose propionate sleeved with shrink polyvinyl chloride containing amber pigments, while the tubing is prepared from polyvinyl chloride (PVC) co-extruded, the outer layer containing amber pigments. These latter sets (A 2000; Amberset) have been shown to prevent the photodegradation of frusemide during exposure to sunlight (Yahya et al., 1986). Although the non-adsorption sets (A 2001; Sureset) inhibit the sorption of glyceryl trinitrate, isosorbide dinitrate and diazepam (Lee, 1986), recent studies in our laboratories have indicated that the sets are not resistant to insulin binding (McElnay et al., 1987).

Several studies have indicated that methotrexate and vinblastine can become involved in adsorption and/or photodegradation reactions. Work by Chatterji and Gallelli (1977), for example, in which reference solutions of methotrexate U.S.P. (pH 8.3) were subjected to a temperature of 85 °C for 10 days or placed under a fluorescent light at room temperature for 6 days indicated that 60% degradation occurred under both experimental conditions. The thermal decomposition rate of methotrexate follows first-order kinetics and increases rapidly above pH 9 (Chatterji and Gallelli, 1978). The major photodegradation products of methotrexate when exposed to fluorescent light are 2,4-diamino-6-pteridine-carbaldehyde, 2,4-diamino-6-pteridinecarboxylic acid and *p*-aminobenzoyl glutamic acid. The photodegradation reaction follows zero-order kinetics, following a lag period (Chatterji and Gallelli, 1978). Further studies by Humphreys et al. (1978) showed that when methotrexate (0.75 mg/ml) was stored in 5% dextrose containing 0.05 mmol/l sodium bicarbonate at 4–5 °C and protected from light, a mean decrease in methotrexate concentration of only 1.4% occurred after 72 h with 6.1% degradation after 1 week. Storage of the solution at room temperature and exposed to light resulted in mean decreases of 6.21% and 14.9% in methotrexate concentrations over the same time periods. A schematic representation of the possible degradation products of methotrexate under the influence of pH, temperature and light has recently been presented by Dyvik et al. (1986).

In an investigation into the stability and compatibility of 13 cytotoxic drugs in glass and plastics containers, methotrexate in 5% dextrose was reported to be stable for up to 24 h (Benvenuto et al., 1981). When methotrexate (1 μg/ml) was prepared in methanol or 80% ethanol there was a respective 23% and 7% sorption to a glass volumetric flask (Chen and Chiou, 1982).

Benvenuto et al. (1981) also studied the stability of vinblastine and vincristine in glass and plastics containers. When the drugs (10 mg vinblastine sulphate and 1 mg vincristine sulphate each in 50 ml of 5% dextrose) were stored in PVC bags and glass containers for 24 h unprotected from light, vinblastine sulphate was relatively stable (a maximum loss of 10% in concentration); vincristine sulphate was stable in glass but by 10 h there was a 10% decrease in drug concentration when stored in the plastic containers.

A 48% loss of vinblastine (1 mg/ml) from normal saline, when placed in an implantable infusion device, has been reported over a 14-day exposure period at 37 °C. During the first 24 h the drug concentration was reduced by 24%. The solutions were in contact with titanium, stainless steel, polypropylene, silicone rubber, teflon and a membrane filter within the infusion system (Keller and Ensminger, 1982).

The pharmaceutical precautions given by the manufacturers suggest that methotrexate should be stored protected from direct sunlight while vinblastine should be stored in a refrigerator (ABPI, 1988/89).

The aim of the present study was to examine the stability (with respect to photodegradation and sorption) of methotrexate and vinblastine when placed in the novel administration sets discussed earlier, i.e. Amberset and Sureset burette administration sets, and to compare this with standard burette sets (A 200 – cellulose propionate burette with PVC tubing).

Materials and Methods

Three types of burette administration set (manufactured by Avon Medicals, Redditch, U.K.) were used in the study, namely the A 200 standard sets, the A 2000 Ambersets and the A 2001 Suresets.

The experimental procedure (with the exception of the drug assay) was essentially the same for both study drugs and was as follows.

(a) Methotrexate

(i) Five Ambersets, 5 standard set burettes and 10 Sureset burettes, 5 of them wrapped in tinfoil, were clamped to retort stands. After removal of the administration tubing the burettes were placed on a bench near the laboratory window so that they were exposed to diffuse daylight/fluorescent tube room lighting (approx. 820 lux).

(ii) Methotrexate (1 g injection; Lederle) was reconstituted with 20 ml of Water-for-Injection B.P. and made to volume (1 litre) with 0.9% Sodium Chloride Injection B.P.

(iii) After closing the fluid outlets aliquots of the stock solution (50 ml) were placed in each burette by way of the medication cap using a 50 ml glass syringe.

(iv) Samples (2 ml) were taken at 0, 1, 4, 8, 12, 24 and 48 h via the drip chamber. These were collected in borosilicate tubes and stored at $-20\,^{\circ}C$ until required for assay. Although infusions are unlikely to be kept clinically for periods in excess of 24 h, a total of 48 h storage was used in the present studies to obtain additional stability data.

(v) The same procedure was repeated for the tubing of the administration sets. The only difference being that: (a) the complete tubing was filled by means of the syringe; all air-bubbles were expelled; and (b) instead of 2 ml samples, 10 drops were removed at each sampling time and used for analysis of methotrexate content.

(vi) A further preliminary study on the effect of sunlight on methotrexate stability was carried out. In this preliminary work 3 Amberset burettes and 6 standard set burettes, 3 of them wrapped in tinfoil, were prepared and investigated as described above. These burettes were, however, exposed to 7 h of continuous sunlight via the laboratory window.

(vii) All samples were assayed for methotrexate content using HPLC. The system used consisted of the Altex 110A pump, a 20 μl Rheodyne loop injection valve, a Spherisorb (5 μm) ODS column (25 cm $\times$ 4.6 mm) and a Perkin-Elmer LC-75 spectrophotometer (303 nm). Peak areas were recorded using a Hewlett Packard 339OA integrator. The mobile phase used was based on the work of Tong et al. (1980) and consisted of 70 : 30 water/methanol with tetrabutylammonium hydrogen sulphate (5 mmol/l) as an ion-pairing agent. This was run at a flow rate of 0.9 ml/min.

To ensure that the assay method was able to differentiate between methotrexate and its breakdown products, a small volume of methotrexate solution, which was left to stand in sunlight for 12 h over a 2-day period, was examined chromatographically.

A standard calibration curve was prepared over the concentration range 0.4–1.0 mg/ml. This calibration curve was then used to calculate the methotrexate content of samples taken during the study. In all cases a standard solution was injected after every 5 samples to ensure that no fluctuations in the chromatogram were taking place, e.g. due to changes in room temperature.

(b) Vinblastine

(i) Burettes and tubing were prepared as for the methotrexate experiments.

(ii) One vial of vinblastine sulphate (10 mg; Eli Lilly) was reconstituted according to the manufacturer's instructions to produce 10 ml (diluting solution is 0.9% sodium chloride with 2% benzyl alcohol). An aliquot of this solution (3 ml) was made up to 1 litre using 5% Dextrose Injection BP (final concentration was therefore 3 μg/ml). The sample collection from the burettes and the administration set tubing was as described for methotrexate; however, in this case, samples were taken at 0, 1, 2, 4, 8, 24 and 48 h. All samples were stored at $2–4\,^{\circ}C$, protected from light, until required for assay.

(iii) Assay of all samples for vinblastine content was by HPLC. The system used was as described for methotrexate, the only difference being the use of an Altex 330 fixed-wavelength UV detector (254 nm) instead of the Perkin-Elmer LC-75 spectrophotometer.

As before, the peak areas were recorded using the Hewlett Packard 339OA integrator. The method used was based on that of Gaj and Sesin (1984) which used a mobile phase consisting of

methanol/water in the ratio 2:1. After preliminary experimentation, a mobile phase consisting of 50:50 water/methanol adjusted to pH 3.0 with glacial acetic acid was found to be optimal for the present work and was run at a flow rate of 1.5 ml/min.

To ensure that the assay method was able to differentiate between vinblastine and its breakdown products, a small volume of vinblastine solution was autoclaved for 1 h and then injected on to the system.

A standard calibration curve of vinblastine over the concentration range 1–3 μg/ml was used to calculate the vinblastine content of samples taken during the study. In all cases a standard solution was injected after every 5 samples to ensure that no fluctuations in the chromatogram occurred.

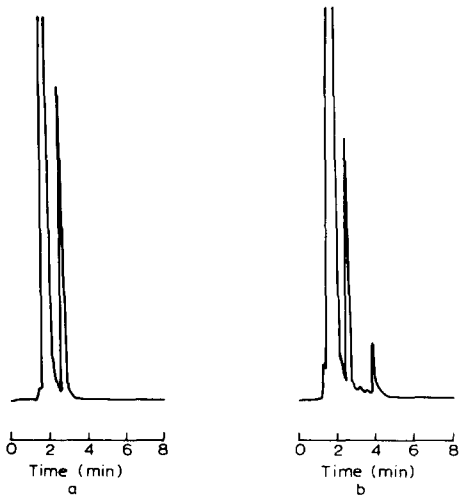

Fig. 2. a: chromatogram of solution of vinblastine (3 μg/ml) in 5% Dextrose Injection B.P. b: chromatogram of above solution after degradation by autoclaving for one hour.

Results and Discussion

(a) Methotrexate

Typical chromatograms of the standard solution of methotrexate before and after decomposition, by placing in sunlight, are shown in Fig. 1. After light exposure, there was a decrease in the height of the methotrexate peak (retention time 3.5 min) while further peaks, due most likely to degradation products, appeared on the chromatogram. The work of Chatterji and Gallelli (1978) and Hansen et al. (1983) suggest that the degradation peaks may be due to N^{10}-methylfolic acid and 2,4-diamino-6-pteridine carboxylic acid. The calibration curve for methotrexate had a correlation coefficient of 0.998 with a C.V. (%) of 0.70% ($n = 6$).

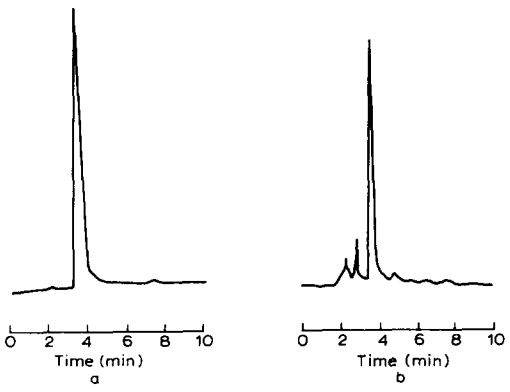

Fig. 1. a: chromatogram of solution of methotrexate sodium (0.5 mg/ml) in 0.9% Sodium Chloride Injection B.P. b: chromatogram of above solution after degradation in sunlight over a 2-day period.

Storage of methotrexate under normal lighting conditions led to little change in drug concentration over the first 24 h of storage (Fig. 3). Statistical analysis of these and all further results are summarised in Table 1. Storage for a further 24 h led to a small decrease (maximum 12%; Sureset). A much more rapid decrease in methotrexate concentration was demonstrated when the drug solution was exposed to sunlight (Fig. 4). An 11% decline in methotrexate concentration occurred in the standard sets in just 7 h. No change in concentration was seen in those cases where methotrexate was protected from light either by tinfoil or by being present in Amberset burettes.

The results obtained for methotrexate stored in the administration tubing were slightly more complex. Storage of methotrexate in the unprotected tubing of the standard set (PVC) and Sureset (polybutadiene) produced a 65.52% and 79.13% drug loss, respectively, after 48 h compared to a loss of 16.38% and 12.07% from Amberset (PVC) and light-protected Sureset, respectively.

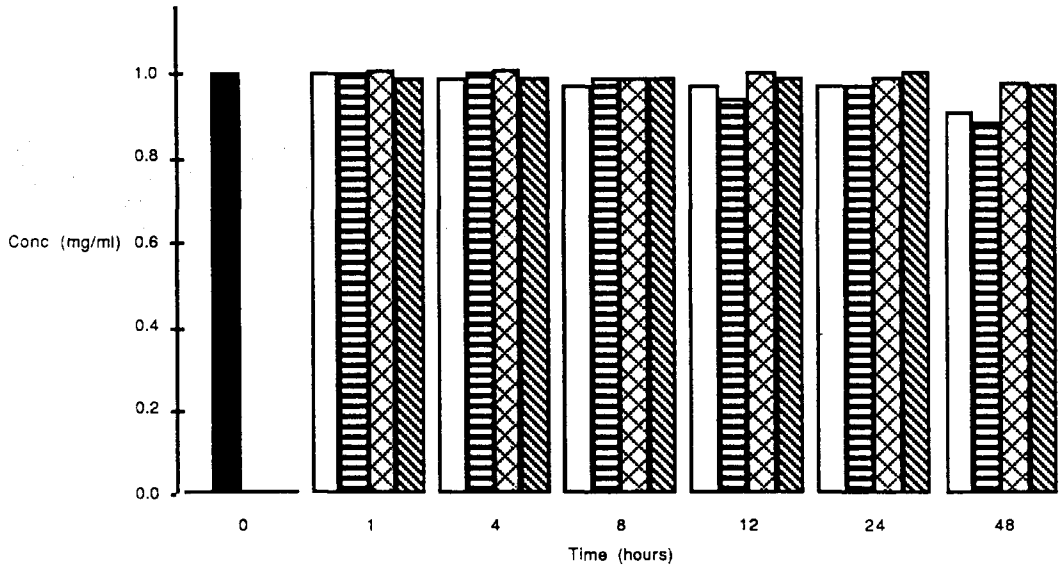

Fig. 3. Concentration vs time profiles for methotrexate when stored in the burettes of standard sets (□), Suresets (▤), Ambersets (▩) and Suresets wrapped in tinfoil (▧) and exposed to diffuse daylight/fluorescent tube room light. (Maximum coefficient of variation within replicate data points was 3.7%.) Control measurement at time 0 (■).

(b) Vinblastine

Typical chromatograms of a standard solution of vinblastine (retention time = 2.6 min) before and after autoclaving for 1 h are given in Fig. 2. One small degradation peak was obtained for the autoclaved sample at a retention time of 4 min. The broad peak at the beginning of the chromatograms was due to dextrose. The calibration curve for vinblastine had a correlation coefficient of 0.998 with a C.V. (%) of 2.40% ($n = 6$).

No degradation peaks appeared on the chromatograms during the storage studies. The Suresets (methacrylate butadiene styrene) produced the best results with respect to maintenance of vinblastine concentration. After 48 h in these sets only a 5.14% loss in initial concentration was found;

TABLE 1

One-factor analysis of variance for all 24-h data (7 h data in case of sunlight study)

	Foil-wrapped Sureset vs Amberset	Foil-wrapped Sureset vs Standard set	Foil-wrapped Sureset vs Sureset	Sureset vs Amberset	Sureset vs standard set	Standard set vs Amberset	Foil-wrapped standard set vs Amberset	Foil-wrapped standard set vs standard set
Methotrexate (burettes)	NS	S	S	NS	NS	NS	NA	NA
Methotrexate (tubing)	NS	S	S	S	S	S	NA	NA
Methotrexate (burettes in sunlight)	NA	NA	NA	NA	NA	S	S	S
Vinblastine (burettes)	S	S	NS	S	S	NS	NA	NA
Vinblastine (tubing)	S	S	NS	S	S	NS	NA	NA

NS = not significant ($P > 0.05$); S = significant ($P < 0.05$); NA = not applicable.

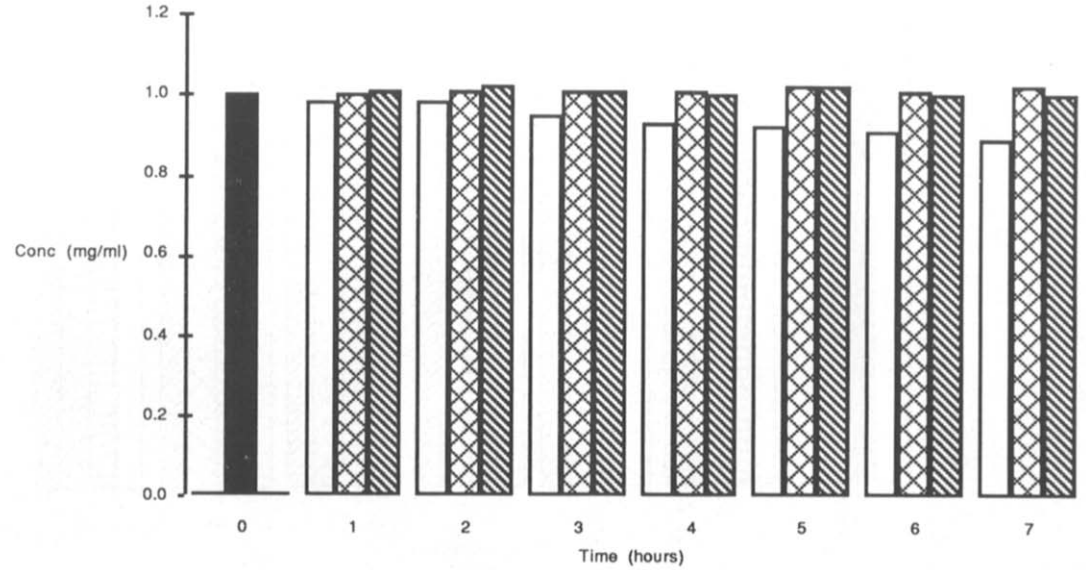

Fig. 4. Concentration vs time profiles for methotrexate when stored in the burettes of standard sets (□), Ambersets (▨) and standard sets wrapped in tinfoil (▨) and exposed to sunlight. (Maximum coefficient of variation within replicate data points was 4.0%.) Control measurement at time 0 (■).

this was reduced to 2.25% when these burettes were wrapped with tinfoil. The burettes manufactured from cellulose propionate did not yield such favourable results. After storage of vinblastine in the standard set and Amberset burettes for 48 h the reduction in vinblastine concentration was

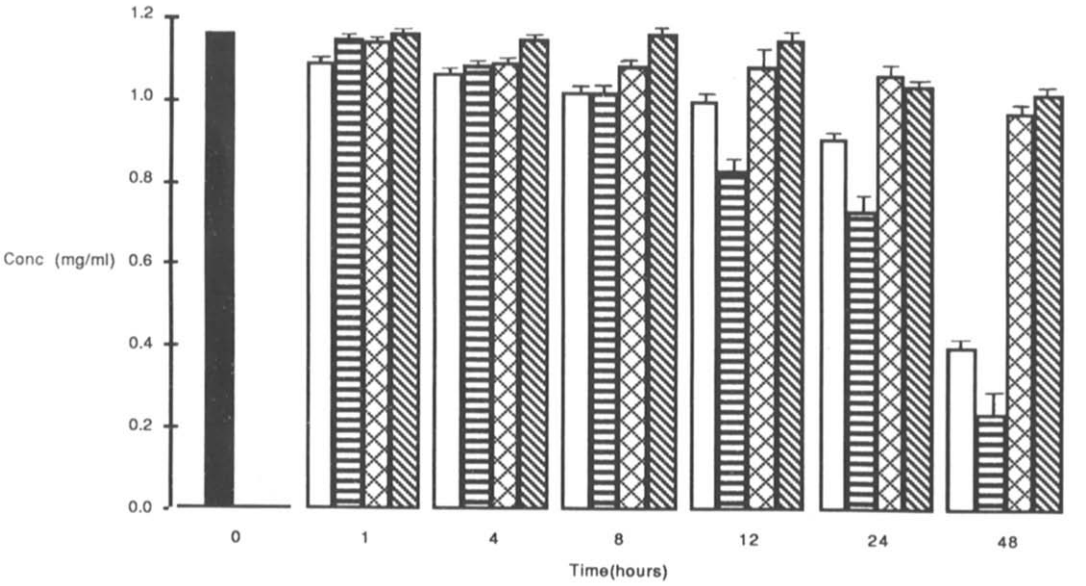

Fig. 5. Concentration (±S.D.) vs time profiles for methotrexate when stored in administration tubing of standard sets (□), Suresets (▤), Ambersets (▨) and Suresets wrapped in tinfoil (▨) and exposed to diffuse daylight/fluorescent tube room light. Control measurement at time 0 (■).

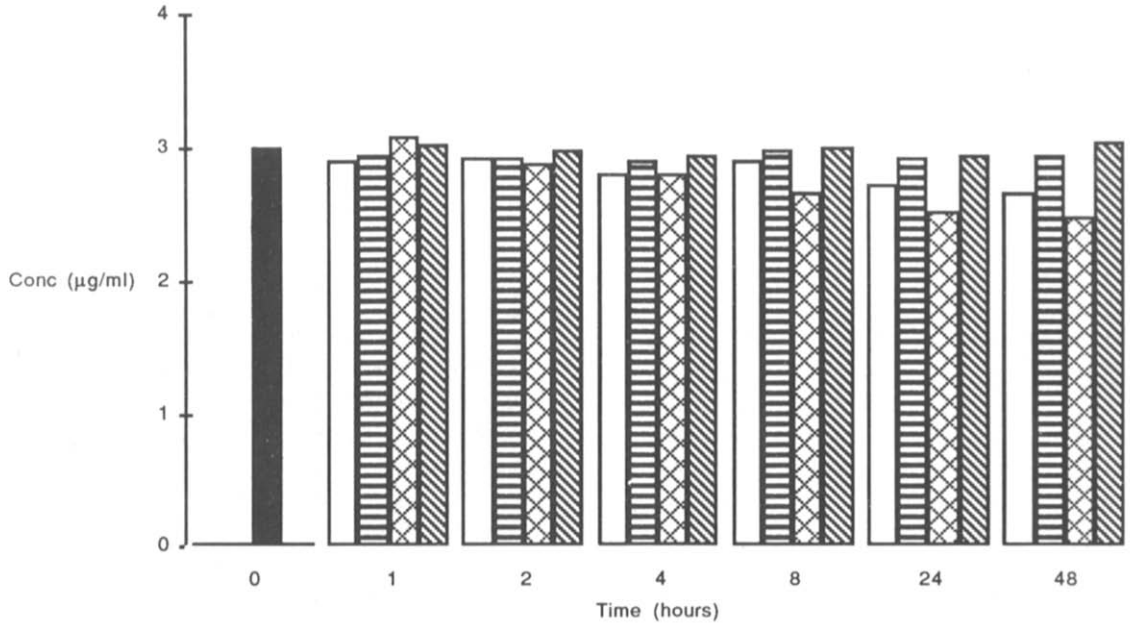

Fig. 6. Concentration vs time profiles for vinblastine when stored in the burettes of standard sets (□), Suresets (▤), Ambersets (▨) and Suresets wrapped in tinfoil (▧) and exposed to diffuse daylight/fluorescent tube room light. (Maximum coefficient of variation within replicate data points was 3.6%.) Control measurement at time 0 (■).

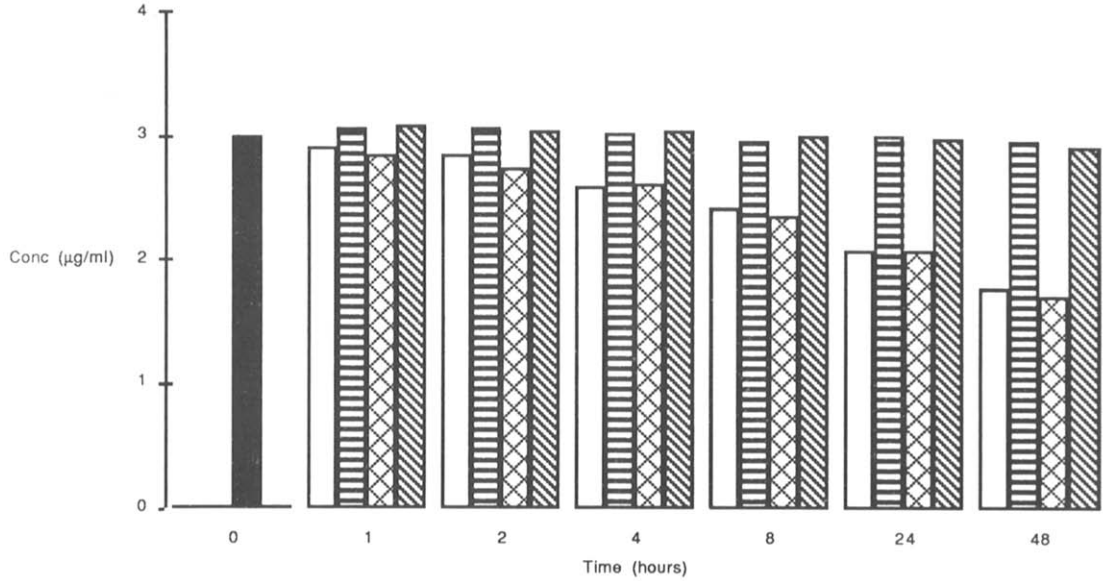

Fig. 7. Concentration vs time profiles for vinblastine when stored in the administration tubing of standard sets (□), Suresets (▤), Ambersets (▨) and Suresets wrapped in tinfoil (▧) and exposed to diffuse daylight/fluorescent tube room light. (Maximum coefficient of variation within replicate data points was 3.6%.) Control measurement at time 0 (■).

14.47% and 20.58%, respectively (Fig. 6; see Table 1 for statistical evaluation). The loss from the Amberset and standard set burettes was exponential in fashion. Butler et al. (1980), investigating the effect of inline filtration on the potency of low-dose vinblastine, reported a 0.7% drug loss from a 5% dextrose solution and a 2.5% drug loss from a normal saline solution when passed through a filter containing a cellulose ester membrane over a 6-h period.

The experiments involving the administration tubing gave very similar results. Again it was the Sureset tubing (polybutadiene) which produced the best results. Storage in contact with this tubing, whether wrapped in tinfoil or not, caused no more than a 6% loss in drug concentration after 48 h (Fig. 7). The tubing made from PVC, i.e. the standard set and the Amberset tubing, caused a reduction in vinblastine concentration of 42.16% and 44.12%, respectively, after 48 h (Fig. 7). Both sets of tubing produced almost identical results. As before, the sorption was greatest during the first 8 h, after which the rate of sorption decreased. The larger surface area-to-volume ratio in the tubing compared to the burettes was probably the main reason why sorption on to the PVC seemed much greater than on to the cellulose propionate. This could explain why the present results contradict the finding of Benvenuto et al. (1981) who reported that vinblastine sulphate in dextrose was stable (less then 10% change in drug content) in PVC bags over a 24-h period at room temperature, unprotected from light.

Conclusions

A general review of the results obtained indicates that photodegradation is the main problem with the storage of methotrexate in the administration sets examined.

This photodegradation did not require direct sunlight but occurred much more rapidly in the presence of direct sunlight. The use of Amberset burettes and tubing or wrapping normal administration sets with tinfoil largely overcame this drug loss by photodegradation.

The studies with vinblastine indicate that photodegradation is not a problem with this drug, but that sorption to PVC and cellulose propionate could be potentially of clinical significance. The use of Suresets would appear to overcome this sorption problem.

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