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Compatibility of paclitaxel in 5% glucose solution with ECOFLAC® low-density polyethylene containers–stability under different storage conditions

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Abstract

The compatibility of paclitaxel with low-density polyethylene containers (ECOFLAC®) was studied under different temperature and light conditions. Solutions of 0.4 and 1.2 mg/ml of paclitaxel in 5% glucose solution were prepared, put into ECOFLAC® containers and stored: (i) at ambient temperature (20–25°C) and in ambient light; (ii) at ambient temperature in the dark; and (iii) at +4°C in the dark. Paclitaxel was assayed by high-performance liquid chromatography after visual inspection of the solutions. The results show that solutions of TAXOL® in 5% glucose should not be stored for more than 5 days in glass or ECOFLAC® containers because a whitish precipitate tends to form, lowering the paclitaxel concentration. The decrease in the paclitaxel concentration observed after chromatographic analysis ranged very widely (from 12 to 83% of the initial concentration). However solutions of TAXOL® diluted in 5% glucose was stable for 5 days in ECOFLAC® containers under all the storage conditions tested. These additive-free low-density polyethylene containers offer the advantage of not releasing DEHP into the paclitaxel solutions.

Keywords: Compatibility; Paclitaxel; Low-density polyethylene containers

1. Introduction

The drug paclitaxel (TAXOL®) is currently of major importance in the treatment of ovary and breast cancer. This agent is of proven clinical value but its pharmaceutical formulation is problematic because of its incompatibility with the materials of perfusion kits. Paclitaxel is almost insoluble in water, and so a mixture of CREMOPHOR® (castor oil) and ethanol is used for the commercial solution. The CREMOPHOR® produces an incompatibility of the drug solution

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with PVC bags, which release di-(2-ethylhexyl) phthalate (DEHP). Accordingly, the supplier recommends using glass or polypropylene bottles, and polyolefin bags, with which TAXOL® is known to be compatible (Waugh et al., 1991; Chin et al., 1994; Husson and Becker, 1995, Xu et al., 1994). Solutions containing 0.3 and 1.2 mg/ml of paclitaxel (in 5% glucose) are chemically and physically stable for 27 h at 25°C.

The availability of ECOFLAC® containers made of low-density polyethylene prompted us to study their compatibility with paclitaxel. ECOFLAC® containers offer numerous advantages; they are flexible, and will collapse like a bag when the contents are drawn off, while being sufficiently stiff to stand up like a bottle. Also, the material produces less particulate contamination than glass or PVC, and contains no additives that liable to migrate into the drug solutions (Anduze-Acher et al., 1997). No additive is used during the manufacture of ECOFLAC® containers, which are hot-formed by the blow-fill-seal process.

We investigated the compatibility of paclitaxel (in a specific formulation, TAXOL®) in 5% glucose solution and ECOFLAC® containers. In parallel we studied the stability of the solutions stored under different light and temperature conditions to evaluate whether solutions of 0.4 and 1.2 mg/ml paclitaxel in 5% glucose solution could be prepared in advance.

2. Materials and methods

2.1. Drug

Paclitaxel (TAXOL®) was supplied by Bristol-Myers-Squibb as an injectable solution in 5-ml vials containing 30 mg of paclitaxel.

2.2. Solvent for dilution and containers

The 5% glucose solution used to dilute the paclitaxel was supplied in ECOFLAC® polyethylene containers (B. Braun Médical) and in glass bottles (B. Braun Médical).

2.3. Design of the stability study

The stability study was conducted with paclitaxel concentrations of 0.4 and 1.2 mg/ml. These concentrations correspond to dosages commonly used in clinical practice. The dilutions were made under a laminar flow hood in 5% glucose solution in ECOFLAC® and glass containers. The glass served as a control, since the compatibility of paclitaxel and glass is established. The total volume of the drug solutions obtained after dilution was 50 ml (for the 0.4 mg/ml solution: 3.3 ml TAXOL® were added to 46.7 ml 5% glucose solution; for the 1.2 mg/ml solution, 10 ml TAXOL®, i.e. the contents of 2 vials, was diluted in 40 ml 5% glucose solution).

The solutions of paclitaxel in glass or ECOFLAC® bottles were stored under different conditions: (i) at ambient temperature (20–25°C) and in ambient light (daylight, out of direct sunlight, on a table in the middle of the laboratory); (ii) at ambient temperature in the dark (wrapped in aluminium foil); and (iii) at +4°C in the dark (in a refrigerator). The supplier recommends that dilute solutions of paclitaxel should not be refrigerated because of AN increased risk of precipitation at low temperatures. These conditions are those any diluted drug solution is liable to encounter in clinical practice before administration to a patient. For each storage condition the preparations in ECOFLAC® were made in triplicate. A single control test was performed for glass (multiple tests were unnecessary because THE compatibility of glass and paclitaxel is established). These experimental conditions are set out in Table 1.

For each preparation, the first sample was taken immediately after dilution and bottling (sample t_0), which served as the baseline. Subsequent samples were taken at the following times; on day 1 after 1, 2, 4 and 6 h storage, and then every day for 4 days. To evaluate the shelf-life of the solutions, an analysis was carried out on day 8 and then every week (such storage times would not be encountered in clinical practice, because dilute solutions would never be prepared so long before administration to a patient).

Table 1
Experimental conditions

Container	Concentration of paclitaxel (mg/ml)	Condition of storage	Number of preparations
ECOFLAC®	0.4	Ambient light and temperature	3
		Dark and ambient temperature	3
		Refrigerator at +4°C	3
	1.2	Ambient light and temperature	3
		Dark and ambient temperature	3
		Refrigerator at +4°C	3
Glass	0.4	Ambient light and temperature	1
		Dark and ambient temperature	1
		Refrigerator at +4°C	1
	1.2	Ambient light and temperature	1
		Dark and ambient temperature	1
		Refrigerator at +4°C	1

The samples were collected in glass tubes. First, a visual inspection of the solutions was made to evaluate their appearance (limpidity, coloration). Second, the solution was analysed by chromatography to determine the concentration of paclitaxel and any breakdown products.

2.4. Visual inspection

The samples were examined against a white background and a black background under unpolarised light.

2.5. Chromatographic assay of paclitaxel

If the solution was limpid, the sample was analysed directly by high performance liquid chromatography (HPLC). If any turbidity or precipitate was visible, the sample was filtered (MILLEX-GS 0.22 μm, Millipore) before chromatographic analysis. We first checked that the filtration of a limpid sample (no turbidity detected by visual inspection) caused no loss of paclitaxel (no fall in paclitaxel concentration before and after filtration by chromatographic analysis of the samples).

Table 2
Stability of solutions of 1.2 mg/ml of paclitaxel in 5% glucose

	Ambient light and temperature		Dark and ambient temperature		Refrigerator at +4°C	
	ECOFLAC® ($n=3$)	Glass ($n=1$)	ECOFLAC® ($n=3$)	Glass ($n=1$)	ECOFLAC® ($n=3$)	Glass ($n=1$)
Actual initial concentration (mg/ml)	1.53 ± 0.16	1.31	1.59 ± 0.07	1.58	1.35 ± 0.03	1.56
Percent initial concentration remaining after storage (mean ± S.D.)						
D_1 (T_{1h})	98.4 ± 1.2	96.2	99.0 ± 0.9	101.3	99.3 ± 4.3	94.9
D1 (T_{2h})	97.5 ± 3.5	98.5	101.8 ± 4.2	98.7	100.0 ± 2.6	100.0
D_1 (T_{4h})	94.0 ± 0.9	100.8	100.8 ± 1.9	100.0	97.8 ± 2.6	102.6
D_1 (T_{6h})	97.8 ± 3.0	100.8	100.2 ± 5.5	102.5	99.3 ± 2.2	100.0
D_2	98.8 ± 5.9	97.7	101.2 ± 4.7	104.4	101.3 ± 4.1	100.0
D_3	97.7 ± 1.1	94.7	100.6 ± 4.7	104.4	99.8 ± 2.3	100.0
D_4	97.7 ± 0.3	96.2	100.9 ± 4.9	102.5	101.2 ± 0.5	100.6
D_5	96.2 ± 1.9	97.7	99.9 ± 4.3	101.9	101.8 ± 4.6	100.0

The assay of paclitaxel was carried out by HPLC using the following apparatus (Merck-Hitachi): a constant flow rate pump (L-6200), a sample injector (L-7200), UV–visible (L-4250), and an integrator (D-2500).

The analysis method used to assay paclitaxel was that recommended by the supplier, Bristol-Myers-Squibb. The chromatography column contained a pentafluorophenyl-type stationary phase (Interchim, 250 × 4 mm i.d., 5 μm). The mobile phase was a mixture (45/55 v/v) of acetonitrile (Carlo Erba) and purified water (B. Braun). The flow rate was 1.5 ml/min. The detection wavelength was set at 227 nm. The standard solutions were prepared by dilution of paclitaxel 6 mg/ml (commercial solution of TAXOL®) in 0.02% acetic acid–methanol (v/v) to obtain concentrations of paclitaxel of 0.3, 0.6 and 1.2 mg/ml. These solutions were used to plot a calibration line with equation $y = ax + b$ (where x is the paclitaxel concentration and y is the area under the paclitaxel peak). Paclitaxel concentrations in experimental samples were obtained by extrapolation from this graph. The chromatography method used was validated specifically to study the stability of paclitaxel according to the recommendations of Trissel (1983). It had to be able to detect and separate paclitaxel from any degradation products. Accordingly, we carried out a deliberate complete degradation of the drug molecule by means of heating (65°C) and light (254 nm) for 3 weeks. The solution obtained was analysed by chromatography to check that there was no interference of degradation products with the paclitaxel.

The average concentrations of paclitaxel were calculated from the different tests performed in ECOFLAC® ($n = 3$). The results are expressed in percentages relative to the initial concentration at t_0 (value taken as 100%). Drug solutions are considered acceptable for use by some if they contain $> 90\%$ of the label claim (Trissel, 1994).

2.6. Chromatographic analysis of DEHP

The column was a LICHROSPHER 100 RP18 endcapped (Merck). The mobile phase was a mixture of acetonitrile–water–tetrahydrofuran (70/15/15 v/v/v). The flow rate was 1 ml/min. The detection wavelength was 254 nm. The standard DEHP was supplied by Prolabo.

3. Results and discussion

3.1. Study of stability over 5 days

3.1.1. Visual inspection of diluted solutions of paclitaxel

Diluted solutions of 0.4 and 1.2 mg/ml paclitaxel in 5% glucose displayed no change in limpid-

Table 3
Stability of solutions of 0.4 mg/ml of paclitaxel in 5% glucose

	Ambient light and temperature		Dark and ambient temperature		Refrigerator at +4°C	
	ECOFLAC® ($n = 3$)	Glass ($n = 1$)	ECOFLAC® ($n = 3$)	Glass ($n = 1$)	ECOFLAC® ($n = 3$)	Glass ($n = 1$)
Actual initial concentration	0.43 ± 0.01	0.44	0.42 ± 0.006	0.45	0.44 ± 0.006	0.43
Percent initial concentration remaining after storage (mean ± S.D.)						
D_1 (T_{1h})	101.6 ± 1.3	100.0	100.8 ± 3.6	100.0	99.2 ± 1.3	102.3
D_1 (T_{2h})	102.3 ± 0.06	102.3	101.6 ± 3.6	100.0	98.5 ± 2.6	102.3
D_1 (T_{4h})	100.8 ± 1.4	100.0	100.8 ± 2.7	100.0	100.0 ± 0.0	100.0
D_1 (T_{6h})	99.2 ± 1.3	97.7	102.4 ± 4.1	97.8	101.5 ± 1.3	102.3
D_2	100.0 ± 0.0	97.7	100.8 ± 2.7	97.8	97.7 ± 0.0	100.0
D_3	100.0 ± 2.3	97.7	100.0 ± 4.1	97.8	99.2 ± 1.3	102.3
D_4	99.3 ± 2.7	100.0	101.6 ± 5.5	100.0	98.5 ± 1.3	97.7
D_5	98.5 ± 1.3	97.7	100.8 ± 4.9	97.8	98.5 ± 1.3	97.7

Table 4
Paclitaxel (%) in different solutions after 8 and 15 days storage

	Day 8		Day 15	
	ECOFLAC®	Glass	ECOFLAC®	Glass
Paclitaxel 0.4 mg/ml				
Ambient light and temperature	99.6	98.6	88.0[a]	92.5
	97.6		77.0[a]	
	99.3		73.0[a]	
Dark and ambient temperature	97.8	95.3	84.9[a]	88.0[b]
	106.4		80.0[a]	
	100.6		82.8[a]	
Refrigerator +4°C	99.3	97.9	99.3	95.8
	97.6		98.6	
	97.9		94.5	
Paclitaxel 1.2 mg/ml				
Ambient light and temperature	66.3[a]	107.5	17.2[a]	80.4[a]
	88.7[a]		62.3[a]	
	96.4		67.7[a]	
Dark and ambient temperature	97.8	100.0	96.6	101.6
	106.4		68.3[a]	
	100.6		84.1[b]	
Refrigerator +4°C	102.3	104.2	99.3	104.0
	103.8		98.4	
	103.5		103.9	

[a] Filtered sample.
[b] Sample that displayed no turbidity on visual inspection (non filtered sample) but nevertheless showed a decrease in paclitaxel concentration of more than 10%.

ity or colour during 5 days storage in glass or ECOFLAC® under any of the temperature and light conditions tested.

3.1.2. Chromatographic assay of paclitaxel

The paclitaxel assay method was validated. Its precision (investigated by injecting a 0.6 mg/ml solution of paclitaxel) was satisfactory with a relative S.D. of 0.2% for intra-day assay variability ($n = 6$) and 0.6% for inter-day variability ($n = 6$). The method displayed high linearity in the calibration range 0.3–1 mg/ml with a correlation coefficient $r = 0.9999$. The equation for the mean calibration was $y = 1.371 \cdot 10^7 x + 2.62 \cdot 10^4$. The recovery of paclitaxel from TAXOL® was satisfactory ($99.6 \pm 0.8\%$). The chromatography method was validated as an analytical method for the stability study of paclitaxel. It provided specific quantitation of the drug itself without interference by any of its breakdown products.

The results reported in Tables 2 and 3 show that paclitaxel diluted in 5% glucose to 0.4 and 1.2 mg/ml was stable for 5 days in ECOFLAC® under any of the storage conditions tested (ambient light, dark, ambient temperature, refrigeration at + 4°C). The paclitaxel concentrations obtained at different sampling times did not vary by more than 6% relative to the initial concentration at t_0. The paclitaxel concentrations determined in ECOFLAC® were comparable to those measured in glass, the control material. Also, the chromatographic analysis showed no secondary peak that might have indicated a breakdown of the paclitaxel.

3.2. Evaluation of the shelf-life of the solutions

After 8 days storage, and more so after 15 days, the results ranged widely. We found that some preparations displayed a white turbidity that evolved into a precipitate. Analysis of the samples after filtration showed a loss of paclitaxel exceeding 10% of the initial concentration at t_0. How-

ever, turbidity appeared haphazardly in the different preparations. Also, the decrease in the paclitaxel concentration observed after chromatographic analysis of the filtered samples ranged very widely (from 12 to 83% of the initial concentration). Some solutions that displayed no turbidity on visual inspection nevertheless showed a decrease in paclitaxel concentration of more than 10% (Table 4). Storage of these preparations for longer than 15 days gradually resulted in the appearance of turbidity in both the glass and the polyethylene containers.

Such appearance of turbidity has already been reported after several days storage of dilute solutions (Waugh et al., 1991; Xu et al., 1994). This effect seems to be related to the presence in the solutions of 6 mg/ml TAXOL® of the castor oil–ethanol mixture (50/50). This may have favoured precipitation when the TAXOL® was diluted in 5% glucose to obtain solutions containing 0.4 and 1.2 mg/ml, given the low solubility of TAXOL® in water.

In this study, precipitation started to occur only after 5 days storage of the diluted solutions, well beyond the storage time usual in clinical practice.

3.3. Analysis of DEHP in the paclitaxel solutions

No DEHP was detected in each solution of paclitaxel in ECOFLAC® after 15 days storage under any of the temperature and light conditions tested. The limit of quantification for DEHP by this chromatography method was 1 μg/ml of solution of paclitaxel in 5% glucose.

4. Conclusion

Paclitaxel at 0.4 and 1.2 mg/ml in 5% glucose is physically and chemically stable in ECOFLAC® for 5 days in ambient light and in the dark, at ambient temperature and in a refrigerator at + 4°C. No DEHP is released from the ECOFLAC® containers into the paclitaxel solutions. The stability of paclitaxel in ECOFLAC® is comparable to that obtained in glass containers.

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References

Anduze-Acher, V., Jaouen-Vakkou, C., Vanheerswynghels, S., Cabrol, E., 1997. ECOFLAC, poche pour perfusion en polyéthylène. Revve de l' ADPHSO 22 (2), 73–81.

Chin, A., Ramakrishnan, R.R., Yoshimura, N.N., Jeong, E.W.S., Nii, L.J., Dimeglio, L.S., 1994. Paclitaxel stability and compatibility in polyolefin containers. Ann. Pharmacother. 28, 35–36.

Husson, M.C., Becker, A., 1995. Médicaments anticancéreux. De la préparation à l'administration. Optimisation Technique & Documentation. APHIF Editions Médicales Internationales.

Trissel, L.A., 1983. Avoiding common flaws in stability and compatibility studies of injectable drugs. Am. J. Hosp. Pharm. 40, 1159–1160.

Trissel, L.A., 1994. Handbook on Injectable Drugs, 8th edn. American Society of Hospital Pharmacists, Bethesda.

Waugh, W.N., Trissel, L.A., Stella, V.J., 1991. Stability, compatibility, and plasticizer extraction of taxol (NSC-125973) injection diluted in infusion solutions and stored in various containers. Am. J. Hosp. Pharm. 48, 1520–1524.

Xu, Q., Trissel, L.A., Martinez, J.F., 1994. Stability of paclitaxel in 5% dextrose injection or 0.9% sodium chloride injection at 4, 22 or 32°C. Am. J. Hosp. Pharm. 51, 3058–3060.