Cancer Chemother Pharmacol (1989) 23: 197–207

Cancer Chemotherapy and Pharmacology



*Review*

# Stability of solutions of antineoplastic agents during preparation and storage for in vitro assays

## III. Antimetabolites, tubulin-binding agents, platinum drugs, amsacrine, L-asparaginase, inteferons, steroids and other miscellaneous antitumour agents

**Andrew G. Bosanquet**

Bath Cancer Research Unit, Royal United Hospital, Combe Park, Bath BA1 3NG, England

**Summary.** The stability of solutions of the antitumour antimetabolites, vinca alkaloids, podophyllotoxins, interferons, steroids and platinum drugs as well as maytansine, asparaginase, amsacrine, flavone-8-acetic acid, mitoguazone, and *N*-phosphonoacetyl-L-aspartate (PALA) is reviewed. Much of the published work has been done with biological, not stability-indicating, assays; thus, the relevant results should be used with caution. With this proviso, almost all of these drugs can be stored in solution for several days at room temperature or 4° C. Most reports also suggest that the drugs that have been tested are stable when frozen in solution. For a number of the drugs, particular precautions are required; for instance, amsacrine should not be mixed with chloride-containing solutions, whereas cisplatin is most stable in solutions containing $>0.1$ *M* chloride.

### Introduction

In the first two reviews of this series [13, 14], general considerations about anticancer drug stability and the methods for its measurement were discussed; details were given as to the stability of the alkylating agents and nitrosoureas [13] as well as the antitumour antibiotics [14]. In this review, the stability of many other antitumour agents is discussed, including the antimetabolites, tubulin-binding agents, platinum drugs and other antitumour agents, among which are some steroids.

It should be borne in mind that the values cited in this review for drug stability represent, for the most part, the chemical stability of the drug. However, the time during which these drugs can be given to patients is often limited by other considerations, and care should be taken to abide by the time constraints listed on package inserts.

### Antimetabolites

The structures of the antimetabolites discussed in this review are shown in Fig. 1. A summary of the stability of these drugs is presented in Table 1.

*Offprint requests to:* AG Bosanquet

The author would be happy to receive letters detailing errors, omissions or additions

*Methotrexate*

The main problem with methotrexate is that it is photolabile [3, 21, 24, 68]. Despite its being used for many years, no reports the drug's photolability appeared in the literature until 1976 [22]; however, when methotrexate was first thoroughly investigated in 1978 [24, 68], this problem seemed to have been recognised. Chatterji and Gallelli [24] found a lag phase to its degradation followed by zero-order kinetics, suggesting a free radical mechanism. The photolability of the drug is increased by drug dilution [3] and the addition of bicarbonate [24].

Methotrexate (at 0.5 mg/ml) is stable during freezing and thawing [76]. The type of container used, whether glass or polyvinylchloride (PVC), does not seem to affect the stability of the drug over 2 days at room temperature [6], but methotrexate dissolved in methanol or ethanol adsorbs to glassware [25], suggesting that these solvents should not be used when working with the drug. Methotrexate binds quite strongly (94%) to serum albumin [82].

A decade ago, methotrexate was only available as $>85\%$ pure 4-amino-10-methyl-folic acid [23, 68]. Now it is 94% pure but still contains significant amounts of other substances; therefore, care should be taken in making up standard solutions.

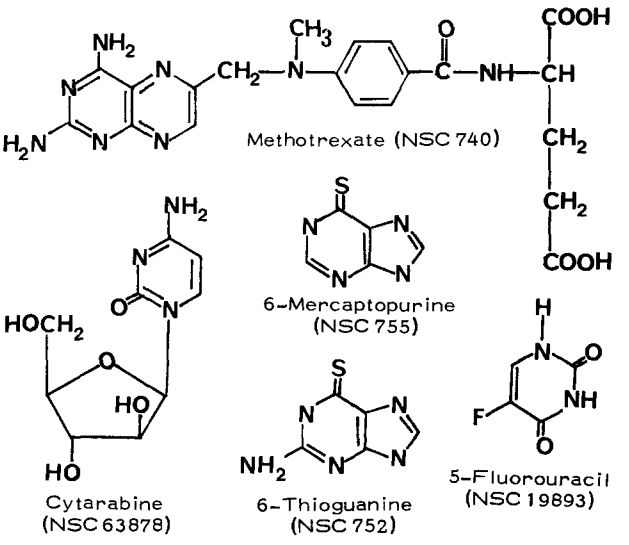



**Fig. 1.** Structures and NSC numbers of the antimetabolites

**Table 1.** Summary of the stability of the antimetabolites and mitotic spindle inhibitors

| Drug | Stability frozen[a] | Stability at 4° C[a] | Stability at room temperature[a] | Stability in medium at 37° C | Stability (RT) dissolved in the i.v. formulation |
|---|---|---|---|---|---|
| **Antimetabolites:** | | | | | |
| Methotrexate | −20° C S 30 days (H) [76][f] | S 7 days (U) [68] | 10 days (H) [24]<br>4 years (dark, H) [59] | – | S 2 years [m][g] |
| 5-Fluorouracil | −20° C S 2 weeks (B) [153]<br>−70° C S 2 weeks (B) [153]<br>−60° C S 37 weeks (B) [48] | S 2 weeks (B) [153] | S 7 days (U) [128] | $t_{1/2}$ > 2 weeks (B) [65] | S 4 years [m] |
| 6-Thioguanine | – | S 24 h [138] | S 24 h [138] | – | S < 24 h at 15 mg/ml [138] |
| 6-Mercaptopurine | −20° C S 52 days (plasma, H) [37] | S 7 days (U) [52, 139] | S 7 days (U) [52, 139] | – | S > 3 weeks at 10 mg/ml [138] |
| Cytarabine | −20° C S 2 weeks (H) [100] | S 2 weeks (H) [100] | 12 days (U, H) [31, 100] | – | S > 2 years [152]<br>3 years (4° C) [m] |
| **Mitotic spindle inhibitors:** | | | | | |
| Vincristine | −60° C S 68 weeks (B) [49] | S 2 weeks (M) [72, 81] | S 24 h (H) [5] | S 2 weeks (B) [50, 65] | S 2 weeks (4° C) [m] |
| Vinblastine | −60° C S 14 weeks (B) [48]<br>−20° C S 30 days (U) [124] | S 30 days (M, U) [124] | S 24 h (H) [6] | S 2 weeks (B, R) [50, 65, 93] | S > 30 days at 1 mg/ml [138] |
| Vindesine | −70° C S 3 weeks (B) [153]<br>−20° C S 3 weeks (B) [153] | S 30 days (M)<br>S 3 weeks (B) [153] | – | – | S 30 days (4° C) [m] |
| Etoposide | −70° C S 3 weeks (B) [153]<br>−20° C S 3 weeks (B) [153]<br>−60° C S 77 weeks (B) [49] | S 3 weeks (B) [153] | S 6 h (M, ⩽400 μg/ml) [70] | $t_{1/2}$ 36 h (B, R) [1, 93] | S 5 years [m] |
| Teniposide | – | S 24 h (M, ⩽100 μg/ml) [139] | S 24 h (M, ⩽100 μg/ml) [70, 138, 139] | – | – |
| Maytanzine | – | S 4 days (U) [138] | S 4 days (U) [138] | – | – |

*5-Fluorouracil*

Fluorouracil is a reasonably stable drug that degrades at high pH (hydrolyses) [120] and under UV light (96, 101], but at high concentrations (50 mg/ml) in amber bottles it is stable for 8 weeks [96]. Adsorption of fluorouracil seems to be negligible [40, 41, 83, 84, 109, 110, 114], although it cannot be quantitatively recovered after having been evaporated to dryness in glass [40, 41]. By biological assay, when frozen it is stable for 2 weeks [153], and it is also reasonably stable at 37° C in medium (including 15% heat-inactivated foetal bovine serum) [65]. Fluorouracil is less cytotoxic at pH 6.8 than at pH 7.4–7.7 (as measured by the inhibition of [$^3$H]-uridine incorporation) [102] as well as in the presence of hepatocytes [2], but its cytotoxicity is not reduced by 25 mg/ml human serum albumin [134].

*6-Thioguanine*

Very little has been published about the stability of 6-thioguanine. Trissel [138] has reported that it is stable at 15 mg/ml for 24 h at 4° C but may form a precipitate at room temperature. When diluted with 150 m*M* NaCl or 5% dextrose, it is stable for 24 h at 4° C or room temperature [138].

*6-Mercaptopurine*

Mercaptopurine is sensitive to light and oxidation [4, 69], degrading to hypoxanthine in light [4]. At 10 mg/ml it is stable for >21 days at room temperature and 4° C [138]. When diluted to 3.3 mg/ml with 150 m*M* NaCl or 5% dextrose, it is stable for 7 days at 5° C [52]. Mercaptopurine frozen in plasma is stable for 52 days [37] and is neither activated nor inactivated by rat hepatocytes [2].

*Cytarabine*

Cytarabine is stable in vitro but is deaminated more rapidly in vivo to inactive uracil arabinoside [79, 103, 104, 106]. Deaminase inhibitors are required to keep the drug stable in plasma [18, 130], and prodrugs have been suggested in an attempt to limit this metabolism in vivo [60, 79].

**Table 1.** (continued)

| Drug | Most stable pH | Adsorption to filters[b] | Protein binding[c] | Effect of light[d] | Drug stability reduced by | References for frozen storage[e] |
|---|---|---|---|---|---|---|
| **Antimetabolites:** | | | | | | |
| Methotrexate | 7 [59] | 0 [110] | 94% [82] | + [3, 21, 24, 68] | Bicarbonate, aluminium [24, 92] | [34, 42, 51, 78, 119, 122, 123, 126, 127, 129, 136, 143, 144, 148] |
| 5-Fluorouracil | <9 [120] | 0 [109, 110] | – | + [95] | UV light, bisulphite [96, 101, 118] | [34, 65, 67, 78, 99, 123, 126, 127, 129, 143, 144, 148, 150] |
| 6-Thioguanine | – | – | – | – | – | [9, 10, 90, 91, 144] |
| 6-Mercaptopurine | – | – | – | + [4, 69] | Oxidation [69] | [9, 37, 129] |
| Cytarabine | 7 [105] | +/– [45, 110] | 13% [18, 133, 140] | 0 [31] | – | [9, 10, 51, 91, 99, 129, 145] |
| **Mitotic spindle inhibitors:** | | | | | | |
| Vincristine | – | + [19, 75 109, 110] | ++ [38] | +/– [138] + [m] | – | [9, 10, 16, 34, 42, 51, 65, 78, 99, 119, 126, 127, 129, 143, 144] |
| Vinblastine | – | 0 [19, 109, 110] | ++ [38] | +/– [138] 0 [m] | – | [9, 10, 34, 42, 65, 99, 119, 122, 123, 129, 143–145, 148, 150] |
| Vindesine | – | – | – | – | – | [9, 10, 36, 129] |
| Etoposide | – | – | – | + [m] | Increased drug concentration, dextrose [39, 138] | [10, 34, 51, 67, 143] |
| Teniposide | – | – | – | – | Increased drug concentration, "plastic" [138, 139] | [10, 99, 143] |
| Maytanzine | – | +++ [109, 110] | – | 0 [138] | – | [129] |

[a] Where possible, values for the stability frozen, at 4° C and at room temperature were determined in experiments with the drug dissolved in 150 m*M* NaCl or similar physiological solution; at room temperature they were usually determined in room light

[b] Drug binding to cellulose ester membranes: 0, negligible (<5%); +/–, uncertain; +, slight (5%–15%); ++, considerable (15%–50%); +++, severe (>50%). Most drugs that bind to cellulose ester also bind to other membrane materials such as PTFE [109, 110]

[c] Drug binding to plasma protein; values are expressed as either percentages or: 0, negligible; +/– uncertain; +, some binding; ++, considerable binding

[d] 0, not photolabile; +/–, uncertain; +, photolabile; ++, severely degraded by light

[e] References were tabulated for cases in which the authors have reported that "all drugs were stored frozen in solution" and the drug was used in their work. However, due to the large numbers of drugs involved, this might conceivably have been an oversimplification for the purposes of writing this paper; moreover, all of the drugs mentioned in a publication may not have always been stored frozen

[f] Drugs are listed as either stable (S) for the time given (assay method), as a figure representing 5% degradation ($t_{0.95}$) or as a $t_{1/2}$. Assay methods are abbreviated as: H, high-pressure liquid chromatography; B, biological assay; U, UV absorption; M, manufacturer's information; R, radioimmunoassay

[g] [m], information obtained from the package insert or directly from the manufacturer

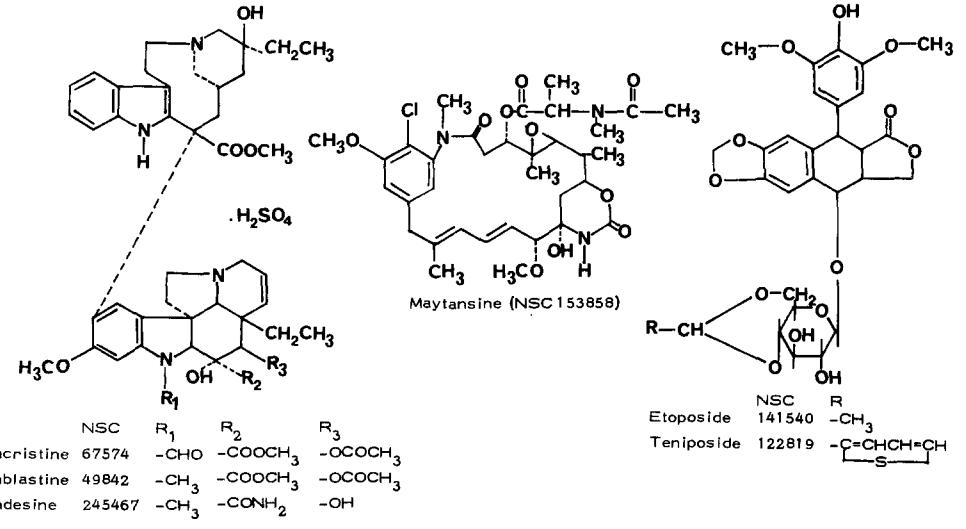



**Fig. 2.** Structures and NSC numbers of the mitotic spindle inhibitors

A constant proportion of the drug (13%–15%) is bound to plasma proteins over a wide concentration range [18, 133, 140], but this does not affect its cytotoxicity [134].

Cytarabine injection formulation can be stored "indefinitely" at room temperature [152] and, under a variety of conditions, more dilute solutions (200–1000 μg/ml) are stable for up to 14 days [31, 53, 77, 100, 105]. In 60 m*M* phosphate buffer (pH 6.9), the $t_{0.95}$ was calculated to be 3 months at room temperature [105]. There is disagreement as to whether the drug is slightly [45], or not at all [110], adsorbed by filters.

**Tubulin-binding agents**

The tubulin-binding agents include the vinca alkaloids vincristine, vinblastine and vindesine, the podophyllotoxins etoposide and teniposide, and maytansine (Fig. 2). The stability of these compounds is summarised in Table 1 and more detailed information is given below.

*Vinca alkaloids*

Originally it was suggested that the vinca alkaloids be used "as soon as possible" after reconstitution [137] and be protected from light [72, 138]. The former suggestion has been superseded by the results of stability studies, drug information sheets no longer mention any influence of light.

Both vincristine and vinblastine bind strongly to α- and β-globulins [38], and their biological activity is increased by 25 mg/ml human serum albumin [134]. According to Ferguson et al. [47], the two drugs are avidly taken up into cultured cells in a pH-dependent process, with a maximum uptake around pH 7.5, but these authors did not mention whether the pH affected cytotoxicity. This avid uptake may explain the observation of Freshney et al. [50] that whereas vinblastine is stable for 24 h in Ham's F12 medium containing 20% foetal bovine serum, the addition of HeLa cells reduces the concentration of unbound drug to about 0.005% of that in controls.

Despite their similarity in structure, vincristine binds significantly (5%–15%) to cellulose ester membranes [19, 75, 109, 110] – and the problem is increased by more dilute solutions [19] – whereas vinblastine does not [19, 109, 110]. The use of polytetrafluoroethylene (PTFE) membranes did not reduce this binding of vincristine [109, 110], whereas an unspecified "treatment" was more successful at doing so [75]. Hyperthermia has only a slight (additive) effect on the cytotoxicity of vindesine [63] and other vinca alkaloids [58].

Concentration-dependent crystallisation is a limiting factor in the stability of etoposide at room temperature [138], and the drug has been observed to precipitate in dextrose solution [39].

**Platinum drugs**

A considerable amount of work has been undertaken on the stability of cisplatin and its analogues (Fig. 3 and Table 2). Unlike that of most drugs, the degradation of these compounds does not usually follow first-order or pseudo-first-order kinetics. The components of the solution tend to form mixtures of different drug species, the proportions of which can often be relatively stable as long as the solution composition remains unchanged [26]. In general, solutions containing the leaving groups (from the

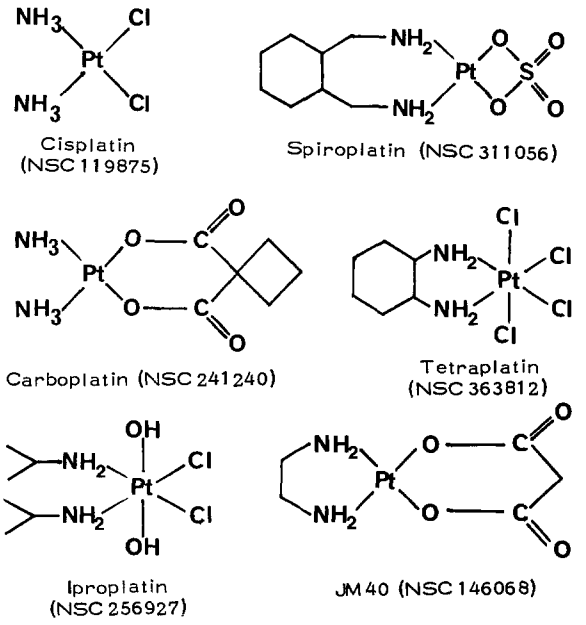



**Fig. 3.** Structures and NSC numbers of the platinum drugs

platinum) seem to confer the best stability on the drug. Thus, chloride is best for cisplatin [43, 56, 86, 94, 146] and tetraplatin [26], malonate is best for JM40 [33], and sulphate is best for spiroplatin [44]. Both water and chloride are good for iproplatin [86, 139] and carboplatin [55, 86, 117].

*Cisplatin*

For the stability of cisplatin, the main consideration is the chloride concentration of the solution; thus, whether the drug is in water [56, 86, 146], dextrose [66] or plasma [86], an increase in the chloride concentration to $\geqslant 0.1$ *M* confers the best stability. Hence, at 100 μg/ml and 37° C, cisplatin in 150 m*M* NaCl is stable for 6 days [86]. In medium (+15% foetal bovine serum), the half-life of the cell-killing ability has been reported to be 18.5 h [65].

Cisplatin has been observed to isomerise slowly to *trans*-diaminodichloroplatinum II, an inactive but toxic form [69, 146], but no details were reported except that UV light seemed to encourage the isomerisation [146], suggesting that solutions of the drug should not be placed in direct sunlight. One group [56] has suggested that normal laboratory lighting is harmful, whereas three others [66, 94, 116] have reported that it is not.

Due to the possibility of precipitation [56], it has been suggested that cisplatin not be refrigerated when the solution concentration exceeds 0.6 mg/ml [29, 39, 137]. The drug is incompatible with aluminium [11, 113] and is neutralised by thiols [125]. It does not bind to cellulose ester membrane filters [109], but does bind to proteins [61, 135], with a consequent, considerable reduction in cytotoxicity [134, 135]. The cytotoxicity of cisplatin to Walker 256 carcinoma cells has also been affected by the pH of the medium, with a minimum cytotoxicity observed at pH 7.0, which increased considerably at pH 7.4 [73]. Increased temperature also increases the cytotoxicity of the drug [62, 64], whereas cell density has no effect [108].

Frozen cisplatin has been successfully stored at high concentrations, showing minimal decomposition over

**Table 2.** Summary of the stability of the platinum, steroid and miscellaneous antitumour drugs

| Drug name | Stability frozen[a] | Stability at 4° C[a] | Stability at room temperature[a] | Stability in 150 m*M* NaCl at 37° C | Stability in medium at 37° C | Stability (room temperature) dissolved in the i.v. formulation |
|---|---|---|---|---|---|---|
| **Platinum drugs:** | | | | | | |
| Cisplatin | −70° C S 3 weeks (B) [153][f]<br>−60° C S 36 weeks (B) [48]<br>−15° C S 30 days (SI) [87] | S 6 days (<0.6 μg/ml) [29, 39, 56, 87, 137] | S 2 days (SI) [26, 87] | S 6 days (H) [86] | $t_{1/2}$ 18.5 h (B) [1, 65] | – |
| Carboplatin | −25° C S 7 days (H) [55] | – | 24 h (SI) [26] | S 7 days (H) [55, 86] | $t_{1/2}$ 94 h (B) [65] | S > 24 h at 15 mg/ml [32, 139] |
| Iproplatin | – | – | S 24 h [26, 139] | S 6 days (H) [86] | – | S 24 h at 5 mg/ml [139] |
| Spiroplatin | – | – | – | – | – | – |
| Tetraplatin | – | – | 4 h (SI) [26] | – | – | – |
| JM40 | – | – | 70 min (H) [142] | 20 h (water)<br>2 h (0.1 *M* NaCl) [33] | – | – |
| **Miscellaneous drugs:** | | | | | | |
| Asparaginase | – | S 7 days (B) [20] | S 2 days (B) [20] | – | – | S > 2 days [20, 115, 138]<br>S > 20 days (37° C) [m][g] |
| Amsacrine | – | – | – | Precipitates [138, 139] | – | S > 2 days at 5 mg/ml [139] |
| Flavone-8-acetic acid | – | – | S 14 days (H) [139] | S 10 days (H, 80° C, dark) [7] | – | S > 2 weeks at 25 mg/ml [139] |
| Interferons, α- & β | "Indefinite" [46, 151] | – | S < 3 h (no carrier protein) [89] | – | – | – |
| Mitoguazone | – | S 7 days [139] | S 7 days [139] | – | – | S > 2 days at 100 mg/ml [139] |
| PALA | – | – | S 2 weeks [139] | – | $t_{1/2}$ > 2 weeks (B) [65] | S > 2 years at 100 mg/ml [139] |
| **Steroid drugs:** | | | | | | |
| Prednisolone | – | – | S 92 days [57] | 6 h (H) [17] | – | – |
| Prednisolone sodium succinate | – | S 12 days [52] | – | – | – | – |
| Prednisone | – | – | Crystalises [57] | – | – | – |
| Dexamethasone | – | – | – | – | – | – |
| Dexamethasone sodium phosphate | Not high concentrations [138] | – | – | – | – | S 3 years [27] [m] |

3 weeks [153] and 36 weeks [48], but Yang and Drewinko [153] claim that it is not as stable when stored at more dilute concentrations.

*Other platinum drugs*

Carboplatin has been found to be stable in 150 m*M* NaCl for 7 days (100 μg/ml, 37° C) [86] and in water for 5 days at 37° C (200 μg/ml) [55]. No degradation has been observed in 5% dextrose at either high [139] or low drug concentrations [32]. Despite these good stability results, 150 m*M* NaCl has been suggested to increase the drug's decomposition to the more toxic cisplatin and is therefore not recommended as a diluent [139]. Carboplatin binds to a small extent to plasma proteins [55, 98], and the half-life of its cell-killing ability in medium is reported to be 94 h [65].

Iproplatin is stable at 40 μg/ml in 150 m*M* NaCl or water for 6 days at 37° C [86] and for at least 1 day at 5 mg/ml at room temperature [139]. Dextrose (5%) does not seem to reduce this stability, but it has been suggested that solutions are protected from light if stored for > 24 h [139]. Spiroplatin readily forms an equilibrium with a number of aquatic species [44]. The drug precipitates in chloride-containing solutions [141].

JM40 is stable for 2 days in water [141] or 5% glucose [142], whereas it is unstable in 150 m*M* NaCl [142], with

**Table 2.** (continued)

| Drug name | Most stable pH | Adsorption to filters[b] | Protein binding[c] | Effect of light[d] | Drug stability reduced by | References for frozen storage[e] |
|---|---|---|---|---|---|---|
| **Platinum drugs:** | | | | | | |
| Cisplatin | – | 0 [109] | +/++ [61, 132, 135] | +/– [56, 66, 94, 116] | <0.1 *M* chloride, UV light, aluminium, thiols [11, 56, 66, 86, 107, 113, 125, 146] | [34, 65, 67, 74, 112, 119, 126, 127, 129, 143–145, 148–150] |
| Carboplatin | – | – | +/– [55, 98] | – | Chloride [26, 139] | [65, 129] |
| Iproplatin | – | – | 0 [98] | +/– [139] | – | – |
| Spiroplatin | – | – | – | – | Chloride [141] | – |
| Tetraplatin | – | – | – | – | <0.1 *M* chloride [26] | – |
| JM40 | ⩾ 7 [33] | – | – | – | Chloride [33] | – |
| **Miscellaneous drugs:** | | | | | | |
| Asparaginase | 5–9 [115] | – | – | – | PVC, dilution, preservatives, shaking [30, 54, 80, 115, 138] | [9, 10, 51] |
| Amsacrine | – | – | 97% [111] | – | Chloride [138, 139] | [10, 51, 143, 144] |
| Flavone-8-acetic acid | <10 [155] | 0 [7] | 0[h] | ++ [7] | Dextrose [139] | [7] |
| Interferons, α- & β- | – | – | – | – | Lack of 5 mg/ml human serum albumin as carrier protein, mechanical stress [46, 151] | [143–145] |
| Mitoguazone | – | – | – | 0 [139] | ? Preservatives [54] | [143] |
| PALA | – | 0 [110] | – | – | – | [65, 126, 129] |
| **Steroid drugs:** | | | | | | |
| Prednisolone | – | – | ++ [17, 71, 88, 97] | ?+ [28] | Citrate [57] | – |
| Prednisolone sodium succinate | – | – | – | + [28] [m] | – | [10, 16] |
| Prednisone | – | – | – | + [28] | – | – |
| Dexamethasone | – | – | – | + [28] [m] | – | – |
| Dexamethasone sodium phosphate | – | 0 [121] | – | + [138] | – | [51, 150] |

[a] Where possible, values for the stability frozen, at 4° C and at room temperature were determined in experiments with the drug dissolved in 150 m*M* NaCl or similar physiological solution; at room temperature they were usually determined in room light

[b] Drug binding to cellulose ester membranes: 0, negligible (<5%); +, slight (5%–15%); ++, considerable (15%–50%); +++, severe (>50%). Most drugs that bind to cellulose ester also bind to other membrane materials such as PTFE [109, 110]

[c] Drug binding to plasma protein; values are expressed as either percentages or: 0, negligible; +/– uncertain; +, some binding; ++, considerable binding

[d] 0, not photolabile; +/–, uncertain; +, photolabile; ++, severely degraded by light

[e] References were tabulated for cases in which the authors have reported that "all drugs were stored frozen in solution" and the drug was used in their work. However, due to the large numbers of drugs involved, this might conceivably have been an oversimplification for the purposes of writing this paper; moreover, all of the drugs mentioned in a publication may not have always been stored frozen

[f] Drugs are listed as either stable (S) for the time given (assay method), as a figure representing 5% degradation ($t_{0.95}$) or as a $t_{1/2}$. Assay methods are abbreviated as: H, high-pressure liquid chromatography; B, biological assay; U, UV absorption; M, manufacturer's information; R, radioimmunoassay

[g] [m], information obtained from the package insert or directly from the manufacturer

[h] Betteridge and Bosanquet, unpublished observation

both chloride and low pH displacing the malonate group [33]. When the drug was dissolved in water at 37° C, a $t_{0.95}$ of 20 h was observed for JM40; its stability was increased by the addition of potassium malonate to the solution [33].

### Other antitumour drugs

The structures of antitumour drugs that are not high-molecular-weight proteins are shown in Figs. 4 and 5. Stability information is given in Table 2, and further details about some of these drugs are recorded below.

*Amsacrine*

Amsacrine cannot be diluted with chloride-containing solutions, as amsacrine hydrochloride is poorly soluble [138, 139]; thus, a lactate buffer is used with the formulated product. The cytotoxicity of the drug is reduced by 25 mg/ml human serum albumin [134], presumably due to its very high protein binding [111]. In contrast to almost all other cytotoxic drugs, the cytotoxicity of amsacrine is *reduced* by treating cells at 42.4° C [63]; this effect is not caused by drug degradation [63].

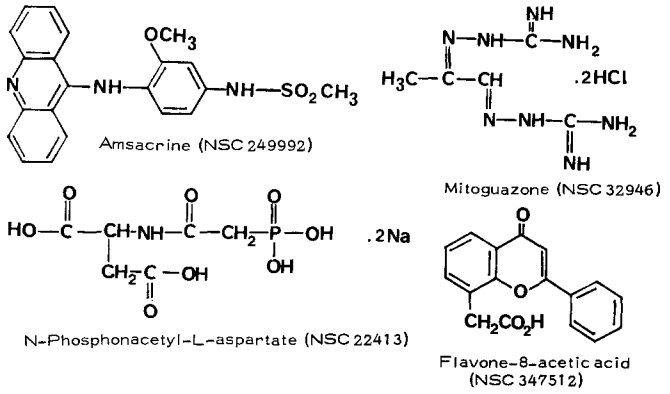



**Fig. 4.** Structures and NSC numbers o the miscellaneous antitumour agents

### *L-Asparaginase*

L-Asparaginase is a rather different sort of cytotoxic drug acting, it is thought, by reducing L-asparagine levels. It has been isolated from a number of different sources, including *Erwinia carotovora, Escherichia coli* [20, 80] and other bacteria [35]. The *E. coli* variety is a tetramer with a molecular weight of 255,000 daltons; each tetramer is capable of being split into subunits weighing about 22,000 daltons [80]. At high concentrations (> 2 mg/ml), L-asparaginase is stable in PBS for 2 days at 20° C [20, 115, 138], but dilution to < 1 μg/ml causes rapid deactivation [30, 154], probably due to adsorption onto vessel surfaces [115]. PVC containers have been found to be worse than glass, polyethylene, polystyrene or steel containers in this respect [115]. The drug's stability is also reduced by preservatives [54] and shaking [115, 138] but increased by non-ionic detergents and polyethylene glycol [115].

### *Interferons*

The interferons are proteins weighing around 20,000 daltons. Their stability is difficult to assess because of the large number of different species known and because accurate determination of their activity is difficult. There is a distinct need for a good interferon assay [12].

The α- and β-interferons are more stable than γ-interferon, the latter being heat- and acid-sensitive [147]. Good stability has been reported for α-interferon at < −10° C [46, 151]. The most important aspect of the stability of these molecules seems to be the presence of 5 mg/ml human serum albumin as a carrier protein [151]. Without this, a solution of β-interferon in 150 m*M* NaCl was only stable for 2 h at room temperature.

### *Steroids*

Prednisone, prednisolone and dexamethasone (Fig. 5) are all derivatives of cortisone normally used in combination with other cytotoxic agents. Prednisolone sodium succinate and prednisolone sodium phosphate are used both for i.v. injection and quite often in vitro due to their increased solubility, but these drugs come with a number of other additives [138]. Prednisolone binds strongly to proteins [17, 88], even at low protein concentrations [71]; surprisingly, this binding is inhibited by hydrocortisone but not by prednisone [88]. Prednisolone does not adsorb to plastic delivery systems [83, 84]. The succinate and phosphate esters are more stable in solution than is prednisolone [17, 52, 57, 85, 131].

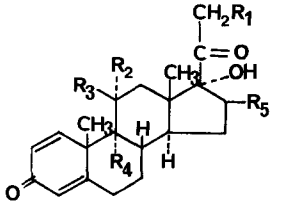
The stability of dexamethasone (sodium phosphate) is probably similar to that of the other cytotoxic steroids [28]. The sodium succinate ester has shown good stability in a number of dosage forms [8].

## Conclusions and recommendations

In general, the stability of a drug should be checked when the drug is first handled, even if the stability of a close analogue is already known; although many of the drugs mentioned in this review are reasonably stable, there are sometimes small but significant differences in stability between close analogues.

The light sensitivity of a number of drugs mentioned here remains unclear, perhaps because initial observations suggested light sensitivity, whereas it was later realised that such an effect was very minor compared with other causes of degradation. We recently reported a similar situation with chlorambucil [15].

*Acknowledgements.* I thank the Cancer Research Campaign for supporting me during the compilation of this work and Sue Forskitt for drawing the structures and for her help in preparing the manuscript.



| | NSC | $R_1$ | $R_2$ | $R_3$ | $R_4$ | $R_5$ |
|---|---|---|---|---|---|---|
| Prednisolone | 9120 | -OH | -H | -OH | -H | -H |
| Prednisolone sodium phosphate | | $-OPO_3Na_2$ | -H | -OH | -H | -H |
| Prednisolone sodium succinate | 9151 | $-OCO(CH_2)_2COONa$ | -H | -OH | -H | -H |
| Prednisone | 10023 | -OH | =O | | -H | -H |
| Dexamethazone | 34521 | -OH | -H | -OH | -F | $-CH_3$ |

**Fig. 5.** Structures and NSC numbers of the steroid antitumour agents

## References


1. Ali-Osman F, Giblin J, Dougherty D, Rosenblum ML (1987) Application of in vivo and in vitro pharmacokinetics for physiologically relevant drug exposure in a human tumor clonogenic cell assay. Cancer Res 47: 3718–3724
2. Alley MC, Powis G, Appel PL, Kooistra KL, Lieber MM (1984) Activation and inactivation of cancer chemotherapeutic agents by rat hepatocytes cocultured with human tumor cell lines. Cancer Res 44: 549–556
3. Battelli G, Marra M, Romagnoli E, Tagliapietra L, Tassara A (1983) Fotosensibilita' di soluzioni acquose di methotrexate e di acido ascorbico. Boll Chim Farm 123: 149–157
4. Benezra SA, Foss PRB (1978) 6-Mecaptopurine. In: Florey K (ed) Analytical profiles of drug substances, vol 7. Academic, New York, pp 343–357
5. Benvenuto JA (1983) Errors in oncolytic agent stability study. Am J Hosp Pharm 40: 1628
6. Benvenuto JA, Anderson RW, Kerkof K, Smith RG, Loo TL (1981) Stability and compatibility of antitumor agents in glass and plastic containers. Am J Hosp Pharm 38: 1914–1918
7. Betteridge RF, Land CP, Bosanquet AG (1988) Stability of flavone-8-acetic acid LM975 in aqueous solutions by high-performance liquid chromatography. J Pharmaceut Biomed Anal 6: 875–878
8. Biles JA (1961) Optical crystallographic properties of some glucocorticoids. J Pharm Sci 50: 464–468
9. Bird MC, Bosanquet AG, Gilby ED (1985) In vitro determination of tumour chemosensitivity in haematological malignancies. Hematol Oncol 3: 1–10
10. Bird MC, Bosanquet AG, Forskitt S, Gilby ED (1986) Semi-micro adaptation of a 4-day differential staining cytotoxicity (DiSC) assay for determinating the in vitro chemosensitivity of haematological malignancies. Leuk Res 10: 445–449
11. Bohart RD, Ogawa G (1979) An observation on the stability of *cis*-dichlorodiammineplatinum(II): a caution regarding its administration. Cancer Treat Rep 63: 2117–2118
12. Bontempo JA (1987) The need for a collaborative effort to develop a common, rapid, automated assay for natural and recombinant interferons. J Parenter Sci Technol 41: 2
13. Bosanquet AG (1985) Stability of solutions of antineoplastic agents during preparation and storage for in vitro assays. General considerations, the nitrosoureas and alkylating agents. Cancer Chemother Pharmacol 14: 83–95
14. Bosanquet AG (1986) Stability of solutions of antineoplastic agents during preparation and storage for in vitro assays: II. Assay methods, adriamycin and the other antitumour antibiotics. Cancer Chemother Pharmacol 17: 1–10
15. Bosanquet AG, Clarke HE (1986) Chlorambucil: stability of solutions during preparation and storage. Cancer Chemother Pharmacol 18: 176–179
16. Bosanquet AG, Bird MC, Price WJP, Gilby ED (1983) An assessment of a short-term tumour chemosensitivity assay in chronic lymphocytic leukaemia. Br J Cancer 47: 781–789
17. Boudinot FD, Jusko WJ (1984) Fluid shifts and other factors affecting plasma protein binding of prednisolone by equilibrium dialysis. J Pharm Sci 73: 774–780
18. Breithaupt H, Schick J (1981) Determination of cytarabine and uracil arabinoside in human plasma and cerebrospinal fluid by high-performance liquid chromatography. J Chromatogr 225: 99–106
19. Butler LD, Munson JM, DeLuca PP (1980) Effect of inline filtration on the potency of low-dose drugs. Am J Hosp Pharm 37: 935–941
20. Cammack KA, Marlborough DI, Miller DS (1972) Physical properties and subunit structure of L-asparaginase isolated from *Erwinia carotovora*. Biochem J 126: 361–379
21. Chahidi C, Morliere P, Aubailly M, Dubertret L, Santus R (1983) Photosensitization by methotrexate photoproducts. Photochem Photobiol 38: 317–322
22. Chamberlin AR, Cheung APK, Lim P (1976) Methotrexate. In: Florey K (ed) Analytical profiles of drug substances, vol 5. Academic, New York, pp 283–306
23. Chatterji DC, Gallelli JF (1977) High-pressure liquid chromatographic analysis of methotrexate in presence of its degradation products. J Pharm Sci 66: 1219–1222
24. Chatterji DC, Gallelli JF (1978) Thermal and photolytic decomposition of methotrexate in aqueous solutions. J Pharm Sci 67: 526–531
25. Chen M-L, Chiou WL (1982) Adsorption of methotrexate onto glassware and syringes. J Pharm Sci 71: 129–131
26. Cheung Y-W, Cradock JC, Vishnuvajjala BR, Flora KP (1987) Stability of cisplatin, iproplatin, carboplatin, and tetraplatin in commonly used intravenous solutions. Am J Hosp Pharm 44: 124–130
27. Coffman HD, Crabbs WC, Joachims GL, Kolinski RE, Page DP (1983) Stability of sterile dexamethasone acetate suspensions and dexamethasone sodium phosphate injections submitted by US hospitals. Am J Hosp Pharm 40: 2165–2169
28. Cohen EM (1973) Dexamethasone. In: Florey K (ed) Analytical profiles of drug substances, vol 2. Academic, New York, pp 163–197
29. Cohen MR (1980) Medication error reports. Error 148 – more on cisplatin storage. Hosp Pharm 15: 158–159
30. Cooney DA, Davis RD (1970) The stability of L-asparaginase with respect to protein denaturants. Biochim Biophys Acta 212: 134–138
31. Cradock JC, Kleinman LM, Rahman A (1978) Evaluation of some pharmaceutical aspects of intrathecal methotrexate sodium, cytarabine and hydrocortisone sodium succinate. Am J Hosp Pharm 35: 402–406
32. Curt GA, Grygiel JJ, Corden BJ, Ozols RF, Weiss RB, Tell DT, Myers CE, Collins JM (1983) A phase I and pharmacokinetic study of diamminecyclobutanedicarboxylatoplatinum (NSC 241240). Cancer Res 43: 4470–4473
33. Cutbush SD, Kuroda R, Neidle S, Robins AB (1983) The antitumor complex ethylenediamine platinum (II) malonate: X-ray structure analysis, and studies of its stability in solution. J Inorg Biochem 18: 213–220
34. Daniels JR, Daniels AM, Luck EE, Whitman B, Casagrande JT, Skinner DG (1981) Chemosensitivity of human neoplasms with in vitro clone formation. Cancer Chemother Pharmacol 6: 245–251
35. Davidson L, Burkom M, Ahn S, Chang L-C, Kitto B (1977) L-Asparaginases from *Citrobacter freundii*. Biochim Biophys Acta 480: 282–294
36. De Vries EGE, Meijer C, Mulder NH, Postmus PE (1987) In vitro chemosensitivity of human lung cancer for vindesine. Eur J Cancer Clin Oncol 23: 55–60
37. Ding TL, Benet LZ (1979) Determination of 6-mercaptopurine and azathioprine in plasma by high-performance liquid chromatography. J Chromatogr 163: 281–288
38. Donigian DW, Owellen RJ (1973) Interaction of vinblastine, vincristine and colchicine with serum proteins. Biochem Pharmacol 22: 2113–2119
39. Dozier N, Ballentine R (1983) Practical considerations in the preparation and administration of cancer chemotherapy. Am J IV Ther Clin Nutr 10 (Sept): 6–8, 10, 13–15
40. Driessen O, Vos D de, Timmermans PJA (1978) Adsorption of fluorouracil on glass surfaces. J Pharm Sci 67: 1494–1495
41. Driessen O, Vos D de, Timmermans PJA (1979) Sensitive gas-liquid chromatographic assay of underivatized 5-fluorouracil in plasma. J Chromatogr 162: 451–456
42. Durie BGM (1984) Experimental approaches to drug testing and clonogenic growth: results in multiple myeloma and acute myelogenous leukemia. Recent Results Cancer Res 94: 93–101
43. Earhart RH (1978) Instability of *cis*-dichlorodiammineplatinum in dextrose solution. Cancer Treat Rep 62: 1105–1106
44. Elferink F, Vijgh WJF van der, Poort SEJM van der, Henzen-Logmans SC, Pinedo HM (1984) Influence of hydrolysis

products of aqua(1,1-bis(aminomethyl)cyclohexane)sulfatoplatinum(II) on toxicity in rats. Cancer Lett 25: 61–69
45. Ennis CE, Merritt RJ, Neff DN (1983) In vitro study of inline filtration of medications commonly administered to pediatric cancer patients. JPEN 7: 156–158
46. Evans S, Grassam P (1986) Considerations for parenteral dosage form development of natural alpha interferon. J Parenter Sci Technol 40: 83–87
47. Ferguson PJ, Phillips JR, Selner M, Cass CE (1984) Differential activity of vincristine and vinblastine against cultured cells. Cancer Res 44: 3307–3312
48. Franco R, Kraft T, Miller T, Popp M, Martelo O (1984) Storage of chemotherapy drugs for use in the human tumor stem cell assay. Int J Cell Cloning 2: 2–8
49. Franco R, Miller T, Kraft T, Popp M, Martelo O (1985) The stability of five chemotherapy drugs stored frozen for use in the human tumor stem cell assay. Int J Cell Cloning 3: 424–426
50. Freshney RI, Paul J, Kane IM (1975) Assay of anti-cancer drugs in tissue culture; conditions affecting their ability to incorporate $^{3}$H-leucine after drug treatment. Br J Cancer 31: 89–99
51. Funa K, Dawson N, Jewett PB, Agren H, Ruckdeschel JC, Bunn PA Jr, Gazdar AF (1986) Automated fluorescent analysis for drug-induced cytotoxicity assays. Cancer Treat Rep 70: 1147–1151
52. Gallelli JF (1967) Stability studies of drugs used in intravenous solutions. part I. Am J Hosp Pharm 24: 425–433
53. Gannon PM, Sesin GP (1983) Stability of cytarabine following repackaging in plastic syringes and glass containers. Am J IV Ther Clin Nutr 10 (June): 11–12, 14–16
54. Garnett W (1971) Diluents for neoplastic drugs. Drug Intell Clin Pharm 5: 261
55. Gaver RC, Deeb G (1986) High-performance liquid chromatographic procedures for the analysis of carboplatin in human plasma and urine. Cancer Chemother Pharmacol 16: 201–206
56. Greene RF, Chatterji DC, Hiranaka PK, Gallelli JF (1979) Stability of cisplatin in aqueous solution. Am J Hosp Pharm 36: 38–43
57. Gupta VD (1979) High-pressure liquid chromatographic evaluation of aqueous vehicles for preparation of prednisolone and prednisone liquid dosage forms. J Pharm Sci 68: 908–910
58. Hahn GM (1982) Hyperthermia and cancer. Plenum, New York, p 84
59. Hansen J, Kreilgård B, Nielsen O, Veje J (1983) Kinetics of degradation of methotrexate in aqueous solution. Int J Pharm 16: 141–152
60. Hayashikawa RH, Nagyvary J (1973) Studies in vitro on some precursors of 1-β-D-arabinosylcytosine. Biochem Pharmacol 22: 609–622
61. Hecquet B, Meynadier J, Bonneterre J, Adenis L, Demaille A (1985) Time dependency in plasmatic protein binding of cisplatin. Cancer Treat Rep 69: 79–83
62. Herman TS (1983) Temperature dependence of adriamycin, *cis*-diamminedichloroplatinum, bleomycin, and 1,3-bis (2-chloroethyl)-1-nitrosourea cytotoxicity in vitro. Cancer Res 43: 517–520
63. Herman TS (1983) Effect of temperature on the cytotoxicity of vindesine, amsacrine, and mitoxantrone. Cancer Treat Rep 67: 1019–1022
64. Herman TS, Henle KJ, Nagle WA, Moss AJ, Monson TP (1984) Effect of step-down heating on the cytotoxicity of adriamycin, bleomycin, and *cis*-diamminedichloroplatinum. Cancer Res 44: 1823–1826
65. Hildebrand-Zanki SU, Kern DH (1986) A rapid bioassay to determine stabilities of anticancer agents under conditions of the clonogenic assay. In Vitro Cell Devel Biol 22: 247–252
66. Hincal AA, Long DF, Repta AJ (1979) Cis-platin stability in aqueous parenteral vehicles. J Parenter Drug Assoc 33: 107–116
67. Hug V, Thames H, Hortobagyi G, Finders M (1986) Chemosensitivities of human clonogenic breast tumor cells. Eur J Cancer Clin Oncol 22: 971–981
68. Humphreys A, Marty JJ, Gooey SL, Bourne DWF (1978) Stability of methotrexate in an intravenous fluid. Aust J Hosp Pharm 8: 66–67
69. IARC (1981) IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, vol 26, IARC, Lyons
70. Issell BF (1982) The podophyllotoxin derivatives VP16-213 and VM26. Cancer Chemother Pharmacol 7: 73–80
71. Iwayama S, Yasaki T, Miyata T, Asano Y (1983) Simply diluting serum before radioimmunoassay does not prevent binding of prednisolone to protein. Clin Chem 29: 1989
72. Jacobs J (1970) Drug stability and compatibility in injections and intravenous infusions. Pharm J 205: 437–442
73. Jauhiainen K, Kangas L, Käpylä H, Alfthan O (1985) Intravesical cytostatics: pH-dependence of antitumour activity. Urol Res 13: 19–21
74. Johnson GE, Glaubiger DL (1983) Correlation of cellular tritiated thymidine incorporation with soft agar clonogenicity in chemosensitivity testing of human neuroblastoma cells. Cancer Treat Rep 67: 163–168
75. Kanke M, Eubanks JL, DeLuca PP (1983) Binding of selected drugs to a "treated" inline filter. Am J Hosp Pharm 40: 1323–1328
76. Karlsen J, Thonnesen HH, Olsen RI, Sollien AH, Skobba TJ (1983) Stability of cytotoxic intravenous solutions subjected to freeze-thaw treatment. Nor Pharm Acta 45: 61–67
77. Keller JH, Ensminger WD (1982) Stability of cancer chemotherapeutic agents in a totally implanted drug delivery system. Am J Hosp Pharm 39: 1321–1323
78. Kern DH, Lee MB (1984) Association between histopathologic factors and sensitivity of human colon cancer to 5-fluorouracil. In: Salmon SE, Trent JM (eds) Human tumor cloning. Grune and Stratton, Orlando, pp 575–584
79. Kirsch LE, Notari RE (1984) Aqueous conversion kinetics and mechanisms of ancitabine, a prodrug of the antileukemic agent cytarabine. J Pharm Sci 73: 896–902
80. Kirschbaum J, Wriston JC Jr, Ratych OT (1969) Subunit structure of L-asparaginase from *Escherichia coli* B. Biochim Biophys Acta 194: 161–169
81. Kirschenbaum BE, Latiolais CJ (1976) Stability of injectable medications after reconstitution. Am J Hosp Pharm 33: 767–791
82. Koch-Weser J, Sellers EM (1976) Binding of drugs to serum albumin. N Engl J Med 294: 311–316
83. Kowaluk EA, Roberts MS, Blackburn HD, Polack AE (1981) Interactions between drugs and polyvinyl chloride infusion bags. Am J Hosp Pharm 38: 1308–1314
84. Kowaluk EA, Roberts MS, Polack AE (1982) Interactions between drugs and intravenous delivery systems. Am J Hosp Pharm 39: 460–467
85. Kreienbaum MA (1986) Stability of betamethasone sodium phosphate, hydrocortisone sodium phosphate, and prednisolone sodium phosphate injections submitted by US hospitals. Am J Hosp Pharm 43: 1747–1750
86. Krull IS, Ding X-D, Braverman S, Selavka C, Hochberg F, Sternson LA (1983) Trace analysis for cis-platinum anti-cancer drugs via LCEC. J Chromatogr Sci 21: 166–173
87. LaFollette JM, Arbus MH, Lauper RD (1985) Stability of cisplatin admixtures in polyvinyl chloride bags. Am J Hosp Pharm 42: 2652
88. Legler UF, Benet LZ (1986) The effect of prednisone and hydrocortisone on the plasma protein binding of prednisolone in man. Eur J Clin Pharmacol 30: 51–55
89. Leong SS, Horoszewicz JS, Mirand EA (1983) The stability of human fibroblast interferon (HuIFN-β). Prog Clin Biol Res 132E: 327–336

90. LePage GA, Whitecar JP Jr (1971) Pharmacology of 6-thioguanine in man. Cancer Res 31: 1627–1631
91. Lihou MG, Smith PJ (1983) Quantitation of chemosensitivity in acute myelocytic leukaemia. Br J Cancer 48: 559–567
92. Lough M, Watson ID, Stewart MJ (1987) Degradation of methotrexate in an automated procedure for extracting it from serum. Clin Chem 33: 1072
93. Ludwig R, Alberts DS (1984) Chemical and biological stability of anticancer drugs used in a human tumor clonogenic assay. Cancer Chemother Pharmacol 12: 142–145
94. Mariani EP, Southard BJ, Woolever JT, Erlich RH, Granatek AP (1980) Physical compatibility and chemical stability of cisplatin in various diluents and in large-volume parenteral solutions. In: Prestayko AW, Crooke ST, Carter SK (eds) Cisplatin. Current status and new developments. Academic, New York, pp 305–316
95. Milewski R, Jones D (1981) Photodecomposition. Hosp Pharm 16: 178
96. Milovanovic D, Nairn JG (1980) Stability of fluorouracil in amber glass bottles. Am J Hosp Pharm 37: 164–165
97. Milsap RL, Plaisance KI, Jusko WJ (1984) Prednisolone disposition in obese men. Clin Pharmacol Ther 36: 824–831
98. Momburg R, Bourdeaux M, Sarrazin M, Roux F, Briand C (1985) In vitro plasma binding of some second generation antitumor platinum complexes. Eur J Drug Metab Pharmacokinet 10: 77–83
99. Morgan D, Freshney RI, Darling JL, Thomas DGT, Celik F (1983) Assay of anticancer drugs in tissue culture: cell cultures of biopsies from human astrocytoma. Br J Cancer 47: 205–214
100. Munson JW, Kubiak EJ, Cohon MS (1982) Cytosine arabinoside stability in intravenous admixtures with sodium bicarbonate and in plastic syringes. Drug Intell Clin Pharm 16: 765–767
101. Niemiec PW Jr, Vanderveen TW (1984) Compatibility considerations in parenteral nutrient solutions. Am J Hosp Pharm 41: 893–911
102. Nissen E, Tanneberger S (1981) Influence of pH and serum on the effectivity of antineoplastic agents in vitro. Arch Geschwulstforsch 51: 152–156
103. Notari RE (1967) A mechanism for the hydrolytic deamination of cytosine arabinoside in aqueous buffer. J Pharm Sci 56: 804–809
104. Notari RE, Chin ML, Cardoni A (1970) Intermolecular and intramolecular catalysis in deamination of cytosine nucleosides. J Pharm Sci 59: 28–32
105. Notari RE, Chin ML, Wittebort R (1972) Arabinosylcytosine stability in aqueous solutions: pH profile and shelflife predictions. J Pharm Sci 61: 1189–1196
106. Notari RE, Witiak DT, DeYoung JL, Lin AJ (1972) Comparative kinetics of cytosine nucleosides. Influence of a 6-methyl substituent on degradation rates and pathways in aqueous buffers. J Med Chem 15: 1207–1214
107. Ogawa GS, Young R, Munar M (1985) Dispensing-pin problems. Am J Hosp Pharm 42: 1042–1045
108. Ohnuma T, Arkin H, Holland JF (1986) Effects of cell density on drug-induced cell kill kinetics in vitro (inoculum effect). Br J Cancer 54: 415–421
109. Pavlik EJ, Nagell JR Jr van, Hanson MB, Donaldson ES, Powell DE, Kenady DE (1982) Sensitivity to anticancer agents in vitro: standardizing the cytotoxic response and characterizing the sensitivities of a reference cell line. Gynecol Oncol 14: 243–261
110. Pavlik EJ, Kenady DE, Nagell JR van, Keaton K, Hanson MB, Donaldson ES, Griffen WO, Flanigan RC (1983) Properties of anticancer agents relevant to in vitro determinations of human tumor cell sensitivity. Cancer Chemother Pharmacol 11: 8–15
111. Paxton JW, Jurlina JL, Foote SE (1986) The binding of amsacrine to human plasma proteins. J Pharm Pharmacol 38: 432–438
112. Pontén J, Larson A, Lindström M (1984) Chemosensitivity tests by a miniclone technique. Med Oncol Tumor Pharmacother 1: 19–23
113. Prestayko AW, Cadiz M, Crooke ST (1979) Incompatibility of aluminum-containing i.v. administration equipment with *cis*-dichlorodiammineplatinum(II) administration. Cancer Treat Rep 63: 2118–2119
114. Quebbeman EJ, Ausman RK, Hamid AAR, Caballero G, Hoffman NE (1984) Drug concentration errors related to sampling from silastic catheters. J Clin Oncol 2: 712
115. Rauenbusch E, Bauer K, Kaufmann W, Wagner O (1970) Isolation and crystallisation of L-asparaginase from *E. coli*. Recent Results Cancer Res 33: 31–38
116. Repta AJ, Long DF, Hincal AA (1979) *cis*-Dichlorodiammineplatinum(II) stability in aqueous vehicles. Cancer Treat Rep 63: 229–230
117. Roos RW, Lau-Cam CA (1986) General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base. J Chromatogr 370: 403–418
118. Rork GS, Pitman IH (1975) Bisulfite-ion-catalyzed degradation of fluorouracil. J Pharm Sci 64: 216–220
119. Ruckdeschel JC, Carney DN, Oie HK, Russell EK, Gazdar AF (1987) In vitro chemosensitivity of human lung cancer cell lines. Cancer Treat Rep 71: 697–704
120. Rudy BC, Senkowski BZ (1973) Fluorouracil. In: Florey K (ed) Analytical profiles of drug substances, vol 2, Academic, New York, pp 221–244
121. Rusmin S, Welton S, DeLuca P, DeLuca PP (1977) Effect of inline filtration on the potency of drugs administered intravenously. Am J Hosp Pharm 34: 1071–1074
122. Salmon SE, Hamburger AW, Soehnlen B, Durie BGM, Alberts DS, Moon TE (1978) Quantitation of differential sensitivity of human-tumor stem cells to anticancer drugs. N Engl J Med 298: 1321–1327
123. Sandbach J, Von Hoff DD, Clark G, Cruz AB Jr, O'Brien M (1982) Direct cloning of human breast cancer in soft agar culture. Cancer 50: 1315–1321
124. Saul VG, Tanneberger S (1975) Stabilität klinisch gebräuchlicher Zytostatika. Arch Geschwulstforsch 45: 259–267
125. Sauter C, Cogoli M, Arrenbrecht S, Marti C (1984) Neutralization of *cis*-dichlorodiammineplatinum II and nitrogen mustard by thiols. Recent Results Cancer Res 94: 232–236
126. Scheithauer W, Clark GM, Moyer MP, Von Hoff DD (1986) New screening system for selection of anticancer drugs for treatment of human colorectal cancer. Cancer Res 46: 2703–2708
127. Schieder K, Bieglmayer C, Breitenecker G, Csaicsich P, Rogan A, Janisch H (1984) Drug sensitivity testing on in vivo and in vitro passages of a human melanoblastoma by use of the human tumor colony forming assay. In: Salmon SE, Trent JM (eds) Human tumor cloning. Grune and Stratton, Orlando, pp 459–475
128. Sesin GP, Millette LA, Weiner B (1982) Stability study of 5-fluorouracil following repackaging in plastic disposable syringes and multidose vials. Am J IV Ther Clin Nutr 9 (Sept): 23–25, 29–30
129. Shoemaker RH, Wolpert-DeFilippes MK, Kern DH, Lieber MM, Makuch RW, Melnick NR, Miller WT, Salmon SE, Simon RM, Vendetti JM, Von Hoff DD (1985) Application of a human tumor colony-forming assay to new drug screening. Cancer Res 45: 2145–2153
130. Sinkule JA, Evans WE (1983) High-performance liquid chromatographic assay for cytosine arabinoside, uracil arabinoside and some related nucleosides. J Chromatogr 274: 87–93
131. Stroud N, Richardson NE, Davies DJG, Norton DA (1980) Quality control of prednisolone sodium phosphate. Analyst 105: 455–461
132. Sykes TR, Stephens-Newsham LG, Noujaim AA (1985) In vitro binding of platinum compounds to human transferrin. Res Commun Chem Pathol Pharmacol 50: 387–394

133. Szekerke M, Horvath M, Hudecz F (1979) Antitumor drug-protein interactions. Binding of 1-β-D-arabinofuranosyl cytosine to albumin and chemically modified albumin. Arzneim-Forsch/Drug Res 29: 19–21
134. Takahashi I, Ohnuma T, Kavy S, Bhardwaj S, Holland JF (1980) Interaction of human serum albumin with anticancer agents in vitro. Br J Cancer 41: 602–608
135. Takahashi K, Seki T, Nishikawa K, Minamide S, Iwabuchi M, Ono M, Nagamine S, Horinishi H (1985) Antitumor activity and toxicity of serum protein-bound platinum formed from cisplatin. Jpn J Cancer Res 76: 68–74
136. Taylor IW, Tattersall MHN (1981) Methotrexate cytotoxicity in cultured human leukemic cells studied by flow cytometry. Cancer Res 41: 1549–1558
137. Trester AK, Nader ML (1982) Guide for the administration and use of cancer chemotherapeutic agents. Am J IV Ther Clin Nutr 9 (Nov): 9–31
138. Trissel LA (1983) Handbook on injectable drugs, 3rd edn. American Society of Hospital Pharmacists, Washington, DC
139. Trissel LA, Davignon JP, Kleinman LM, Cradock JC, Flora KP, Quinn FR, Vishnuvajjala R, Morgan JM (1987) NCI investigational drugs – pharmaceutical data. National Institutes of Health, Bethesda
140. Van Prooijen HC, Vierwinden G, Wessels J, Haanen C (1977) Cytosine arabinoside binding to human plasma proteins. Arch Int Pharmacodyn 229: 199–205
141. Vermorken JB (1984) Clinical experience with platinum analogues in The Netherlands. Pharm Weekbl 119: 1161–1166
142. Vijgh WJF van der, Elferink F, Postma GJ, Vermorken JB, Pinedo HM (1984) Determination of ethylenediamineplatinum(II) malonate in infusion fluids, human plasma and urine by high-performance liquid chromatography. J Chromatogr 310: 335–342
143. Von Hoff DD, Casper J, Bradley E, Sandbach J, Jones D, Makuch R (1981) Association between human tumor colony-forming assay results and response of an individual patient's tumor to chemotherapy. Am J Med 70: 1027–1032
144. Von Hoff DD, Clark GM, Stogdill BJ, Sarosdy MF, O'Brien MT, Casper JT, Mattox DE, Page CP, Cruz AB, Sandbach JF (1983) Prospective clinical trial of a human tumor cloning system. Cancer Res 43: 1926–1931
145. Von Hoff DD, Clark GM, Weiss GR, Marshall MH, Buchok JB, Knight WA III, LeMaistre CF (1986) Use of in vitro dose response effects to select antineoplastics for high-dose or regional administration regimens. J Clin Oncol 4: 1827–1834
146. Vrana O, Kleinwachter V, Brabec V (1984) Polarographic activity of the antitumor drug *cis*-dichlorodiammineplatinum(II). The effect of hydrolysis and trans-isomerization of the drug. Experientia 40: 446–451
147. Walsh JP (1985) The interferons. Med Lab Sci 42: 346–351
148. Weisenthal LM, Dill PL, Kurnick NB, Lippman ME (1983) Comparison of dye exclusion assays with a clonogenic assay in the determination of drug-induced cytotoxicity. Cancer Res 43: 258–264
149. Weisenthal LM, Lalude AO, Miller JB (1983) In vitro chemosensitivity of human bladder cancer. Cancer 51: 1490–1496
150. Weisenthal LM, Marsden JA, Dill PL, Macaluso CK (1983) A novel dye exclusion method for testing in vitro chemosensitivity of human tumors. Cancer Res 43: 749–757
151. WHO Expert Committee (1985) Biological standardization. WHO Tech Rep Ser 725: 46–49
152. Wolfert RR, Cox RM (1975) Room temperature stability of drug products labeled for refrigerated storage. Am J Hosp Pharm 32: 585–587
153. Yang L-Y, Drewinko B (1985) Cytotoxic efficacy of reconstituted and stored antitumor agents. Cancer Res 45: 1511–1515
154. Yoshimoto T, Takahashi K, Ajima A, Matsushima A, Saito Y, Tamaura Y, Inada Y (1985) Activation and stabilization of asparaginase by anti-asparaginase IgG and its Fab. FEBS Lett 183: 170–172
155. Zaharko DS, Grieshaber CK, Plowman J, Cradock JC (1986) Therapeutic and pharmacokinetic relationships of flavone acetic acid: an agent with activity against solid tumors. Cancer Treat Rep 70: 1415–1421

Received March 1, 1988/Accepted November 8, 1988