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The use of a new laser particle sizer and shape analyser to detect and evaluate gelatinous microparticles suspended in reconstituted anthracycline infusion solutions

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Abstract: The anthracyclines are an important group of antitumour drugs; the best known anthracyclines are doxorubicin (Adriamycin^R) and epirubicin (Pharmorubicin^R), both of which are very active against a wide range of solid tumours and haematological malignancies. They are marketed as lyophilized formulations that need to be reconstituted for administration with water for injections or sodium chloride injection. With the aim of reducing the risks of contamination during reconstitution (spillage, spray formation, etc.) and of enhancing the rate of dissolution (that is otherwise slow because of the formation of conglomerates and gelatinous masses), a new formulation (rapid dissolution formula, RDF) containing parabens (hydroxybenzoate esters) as anti-aggregants has been developed; the formulation is a freeze-dried product and is characterized by a practically instantaneous and complete reconstitution. A valid estimate of the completeness of dissolution has been objectively achieved by means of an instrument (Galai CIS-1) that acts both as a particle sizer and a shape analyser; the instrument is equipped with a rotating laser system that defines a toroidal–cylindrical space inside the solution in which every moving particle is measured and, at the same time, visualized on a monitor by an electronically driven video microscope. The instrument has been applied with very satisfactory results to the visualization of the reconstitutional behaviour of commercial lots of Adriamycin and Pharmorubicin lyophilized products, reconstituted at a concentration of 2 mg ml^{-1} with sodium chloride injection.

Keywords: *Adriamycin; Pharmorubicin; rapid dissolution formula; laser scattering; particle size determination; gelatinous lumps.*

Introduction

The anthracyclines are well known for their cytotoxic properties and have been widely studied for more than 20 years for their clinical antineoplastic activity. The best known anthracycline developed by Farmitalia Carlo Erba is doxorubicin (Adriamycin^R) (Fig. 1), [1], which has been found to be very active against a wide range of solid tumours and haematological malignancies [2–12].

Subsequent studies, aimed at the improvement of the therapeutic index of anticancer drugs, led to the development of another anthracycline, epirubicin (Pharmorubicin^R) (Fig. 2) that is active against neoplastic diseases but has a lower general toxicity. Doxorubicin and epirubicin have been

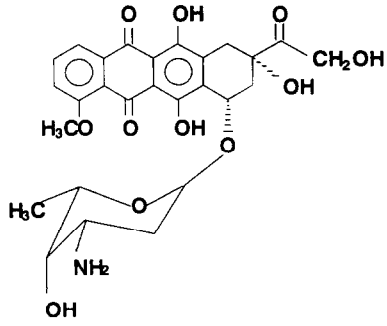

Figure 1
Doxorubicin.

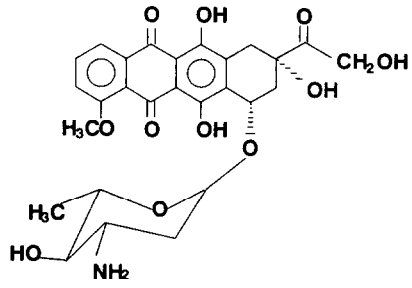

Figure 2
Epirubicin.

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Table 1
Lyophilized typical formulations (50 mg vial)

Adriamycin freeze-dried	
Doxorubicin hydrochloride	50.0 mg
Lactose monohydrate	250.0 mg
Pharmorubicin freeze-dried	
Epirubicin monohydrate	50.0 mg
Lactose monohydrate	250.0 mg

Table 2
Rapid dissolution formulations (50 mg vial)

Adriamycin RDF	
Doxorubicin hydrochloride	50.0 mg
Methyl parahydroxybenzoate	5.0 mg
Lactose monohydrate	250.0 mg
Pharmorubicin RDF	
Epirubicin monohydrate	50.0 mg
Methyl parahydroxybenzoate	10.0 mg
Lactose monohydrate	250.0 mg

marketed as lyophilized formulations (Table 1) that need to be reconstituted for administration with water for injections or sodium chloride injection; the latter is widely preferred in worldwide hospital practice since it allows direct intravenous infusion.

Nevertheless, the use of these lyophilized formulations has generated some technical problems since the handling of such preparations exposes pharmacists, medical staff and nurses to significant risks of contamination because of the reconstitution process (possible spillage, spray formation, etc.). Furthermore, reconstitution with sodium chloride injection takes place comparatively slowly owing to the formation of conglomerates and gelatinous masses, whose complete dissolution requires continuous and vigorous shaking often by hand for a prolonged period of time (up to 5 min or even longer). This may be due to the tendency of anthracycline molecules to associate by forming dimeric or polymeric aggregates which probably lower the wettability of the drug in the freeze-dried state and consequently decrease the dissolution rate.

New formulations

The discovery by Farmitalia Galenical R&D workers of the anti-aggregant power of parabens (hydroxybenzoate esters) against the natural tendency of molecules of the anthracycline compounds to associate to form polymers, led to the development of new patented formulations for doxorubicin and epirubicin, known as Adriamycin rapid dissolution formula (RDF) and Pharmorubicin rapid dissolution formula (RDF), respectively; the compositions are reported in Table 2. The new RDF formulations are characterized by a practically instantaneous complete reconstitution, thus achieving not only a dramatic time saving but also a reduction of the potential hazard to personnel involved with the prolonged handling of these drugs.

Although the reconstitution times of the two formulations reveal a clear difference between the original products (without methylparaben in the composition) and the new products (RDF) [13, 14], it is considered that this parameter is not the only one to be taken into account in examining the advantages of the new RDF preparations in respect of safety in intravenous administration.

In the authors' experience, evaluation of the completeness of reconstitution with sodium chloride injection of the formulations without methylparaben is difficult owing to the formation of transparent gelatinous lumps of undissolved product; these lumps are not easily detectable against the dark red background of the solution by visual inspection alone. The difficulty of obtaining a valid estimate of the complete dissolution of the drug product has been recently highlighted in a test carried out in five oncological centres in the USA, where the hospital pharmacists were requested to reconstitute, with sodium chloride injection, three commercially available freeze-dried preparations containing doxorubicin; the results are reported in Table 3.

The scattering of the data can be correlated in part with the differences in dissolution technique in the hospitals tested but also shows up the importance of individual judgement in the estimation of the completeness of reconstitution. The present studies therefore have

Table 3
Mean (n = 20) dissolution times in minutes at 5 min

	Test sites				
Test sites	1	2	3	4	5
Adriamycin RDF (doxorubicin HCl, USP)	3.2	0.1	2.2	1.4	0.6
Rubex (Bristol)	23.6	5.8	25.9	15.1	4.0
Doxorubicin from Cetus	36.8	6.8	25.8	14.0	4.7

been aimed at the development of an instrumental technique capable of giving an objective evaluation of the extent of reconstitution achieved. The laser scattering instruments available on the market until now have not been able to visualize the shape and size of such gelatinous microparticles since these systems are able to provide information only about the distribution of suspended particles, ideally considered spherical, with a maximum diameter of about 1000–1600 μm.

The recent commercial availability of an instrument that acts both as a particle sizer and a shape analyser (Galai CIS-1, manufactured by Galai Production Ltd, Migdal Haemek, Israel) has helped in solving this visualization problem.

The Galai CIS-1 particle sizer and shape analyser

This instrument (Fig. 3) is equipped with a rotating laser system that defines a toroidal-cylindrical space inside the solution in which moving particles are measured and their size determined by quenching of the laser light emerging at 180°C; a synchronized flash takes pictures of anything passing through the focus of a microscopic video system, allowing visualization on a monitor by an electronic image-capturing card. On the screen are clearly seen the images of all particles in movement in front of the synchronous flash, independent of their size.

The Galai instrument can be used for the quantitative and qualitative examination of particles dispersed in a liquid (liquid dispersion) or a gas (aerosol or airborne particles) or of particles smeared on a surface (solid sample measurement), with different types of measurement cells.

In addition to its use in the determination of particle-size distribution in medicines, the instrument can be used as a shape analyser in the evaluation of the stability of opaque emulsions, such as cosmetic products, and in studies of variations in the dimensions of multilamellar liposomes in suspension during storage. Also the ratios between excipients and the drug substance can be measured in finished pharmaceutical products directly in the solid state;

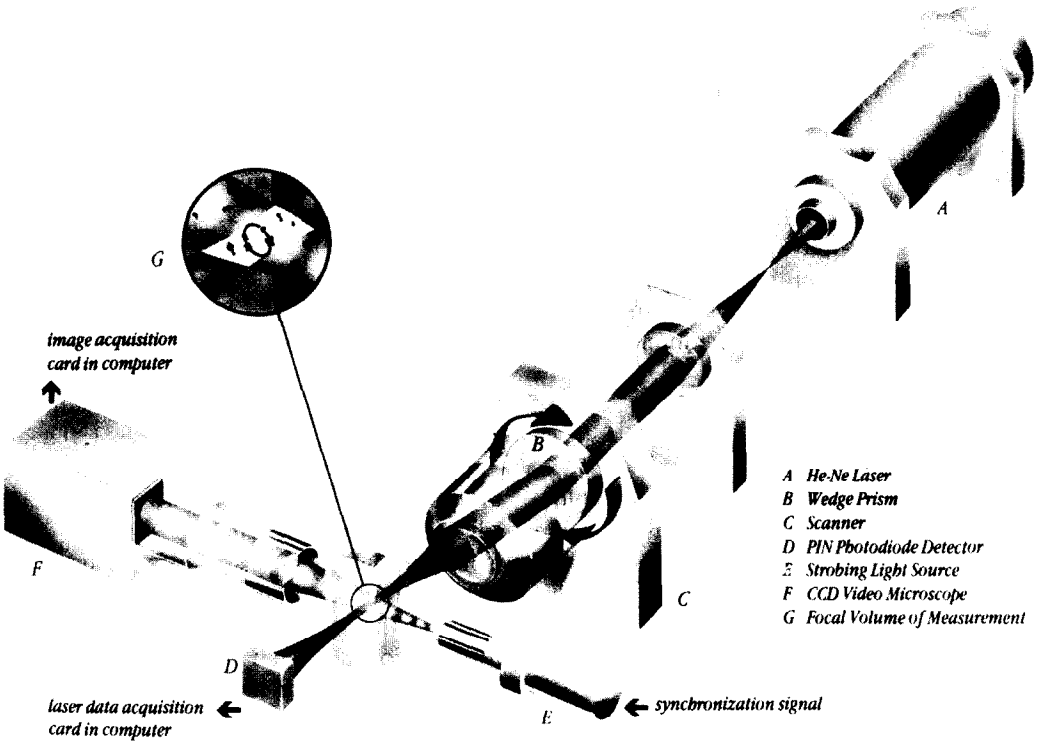

Figure 3
Schematic diagram of Galai CIS-1 particle sizer and shape analyser.

useful information can be obtained on the shape of the particles at the various stages in the manufacture of the product.

This new instrument has been applied to the visualization of reconstitutional behaviour in a comparison of commercial lots of Adriamycin and Pharmorubicin lyophilized products, reconstituted at a concentration of 2 mg ml^{-1} with sodium chloride injection.

Experimental

Apparatus

The Galai CIS-1 particle size and shape analyser (Fig. 3) used for this study was equipped with a CCD (charge coupled diode) video microscope with a synchronized flash to capture dynamic images of the particles in suspension, and with Shape Analysis Galai software, version 4.2 T, to obtain two-dimensional geometric and densitometric information. During the analyses, the CCD camera parameters were set up on the computer as follows: F1 = cursor size, 15; F2 = system calibration and camera factor, 2.412 and 0.808; F3 = measurement units, μm; F4 = microscope magnification, 3.11 (lens D, for the range 10–1200 μm); F5 = distribution size, 60; F6 = colour programming, linear; F7 = light correction, off; F8 = external camera frame, X1 = 6 X2 = 507 Y1 = 19 Y2 = 463; F8 = internal camera frame, X1 = 56 X2 = 457 Y1 = 69 Y2 = 413; F9 = image monitor type, B&W.

The synchronous flash was set in the slow position. The images on the screen were taken at preset times, with continuous stirring (speed of stirring set at position 2 = medium), using a Nikon F3 camera.

Preparation of samples

After the addition of 25 ml of sodium chloride injection, the vials under analysis were shaken by hand for exactly 30 s; then samples (about 2.0 ml) of the reconstituted solutions were transferred directly into disposable 4.5-ml clear polystyrene cuvettes, equipped with plastic stirrers, for examination.

This reconstitution procedure was then repeated on subsequent vials, which were shaken by hand for exactly 1 and 3 min, respectively, and the solutions examined as previously described.

Batches examined

A: Doxorubicin 50 mg, Cetus, Emeryville, CA — Batch No. P9A233. B: Rubex™ 50 mg, Bristol, Evansville, IN — Batch No. A9F10A. C: Adriamycin 50 mg, Farmitalia Carlo Erba, Milano — Batch No. 9005AC. D: Adriamycin RDF 50 mg, Farmitalia Carlo Erba, Milano — Batch No. 9010BC. E: Pharmorubicin 50 mg, Farmitalia Carlo Erba, Milano — Batch No. 9006DC. F: Pharmorubicin 50 mg, Farmitalia Carlo Erba, Milano — Batch No. 9014DC. G: Pharmorubicin RDF 50 mg, Farmitalia Carlo Erba, Milano — Batch No. 9002JC. H: Pharmorubicin RDF 50 mg, Farmitalia Carlo Erba, Milano — Batch No. 9010JC.

Results and Discussion

The results are presented in Figs 4–11 as the images appeared on the screen after preset times. A comparison of the images reveals very marked differences between the preparations without methylparaben and the RDF preparations with methylparaben.

If 1–3 min is considered to be a reasonable time for pharmacists and nurses to reconstitute a single vial by hand-shaking, the pictures clearly highlight the fact that only the RDF preparations, both for Adriamycin and Pharmorubicin (Figs 7 and 10), dissolve completely; in contrast all the other formulations (Figs 4–6 and Figs 8 and 9) still contain in suspension very large masses and aggregates (500–600 μm or larger) of irregular shape.

After 3 min, the RDF formulations show a practically clear screen, and therefore complete reconstitution [Fig 7(c) for Adriamycin RDF and Fig. 10(c) and 11(c) for Pharmorubicin RDF]; these images are quite different from those obtained with the other solutions [Figs 4(c), 5(c), 6(c) and 9(c)]. Furthermore, for reduced hand-shaking times (30 s and 1 min) the monitor shows that the RDF preparations present only few small particles (diameter not exceeding 100 μm) in comparison with the large masses existing in the other formulations. In addition, a quantitative evaluation of the reconstitutional behaviour of the formulations is given by the particle-size distributions reported in Fig. 12 for Adriamycin preparations (FICE old formulation, FICE RDF, Bristol "Rubex®" and Cetus doxorubicin hydrochloride injection) and in Fig. 13 for Pharmorubicin preparations (FICE old formulation and FICE RDF).

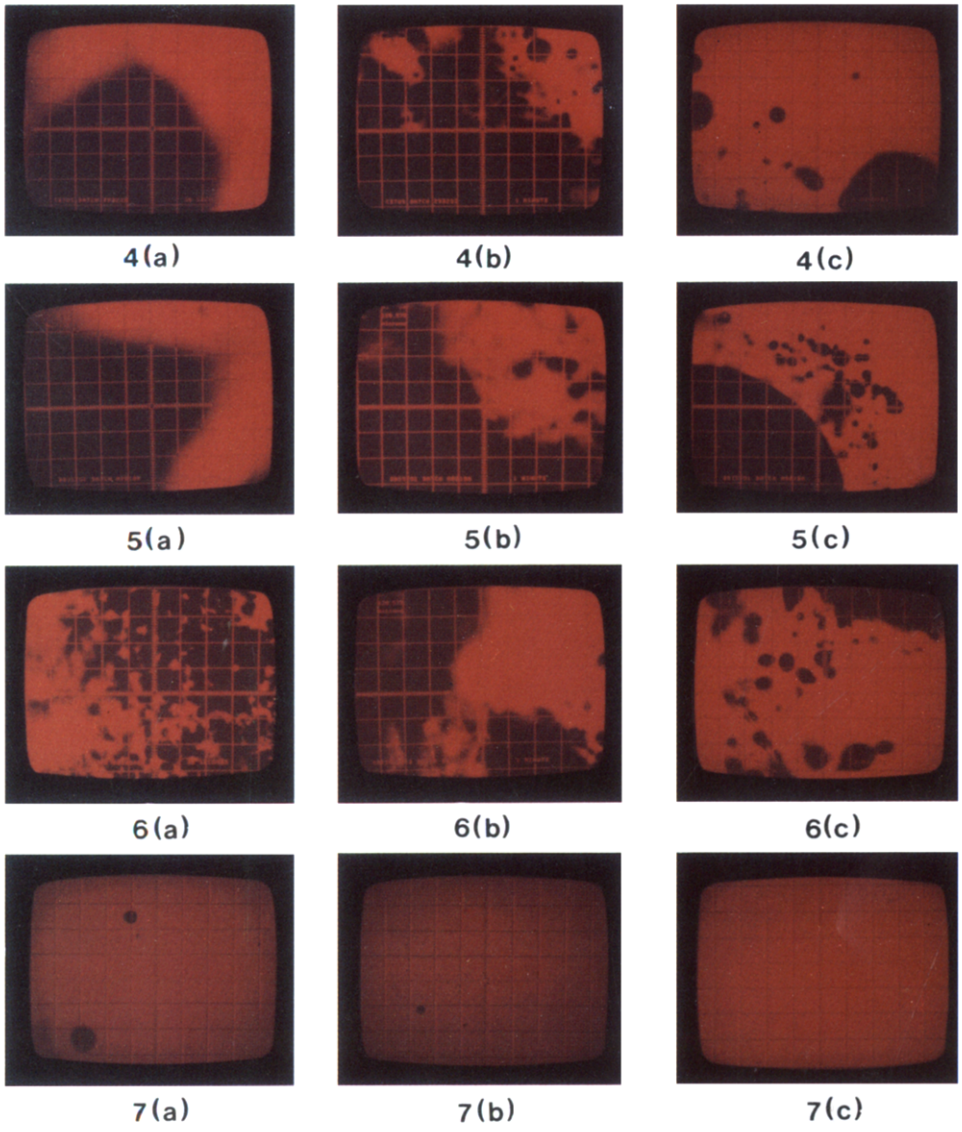

Figure 4
Doxorubicin 50 mg Cetus — Batch A No. P9A233 (a) after 30 s; (b) after 1 min; and (c) after 3 min.
Scale: 1 grid = 120. 579 μm.

Figure 5
Rubex™ 50 mg Bristol — Batch B No. A9F10A (a) after 30 s; (b) after 1 min; and (c) after 3 min. Scale as in Fig. 4.

Figure 6
Adriamycin 50 mg FICE — Batch C No. 9005AC (a) after 30 s; (b) after 1 min; and (c) after 3 min. Scale as in Fig. 4.

Figure 7
Adriamycin RDF 50 mg FICE — Batch D No. 9010BC (a) after 30 s; (b) after 1 min; and (c) after 3 min. Scale as in Fig. 4.

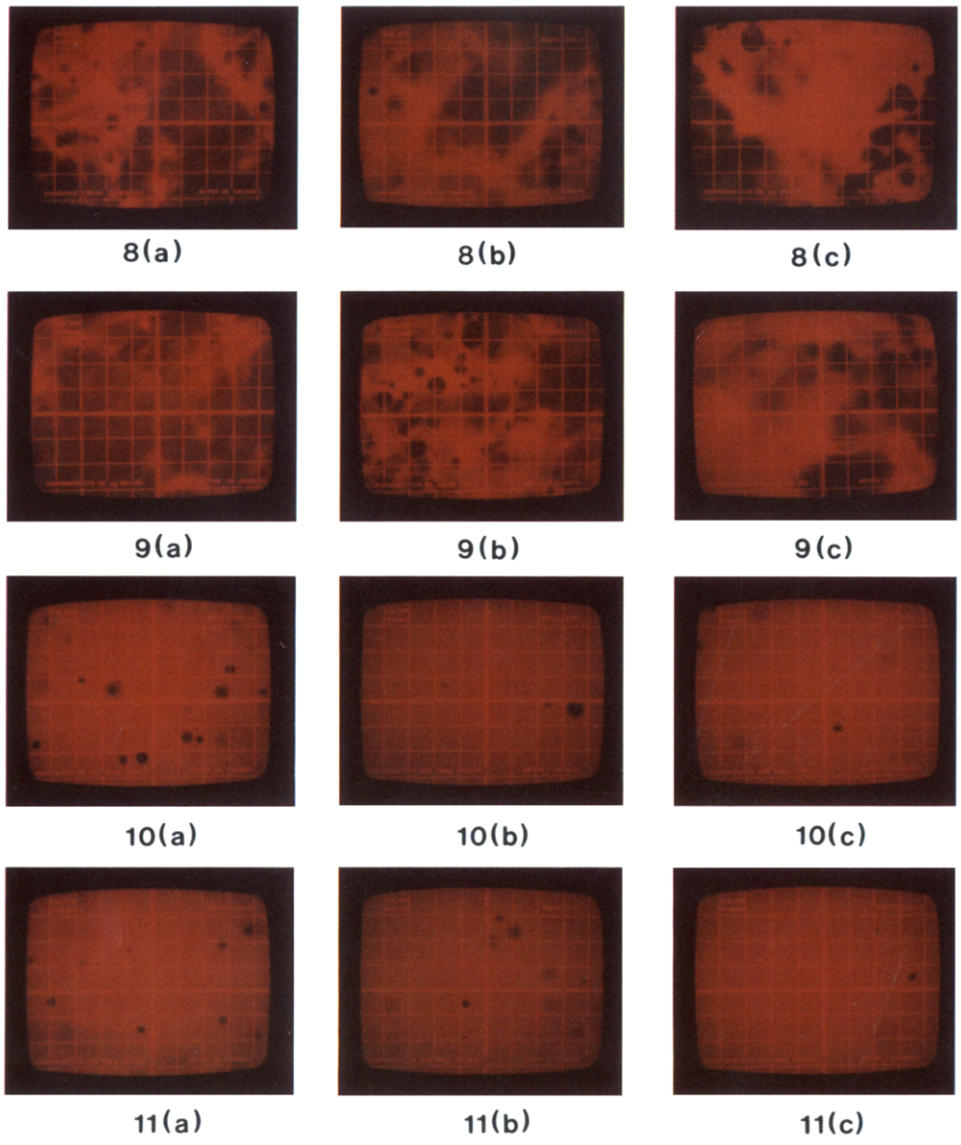

Figure 8
Pharmorubicin 50 mg FIC — Batch E No. 9006DC (a) after 30 s; (b) after 1 min; and (c) after 3 min. Scale as in Fig. 4.

Figure 9
Pharmorubicin 50 mg FICE — Batch F No. 9014DC (a) after 30 s; (b) after 1 min; and (c) after 3 min. Scale as in Fig. 4.

Figure 10
Pharmorubicin RDF 50 mg FICE — Batch No. G 9002JC (a) after 30 s; (b) after 1 min; and (c) after 3 min. Scale as in Fig. 4.

Figure 11
Pharmorubicin RDF 50 mg FICE — Batch H No. 9010JC (a) after 30 s; (b) after 1 min; and (c) after 3 min. Scale as in Fig. 4.

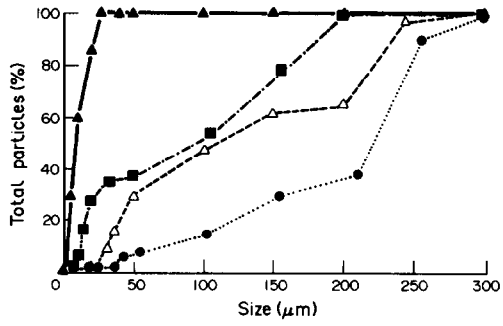

Figure 12
Adriamycin particle size distribution. Equilibrium time, 2 min (Galai equipment). ▲, Adriamycin RDF FICE; △, Rubex Bristol; ■, Adriamycin FICE; ●, doxorubicin Cetus.

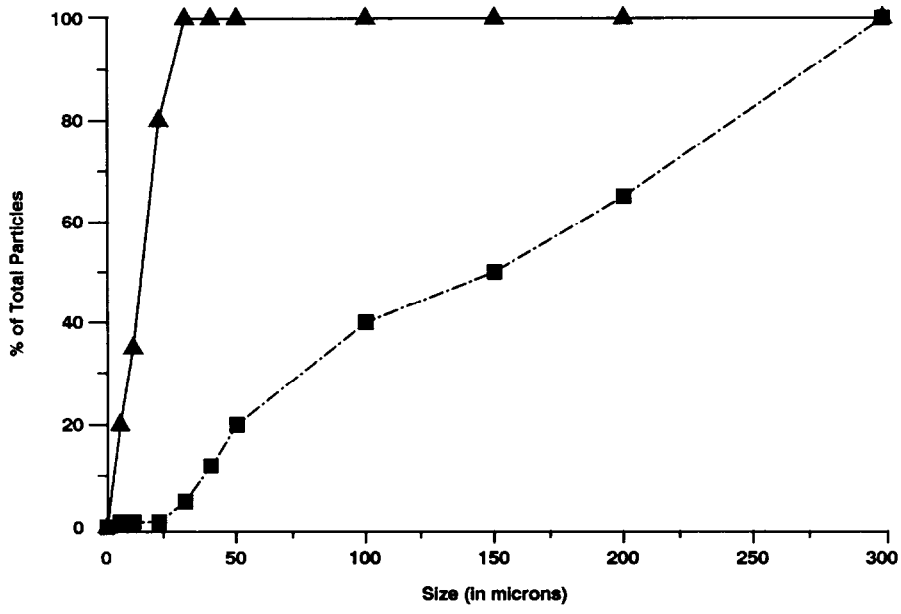

Figure 13
Pharmorubicin particle size distribution. Equilibrium time, 2 min (Galai equipment). ■, Pharmorubicin FICE; ▲, Pharmorubicin RDF FICE.

Conclusions

The analyses performed on the Galai CIS-1 particle size and shape analyser have permitted the study of the physical phenomena that are involved in the reconstitution of two different commercial formulations of Adriamycin and Pharmorubicin, using sodium chloride injection and hand-shaking up to 3 min, as frequently occurs in hospital practice.

The data obtained reveal marked differences in the reconstitutional behaviour between the RDF (of both Adriamycin and Pharmorubicin) and the other preparations formulated without methylparaben.

These differences were not observable by the simple determination of the time for reconstitution or by simple visual inspection since most of the suspended gelatinous microparticles in the solution are not visible to the human eye.

The new technique for shape analysis allowed the objective comparison of the complete reconstitution of the RDF formulations with the incomplete reconstitution observed with the original formulations without methylparaben.

The complete and fast dissolution achieved represents an important result in respect of time saving and of a marked reduction in the

potential hazards for the personnel involved in handling the drug product since the risk of spillage increases with the hand-shaking time.

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References

[1] F. Arcamone, Academic Press (1981).
[2] M. Ghione, J. Fetzer and H. Maier (Eds) Adriamycin Symposium of Frankfurt/Main. Springer, New York (1974).
[3] W.B. Pratt and R.W. Ruddon, in *The Anticancer Drugs*, pp. 155–170. Oxford University Press, Oxford (1979)
[4] R.T. Dorr and W.L. Frits, in *Cancer Chemotherapy Handbook*, pp. 373–378 and 388–401. Elsevier, Amsterdam (1980).
[5] C.E. Myers, Anthracyclines. In *Pharmacological Principles of Cancer Treatment* (B. Chabner, Ed.), pp. 416–434. W.B. Saunders, Philadelphia (1982).
[6] J.R. Brown, *Prog. Med. Chem.* **15**, 125–164 (1978).
[7] J.R. Brown and S.H. Imam, *Prog. Med. Chem.* **21**, 170–236 (1984).
[8] S.K. Carter, *J. Natl. Cancer Inst.* **55**, 1265–1273 (1975).
[9] P.M. Wilkinson, in *Clinical Pharmacology of Antineoplastic Drugs* (H.M. Pinedo, Ed.), Vol. 1, pp. 209–223. Elsevier/North Holland Biomedical Press, New York (1978).
[10] H.L. Davis and T.E. Davis, *Cancer Treat. Rep.* **63**, 809–816 (1979).
[11] A. Di Marco, *Expt Clin. Res.* **9**, 751–765 (1983).
[12] G. Aubel-Sadron and D. Londos-Gagliardi, *Biochimie* **66**, 333–353 (1984).
[13] A. Murphy, S. Maltby and A.P. Launchbury, *Int. J. Pharm.* **38**, 257–259 (1987).
[14] T. Baumann, M. Smythe, K. Kaufmann, Z. Miloboszeswi, J. O'Malley and R. Fudge, *Am. J. Hosp. Pharm.* **45**, 1667 (1988).

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