

Spectral analysis of doxorubicin and indirect quantification of its DNA intercalation

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Doxorubicin (Dox) is a cytostatic drug of first choice in many malignancies, but its high efficacy in cancer treatment is however restrained by its unwanted side-effects. To eliminate such complications, high effort is spent to design new drug forms, with optimized pharmacokinetics, pharmacodynamics and eventually targeted to site of tumors (1, 2). Macromolecular systems based on linear polymers, nanoparticles, liposomes, dendrimers and many other systems were tested up today as potential candidates (2, 3). To optimize such systems, intracellular pathway, rate of accumulation inside cells and translocation of active compound to target compartments was necessary to analyze. An intrinsic fluorescence of doxorubicin seemed to be a great tool to analyze that. However, situation is much more complicated than it seems to be. It was documented that Dox releases spontaneously in aqueous media degradation product D* described by Fiallo et al (4) whose fluorescence yield is about 30 times stronger compared to parent drug. This contamination was detected also in some of polymeric conjugates bearing doxorubicin bound via amidic bond. Because of its enormous fluorescent activity, this compound almost completely overlaps doxorubicin fluorescence.

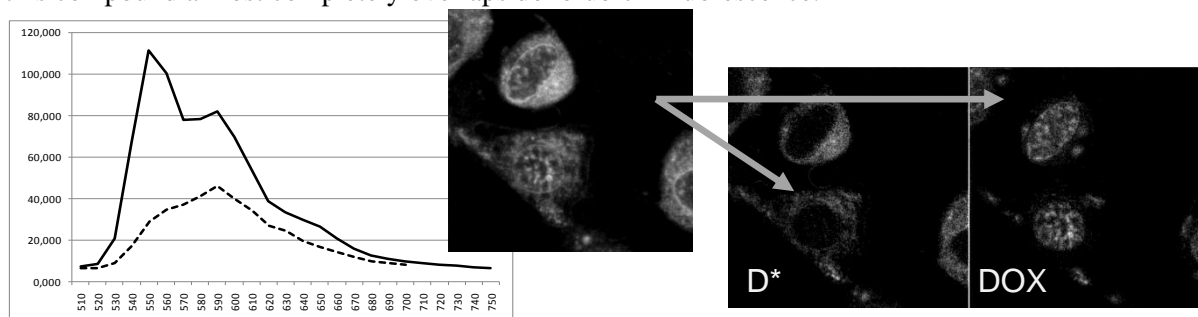


Fig.1 A: spectral characteristic of doxorubicin (- -) and D* (—). **B:** spectral unfixing in cells treated with Dox/D* (5:1).

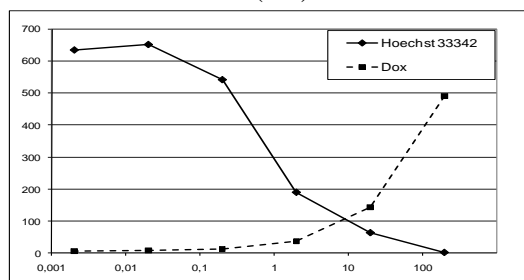


Fig. 2 Flow cytometry analysis of the proportion of doxorubicin fluorescence to fluorescence intensity of Hoechst 33342. Cells were incubated with doxorubicin and counter-stained with Hoechst 33342. Decrease of Hoechst fluorescence is directly proportional to increase of doxorubicin concentration.

To directly analyze accumulation of doxorubicin or doxorubicin bearing drug delivery systems by flow cytometry is problematic. Because major cytotoxic effect of doxorubicin is targeted against nuclear DNA, it could be an advantage to measure instead of increase of doxorubicin fluorescence, intercalation competition with Hoechst 33342, detectable as decrease Hoechst staining. Indirect detection of doxorubicin uptake is more sensitive, compared to direct detection of doxorubicin fluorescence and furthermore there is no doubt about intranuclear localization of doxorubicin.

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