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Is dialysis a reliable method for studying drug release from nanoparticulate systems?—A case study

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ABSTRACT

The kinetics of in vitro drug release from nanoparticulate systems is extensive, though uncritically, being studied by dialysis. Evaluating the actual relevance of dialysis data to drug release was the purpose of this study. Diclofenac- or ofloxacin-loaded chitosan nanoparticles crosslinked with tripolyphosphate were prepared and characterized. With each drug, dynamic dialysis was applied to nanoparticle dispersion, solution containing dissolved chitosan·HCl, and solution of plain drug. Drug kinetics in receiving phase (KRP), nanoparticle matrix (KNM) and nanoparticle dispersion medium (KDM) were determined. Release of each drug from nanoparticles was also assessed by ultracentrifugation. Although KRP data may be interpreted in terms of sustained release from nanoparticles, KNM and KDM data show that, with both drugs, the process was in fact controlled by permeation across dialysis membrane. Analysis of KRP data reveals a reversible interaction of diclofenac with dispersed nanoparticle surface, similar to the interaction of this drug with dissolved chitosan·HCl. No such interactions are noticed with ofloxacin. The results from the ultracentrifugation method agree with the above interpretation of dialysis data. This case study shows that dialysis data from a nanoparticle dispersion is not necessarily descriptive of sustained-release from nanoparticles, hence, if interpreted uncritically, it may be misleading.

1. Introduction

A tremendous effort has been and is currently being devoted to the research in the field of pharmaceutical nanotechnology. In vitro drug release has commonly been determined to characterize medicated nanoparticulate systems, along with other properties, such as, e.g., particle shape, size, zeta-potential, drug encapsulation efficiency, etc. The dynamic dialysis method has extensively been used to measure the release kinetics. A porous membrane of 12 kDa (or less) MW cut-off (MWCO) has usually been used to separate the donor phase, containing the medicated nanoparticulate system, from the receiving phase, where sink conditions for the drug with respect to the donor phase were maintained. The receiving phase was generally analyzed for the drug and the rate of drug appearance in this phase was generally taken as the rate of drug release from nanoparticles (see, e.g., Essa et al., 2011; Hao et al., 2011; Jain et al., 2011; Kulhari et al., 2011; Nagarwal et al., 2011; Pandita et al., 2011; Saremi et al., 2011; Tan and Liu, 2011; Wang et al., 2011a, 2011b; Xu et al., 2011). The majority of these articles report a release pattern characterized by a short-lasting burst release followed by a longer-lasting sustained release. On this basis hypotheses on release mechanism and drug location in nanostructures were made.

However, the following concepts ought to be given consideration:

Drug appearance in the receiving phase of dialysis is the result of a sequence of two steps: (1) drug release from the nanoparticulate matrix into the dispersion medium (donor phase of dialysis), and (2) drug permeation across the dialysis membrane. Assuming drug transport from donor to acceptor being controlled by step (1) would imply assuming no significant resistance to drug transport being opposed by the dialysis membrane. This has generally been taken for granted, in fact, the only experimental support for a sustained release from nanoparticles has been the finding that with the nanoparticle dispersion the dialysis was slower than with the solution of the free drug (see, e.g., Tan and Liu, 2011). Nevertheless, we point out that a lower dialysis rate in the presence than in the absence of dispersed nanoparticles could also be found if drug transport were controlled by step (2) and the drug molecules, after a comparatively rapid step (1), were involved in an equilibrium interaction with the dispersed nanoparticles. This interaction would lower the drug thermodynamic activity in the donor solution, hence, the activity gradient in the membrane, hence, the dialysis rate. In this event the assumption of a sustained release from nanoparticles would be misleading. Substantially similar concepts as the above were illustrated in the past by Washington (1989)

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who hypothesized an equilibrium drug partitioning between the colloid and its dispersion medium, and theoretically demonstrated that, in this case, the rate of drug transfer into the receiving sink is regulated by both the membrane first-order rate constant and the equilibrium distribution constant, with no contribution from the release rate constant. Despite this, over the year 2011, of about 90 literature articles reporting on in vitro drug release from nanoparticles, in nearly 40 cases the dynamic dialysis method was used to measure the release kinetics.

For these reasons we have deemed it important to assess the rate-controlling step of the above-described release/permeation process experimentally, in order to either confirm or doubt the reliability of the dialysis method.

To this purpose we have carried out a case study where each of two drugs having different physicochemical properties, namely, diclofenac (MW 296; pK_a 4.0 according to Khazaeinia and Jamali, 2003; log P 4.40 according to Kourounakis et al., 1999) and ofloxacin (MW 361; zwitterion; log P 0.47 according to Zlotos et al., 1998) has been entrapped into ionotropically crosslinked chitosan nanoparticles, prepared and characterized after a previous report (Sandri et al., 2007) with some modifications. The drug-loaded nanoparticles have been collected by ultracentrifugation, re-dispersed into aqueous polysorbate 80 (case of diclofenac) or water (case of ofloxacin), and the dispersion has been subjected to dynamic dialysis using a membrane with an MWCO of 12 kDa. The MWCOs reported in the literature are like this (Aji Alex et al., 2011; Das and Suresh, 2011; Hao et al., 2011; Jain et al., 2011; Jingou et al., 2011; Kakkar et al., 2011; Nagarwal et al., 2011; Panchamukhi et al., 2011; Pandita et al., 2011; Pathan et al., 2011; Sahu et al., 2011; Saremi et al., 2011; Seju et al., 2011; Wang et al., 2011c; Wu et al., 2011) or smaller (Das et al., 2011; Essa et al., 2011; Kurmi et al., 2011; Thamake et al., 2011; Tian et al., 2011; Wang et al., 2011a, 2011b; Zhang et al., 2011), which implies a similar or higher resistance to drug transport. With both drugs the cumulative drug fraction appearing in the receiving phase has been plotted against time, in analogy with literature data obtained by the dialysis method. Also determined have been the plots of the drug fraction in the donor solution and that in the nanoparticle phase as a function of time, in order to investigate the rate-limiting step. Finally, the release of either drug from chitosan nanoparticles has been studied by the second more applied method in the year 2011 (15 articles), based on the separation of the nanoparticle phase from the dispersion medium by ultracentrifugation (Fan et al., 2011; Gupta et al., 2011; Keawchaoon and Yoksan, 2011; Kumari et al., 2011; Mahjub et al., 2011; Nair et al., 2011; Pandev et al., 2011; Saboktakin et al., 2011a, 2011b; Sanna et al., 2011; Song et al., 2011; Thomas et al., 2011; Yeh et al., 2011; Zhou et al., 2011). The relevant results have been compared with those obtained by the dialysis method.

2. Materials and methods

2.1. Solubility determination

The solubility of diclofenac free acid (Labochim, Milan, Italy) in aqueous 0.5% polysorbate 80 (Sigma) was evaluated. Excess drug was shaken in the solvent at 37 °C. Periodically, aliquots of suspension were withdrawn, filtered (0.45 μm pore size) in a controlled-temperature atmosphere and analyzed spectrophotometrically (284 nm) after appropriate dilution with the same solvent, until equilibrium was attained. This required less than 8 h.

2.2. Preparation of a micronized chitosan·HCl powder

Commercial chitosan minimum 90% deacetylated from shrimp shell (Chito-clear FG90, Primex, Drammen, Norway), having an average viscometric molecular weight of 590 kDa (Zambito et al., 2008), was converted into a micronized chitosan·HCl powder by making an aqueous chitosan suspension (12 g in 2000 ml) to pH 4.7 with 1 N HCl (about 43.5 ml) and spray-drying the resulting solution (Mini Spray Dryer BÜCHI B-191, inlet and outlet air temperatures, 160 °C and 75 °C, respectively; spray nozzle, 0.7 mm; feed flow, 8 ml/min).

2.3. Preparation of medicated nanoparticles from chitosan·HCl

In order to tentatively optimize the conditions for preparation of ionotropically crosslinked particles in the nano-size range, 100 μl aliquots of 1 mg/ml sodium tripolyphosphate (Sigma) in aqueous 0.5% (w/v) polysorbate 80 were consecutively added to 10 ml of 1 mg/ml chitosan·HCl in aqueous 0.5% (w/v) polysorbate 80 until clouding of solution. Addition of tripolyphosphate aliquots was continued after clouding while measuring particle size by light scattering (Coulter, N4 Plus) after each addition. The first addition after clouding caused a decrease of nanoparticle size, whereas the successive additions caused a size increase due to particle aggregation. Therefore addition of tripolyphosphate aliquots until clouding followed by addition of further 100 μl and size checking was established as the norm to obtain chitosan nanoparticles similar to those described in the previous report (Sandri et al., 2007). To prepare diclofenac- or ofloxacin (Sigma)-loaded nanoparticles, the 100 μl tripolyphosphate aliquots were added, following the above procedure, to the chitosan·HCl-polysorbate 80 solution containing 0.1 mg/ml diclofenac or ofloxacin. The total tripolyphosphate volume used to prepare each medicated nanoparticle batch was in the range of 0.9–1.5 ml, for diclofenac, and 0.6–0.9 ml, for ofloxacin. Immediately after preparation, each nanoparticle dispersion was centrifuged at 10,500 rpm and 14 °C for 1 h (Virtis adVantage ES-53) and the supernatant spectrophotometrically analyzed for the drug, after appropriate dilution, at 284 nm (diclofenac) or 286 nm (ofloxacin), to determine the entrapment efficiency (EE) according to the following equation:

$$EE = [(M_t - M_s)/M_t] \times 100$$

where M_t is the total drug mass used for nanoparticle preparation, and M_s is the drug mass found in the supernatant.

2.4. Kinetic measurements

For the kinetic measurements, 5 batches of diclofenac- or ofloxacin-loaded nanoparticles, prepared and analyzed for drug content and particle size as described above, were pooled, ultracentrifuged, the supernatant was spectrophotometrically analyzed to calculate the drug content in nanoparticles, the sediment redispersed, by vortexing, in an appropriate volume (5 ml for the dialysis method, 100 ml for the ultracentrifugation method) of aqueous 0.5% (w/v) polysorbate 80 (case of diclofenac) or water (case of ofloxacin), and particle size checked again. The resulting dispersion was used for the kinetic measurements by one of the methods described below.

2.4.1. Dynamic dialysis method

A porous regenerated cellulose membrane (MWCO 12 kDa, Sigma), pre-soaked at least 24 h in aqueous 0.5% (w/v) polysorbate 80 or water, for dialysis of diclofenac or ofloxacin, was mounted into a dynamic dialysis cell and apparatus, described in detail by Bottari et al. (1975). At time $t = 0$, 5 ml of diclofenac- or ofloxacin-loaded nanoparticles, obtained as described under Section 2.4 was placed in the donor compartment of the cell and stirring of donor and acceptor phases was started, while maintaining the thermostat temperature at 37 °C. The drug mass introduced in the