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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

cell via nanoparticles for each of 3 runs ranged from 0.544 to 0.674 mg (diclofenac) or 0.365 to 0.551 mg (ofloxacin). The volume of acceptor phase (aqueous 0.5% (w/v) polysorbate 80, with diclofenac, or water, with ofloxacin) was 200 ml. Drug transport across the membrane was assessed by spectrophotometrically analyzing the receiving phase at intervals and calculating the drug fraction appeared in the receiving phase at time *t*. At the end of each run the donor was checked for mean size of dispersed nanoparticles. For comparison, kinetic data were also obtained, using the same membrane and procedure, for the plain 0.1 mg/ml drug solutions and these solutions containing 1 mg/ml dissolved chitosan·HCl. The medium was aqueous 0.5% (w/v) polysorbate 80, with diclofenac, or water, with ofloxacin.

In some experiments the dialysis was stopped after an established time from the start, and the drug fraction contained in each of nanoparticle matrix, nanoparticle dispersion medium, and acceptor medium was determined. This procedure was repeated, running the dialysis for different times, to construct the plots of the drug fraction in each phase vs. time. Ultracentrifugation of donor phase followed by spectrophotometric analysis of supernatant was carried out to determine the drug fraction in dispersion medium and calculate that in nanoparticle matrix. With diclofenac, the drug fraction in such a matrix was determined by the following procedure. The sediment from centrifugation was suspended in 5 ml water, the suspension made to pH 8 with 0.1 N NaOH and stirred 24 h to extract diclofenac from matrix as the sodium salt. Then the suspension was ultracentrifuged and the supernatant spectrophotometrically analyzed for sodium diclofenac at 280 nm, to measure the drug mass extracted from nanoparticle matrix. With ofloxacin, the drug fraction in such a matrix could not be determined, so it was calculated, knowing the drug mass in receiving phase, that in nanoparticle dispersion medium, and total drug mass used for the experiment.

2.4.2. Ultracentrifugation method

In this case the volume used to re-disperse the sediment from ultracentrifugation of nanoparticle dispersion, referred under Section 2.4 was 100 ml to ensure sink conditions for drug release. After particle size checking this dispersion was kept at 37 °C under magnetic stirring. Hourly, 5-ml samples were withdrawn, checked for particle size, ultracentrifuged in the usual conditions, and the supernatant analyzed for the drug.

3. Results and discussion

3.1. Characteristics of nanoparticles

For preparation of nanoparticles, polysorbate 80 was added to the dispersion medium to limit particle size and prevent aggregation. The size of diclofenac- or ofloxacin-loaded nanoparticles is seen in Table 1. In either case the size is similar to that of chitosan nanoparticles of the same nature as the present ones, that were prepared and characterized in a previous work, where they were shown, by confocal laser scanning microscopy, to be well-defined spherical particles (Sandri et al., 2007). In Table 1 are also found the respective EE values. These are rather low, yet sufficient to carry out the present study which, in fact, does not propose a new pharmaceutical system, but rather, it discusses the correct interpretation of dynamic dialysis data. Five batches of each medicated nanoparticle formulation, prepared in the same conditions, were pooled to carry out the size measurements or the kinetic experiments, so the relevant results are supposed to be representative of the formulation under study. The size data in Table 1 refer to just-prepared dispersed nanoparticles. Following ultracentrifugation and re-dispersion the nanoparticles, loaded with either diclofenac or ofloxacin, showed no significant size change. The size also remained substantially unchanged after the kinetic studies, then particle size was not considered an effective process variable.

Table 1
Particle size and encapsulation efficiency (EE) of chitosan nanoparticles (mean ± SD; $n \geq 6$).

Drug in particles	Particle size (nm)	EE (%)
Diclofenac	782.0 ± 183.6	12.2 ± 5.7
Ofloxacin	716.9 ± 123.6	9.16 ± 1.86

3.2. Kinetic studies by dynamic dialysis

3.2.1. Kinetics of drug transport into receiving phase

Sink conditions in the receiving phase are normally maintained to make back diffusion from receiving to donor phase negligible. This is generally accomplished by keeping the thermodynamic activity of permeant in the acceptor negligible compared to that in the donor. When the permeant is completely dissolved in the donor, and the medium is the same in both donor and acceptor compartments sink conditions are virtually realized when the permeant concentration in the acceptor is made not to exceed 10% of that in donor. This was in fact the situation in the present dialysis experiments with the plain drugs, where the medium in either compartment was 0.5% aqueous polysorbate 80 (case of diclofenac) or water (case of ofloxacin) and the donor contained around 0.1 mg/ml drug. In either case such a concentration was below saturation, as the diclofenac solubility in 0.5% polysorbate 80, determined as described in the methods section, was 0.2 mg/ml, and the ofloxacin water solubility, determined by Zhang and Wang (2008) at 35 °C, was 3.4 mg/ml. Therefore the permeated drug was let to accumulate in the receiving phase up to no more than 80% of the total drug amount used in the experiment. At this uppermost limit, since the volume of receiving phase was 40-fold larger than that of the donor the drug concentration in the acceptor reached about 10% of that in the donor, and sink conditions were respected throughout the experiment. When the drug experienced some binding in the donor phase (e.g., with chitosan·HCl, or nanoparticles), which lowered its activity coefficient, the compliance with sink conditions was checked a posteriori for each case, at each time point. To this purpose the drug concentration in the donor, free from binding, was calculated from dialysis data, as will be illustrated in Sections 3.2.2 and 3.2.3, and compared with the concentration in the receiving phase at the corresponding time. All reported data were obtained under sink conditions.

3.2.1.1. Case of diclofenac. From Fig. 1 it appears that the drug transport rates were lower in the case where the donor contained dispersed nanoparticles or molecular chitosan·HCl than in that where it contained the plain drug. These data, if interpreted in the light of the relevant literature so far, would indicate sustained drug release from nanoparticles. More precisely, though, they are indicative of some type of interaction of diclofenac with nanoparticles or molecular chitosan·HCl. The latter interaction can easily be conceived as an equilibrium binding between dissolved diclofenac and molecular chitosan·HCl, whereas two alternative interpretations can be given for the interaction between diclofenac and nanoparticles. The first one hypothesizes a burst release of drug from nanoparticles into their dispersion medium, and establishment of a quasi-stationary equilibrium in donor phase between free drug and drug bound to nanoparticles. In this case the dialysis membrane would be the rate-limiting barrier to mass transport into the receiving phase (Washington, 1989). The other hypothesis, i.e., the one that is given credit in the recent literature, considers that the drug, entrapped in the nanoparticle matrix, is released there from in a sustained manner. This would imply sink conditions both in

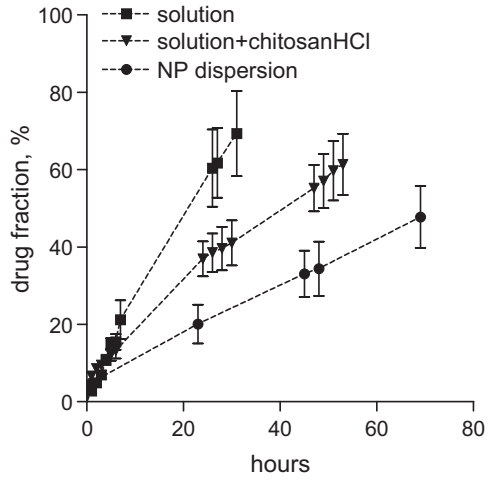

Fig. 1. Diclofenac mass fraction appearing in receiving phase of dialysis, plotted vs. time. Donor phase: drug solution; drug solution containing chitosan·HCl; nanoparticle (NP) dispersion. Means ± SD of 3 runs.

the donor phase at contact with nanoparticles and in the receptor phase beyond the membrane. The membrane would oppose negligible resistance to mass transport compared to the nanoparticle matrix. Further evidence is required to discern between the two hypotheses.

3.2.1.2. Case of ofloxacin. In this case the donor and acceptor phases contained water as the medium because polysorbate 80 would interfere with the UV analysis of drug in the sink. As a consequence, however, the experiment was protracted for only two hours because it was observed that ofloxacin nanoparticles would aggregate after longer times in water. Over this term about 70% drug from plain drug solution crossed the membrane into the sink. A comparison of data in Fig. 2 for the plain ofloxacin with corresponding data in Fig. 1 for plain diclofenac shows that the permeation of the latter was much slower. This is ascribed to solubilization of diclofenac into the micelles of polysorbate 80 (CMC, 0.0157 mg/ml, according to Chou et al., 2005), which was present in the medium of diclofenac but not in that of ofloxacin. As appeared in Fig. 2, the transmembrane drug transport data for the solution containing molecular chitosan·HCl are not significantly different from those for the plain drug, indicating the absence of any

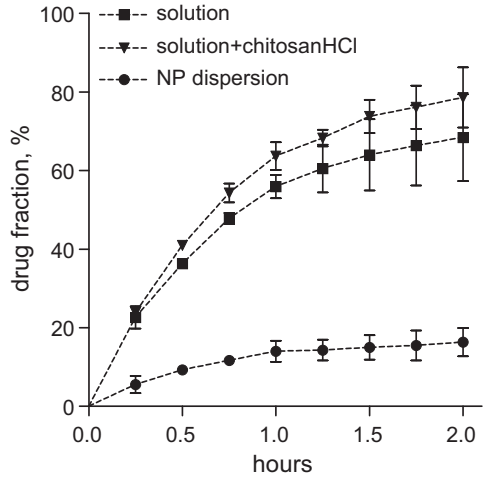

Fig. 2. Ofloxacin mass fraction appearing in receiving phase of dialysis, plotted vs. time. Donor phase: drug solution; drug solution containing chitosan·HCl; nanoparticle (NP) dispersion. Means ± SD of 3 runs.

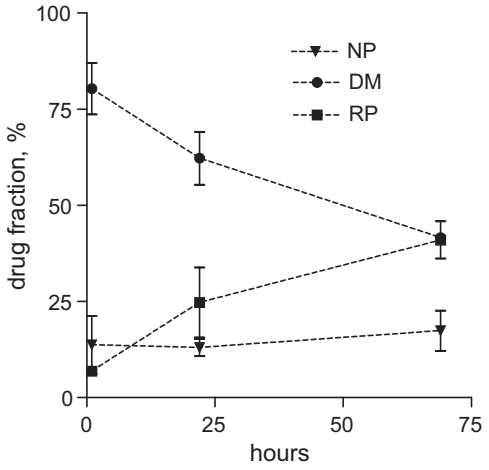

Fig. 3. Diclofenac kinetics in each phase of dialysis: nanoparticle (NP) phase; nanoparticle dispersion medium (DM); receiving phase (RP). Means ± SD of 3 runs.

significant ofloxacin–chitosan·HCl interaction in solution. On the other hand nanoparticles remarkably depressed such a transport, which points to a comparatively strong interaction of ofloxacin with the nanoparticle matrix.

3.2.2. Kinetic analysis of phases

In an attempt to detect the rate-limiting factor of the nanoparticle dialysis process, this was stopped at different times from the start and the drug fractions contained in the nanoparticle phase, the nanoparticle dispersion medium, and the receiving phase were determined at each stop, after separation of nanoparticles from dispersion medium. The changes in time of such drug fractions can be visualized in Figs. 3 and 4 for nanoparticles loaded with diclofenac and ofloxacin, respectively.

3.2.2.1. Case of diclofenac. Data in Fig. 3 show that in just 1 h time most of the drug mass (about 80%) was released from the nanoparticle matrix into the dispersion medium in the donor phase, while only about 7% crossed the membrane into the receiving phase. Over the course of experiment (70 h) about 40% of the drug mass passed from donor to acceptor medium, while no significant decrease of the drug mass entrapped in the nanoparticle matrix (about 15%) could be detected. The data altogether indicate that over the time of experiment subsequent to the first hour the release from

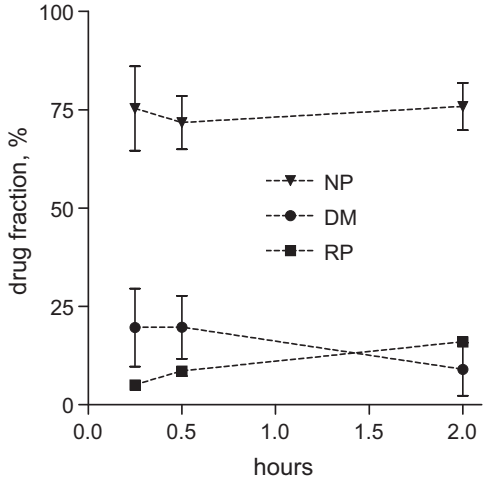

Fig. 4. Ofloxacin kinetics in each phase of dialysis: nanoparticle (NP) phase; nanoparticle dispersion medium (DM); receiving phase (RP). Means ± SD of 3 runs.

nanoparticles was practically insignificant and drug transport from donor to acceptor phase was governed by permeation across the dialysis membrane. The dialysis being faster with the plain drug than in the presence of nanoparticles, as appears in Fig. 1, is ascribed to drug in donor solution interacting with nanoparticles. This point will be resumed later on.

3.2.2.2. Case of ofloxacin. The data for this case, seen in Fig. 4, agree with those presented in Fig. 2 in indicating that only about 25% of the whole drug load was released from the nanoparticle matrix in two hours, while about 75% remained entrapped in matrix. As in the case of diclofenac, the ofloxacin fraction entrapped in nanoparticles showed no significant decrease over the time in which the dispersed system remained in the dispersed state, and hence, could be studied. Unfortunately, such a time lasted only two hours, sufficient, nevertheless, to evidence a specular correspondence between decrease of drug fraction in dispersion medium (donor phase) and increase of such a fraction in receiving phase. The data in Fig. 4 indicate that, at least within the first two hours of process, the drug kinetics in the receiving phase were controlled by permeation across the dialysis membrane and not by release from nanoparticles. This is a further instance of a process that is in fact permeation but could be interpreted as a release process on the basis of data obtained from analysis of only the receiving phase.

3.2.3. Study of drug interaction with nanoparticle surface

Relevant data in Figs. 3 and 4 show a virtually time-independent drug fraction entrapped within the nanoparticle matrix. As described earlier in this report, such a fraction was measured by a procedure consisting in separation of dispersed nanoparticles by ultracentrifugation and analysis of sediment and/or supernatant. Such a procedure, however, is not expected to allow assessment of the drug fraction reversibly interacting with the dispersed nanoparticles at their surface, because this is enormously larger than that of their sediment. The following treatment of dialysis data is aimed at allowing such an assessment.

It is now common knowledge that molecular permeation across a membrane from a stirred donor into a stirred acceptor phase under quasi-stationary and sink conditions, as in the present case with plain either diclofenac or ofloxacin, is a first-order process, the rate of which is expressed by the following equation (see, e.g., Flynn et al., 1974; Bottari et al., 1975):

$$\frac{dC_d}{dt} = -K_m C_d \quad (1)$$

where C_d is the drug concentration in donor phase at time t, and K_m is the dialysis rate constant.

Integration of Eq. (1) yields the following equation, where C_{d0} is the drug concentration in donor phase at time $t = 0$:

$$\ln\left[\frac{C_d}{C_{d0}} \times 100\right] = 4.605 - K_m t \quad (2)$$

If the drug molecules in donor phase experience some equilibrium interaction, Eq. (1) is modified as follows (Bottari et al., 1975):

$$dC_d/dt = -K_m C_{df} \quad (3)$$

where C_{df} represents the drug concentration free from binding at time t. By dividing both members of Eq. (3) by C_d and integrating under the assumption that the free/total drug ratio in donor phase ($F_f = C_{df}/C_d$) is constant with varying C_d, the following equation is obtained:

$$\ln\left[\frac{C_d}{C_{d0}} \times 100\right] = 4.605 - K_m F_f t \quad (4)$$

The dialysis data for plain drug, drug in the presence of chitosan·HCl, drug-loaded nanoparticles are reported as

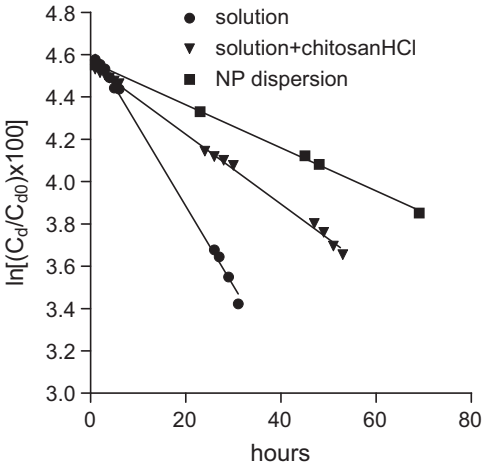

Fig. 5. Ln[$(C_d/C_{d0}) \times 100$] vs. time plots for diclofenac, derived from data in Fig. 1.

$\ln\left[(C_d/C_{d0}) \times 100\right]$ vs. t in Figs. 5 and 6 for diclofenac and ofloxacin, respectively. In the case of nanoparticles a time-independent fraction of total diclofenac or ofloxacin mass appears, from Fig. 3 or 4, to be entrapped within the nanoparticle matrix. In either case the entrapped mass was subtracted from the total drug mass in donor phase, to calculate the effective value of C_d, i.e., the concentration of the drug portion actually variable in time. The data for ofloxacin in the presence of molecular chitosan·HCl are not reported in Fig. 6 because they appear in Fig. 2 not to be significantly different from those for the plain drug.

All plots reported in Figs. 5 and 6 are significantly linear, as demonstrated by the relevant r^2 values, listed in Table 2. The data for the plain drugs are supposedly described by Eq. (2). The dialysis rate constant, that is the modulus of the slope of the relevant straight line, for ofloxacin is about 14-fold higher than for diclofenac, essentially because the latter drug is slowed down by an interaction with the polysorbate 80 micelles, absent in the case of ofloxacin. In fact, Llinas et al. (2007) reported a value of 0.001 mg/ml for the diclofenac water solubility at 25 °C, whereas we have determined a value of 0.2 mg/ml for diclofenac solubility in 0.5% aqueous polysorbate 80 at 37 °C, corresponding to a 200-fold increase essentially ascribable to micellar solubilization.

The log-linear plots in Fig. 5 and the relative rate constant values in Table 2 show that the dialysis of diclofenac is further slowed down by the presence of molecular chitosan·HCl or dispersed nanoparticles. This is presumably due to reversible molecular

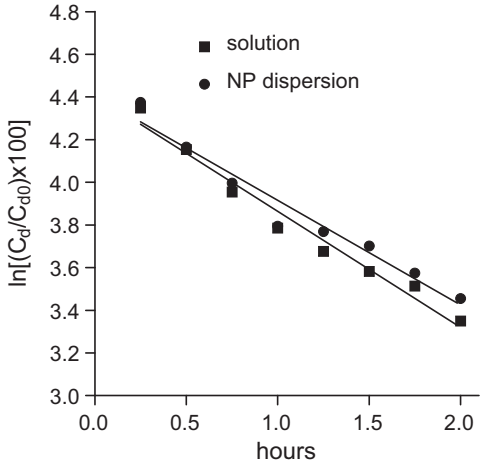

Fig. 6. Ln[$(C_d/C_{d0}) \times 100$] vs. time plots for ofloxacin, derived from data in Fig. 2.

Table 2
Parameters from analysis of data in Figs. 5 and 6 by Eqs. (2) and (4), respectively.

Drug	Substrate	Rate constant ± SD[a] ($\times 10^2$ h^{-1})	r^2	Interaction[b] (%)
Diclofenac	–	3.77 ± 0.06	0.998	–
	Chitosan·HCl	1.65 ± 0.02	0.998	56.2
	Nanoparticles	1.02 ± 0.02	0.999	72.9
Ofloxacin	–	54.38 ± 3.69	0.973	–
	Nanoparticles	49.05 ± 4.23	0.957	ns[c]

[a] Modulus of straight line slope.
[b] Reversibly bound drug fraction.
[c] Not significant.

interactions of diclofenac with the dissolved polymer or the dispersed nanoparticle surface. The significant linearity of plots confirms that in both cases Eq. (4) is obeyed and F_f is actually constant with varying C_d and t. The respective F_f values could be calculated from the ratios of the respective rate constants to the rate constant for the plain diclofenac. From here, the values of drug fraction reversibly interacting with dissolved chitosan·HCl or dispersed nanoparticle surface, reported in Table 2, were readily derived.

The data in Fig. 6 and Table 2 for ofloxacin, if compared with those presented in Fig. 4, point to no significant drug interaction with nanoparticles other than the apparently irreversible entrapment within the nanoparticle matrix.

The above data for diclofenac and ofloxacin indicate a correlation between the drug interaction with the dispersed nanoparticle surface and that with the dissolved molecular chitosan·HCl. Either such interactions are both significant (case of diclofenac) or neither is significant (case of ofloxacin). This suggests a qualitative similarity between the diclofenac interactions with the dispersed nanoparticle surface and that with the dissolved molecular chitosan·HCl.

3.3. Release studies by ultracentrifugation

3.3.1. Sink conditions

In each release experiment nanoparticles containing around 0.5 mg drug were dispersed in 100 ml of receiving phase. Supposing complete drug release from nanoparticles into dispersion medium, this would at most attain 2.5% (case of diclofenac) or 0.17% (case of ofloxacin) of saturation. Then sink conditions for drug release can safely be assumed in both cases.

3.3.2. Release of diclofenac

The samples withdrawn from the nanoparticle dispersion and analyzed, following ultracentrifugation, after 1 h from the start of experiment, and subsequently up to 24 h yielded readings of supernatant not significantly different from one another and corresponding to a range of released drug fraction of 80–85%. This was the case of at least 3 replicates of the experiment. These findings agree with the data shown in Fig. 3, indicating an apparently time-independent drug fraction of 15–20% remaining entrapped in the nanoparticle matrix ever after the first hour of experiment. The drug fraction in the supernatant of centrifugation would be much smaller if the drug mass, found by the dialysis studies to be interacting with the dispersed nanoparticle surface, were contained in the sediment. It should be considered, however, that the formation of the sediment could have resulted in a dramatic drop of such a surface, and hence, of the drug mass reversibly bound to it. At any rate, the release kinetics resulting from ultracentrifugation do not correspond with the kinetics of drug appearance in the receiving phase of the nanoparticle dialysis, shown in Fig. 1. Indeed, the latter data is not determined by drug release from nanoparticles, but rather, by drug permeation across the dialysis membrane.

3.3.3. Release of ofloxacin

As in the case of diclofenac, the release experiment was repeated at least 3 times with similar results. The samples of ofloxacin-loaded nanoparticles, withdrawn from their aqueous dispersion after 1 h from the start and thereafter at subsequent times over 24 h, contained not significantly different drug amounts in the supernatant, and this corresponded to about 25% of total drug. Then, in agreement with relevant data in Fig. 4, an apparently time-independent drug fraction of about 75% remained entrapped within the nanoparticle matrix. In the case of ofloxacin, the drug kinetics in the receiving phase of dialysis of nanoparticles, shown in Fig. 2, approximately correspond with the release data obtained by the ultracentrifugation method. However, it has been demonstrated earlier in this report, on the basis of data presented in Fig. 4, that such kinetics were controlled by the dialysis membrane and not by drug release from nanoparticles.

4. Conclusions

The present study has shown, on an experimental basis, that an uncritical use of the dynamic dialysis method to assess drug release from nanoparticulate systems may be misleading. Indeed, dialysis data obtained from a nanoparticle dispersion is not necessarily descriptive of the kinetics of drug release from this system, even if the process is more sustained and substantially slower with the nanoparticles than with the plain drug solution. In fact, in the first of the two cases analyzed in this report (diclofenac as the drug) release from nanoparticles into their dispersion medium occurred almost entirely within the first hour of experiment, while subsequently, the kinetics of drug appearance in the receiving phase of dialysis were controlled by permeation of burst-released drug across the dialysis membrane. The permeation rate was significantly lower than with the plain drug solution because of a reversible interaction of the drug with the dispersed nanoparticle surface.

In the second case analyzed (ofloxacin as the drug) the surfactant was excluded from the dispersion medium for analytical reasons. In the absence of surfactant, however, the nanoparticles underwent aggregation shortly after two hours, which limited the duration of the study. This nevertheless was sufficient to show evidence of a burst-release to the dispersion medium of a comparatively small drug fraction, which passed into the receiving phase with membrane-controlled kinetics. Not even in this case could we observe a release-controlled dialysis because a substantial drug fraction remained entrapped into the nanoparticles in an apparently irreversible fashion.

The findings with the ultracentrifugation method agree with the above interpretation of kinetic processes.

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