Review Article

A Review of In Vitro Drug Release Test Methods for Nano-Sized Dosage Forms

Susan D’Souza

Sunovion Pharmaceuticals Inc, Marlborough, MA 01752, USA

Correspondence should be addressed to Susan D’Souza; dr_ssdssouza@yahoo.com

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This review summarizes the methods used to study real-time (37°C) drug release from nanoparticulated drug delivery systems and establish an IVIVC. Since no compendial standards exist, drug release is currently assessed using a variety of methods including sample and separate (SS), continuous flow (CF), dialysis membrane (DM) methods, and a combination thereof, as well as novel techniques like voltametry and turbidimetry. This review describes the principle of each method along with their advantages and disadvantages, including challenges with set-up and sampling. The SS method allows direct measurement of drug release with simple set-up requirements, but sampling is cumbersome. With the CF method, sampling is straightforward but the set-up is time consuming. Set-up as well as sampling is easier with the DM, but it may not be suitable for drugs that bind to the membrane. Novel methods offer the possibility of real-time drug release measurement but may be restricted to certain types of drugs. Of these methods, Level A IVIVCs have been obtained with dialysis, alone or in combination with the sample and separate technique. Future efforts should focus on developing mathematical models that describe drug release mechanisms as well as facilitate formulation development of nano-sized dosage forms.

1. Introduction

Ever since reports documenting the utility of polycyanocrylate and poly-ε-caprolactone nanocapsules for ocular administration were published over two decades ago, several publications have highlighted the benefits of using nano-sized dosage forms for medical and imaging purposes [1–3]. Indeed, advantages such as improved drug solubility and stability, enhanced performance as well as increased efficacy have been well established with nanoparticulate preparations [4]. The increasing interest in nanotechnology based drug delivery systems has been a key factor in the design and development of numerous novel dosage forms and complex delivery therapies such as liposomes, nanoemulsions, nanocrystals, polymeric nanoparticles, solid lipid nanoparticles, nanofibers, and dendrimers, to treat a variety of disease states [5–7]. As an example, researchers have investigated nanoparticles of Fenofibrate for the treatment of hypercholesterolemia and Cyclosporine nanoparticles against cancer [8, 9]. Unsurprisingly, several nanoparticulate preparations are currently undergoing clinical investigation for the delivery of a wide range of therapeutics like antibiotics, antigens, cytostatics, and so forth, via the intramuscular, subcutaneous, oral, and intravenous route [10, 11]. A few examples of currently marketed nanotechnology based dosage forms are listed in Table I.

By the ISO definition, the size for these formulations can range between 1 and 100 nm. Since sizes below 10 nm have a greater propensity for renal clearance and tissue extravasations, and larger sizes are quickly opsonized via the macrophages of the reticuloendothelial system, the 10–100 nm size range is considered optimal for nanoparticulate preparations [12]. Hence, for medical and therapeutic purposes, a range of less than 10–100 nm (in at least one dimension) for nanoparticulates appears to be generally accepted, with a few exceptions where sizes greater than 100 nm may be applicable [4, 13]. Due to their small size, nano-sized dosage forms possess an unusually large surface-to-volume ratio that alters the chemical, physical, and biological properties of the dosage form allowing them to cross cell and tissue
Table 1: List of a few currently marketed nanoparticulate products.

<table>
<thead>
<tr>
<th>Brand Name</th>
<th>API</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daunoxome</td>
<td>Daunorubicin</td>
<td>Kaposi’s sarcoma</td>
</tr>
<tr>
<td>Depocyt</td>
<td>Cytarabine</td>
<td>Lymphomatous meningitis</td>
</tr>
<tr>
<td>Myocet</td>
<td>Doxorubicin</td>
<td>Breast cancer</td>
</tr>
<tr>
<td>Oncaspars</td>
<td>PEG-asparaginase</td>
<td>Acute Lymphoblastic Leukemia</td>
</tr>
<tr>
<td>Restasis</td>
<td>Cyclosporine</td>
<td>Chronic dry eye disease</td>
</tr>
<tr>
<td>Triglide</td>
<td>Fenofibrate</td>
<td>Hypercholesterolemia</td>
</tr>
<tr>
<td>Emend</td>
<td>Aprepitant</td>
<td>Chemotherapy induced nausea and vomiting</td>
</tr>
</tbody>
</table>

Without exception, in vitro release testing is an important analytical tool that is used to investigate and establish product behavior during the various stages of drug product development, as well as life cycle management. When designed appropriately, an in vitro release profile can reveal fundamental information on the dosage form and its behavior, as well as provide details on the release mechanism and kinetics, enabling a rational and scientific approach to drug product development. Understandably, for complex dosage forms like nanoparticulates, in vitro release testing assumes greater significance.

Despite the great strides in design and development of nano-sized dosage forms, no compendial or regulatory standards exist for in vitro release testing. Although there have been attempts to use the existing USP apparatus for in vitro drug assessment of nanoparticles, the set-ups were designed primarily for oral and transdermal products and as such pose many challenges during a release study. Hence, several in vitro release methods, both compendial and noncompendial, have been utilized and reported. Certainly, the area of in vitro testing for nanoparticulates lags behind the advances realized in drug product development.

Given the urgent need for improved product safety while maintaining quality of novel dosage forms like nanoparticles, the past decade witnessed a series of international in vitro release workshops co-sponsored by the AAPS (American Association of Pharmaceutical Scientists), US FDA (Food and Drug Administration), FIP (Federation International Pharmaceutique), and several other scientific groups and agencies [22–25]. The outcomes of these workshops were published as position papers with the general consensus that dissolution or in vitro release testing was an important tool in pharmaceutical development and quality control for a plethora of dosage forms, both traditional and novel. Further, the attendees highlighted the fact when compared to traditional formulations, the characteristics of novel/special dosage forms including site and mode of administration was vastly different, and, hence, appropriate consideration should be taken during selection of the apparatus, release medium, agitation (flow rate), and temperature. In summary, the workshops concluded that, along with a few other novel dosage forms, nanoparticulate preparations were categorized as those “dosage forms requiring more work before a (dissolution) method can be recommended” [22, 23].

In a separate endeavor, the Nanotechnology Risk Assessment Working Group in the Center for Drug Evaluation and Research (CDER) within the US FDA, published a regulatory note outlining the risk assessment and management process on a hypothetical nanomaterial drug, to be administered orally [4]. In this example, the Working Group identified “dissolution/release rate” as a risk factor that may impact the evaluation of quality, safety, and efficacy of the drug product containing nano-sized drug, that is, during ingestion and in vitro release/dissolution. Hence, the utility of in vitro release testing, from a drug product development as well as regulatory standpoint, cannot be ignored.

Thus, the purpose of this review is to provide the reader with a summary of existing in vitro release methods for nano-sized dosage forms along with their strengths and
shortcomings. It is hoped that this review will serve as a
guideline for developing suitable techniques for assessment
of exploratory formulations, clinical and commercial dosage
forms, and establishment of an in vitro in vivo correlation
(IVIVC).

2. In Vitro Release Methods

Much attention has been devoted to the development of suit-
able apparatus to assess in vitro release from nanoparticulates.
However, additional considerations, like release media selec-
tion, agitation, and so forth, cannot be overlooked. In contrast
to oral dosage forms where release media typically mimics
pH of the gastrointestinal tract, selection of release media for
nano-sized dosage forms will vary depending on the site of
administration as well as the site of action of the formulation,
and thus simulation of in vivo conditions may be difficult.
In general, selection of release media for nanoparticulate
preparation is generally based on drug solubility and stability,
assay sensitivity, and the method used. Though maintenance
of sink conditions is preferable, nonsink conditions have
been employed. Agitation, frequently utilized to prevent
aggregation of dosage forms during an in vitro release study,
will depend on the apparatus used. Similarly, sampling and
buffer replacement (total or partial) techniques are also based
on the type of in vitro method used.

As such, drug release from nano-sized dosage forms
can be assessed using one of the following three categories,
namely, sample and separate (SS), continuous flow (CF), and
dialysis membrane (DM) methods. More recently, apparatus
that combine the principles of either the SS and DM or CF and
DM have also been reported. Lastly, a few novel methods that
use voltametry, turbidimetry, and so forth are discussed. For
each of these methods, a brief description is provided along
with adaptations, additional considerations, advantages, and
disadvantages.

2.1. Sample and Separate. In the SS method, the nanopartic-
ulate dosage form is introduced into the release media that
is maintained at a constant temperature, after which drug
release is assessed by sampling of the release media (filtrate or
supernatant) or the nanoparticles. From literature, there are
several adaptations to the SS method, with differences noted
in set-up, container size, mode of agitation, and sampling
techniques.

Commonly reported set-ups include USP I (basket), USP
II (paddle), or vials and generally depend on the volume of
release media used in the in vitro release study [7, 38–40]. For
instance, vials were used when the volume of release media
was small (1–15 mL) and in vitro release vessels were employed
with larger volumes (600–900 mL) [5, 7, 38, 40, 41].

In addition to determining the type of set-up, container
size also influences the mode of agitation used in an in vitro
release study with the SS method. With small sized dosage
forms like nanoparticles, agitation of the release media is
critical to the in vitro release process as it reduces the potential
for aggregation and enhances wetting, thereby reducing the
impact of these factors on the in vitro release rate [38]. While
agitation of the release media is easily accomplished via the
USP I or USP II apparatus, media contents in vials have been
agitated using alternate techniques. For example, Danhier
et al. and Li et al. used magnetic stirrers with Paclitaxel and
BSA nanoparticles, respectively, while Prabha et al. reported
the use of orbital shakers with DNA nanoparticles [42–44].

Drug release is monitored by physically separating the
nanoparticles from the release media, followed by analysis of
the former or the latter [7, 41]. While several authors have
documented the use of syringe filters to achieve physical
separation between the release media and nanoparticles,
the small size of nanoparticles has necessitated the use of
high energy separation techniques like centrifugation,
ultracentrifugation, and ultrafiltration [40, 42, 45–47]. For
filtration, syringe filters with pore size as large as 0.45 μm
have been used for withdrawing supernatant to monitor
drug release of small molecules such as Celecoxib [40]. In
comparison, high energy separation techniques have been
reported with larger biomolecules like Insulin, BSA (Bovine
Serum Albumin), and DNA [43, 44, 48]. Once separated,
drug release is generally monitored by sampling a part of
the supernatant, or decanting the entire supernatant content
at periodic intervals [7, 49]. In other instances, separation
was followed by analysis of the nanoparticles (destructive
technique) [41]. After sampling, an equal amount of fresh
release media or buffer is added to the set-up so that sink
conditions are maintained for the duration of the in vitro
release study.

In general, the SS method provides a direct approach to
monitor drug release. With this method, most sample set-
ups, agitation modes, and sampling techniques are reasonably
straightforward and simple. However, due to the small size
of nanoparticulate dosage forms, several practical challenges
have been noted. For instance, aggregation of nanoparticles
during in vitro release appears to be a key concern. Addi-
tionally, while sampling techniques like filtration seem fairly
reasonable in principle, clogging of filters during sampling,
adsorption of drug to the filter, and so forth, have been
reported [50]. Indeed, difficulty in physical separation even
with high energy techniques, continued drug release during
the high energy separation process, and so forth, are some of
the challenges often observed during sampling [38, 45].
Although sink conditions are recommended with the SS
method, nonsink conditions have been reported to be more
discriminatory with poorly soluble drugs [51]. Nevertheless,
as with microparticulate dosage forms, the SS method offers
researchers a simple and straightforward approach to moni-
tor in vitro release from nano-sized dosage forms.

2.2. Continuous Flow. In the CF method, drug release from
the nanoparticulate dosage form is monitored using the
USP IV apparatus or a modification thereof. Drug release
occurs as a result of buffer or media constantly circulating
through a column containing the immobilized dosage form
and is monitored by collecting the eluent at periodic intervals
(Figure 1).
Unlike the widely used SS method, only a few examples of the CF method have been reported for nano-sized dosage forms [38, 52]. For example, the release profiles of amorphous nanoparticles and an unprocessed crystalline form of Cefuroxime Axetil, a BCS II cephalosporin antibiotic, were evaluated using the CF method as well as other methods described below [38]:

(a) USP I (basket): 900 mL buffer at 100 rpm;
(b) USP II (paddle): 900 mL buffer at 100 rpm;
(c) USP IV (flow through cell): 900 mL buffer at a flow rate of 1.6 mL/min (peristaltic pump, closed loop) through a cell (internal diameter = 25 mm) and 0.2 μm membrane disc filter;
(d) dialysis bag (MWCO 12kDa, inner volume = 7 mL) placed into a USP II (paddle) in vitro release tester (outer volume = 900 mL, paddle rpm = 100).

The results revealed that complete drug release was achieved only with the USP II paddle and USP IV apparatus, release profiles with the USP IV method being well separated. Immobilization of the nanoparticles was thought to be a factor contributing to the discriminatory results obtained with the USP IV apparatus.

Similarly, in an experiment on nano- and micro-particle loaded strip films of another BCS II drug, Griseofulvin, in vitro release was performed using two methods [52]:

(a) USP I (basket): 500 and 900 mL media at 50, 100, and 150 rpm;
(b) USP IV (flow through cell): 100 mL media at flow rates of 4, 8, and 16 mL/min (peristaltic pump, closed loop) through a cell (internal diameter = 22.6 mm) and 0.2 μm membrane disc filter with the strip film loaded in 6 different configurations.

While complete release was observed only with USP I and configuration B (strip film positioned in the cell on top of round glass beads) of the USP IV method, results with configurations A (strip film positioned in the cone section of the cell without round glass beads) and C (strip film sandwiched between round glass beads) of USP IV at the highest flow rate were more discriminatory than all the other release profiles obtained in the in vitro study.

In comparison to nanoparticulate dosage forms where literature on the use of USP IV methodology is sparse, several salient aspects of the CF method can be inferred from its use in microparticulate dosage forms [21]. As an example, flow rates used in the CF method depend on the type of pump (peristaltic versus syringe) as well as the filters used (membrane versus ultrafilters). Low flow rates have been known to be a key factor causing slow or incomplete release from dosage forms [21]. In general, the availability of automated equipment has simplified routine sampling and media replacement with the CF method in closed (recirculating media) and open (nonrecirculating media) loop systems. However, the CF methods suffer from several disadvantages that include instrument costs, difficulty in set-up, filter clogging, adsorption to the filter and glass beads, and difficulty in maintaining a constant flow rate leading to wide variability in results.

2.3. Dialysis Method. Of all the methods used to assess drug release from nano-sized dosage forms, the dialysis method (DM) is the most versatile and popular. In this method, physical separation of the dosage forms is achieved by usage of a dialysis membrane which allows for ease of sampling at periodic intervals. As with the other methods, several adaptations of the DM have been reported in literature with key differences in set-up, container size, and molecular weight cut-off (MWCO).

Of the variety of DM set-ups used, the most commonly cited is the dialysis bag (regular dialysis), other adaptations being the reverse dialysis and side-by-side dialysis set-up [26, 53, 54] (Figures 2–4). With the regular dialysis technique,
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Sampling from inner compartment

Dialyzer cap

Inner compartment containing release media

Dialysis membrane

Outer compartment containing nanosuspension

Stir bar

Figure 3: "Reverse dialysis" set-up.

Stir bar

Nanoemulsion in donor compartment

Dialysis membrane

Receiver compartment

Figure 4: "Side-by-side dialysis" set-up (adapted from [26]).

the nanoparticles are introduced into a dialysis bag containing release media (inner media compartment) that is subsequently sealed and placed in a larger vessel containing release media (outer media compartment), agitated to minimize unstirred water layer effects [32, 55]. In general, the volume enclosed in a dialysis bag (inner media) is significantly smaller than the outer media. For instance, inner media volumes reported in literature range from 1 to 10 mL, whereas the outer media volume is much greater, typically around 40–90 mL [32, 53, 55]. Thus, container size will depend on the total volume of release media required for the in vitro release study. In the regular dialysis technique, drug released from the nanoparticles diffuses through the dialysis membrane to the outer compartment from where it is sampled for analysis (Figure 2). In contrast, with the reverse dialysis set-up, the nanoparticles are placed in the outer compartment (agitated to minimize the unstirred water layer) and the inner compartment is sampled for drug release (Figure 3) [54, 56, 57]. Other adaptations of the DM include the side-by-side dialysis set-up where donor and receiver cells, both containing equal volumes of media agitated with a magnetic stirrer, are separated by a dialysis membrane, and sampling occurs from the receiver cell (Figure 4) and a vertical Franz diffusion cell [26, 58, 59].

As with other methods, drug solubility in the release media is essential to its transport across the dialysis membrane. Apart from release media, the importance of selecting an appropriate molecular weight cut-off (MWCO) for the dialysis membrane cannot be overlooked. Indeed, the basic premise of the DM is that drug that is released from the dosage form will diffuse rapidly from one compartment, through the membrane, and enter the second compartment from where it is sampled for analysis. Thus, membranes with a sufficiently high membrane MWCO are often selected for in vitro release studies so that drug transport is not a limiting factor. As an example, in a study with 3 dosage forms of Indomethacin (e.g., nanoparticles and nanocapsules using poly-e-caprolactone, and a submicron emulsion), Calvo et al. noted that nearly 85% of drug was released within 2 hours and complete release was achieved in 4 hours when a 12 kDa membrane was used [54]. However, the rationale for selecting a MWCO is rather subjective. For instance, a MWCO as high as 10–14 kDa was used to study drug release of small molecules like Risperidone and Indomethacin, and a MWCO as low as 1 kDa though suitable for assessing in vitro release of a large molecule, pDNA, proved to be limiting for the diffusion of Cefuroxime Axetil, a cephalosporin antibiotic [32, 38, 55, 60]. In general, the MWCO should be sufficiently large to permit drug transport.

The ease of set-up and sampling with the DM make it a very simple and straightforward technique to study drug release from a wide variety of nano-sized dosage forms like nanospheres, liposomes, emulsions, nanocapsions, and so forth [53–55]. However, issues have been reported with the regular dialysis method. If incorrectly sealed, leakage of media and dosage form may occur from both ends of the dialysis bag set-up. Incomplete release data may be observed if nonsink conditions exist or equilibration times are high [38]. On the other hand, the difference in equilibration times can be exploited as a discriminatory tool to distinguish release behavior between fast and slow releasing dosage forms [50]. Another factor to be considered is that the DM cannot be used with drugs that bind to the dialyzing membrane. As stated in a review paper on microparticulate dosage forms, it is recommended that the suitability of the dialyzing membrane be assessed prior to use [21, 61].

2.4. Combination Methods. A few publications have modified the set-ups used in the SS, CF, and DM methods to evaluate drug release from nano-sized dosage forms. In most of these reports, the set-up of the SS method is combined with a dialyzer to allow ease of sampling. In other publications, the DM and the CF set-up is used to assess in vitro release from nanoparticulates.

As an example, Mottaleb et al. compared release profiles of Ibuprofen from liposomes and lipid nanocapsules and nanoparticles using the regular dialysis method (dialysis bag) and modified USP I (basket) set-up where the shaft was affixed with a glass basket having a dialysis membrane at the bottom, that is, glass basket dialysis (GBD), and placed in a larger vessel (Figure 5) [27]. Sampling was performed from the outer vessel. Unlike the dialysis bag that had a large
membrane surface area, the GBD was able to discriminate differences between release profiles from different formulations (lipid nanocapsules, nanoparticles, liposomes). In another study, Lobenberg et al. compared drug release from Rifampicin and Moxifloxacin hydrochloride nanoparticles with the regular dialysis method (dialysis bag) as well as an adaptation of USP I (basket), where a cylinder containing a dialysis membrane at the bottom was attached to the basket shaft [62]. Results from this study showed that modified USP I set-up was more discriminatory in nature and could be attributed to the reduced surface area of the dialysis membrane. In yet another modification of the USP I (basket) set-up, a dialysis bag containing nanostructured lipid carriers of Artemether was placed inside the basket and drug release monitored over time [63]. Though most studies have utilized the USP I set-up, others have used the USP II (paddle) set-up in combination with the DM. For example, Cao et al. affixed dialysis bags containing Silybin meglumine nanoparticles onto a USP II (paddle) apparatus and assessed drug release in five different release media [33, 35].

A combination of a DM, previously reported by Kostanski et al., and CF method was utilized to assess drug release from 5-Fluorouracil loaded BSA nanoparticles [28, 64]. The setup comprised a glass tube (with a dialysis membrane at the bottom) that was placed into a larger vessel containing buffer (Figure 6). Sampling as well as buffer replacement was performed from the larger vessel with the aid of two pumps having the same flow rate. The combined DM-CF set-up was able to demonstrate sustained levels of 5-Fluorouracil and the absence of burst release from the BSA nanoparticles. An alternate dialysis-CF method utilized a dialysis adaptor inside the CF set-up (Figure 7) [29].

### 2.5. New Methods.

Apart from the commonly reported SS, CF, and DM methods, a few alternate approaches have been utilized to monitor drug release. A majority of these appear to be targeted towards electroactive drugs. While the techniques may differ in principle or application, they possess a few common elements in that physical separation of the dosage form from the release media is not required. Additionally, these techniques offer the possibility of measurement under real-time conditions.

As such, electrochemical methods offer the possibility of rapid in situ measurements while avoiding the interference caused by the presence of undissolved dosage form in the release media. In one such approach, a potentiometric drug selective electrode was used to monitor real-time release of Procaine hydrochloride from pH-responsive nanogels [65]. In another report by Mora et al., a highly sensitive repetitive square-wave voltammetric technique was utilized to measure the redox reaction of Doxorubicin hydrochloride (Figure 8) [30]. Similarly, differential pulse polarography was used to assess continuous drug release from Piroxicam, Chloramphenicol, and Diazepam [66, 67]. Apart from the fact that only electroactive drugs can be measured by the above methods, these methods suffer from additional drawbacks. For example, the sensitivity as well as responsiveness of each technique varies greatly. Also, the sensors used may be specific to a particular drug.

Non-electrochemical methods like calorimetry, turbidimetry, and laser diffraction have also been evaluated as in vitro release methods. Solution calorimetry was employed by Kayaert et al. during in vitro release measurement of Naproxen, Cinnarizine, and Compound A (investigational
API) from nanocrystals [68]. The principle of this technique is based on detection of the net proportion of heat change during in vitro release. Challenges with calorimetric measurements include long equilibration times. Further, the heat produced by all the processes needs to be considered. Turbidimetric and laser diffraction approaches utilizing the change light scattering properties of nanoparticles dispersed in release media have also been utilized [69, 70]. Concerns with these methods include the long equilibration times, limited range of particle size, and initial concentration of samples that can be used.

Helle et al. designed a novel online system for evaluating the drug release from Indomethacin and Beclomethasone dipropionate nanoparticles. In this method, nanoparticles are packed into small column which is connected to multiport modulation valve attached to an HPLC system allowing for rapid analysis [71]. Other new methods reported in literature include a vertical diffusion Franz cell and an in vitro lipolysis model [37, 72]. Microdialysis, another set-up utilized to measure drug release from nanoparticles, is based on passive diffusion of drug through a concentration gradient across a semipermeable membrane (Figure 9) [31, 47]. Drug release can be measured in real-time conditions using analytical methods like HPLC. However, care should be taken when surfactants are used in the release media, as recovery may be affected.

3. Modeling Drug Release

Very few publications have attempted to describe drug release profiles from nanoparticulate dosage forms using mathematical models. This is not surprising given that nano-sized dosage forms are complex and drug release evaluations are not straightforward. However, a few authors have discussed the importance of mathematical models and endeavored to characterize drug release. For instance, Barzegar-Jalali et al. suggested the use of reciprocal powered time (RPT) as general model to analyze the complex nature of drug release from nanoparticles, with alternate approaches being the Weibull and log-probability models [73]. In another report, Zeng et al. developed a three-parameter model that considers reversible drug-carrier interaction as well as diffusional drug release from liposomes [74, 75].

Use of mathematical models to describe drug release profiles offers several advantages. It permits the elucidation of drug release mechanisms and can be used to guide formulation development efforts. Further, model parameters can serve to represent as well as compare in vitro release profiles. However, caution must be used when applying mathematical models to complex drug release mechanisms.

4. IVIVC

The US FDA defines IVIVC as "A predictive mathematical model describing the relationship between an in vitro property of an extended release dosage form (usually the rate or extent of drug in vitro release or release) and a relevant in vivo response, for example, plasma drug concentration or amount of drug absorbed" [76]. Thus, establishment of an IVIVC, described as a correlation between in vitro release and in vivo behavior, enhances the utility of an in vitro study. From the 1997 FDA guidance document, an IVIVC will reduce the regulatory burden by paring the number of in vivo studies needed for product approval. Additionally, it promotes the setting of clinically relevant in vitro release specifications [76].

The FDA guidance also categorizes IVIVCs into three levels. Level A represents a linear or nonlinear point-to-point relationship between the in vitro and in vivo release profiles. If the relationship is nonlinear, suitable modeling or scaling is required. Of the three IVIVC levels, Level A describes the highest correlation and is most commonly used to obtain a biowaiver. On the other hand, a Level B correlation is a comparison of summary parameters such as the mean in vitro dissolution time with mean in vivo dissolution time or mean residence time (MRT). Since several in vivo curves will produce a similar MRT value or mean in vitro dissolution time, Level B correlations are not as discriminatory as Level A, but acceptable for an IVIVC. A Level C correlation describes a relationship between an in vitro release parameter (e.g., % dissolved at a particular time) and a pharmacokinetic parameter (e.g., $C_{\text{max}}$). However, a Level C correlation is not descriptive of the complete shape of the in vivo release profile and, though acceptable, seldom used.

As such, the FDA guidance is intended for oral extended release products. However, principles of the FDA guidance
have been used to demonstrate an IVIVC from several nonoral dosage forms, including nano-sized preparations. Most published literature on IVIVC compares the in vitro release behavior with the in vivo absorption profile, the latter calculated by the FDA recommended Wagner-Nelson method [76, 77]. Once the fraction absorbed is computed, a correlation is obtained by comparing it with the in vitro release profile and the type of IVIVC (Level A, level B, etc.) is determined.

Over the last decade, a few reports on IVIVC from nano-sized dosage forms have been published (Table 2). In some instances, a rank order correlation was observed while, in others, a Level A IVIVC was established with some or all of the formulations evaluated in the study. As an example, a rank order correlation was observed with three preparations of Fenofibrate with drug release and absorption from 100 nm lipid matrix particles > microparticles > control (crystalline suspension) [37]. In another study on Fenofibrate nanosuspensions, Xu et al. attempted to elucidate the absorption mechanism using in situ gut perfusion model [36]. Data analysis between in vitro dissolution (P), in situ intestinal absorption (F), and in vivo absorption (Fw) demonstrated a Level A correlation (R^2 > 0.95). As a result, the authors concluded that the in situ intestinal perfusion model could be a predictor of in vivo pharmacokinetic behavior for the nanosuspension.

In a recent report, Kumar et al. investigated the relationship between in vitro and in vivo release from Indomethacin gelatin nanoparticles administered orally to Wistar albino rats [32]. Study results indicated that a good correlation was obtained by plotting the fraction absorbed in vivo (Wagner-Nelson method) against the fraction released, the latter performed using regular dialysis (dialysis bags) (Figure 10). The Wagner-Nelson method was also used in another publication where the in vivo absorption from Silybin meglumine hollow-sphere mesoporous silica nanoparticles, dosed orally to beagle dogs, was compared with in vitro results obtained using a combination USP I (paddle) dialysis bag set-up [33]. Of the five different release media used in the study, a Level A IVIVC (R^2 > 0.97) was obtained with 0.06 M Na2CO3 and 0.08 M Na2CO3 solutions (Figure 11). In another study with Silybin, an IVIVC was achieved with a 72-hour release formulation of Silybin (72-hour SLB) that consisted of a solid dispersion, gel matrix, and porous silica nanoparticles (PSNs) (Table 3) [35]. Similar to the earlier study, the authors compared in vivo release from beagle dogs with in vitro release assessed using a combination USP 1 (paddle) dialysis bag set-up. In another paper, Tiwari et al. demonstrated a Level A

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**Table 2: Examples of IVIVC with nano-sized dosage forms in animal models.**

<table>
<thead>
<tr>
<th>Drug and dosage form</th>
<th>In vitro release set-up</th>
<th>In vivo studies</th>
<th>IVIVC type</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Indomethacin gelatin nanoparticles</td>
<td>Dialysis bags</td>
<td>Wistar Albino rats</td>
<td>Level A</td>
<td>[32]</td>
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<tr>
<td>Silybin meglumine hollow-sphere mesoporous silica nanoparticles</td>
<td>Combination (USP I—dialysis bag)</td>
<td>Beagle dogs</td>
<td>Level A</td>
<td>[33]</td>
</tr>
<tr>
<td>Silybin 72-hour SLB and PSNs (porous silica nanoparticles)</td>
<td>Combination (USP I—dialysis bag)</td>
<td>Beagle dogs</td>
<td>Level A</td>
<td>[35]</td>
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<td>Simvastatin nanostructured lipid carriers and nanoparticles</td>
<td>Dialysis bag</td>
<td>Balb/C mice</td>
<td>Level A</td>
<td>[34]</td>
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<tr>
<td>Fenofibrate nanosuspension</td>
<td>USP I apparatus</td>
<td>Sprague Dawley rats</td>
<td>Level A</td>
<td>[36]</td>
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<td>In vitro lipolysis model</td>
<td>Sprague Dawley rats</td>
<td>Rank order</td>
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</tbody>
</table>

**Table 3: Level A IVIVC with two Silybin preparations (data from reference [35]).**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% absorbed equals</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>72-hour SLB</td>
<td>1.1379 x % dissolved + 8.8688</td>
<td>0.9831</td>
</tr>
<tr>
<td>Silybin PSNs (porous silica nanoparticles)</td>
<td>0.8915 x % dissolved − 10.152</td>
<td>0.9831</td>
</tr>
</tbody>
</table>

**Figure 10:** Level A IVIVC from Indomethacin gelatin nanoparticles using regular dialysis (redrawn from [32]).
correlation between in vitro release (dialysis bag) and percent drug absorbed (Wagner Nelson method) for Simvastatin nanostructured lipid carriers as well as nanoparticles ($R^2 > 0.94$) (Figure 12) [34].

5. Conclusions

For novel dosage forms like nanoparticles where no regulatory or compendial standards exist, in vitro drug release assessment assumes greater significance in serving as an indicator of product quality and performance. A plethora of methods have been used, each with their advantages and drawbacks with respect to ease of set-up, sampling, and rapid buffer replacement. Ideally, an in vitro release method should simulate in vivo conditions, release mechanisms, and enable the establishment of an IVIVC. Future research should focus on developing relevant mathematical models to predict drug release behavior as well as release mechanisms applicable to a wide range of nano-sized dosage forms.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this paper.

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