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Testing the In Vitro Product Performance of Mucosal Drug Products: View of the USP Expert Panel

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ABSTRACT

Performance testing of mucosal drug products presents the user with a multitude of challenges. Not only are there many different dosage forms to be distinguished, but also a wide variety of administration routes. The target action effect (local or systemic) is another factor to be considered. Thus, it quickly becomes apparent that there will never be a universal performance test, but the question arises just as quickly whether the method to be used should rather depend on the dosage form or the place of application, or even whether decisions must be made on an individual basis. This *Stimuli* article is one of a series of *Stimuli* articles on product performance testing, which focuses on methodological approaches and challenges in the field of performance testing of mucosal drug products. The article should be viewed as a supplement to, but also a critical discussion of the methods listed in USP general chapter *Mucosal Drug Products—Performance Tests* <1004>. With consideration of major physiologic aspects at the site of administration and the types of dosage forms to be studied, limitations of the methods described here and the need for methodologic updates or innovations are identified. Furthermore, suggestions are made for future activities, all aimed at developing robust, discriminatory, and meaningful test methods for the wide variety of mucosal drug products.

INTRODUCTION

The development and application of appropriate in vitro performance tests is one of the cornerstones of quality assurance of pharmaceutical dosage forms. In recent decades, there has been a considerable increase in the number of corresponding test methods in international pharmacopoeias, including the United States Pharmacopoeia (USP). It is noteworthy that even though all of the test methods developed to date have basically had the same objective, namely, to ensure the quality, safety, and efficacy of the medicinal product in question, older methods are often relatively simple and, in some cases, do not really show a direct link to the administration site and drug target action site. Nevertheless, they serve the purpose for which they were designed, which is to ensure critical quality measures for the products they were designed for. However, the question arises as to whether the methods in question also accomplish this when applied to similar products intended for the same application.

For many years, we have witnessed a growing number of new pharmaceutical entities and the development of novel dosage forms and generic medicines for a wide range of indications. Along with the increase in novel dosage forms and generic product development there is also an increase in the knowledge about the physiological conditions that may have an influence on the in vivo performance of a pharmaceutical drug product at the site of application and/or the site of drug release. Therefore, it seems reasonable to reconsider existing in vitro performance tests regarding their capabilities and significance, to identify possible methodological gaps, and to think about modern methods that are meaningful,

differentiated, robust, and standardizable and, in the best case, not only provide information on quality but can also provide valuable information on the performance of the drug product (using in vitro or in vivo methods) under investigation.

This article is the sixth in a series of *Stimuli* articles from the USP Expert Panel on New Advancements in Product Performance Testing (EP-NAPPT) and was prepared by the Mucosal Drug Products subgroup. It aims to raise awareness of current practices and new developments in the evaluation of mucosal drug products. The basis for this article is *USP* general chapter *Mucosal Drug Products—Performance Tests* <1004>, which contains the current compendial product performance tests for drugs intended to be delivered to the body via the mucosal route.

The objective of this article is to:

- Evaluate current testing methods and perform a gap analysis that identifies the limitations and analytical challenges of current methods
- Indicate whether there is a need to update existing methods or to implement new performance tests for the various subtypes of mucosal drugs
- Propose methodological approaches for new product performance testing
- Facilitate public comments from users and regulators
- Gather comments from users and regulators and then draft new compendial chapters or update existing compendial chapters

MUCOSAL DRUG DELIVERY

Mucosal drug products deliver active pharmaceutical ingredients to the body via a vast variety of mucous membranes. These include the otic, ophthalmic, nasal, oropharyngeal, urethral, vaginal, rectal, and, in principle, also the pulmonary mucosa. However, USP clearly delineates the latter site of administration from all others and discusses dosage forms that refer to the pulmonary route of administration in *USP* test chapter *Inhalation and Nasal Drug Products—General Information and Product Quality Tests* <5>. The group of mucosal drug products discussed in *USP* chapters *Mucosal Drug Products—Product Quality Tests* <4> and <1004> is therefore limited to the remaining seven mucosal surfaces, with consideration given to products with both local and systemic effects. When considering the specified application sites for mucosal drug products, it quickly becomes apparent that the mucous membranes in question are located in very different locations of the body, which can differ significantly in their structure and function. It is basically a logical consequence that the different sites of application and therapeutic modalities result in quite different requirements for the mucosal drug products to be administered, which in turn directly indicates that the individual performance tests will probably have to be designed differently if meaningful results are to be obtained. These requirements also include whether the drug product is for a local or a systemic effect and whether the dosage form should demonstrate a rapid, delayed, or sustained drug release. As a rule, product performance tests are in vitro drug release studies. However, as noted in *USP* <1004>, consideration should also be given to whether alternative testing strategies (in light of the latest developments in the field) can provide the desired information.

GAP ANALYSIS

To assess the current state of science, to evaluate the possible need for novel in vitro methods, and also to evaluate the need for standardization of existing performance tests, the Mucosal Drug Products Subgroup of the EP-NAPPT performed a gap analysis for each individual subgroup of mucosal dosage forms. In addition to the general performance tests monographed in the *USP* chapters *Dissolution* <711>, *Drug Release* <724>, and *Semisolid Drug Products—Performance Tests* <1724>, individual product-specific USP performance tests, performance tests recommended by the Division of Bioequivalence of the US FDA's Office of Generic Drugs and listed in the FDA's Dissolution Methods Database, and methods listed in the scientific literature for the respective dosage forms were reviewed. In the following sections, the results of this gap analysis are discussed for each individual administration route.

Ophthalmic Route

Background

As stated in the introductory section of *USP* chapter *Ophthalmic Products—Quality Tests* <771> , the routes of administration of ophthalmic products fall into three general categories: topical, intraocular injections, and extraocular injections. Taking a more detailed look, a variety of individual routes of administration can be distinguished, including the topical, subconjunctival, subtenonal, subretinal, subchoroidal, intracorneal, intrascleral, suprachoroidal, intravitreal, intracameral, juxtascleral, and retrobulbar administration. Accordingly, ophthalmic products are administered to the eye in a

wide variety of dosage forms, including but not limited to solutions, suspensions, ointments, gels, emulsions, strips, injections, inserts, and implants. Currently, there are approximately 710 ophthalmic products including 470 generic versions and 320 discontinued products listed in the FDA's *Orange Book* (Approved Drug Products with Therapeutic Equivalence Evaluations).

The focus of this *Stimuli* article is on topically administered ophthalmic products. Whereas intra- and extraocular injections are administered through external boundary tissue, topical drug products are intended to be administered to an ocular surface component, such as the eyelid, conjunctiva, or cornea, and can produce local or systemic effects.

The anatomy and physiology of the eye are extremely complex. The eyeball, which weighs on average about 7.5 g and is about 24 mm long, consists of an outer layer with the cornea as the most anterior tissue layer of the eye, a middle and an inner layer as well as three internal sections, i.e., the anterior and posterior eye chamber and the vitreous body. In addition, various adnexa, especially upper and lower eyelid, lacrimal gland, and the lacrimal drainage system are important for ocular physiology and can significantly affect topical ocular drug therapy. Typical indications for topically applied ophthalmic drug products are the treatment of dryness and irritation of the eye, high intraocular pressure for glaucoma, and inflammation of the conjunctiva (conjunctivitis) and cornea (keratitis). Many of the drugs administered in this way are intended to act in the precorneal area of the eye or in the anterior part of the inner eye, but topical ophthalmic instillation is currently also being discussed as a strategy for delivering drugs to the back of the eye (*1*).

Administration of topical ophthalmic drug products to the cornea means application onto a membrane covered with a very thin film of tear fluid. Tear fluid is a buffered liquid containing a variety of components with a mean pH value of 7.2–7.4 and is approximately iso-osmolar with blood. The average volume of tear fluid available in the precorneal space is small, averaging 7 µL, of which approximately 1 µL is distributed over the cornea and 3 µL is located in each of the tear margins (*2*, *3*). Most of the tear fluid is produced in the lacrimal glands and drains into the nasal cavity via lacrimal ducts at the corner of the eye. Fluid hydrodynamics are influenced by blinking, among other factors. The average turnover rate of tear fluid is reported as about 15% × min^{-1} (*4*). The maximum amount of fluid that can be held in the cul-de-sac is 25–30 µL (*3*). Particularly for liquid formulations, the dose volume that can be administered is thus very low. Application of a drug product into the precorneal area typically induces tear flow. Therefore, not only the applicable dose but also the precorneal residence time of drugs after application is very limited, which severely limits not only the local availability but also the amount of drug that could penetrate through the cornea. Consequently, the ocular availability of topically applied drugs is usually low. With this information, it should be evident that the unique anatomy and physiology of the eye, and the numerous modes of drug delivery to the eye, pose a major challenge to the development of performance tests for topical ophthalmic drug products.

Performance Tests

Ophthalmic dosage forms include emulsions, gels, inserts, lenses, implants, ointments, solutions, and suspensions. Conventional dosage forms (e.g., solutions, suspensions, emulsions, and ointments) cover approximately 97% of the marketed topical ophthalmic products approved by the FDA. Ocular implants are typically administered by the intravitreal route and are thus discussed in the *Stimuli* article *In-Vitro Product Performance of Parenteral Drug Products: View of the USP Expert Panel* (*5*). The same applies to all liquid formulations for intra- or extraocular injection. Information on performance tests for ophthalmic products that have an extended-release (ER) mechanism (beyond 1 day), for which the dissolution or drug release rate is rate limiting for absorption and is expected to provide a controlled therapeutic response is provided in *USP* general chapter *Ophthalmic Products—Performance Tests* <1771>. The performance tests for all other ophthalmic drug products are listed in *USP* <1004>. *USP* <1724> is referenced for testing emulsions, gels, and ointments, i.e., official apparatuses such as the immersion cell apparatus and the vertical diffusion cell, which are commonly applied in performance testing of topical drug products, can be used. For emulsions, also *USP* Apparatus 2, the paddle apparatus can be used, and drug release of suspensions can be assessed with either the paddle apparatus or a miniaturized version thereof. The test conditions are not further specified. Although these test methods might be appropriate for quality testing of selected topically applied suspensions, emulsions, ointments, and inserts, it is questionable whether they would constitute meaningful performance tests. Furthermore, it should be noted that the currently described in vitro performance tests are considered inadequate for in situ forming gels and mucoadhesive formulations because these dosage forms interact with the mucosal membrane to exert their function or effect

in vivo, which can hardly be simulated with the standard set-ups described.

Another point to consider is that many drug products applied in the precorneal area of the eye are formulations that must show a rapid drug release because of the precorneal clearance. If differences in product quality that affect in vivo performance are to be detected in in vitro release testing (IVRT), the rate of drug release within a short release time period must be monitored as accurately as possible. Conventional sample-and-separate methods are often not suitable for this purpose. In standard set-ups such as the paddle apparatus with automated or manual sampling, one would simply not be able to take a sufficient number of samples within the time period of interest, and in diffusion-controlled test models, diffusion rather than release would be the rate-determining step for such rapidly releasing formulations. Accordingly, as part of method development and standardization, consideration must be given to the development of a fit-for-purpose and robust IVRT method that can reliably capture the released drug fraction even over very short time periods, to detect and distinguish variations in product quality and performance. For complex products (e.g., emulsions, suspensions, liposomes, drug-protein complexes), which are not limited to ophthalmic drug products only, an adaptive perfusion method, representing a pressure-driven separation method based on the principle of tangential flow filtration has recently been proposed for this purpose (*6*). It would certainly be valuable to evaluate this or similar methods for their universal applicability in in vitro performance tests for such dosage forms.

Table 1. Ophthalmic Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004> and <1771>

Dosage Form, <4>	Performance Test, <1004> and <1771>
Emulsions	<711>, Apparatus 2, <1724> VDC
Gels	<1724>
Inserts and Lenses	<711>, <724>
Implants	<711>, <724>
Ointments	<1724>
Solutions	—
Strips	—
Suspensions	<711> Apparatus 2*

**Standard or miniaturized version.*
VDC, vertical diffusion cell.

Interestingly, despite the many ophthalmic drug products available on the market, USP does not currently list a single product-specific monograph that includes a requirement for an IVRT. The same situation is seen when reviewing the current FDA Dissolution Methods Database. Here, too, there are no specifications for an IVRT for an ophthalmic drug product.

Biorelevance of In Vitro Test Conditions

Current performance testing for topical ophthalmic products is not biorelevant due to the lack of consideration of available fluid volumes, composition, and dynamics (e.g., precorneal clearance) in the IVRT design. When it comes to studying topically applied dosage forms for drug delivery into the anterior part of the inner eye, it would be important to assess drug permeation through the cornea. Hence, this would have to be appropriately assessed in the IVRT. Currently, there is limited guidance on methods for evaluating corneal and conjunctival drug penetration in diffusion cell and permeation assays, e.g., criteria for tissue selection, tissue preparation, membrane loading with drug, receptor or dissolution media, and agitation or flow rate to be used in such experiments. In some areas, such as the development of artificial tears for in vitro studies, some progress has already been made in the past (*7, 8*). However, the focus of most of the reported studies was not on the development of an IVRT. Nevertheless, the method designs used in these studies as well as the experience gained, could be helpful for the discussion regarding the development of biorelevant in vitro performance tests for topically applied ophthalmic drug products. Although it is expected that there will be more efforts in the future regarding the development of biorelevant IVRTs for topically applied ophthalmic drug products, it should also be noted that for this sensitive application area in particular, there will invariably be differences between in vitro and in vivo conditions, so there is always the chance that performance tests may not be sensitive enough to detect differences in critical material properties and changes in critical manufacturing parameters that would affect in vivo performance.

In Vitro-In Vivo Correlation (IVIVC)

Just as there is a lack of biorelevant performance tests for topical ophthalmic drug products, there is also a lack of those that allow the development of IVIVCs for different ophthalmic dosage forms based on their routes of administration or the regions to which they are to be delivered. There are a few initial approaches in the literature that have been used, for example, to establish an IVIVC between the in vitro release of ocular inserts and their in vivo drug release in the conjunctival sac of rabbits (*9*). However, this was a formulation with release that probably depends little on the conditions prevailing at the site of application; the latter were not addressed in all relevant details the in vitro experiment and the amount of drug released was extrapolated solely from

the unreleased fraction of the administered dose (*9*). As the drug clearance and the distribution of the drug into different eye tissues were not taken into consideration, this developed IVIVC may not be able to predict in vivo performance of these ocular inserts (*10*). Overall, although a few attempts towards establishing IVIVCs have been reported, it can thus be concluded that there is a lack of suitable methods for IVIVC.

Otic Route

Background

The ear is divided into three parts: the external ear, middle ear, and inner ear. The external ear is composed of the pinna and external auditory meatus (ear canal). The ear canal is approximately 0.7 cm in diameter and 2.5-cm long (*11*) and provides passage from the outside to the tympanic membrane, which separates the external ear from the middle ear. The surface of the ear canal is lined with skin. The skin in the ear canal has short hairs and apocrine and sebaceous glands that produce ear wax. Earwax consists of fatty acids, fatty alcohols, squalene, and cholesterol and sometimes dead skin cells and hairs (*12*). The pH in a healthy ear canal is slightly acidic (pH 5–6) and increases with disease (*13*, *14*). The outer epidermal layer is continuous with the epidermis of the external canal. The diffusion of drugs administered to the ear canal into the air-filled middle ear cavity (and the inner ear) is controlled by the tympanic membrane (*15*). The tympanic membrane is a thin, cone-shaped membrane with a surface area of about 80 mm^2 and a thickness of 100–150 µm, depending on the location within the membrane. The tympanic membrane has a low permeability to most substances and its outer epidermal layer has similar properties to the stratum corneum (*15*). The shape of the external canal does not allow clear visualization of the tympanic membrane for monitoring drug application to the middle ear. Infection, trauma, or rapid pressure changes may cause perforation of the tympanic membrane. This creates a connection between the external auditory canal and the middle ear, and drugs from ototopical application can enter the middle ear. Diseases of the external ear requiring topical treatment are mainly associated with skin disorders. However, noninvasive trans-tympanic delivery of drugs to the middle ear has also gained interest, such as for instance for the administration of protective agents to treat drug-induced ototoxicity (*16*) or of antibiotics for otitis media treatment (*17, 18*). Topical antibiotics, corticosteroids, and anesthetics are commonly used for ototopical treatment and topical solutions (e.g., ear drops), suspensions, and ointments are typical dosage forms that are applied to the skin at the pinna, ear canal, and tympanic membrane.

The FDA *Orange Book* currently lists approximately 73 otic drug products including 54 generic versions and 40 discontinued products in the US market.

Performance Tests

Dosage form classifications of otic products can be found in *USP* general chapter *Pharmaceutical Dosage Forms* <1151>. Most of the marketed drug products are topical otic solutions (drops) and other dosage forms are suspensions and ointments. Because of the site of application and the nature of the dosage forms, the general test methods for otic drug products are similar to those for other topical products such as topical suspensions or ointments (see Table 2). For example, in vitro release set-ups for topical dermatological dosage forms such as the vertical diffusion cell or immersion cell apparatus can be used.

Table 2. Otic Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Ointments	<1724>
Solutions	—
Suspensions	<711>, Apparatus 2*

**Standard or miniaturized version.*

The FDA Dissolution Methods Database lists three otic suspension products (ciprofloxacin hydrochloride and hydrocortisone, ciprofloxacin and dexamethasone, and finafloxacin), but no in vitro release method is provided for these products. It is stated that methods will need to be developed to characterize in vitro release of these products. Beyond that, recommendations on performance tests for otic products are currently not available in the literature.

Methodological Standardization

As there are no official release testing methods for topically applied otic drug products so far, the question of how to standardize them does not arise. At the same time, however, this provides an opportunity to develop standardized methods from scratch that are preferably biorelevant, whereby a distinction must certainly be made between products that are generally applied in the ear canal and those that are applied directly to the tympanic membrane.

Biorelevance of In Vitro Test Conditions

The test procedures for otic drug products present similar issues as those for topical dermatological dosage forms. In both cases, the formulations are not in contact with

(a significant amount of) liquid after application, so that all methods in which the active ingredient release from the dosage form is investigated in direct contact with an aqueous medium are rather questionable because such conditions are very different from those at the application site. Thus, the development of biorelevant test methods can certainly be guided by methods for topical drug products for cutaneous application. However, situations specific to the ear such as the presence of earwax and the enclosing environment in the ear canal cannot be easily represented. In addition, when it comes to developing biorelevant in vitro performance tests for preparations for trans-tympanic drug delivery, one should keep in mind that the healthy middle ear is an air-filled space and that there is no liquid volume available for the active ingredient penetrated through the tympanic membrane in which it can disperse. Overall, it is important to discuss whether IVRT is the ultima ratio in the case of topically applied otic drug products when it comes to developing a meaningful performance test.

In Vitro-In Vivo Correlation

Currently, no biorelevant performance tests exist for otic drug products. As there has been no objective to predict the bioavailability of otic drug products on the basis of IVRTs, there also have been no approaches to establish IVIVC for such formulations.

Nasal Route

Background

The nose belongs to the upper airways and has, among other things, the task of warming, cleaning, and humidifying the air we breathe. Anatomically, it is divided into the external nose and the internal nose, and the two nostrils form the entrance to the inner nose. This comprises the nasal cavity, which is separated by the nasal septum into two, ideally symmetrical halves, the left and right nasal cavities. Inside them, the nose is lined with a well perfused mucous membrane with a surface of about 0.01 m^2. The main part of this mucosa, the respiratory mucosa, consists of three tissue layers: the top layer of which is the multilayer ciliated epithelium and contains glands that produce the nasal mucus (~40 mL × h^{-1}) protecting the nasal mucosa from drying out. Slightly different information on nasal mucosal pH can be found in the literature, which among other factors can certainly be attributed to the method used for determining the pH value (*19*–*21*). Overall, however, all studies indicate that the nasal mucus in healthy conditions is approximately neutral or very slightly acidic, whereby it was observed that the pH value increases with increasing distance from the nostrils (*20*). The nasal mucus is permanently moved towards the nasopharynx by the concertized back-and-forth movement of the fine cilia of the epithelium. The respiratory nasal mucosa typically represents the site of application for topically administered mucosal dosage forms, whereas the olfactory mucosa, located in the uppermost part of the nasal concha, is reached only by gases, vapors, and aerosol particles.

Most topically applied nasal medicines are used to achieve a local effect, e.g., in the treatment of colds, with vasoconstrictive agents for decongestion or immunologically active drugs predominating. However, intranasal administration also represents an interesting route of administration to deliver drugs into the bloodstream. The bypass of the hepatic first-pass effect, noninvasive application, good bioavailability, and rapid onset of action theoretically offer several advantages for selected drugs, which is why an increase in research activities in this area has been observed in the recent past. To date, 162 nasal drug products including 100 generic versions and 57 discontinued products reached the US market (FDA *Orange Book*).

Performance Tests

The currently official version of *USP* chapter <4> classifies nasal drug products into aerosols, gels (jellies), ointments, sprays, and solutions. The performance tests of these dosage forms are described in *USP* <1004>. Performance tests for nasal aerosols and nasal sprays are largely concerned with droplet or particle size distribution and aerodynamic size distribution. Accordingly, the procedures in the *USP* general chapter *Inhalation and Nasal Drug Products: Aerosols, Sprays, and Powders—Performance Quality Tests* <601> can be applied to products administered by mucosal routes. Performance tests of these formulations are thus part of the *Stimuli* article, *Testing the In Vitro Product Performance of Inhalation and Nasal Drug Products: Views of the USP Expert Panel* (*22*), and will thus not be discussed in the present *Stimuli* article. Aerosols, sprays, and solutions represent the vast majority of nasal drug products currently on the market, whereas only two gels and one ointment for nasal use are listed in the *Orange Book*. The general test methods for the latter two formulation types are similar to those for other topical gels or ointments (see Table 3). For example, in vitro release set-ups for topical dermatological dosage forms such as the vertical diffusion cell or immersion cell apparatus can be used. Beyond this general information, USP does currently not contain any product-specific monographs with information on in vitro performance testing of nasal gels and ointments. The FDA's Dissolution Methods Database also does not list

any product-specific IVRTs for nasal drug products, and no official recommendations on performance testing for nasal drugs can be found in the literature either.

Table 3. Nasal Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Aerosols	<601>
Gels (jelly)	<1724>
Ointments	<1724>
Sprays	<601>
Solutions	<601>

Methodological Standardization

As there are no official test methods for assessing the active ingredient release of nasal gels and ointments to date, the question of their standardization cannot arise. However, it should be clear that in future developments, care should be taken to establish methods that are as biorelevant and standardized as possible.

Biorelevance of In Vitro Test Conditions

For nasal drug products, there are similar issues as already discussed for ophthalmic and otic drug products. Here, too, the formulations will not be immersed in liquid after application, so that all methods in which the release of active ingredient from the dosage form is investigated in direct contact with large volumes of aqueous media are out of the question. There are already some published approaches in which electrolyte solutions of different compositions with slightly acidic pH have been used to simulate the composition of nasal mucus in an in vitro release experiment. In the corresponding experimental designs, a dialysis-based test design was used in which very small media volumes were employed (*23*–*25*). This could be a possible step toward more biorelevant test methods. A starting point for developing biorelevant test methods for nasal drug products could certainly also be based on methods for topical drugs for cutaneous application, but in any case, it should be borne in mind that, to circumvent mucociliary clearance by increasing retention time at the nasal mucosal surface, research is also increasingly being directed towards mucoadhesive preparations for nasal application, and a distinction must certainly be made between "ordinary" and mucoadhesive preparations when developing a biorelevant performance test.

In Vitro-In Vivo Correlation

Currently, there is no biorelevant performance test for semisolid nasal drugs. Therefore, it is understandable that no approaches to establish an IVIVC for such formulations have been published so far.

Oropharyngeal Route

Background

Oropharyngeal drug products are a class of mucosal drug products that deliver drugs to the mucosal surfaces within the oral cavity and are intended for either local or systemic action. Among the 100–200 cm^2 surface area of the intra-oral mucosa, the buccal (cheeks, gingivae, and inner lips) and sublingual (floor of the mouth and ventral side of the tongue) regions are most permeable for drug uptake or absorption through the nonkeratinized, 0.1- to 0.6-mm-thick stratified squamous epithelial cell barriers, and thereby, have most often been used for systemic drug delivery (*26*, *27*). Although the surface area of these main application sites (~80 cm^2) is smaller, when compared to the gastrointestinal (GI) tract (~200 m^2) or skin (~2 m^2) region, the buccal and sublingual mucosa represent highly vascularized regions that have direct access to the systemic blood circulation via the jugular vein. As a result, bioavailability of buccally or sublingually administered drugs can be high because hepatic first-pass metabolism is bypassed, whereas absorption can be rapid for certain lipophilic, low-molecular-weight drugs, as with injection (*26*, *27*). However, salivary fluid (pH 6.5–7.7) (*28*) is constantly secreted at 1–2 L/day or 0.7–1.4 mL/min, which eliminates dissolved or dispersed drugs to the GI tract by swallowing, and thereby reduces absorption via the oral mucosa. Swallowed drugs can then be absorbed from the GI tract, which may complicate interpretations of the systemic pharmacokinetic (PK) profiles (*26*, *27*). To date, about 210 oropharyngeal drug products including generic products are listed in the *Orange Book*.

Performance Tests

According to *USP* <4>, oropharyngeal drug products are classified into buccal patches, films, gels, gums, lozenges, ointments, solutions (rinses), sprays, and tablets. Note, however, that a revision for <4> has just been proposed in *PF* 48(5) for this classification. The performance tests of these dosage forms are described in *USP* <1004> as per the dosage form types across mucosal drug products rather than the routes of administration. As shown in Table 4, the performance tests for oropharyngeal drug products concern assessments of drug dissolution or release from dosage forms determined by the methodologies in existing general chapters for other (e.g., oral or transdermal) drug products with adaptations, and otherwise, are left unstipulated. *USP* chapters <711> and <1724> are referenced for films, gels, lozenges, ointments, and tablets, and drug release testing devices

described in *European Pharmacopoeia* (*EP*) chapter 2.9.25, Dissolution Test for Medicated Chewing Gums are referred to for gums. However, because the test methods in these chapters were not developed and validated for oropharyngeal, but other (e.g., oral or transdermal) drug products, relevant methodological adaptations are needed, as guided by the *USP* general chapter *The Dissolution Procedure: Development and Validation* <1092>. For drug release testing of sublingual tablets and buccal tablets, USP Apparatus 1 and 2, the basket and the paddle apparatus or mini-basket or mini-paddle apparatuses may be used. Lozenges may be tested with basket or paddle apparatus at high agitation (175 rpm) or with USP Apparatus 3 (reciprocating cylinder apparatus), whereas USP Apparatus 5 (paddle over disk) or a mini-basket may be used for testing films. The use of miniaturized equipment serves to reduce the dissolution or release medium volumes to less than 500 mL, given a smaller fluid volume available in the oral cavity. By contrast, no product performance test is specified for solutions and sprays, presumably because dissolution or release should not in theory be a concern for these solution products.

As shown in Table 5, for a handful of oropharyngeal products, relevant tests are specified in product specific *USP* monographs. In addition, the FDA's Dissolution Methods Database also provides information on dissolution or release test methods for certain drugs formulated in oropharyngeal products. Table 6 summarizes the methodological details of the methods listed therein.

Table 4. Oropharyngeal Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Buccal patches	<724> Apparatus 5
Films	<724> Apparatus 5, Apparatus 1[a]
Gels	<1724>
Gums	European Pharmacopoeia
Lozenges	<711> Apparatus 1[b], Apparatus 2[b], Apparatus 3
Ointments	<1724>
Solutions (rinses)	—
Sprays	—
Tablets (buccal, sublingual)	<711> Apparatus 1[a], Apparatus 2[a]

[a]*Miniaturized version.*
[b]*At high agitation (175 rpm).*

Methodological Standardization

The product-specific *USP* monographs listed in Table 5 stipulate the *USP* disintegration test or dissolution test for eight oropharyngeal drug products as a performance test. However, according to *USP* <1004>, the *USP* disintegration test is not specified as a performance test for buccal and sublingual tablets. By contrast, the methods listed in the FDA's Dissolution Methods Database vary for a given drug or a given dosage form type, and no standardization has been made to date. In line with *USP* <1004>, the basket and the paddle apparatus, and USP Apparatus 5 (paddle over disk) are most frequently suggested, whereas the reciprocating cylinder apparatus is indicated for mini-lozenges of nicotine polacrilex and sublingual tablets

Table 5. Methodological Details of Disintegration or Dissolution Tests for Drugs Formulated in Various Oropharyngeal Drug Products, Found in USP Drug Product Monographs

Dosage Form	Drug	Apparatus	Medium	pH	Volume (mL)	Agitation (rpm)	Duration (min)
Lozenge	Clotrimazole	USP 2[a]	HCl (0.1 N)	1	500	50	45
	Zinc; Vitamin C	USP 2[b]	HCl (0.1 N)	1	900	75	60
Tablet (buccal)	Methyltestosterone	Disintegration[c]	Water	—	—	—	30
Tablet (sublingual)	Buprenorphine and Naloxone	USP 1[a]	Water	—	500	100	10
	Isosorbide dinitrate	USP 2[a]	Water	—	900	50	20
	Isosorbide dinitrate	Disintegration[c]	Water	—	—	—	2
	Nitroglycerin	Disintegration[c]	Water	—	—	—	2
	Ergotamine tartrate	Disintegration[c]	Water	—	—	—	5

[a]*Dissolution <711>.*
[b]*Disintegration and Dissolution of Dietary Supplements <2040>.*
[c]*USP Disintegration <701>, (for uncoated tablets)*
HCL: hydrochloric acid.

Table 6. Methodological Details of Dissolution or Release Performance Tests for Drugs Formulated in Various Oropharyngeal Drug Products, Found in the FDA's Dissolution Methods Database

Dosage Form	Drug[a]	Apparatus	Medium	pH	Volume (mL)	Agitation	Duration
Film (buccal)	Buprenorphine	USP 1 (100 mL)	Phosphate buffer	4.5	60	100 rpm	60 min
	Fentanyl	USP 1 (100 mL)	Phosphate buffer	6.4	60	100rpm	45 min
	Fentanyl	USP 1 (100 mL)	Phosphate buffer	6.5	100	100rpm	45 min
Film (sublingual)	Apomorphine	USP 5	Bis-Tris buffer	6.4	500	75 rpm	20 min
	Buprenorphine and Naloxone	USP 5 (56 mm; 40 mesh disk)	Acetate buffer	4.0	900	100 pm	10 min
Gum	Nicotine	EP	Phosphate buffer	7.4	20	60 cycles/min	30 min
Lozenge	Nicotine	USP 1	Phosphate buffer	7.4	900	100 rpm	8 h
	Nicotine	USP 3	Phosphate buffer	7.4	250	20 rpm	90 min
	Fentanyl	USP 2	Phosphate buffer	4.5	500	175 rpm	40 min
Tablet (buccal)	Acyclovir	USP 1	Phosphate buffer	6.0	1000	60 rpm	12 h
	Miconazole	USP 1	0.5% SDS in water	6.5	1000	60 rpm	12 h
	Fentanyl	USP 2 (small volume)	Phosphate buffer	7.0	100/200	100 rpm	20 h
	Testosterone (ER)	USP 2 (sinker)	0.5% SDS in water	—	1000	60 rpm	24 h
Tablet (sublingual)	Buprenorphine	USP 1	Water	—	500	100 rpm	15 min[b]
	Asenapine	USP 2	Acetate buffer	4.5	500	50 rpm	5 min
	Buprenorphine/Naloxone	USP 2	Water	—	500	100 rpm	20 min
	Fentanyl	USP 2	Phosphate buffer	6.8	500	50 rpm	20 min
	Nitroglycerin	USP 2	Phosphate buffer	6.5	500	50 rpm	10 min
	Zolpidem	USP 2	Phosphate buffer	6.8	900	75 rpm	15 min
	Zolpidem	USP 2	Simulated intestinal fluid	6.8	500	50 rpm	15 min
	Sufentanil	USP 3	Acetate buffer	4.5	50	50 rpm	15 min

[a]Drugs may be in different chemical forms, e.g., salt or polacrilex.
[b]Or until 80% of the labeled content is dissolved.
SDS: Sodium dodecyl sulfate; ER: Extended release.

of sufentanil (Table 6). Both the dissolution or release medium (water, buffer, or simulated body fluid), pH (4.0–7.4), volume (20–1000 mL), and agitation speed (20–170 rpm) vary between test methods. In this regard, the volume of the dissolution or release medium is certainly of particular interest for adaptations, as a volume >500 mL, as described in *USP* <711> and <724> for testing oral dosage forms, is probably of little relevance when trying to represent fluid conditions in the oral cavity. In fact, *USP* <1004> mentions the use of a mini-basket or mini-paddle apparatus to accommodate smaller fluid volumes (e.g., 20–100 mL, as suggested for some products) given the limited volume of oral mucosal fluid in the oral cavity. The duration of the dissolution or release tests also varies. Fast-acting drugs (e.g., sublingual tablets) are tested for 5–20 min, whereas other products are tested for up to 24 h. It is therefore clear that these methodological differences in dissolution or release testing need to be resolved by standardization, presumably according to the type of dosage form.

Biorelevance of In Vitro Test Conditions

Because, as mentioned above, the USP dissolution or drug release apparatuses were developed and validated for testing the performance of other (e.g., oral, or transdermal) dosage forms rather than oropharyngeal drug products, methodological adaptations are required. Accordingly, a lack of biorelevance is evident. The use of more than 500 mL of dissolution or release medium is likely to maintain "sink" conditions in many cases, but this should not necessarily be the case for drugs with low solubility, e.g., lipophilic drugs. Smaller media volumes (e.g., 20–100 mL) can be used with mini-basket and mini-paddle systems. The smaller media volumes may still be larger than the fluid volume available in the oral cavity within a short time for rapidly dissolving or releasing dosage forms, such as sublingual tablets, films, and even lozenges. The composition and pH of the dissolution or release medium should be chosen to reflect the fluid at the site of drug release, i.e., oral mucosal fluid or saliva. For some products, such as buccal tablets, gels, and ER

ointments, drug release should occur only on the surfaces that come into contact with the oral mucosa, and this must be considered during testing. At the same time, for release tests of such dosage forms, no rationale arises in terms of agitation of the medium to simulate the dynamics of oral mucosal fluid. However, there are also dosage forms, such as lozenges, where mechanical stress in particular can have a direct influence on the dissolution behavior, which is why in these cases one should also consider the influence of the mixing of the medium or, for example, the agitation rate in devices such as the reciprocating cylinder apparatus. Finally, the residence time of drugs and dosage forms in the oral cavity is not considered in current compendial test methods. After dissolution or release, dissolved or dispersed drugs may be rapidly excreted from the oral cavity via salivary clearance, and if this is the case, such drugs no longer exert local therapeutic effects or, in the case of systemically active drugs, may no longer be absorbed through the oral mucosa. Meanwhile, a need of biorelevance can even be debatable, especially for rapidly dissolving or releasing dosage forms, if drug dissolution or release anyway occurs in a short period, unaffected by compositions and conditions of surrounding media.

For assessing drug release of gums, *USP* <1004> endorses the official dissolution test for medicated chewing gums (2.9.25) from the *EP*. The two official apparatuses, Apparatus A and B, are both closed chamber systems with horizontal and vertical oscillatory pistons, respectively, to reflect deformation of gums and masticatory actions of subjects. The recommended release medium is 20 mL of phosphate buffer at 37°, as is also found for nicotine gum in the FDA's Dissolution Methods Database (Table 6). Nevertheless, other parameters such as distance between upper and lower chewing surfaces, rotation angle, and chewing frequency, as well as sampling volume and duration still need to be rationally chosen. Indeed, some of them have been shown to affect drug release from gum products (*29*, *30*). In 2015, a *Stimuli* article (*31*) reported a multilaboratory study to test two nicotine gum products on the US market using Apparatus A and B of the *EP*. Despite applying identical procedures and set-ups, variability in the drug release profiles was high and the results for a given product differed between the two apparatuses. These few experiments are certainly not nearly sufficient to identify a method as suitable or unsuitable. In fact, evaluation of new methods requires a much larger number of experiments with different drug products and careful selection and control of experimental conditions, including performance verification tests with reference standards. Hence, *USP* has not yet published its own performance tests for medicated chewing gums.

As stated before, no performance (dissolution or release) test is stipulated for oral spray products. This is presumably because the marketed products deliver an aliquot of drug in solution to a defined location within the oral cavity, e.g., into the mouth over the tongue, and precipitation on the oral mucosal surface would not be expected by virtue of a low dose and a decent aqueous solubility. However, this presumption may or may not be true, if a low solubility drug is formulated or a suspension spray product becomes available in the future. Therefore, even at this point, there should be thought ahead about what the design for a biorelevant and meaningful in vitro performance test for such products could be.

Oropharyngeal drug products are used not only for systemic disease treatments but also for topical local disease treatments. Examples for such topical local treatments are liquids (solutions and suspensions), semisolids (gels, creams, and pastes), chewing gums, and films (patches and strips), to treat oral mucositis, candidiasis, infection, pain relief, or anaesthesia, as well as lozenges to treat sore throat (*32*). However, the current *USP* dissolution or release tests for oropharyngeal drug products (Table 5) may or may not be appropriate to examine local action performance of these products. This is in fact true for many locally acting drug products administered through different routes (e.g., skin, eye, ear, nose, and lung), recognizing that local drug concentrations and profiles are of importance, rather than systemic counterparts. Nevertheless, the dissolution or release profiles may provide some information on the behavior of the products in the oral cavity, whereas the lack of biorelevance and assessment of the residence time of the active ingredient in the current tests may prevent accurate prediction of local drug concentrations and thus performance. Depending on the nature of the locally active dosage form, new methods to be established will certainly not have to meet the same requirements in all cases. For example, testing for some dosage forms, such as locally acting oropharyngeal gels and ointments (*33*), will likely involve use of the vertical diffusion cell for topical and transdermal drugs described in *USP* chapter <1724>. However, it should be noted that there are efforts on the horizon to demonstrate local bioavailability of oropharyngeal dosage forms using appropriate in vitro release testing in generic drug product evaluation. A detailed example of the development of a suitable method for a locally acting lozenge formulation comprising the use of a simulated salivary fluid and an apparatus that mimics fluid exchange in the oral cavity as well as mechanical forces that can act on the dosage form has recently been published (*34*, *35*). The basic considerations for the design

of the cited method can certainly provide a basis for the development of other biorelevant methods, but it should also be clear that the test conditions based on a successful study are not necessarily transferable to all types of lozenges. Here, as with many other oropharyngeal drug products, there is still room to develop standardized, robust, but meaningful performance tests.

In Vitro-In Vivo Correlation

Because current performance tests for oropharyngeal drugs are not biorelevant, IVIVC has not been widely practiced. For systemic applications, challenges are foreseeable for many products when swallowed drugs are absorbed from the gastrointestinal tract, as systemic PK profiles are composed of the fraction of dose absorbed via the oral mucosa and the gastrointestinal mucosa. For example, for a fentanyl buccal lozenge, approximately 25% of the dose is absorbed through the oral mucosa with the remaining 75% being absorbed through the gastrointestinal tract, which is reflected in double peaks in the resulting plasma profiles (*36*). In contrast, for a rapidly dissolving fentanyl buccal tablet, approximately 50% of the administered dose is reported to be absorbed through the buccal mucosa, whereas the remainder is swallowed and absorbed in the gastrointestinal tract (*37*). With this mixed absorption, it is uncertain whether dissolution or release-based performance tests alone are sufficient to predict systemic PK profiles and thus product performance. It may be that drugs are dissolved or released, as predicted by in vitro performance tests, but are readily removed from the oral cavity by salivary clearance, so no local effects are expected. This indicates that in the future it will presumably be difficult to implement standardized methods that allow for an IVIVC for every type of oropharyngeal dosage form.

Vaginal Route

Vaginal drug products are particularly appropriate for drugs associated with women's health issues but may also have applications in general drug delivery within the female population. Whereas historically vaginal drug products have been administered primarily for local effects, for example, to treat infections of bacterial, fungal, or viral origin, or to administer contraceptive or labor-inducing agents, the vaginal route of administration has recently gained more interest because it is also well suited for the administration of a number of drugs with systemic effects (*38*). Systemic administration of drugs via the vaginal mucosa can have several advantages. These include the avoidance of multiple side effects that may result from oral or parenteral administration of the corresponding drugs, but especially the bypass of hepatic first-pass metabolism (*39*).

The vagina represents a slightly S-shaped muscular canal of about 10 cm in length. This canal is collapsed so that the anterior and posterior vaginal walls are in contact (*40*). The vaginal wall as such is about 3 mm thick and consists of three layers. The uppermost layer, i.e., the vaginal epithelium consists of stratified, nonkeratinized squamous epithelial cells, the thickness of which is subject to constant change related to the female menstrual cycle (*41*). The inner mucosa of the vagina also has numerous folds called rugae that provide extensibility, support, and increase the surface area of the vaginal wall. Under the squamous epithelium lies a very elastic layer of connective tissue (lamina propria), permeated by veins, due to which the vagina has excellent elasticity, allowing sexual intercourse and childbirth, but also the administration of vaginal dosage forms.

Similar to the thickness of the vaginal epithelium, the amount and composition of vaginal fluid changes during the menstrual cycle and with age. Estrogen and sexual stimulation increase the secretion of vaginal fluid (*38*). Although women of reproductive age produce 3–4 g of vaginal fluid per hour, this decreases to about half after menopause (*41*). Vaginal fluid as such does not exist; rather, it is a mixture of different secretions, such as cervical secretions and mucus, endometrial and oviductal fluid, transudate from blood vessels containing exfoliated vaginal cells and leukocytes, and microorganisms and their metabolites (*38*, *40*, *41*). Accordingly, it contains a variety of components, such as inorganic and organic salts, mucins, proteins, carbohydrates, urea, and fatty acids (lactic and acetic acids) (*38*). The pH conditions in the vagina are determined by the bacterial flora present. Under normal conditions, i.e., vaginal eubiosis, lactobacilli convert glycogen from exfoliated cells into lactic acid, thus maintaining a buffered acidic environment in the pH range of about 3.5–4.5 (*38*, *40*–*42*). During menstruation, but also due to frequent sexual intercourse, an increase in vaginal pH can be recorded because both ejaculate and vaginal transudate are alkaline. Moreover, vaginal dysbiosis (e.g., bacterial vaginosis) can also lead to noticeable changes in vaginal pH (*41*, *43*). Overall, the parameters that can affect intravaginal drug release or dissolution are quite complex, which may place special demands on the in vitro performance tests to be established, especially when it comes to predicting performance under typical application conditions. A variety of vaginal dosage forms are currently on the market or in clinical development. To date, in the United

States, 145 vaginal drug (48 reference listed drugs (RLD) and 97 generic drug products) products of which 71 have been discontinued have been approved for clinical use (FDA *Orange Book*).

Performance Tests

According to *USP* <4>, vaginal drug products are classified into creams, foams, gels, and inserts. As shown in Table 7, for performance testing of vaginal gels, reference is made to the methods for determining drug release from semisolid dosage forms described in the existing *USP* <1724>. However, because the test procedures in this general chapter were developed and validated for semisolid formulations for cutaneous application rather than for vaginal drug products, appropriate methodological adaptations are required, such as those described in *USP* <1092>. The same applies to vaginal inserts, for which reference is made to *USP* <711> and the use of the basket or the paddle apparatus, which are devices originally developed for dissolution testing of oral dosage forms. For foams, *USP* <1004> does not specify performance tests aimed at investigating the release of the active ingredient, presumably because these are preparations in which the active ingredient is dissolved, and the release of the active ingredient is ensured by the nature of the preparation.

Table 7. Vaginal Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Creams	<1724>
Foams	—
Gels	<1724>
Inserts	<711> Apparatus 1, Apparatus 2

Although the *USP* contains several individual monographs for vaginal drug products (six vaginal creams, two vaginal suppositories, and six vaginal inserts), only one of these monographs, that for *Estradiol Vaginal Inserts*, describes a product-specific drug release test, which must be performed in 500 mL of phosphate buffer, pH 4.75 in the basket apparatus over a test period of 10 h. For all other vaginal inserts, a disintegration test according to *USP* Disintegration <701> is required instead of a dissolution test (Table 8). Interestingly, however, <701> does not contain specific information on how to determine the disintegration of vaginal inserts, so consideration should be given to modifying the method at this point if necessary.

The FDA's Dissolution Methods Database contains several individual dissolution or drug release test methods for certain drugs formulated into vaginal drug products. Table 9 summarizes the methodological details for each. Although this database does not include in vitro performance tests for semisolid preparations for vaginal application, it does include test methods for vaginal inserts and tablets, which are referred to as inserts in the *USP*. In addition, it lists methods for dosage forms such as vaginal rings and vaginal suppositories that are not listed in *USP* chapters <4> and <1004> and also indicates the need to develop in vitro methods to characterize in vitro release of these two dosage form types.

Methodological Standardization

The dissolution method in the *USP* for estradiol inserts (Table 8) as well as most of the FDA-approved test methods (Table 9) specify the use of compendial equipment, such as the basket or the paddle apparatus or a slightly modified basket apparatus (Palmieri basket [*44*] in combination with relatively large volumes (500–900 mL) of aqueous media with varying pH (4.5–7.4) and composition (water, hydrochloric acid, phosphate buffer). In cases where the formulation contains poorly soluble drugs, artificial surfactants such as sodium dodecyl sulfate (SDS) are added to the medium to provide sink conditions. Agitation in the basket or paddle set-up ranges from 40 and 100 rpm and the test duration varies between 30 min and 12 h. For one of the dosage forms, a vaginal ring containing estradiol, a noncompendial in vitro set-up, i.e., an incubator shaker, is used. In this set-up, the vaginal ring is immersed in 250 mL of 0.9% saline solution at pH 6.5 and agitated at 130 rpm for a test duration of 45 days. For selected drug products, such as a dinoprostone suppository or an ethinylestradiol and etonogestrel ring, for which a suitable in vitro performance test does not yet

Table 8. Methodological Details of Disintegration or Dissolution Tests for Drugs Formulated in Various Vaginal Drug Products, Found in USP Drug Product Monographs

Dosage Form	Drug	Apparatus	Medium	pH	Volume (mL)	Agitation (rpm)	Duration
Insert	Nystatin	Disintegration[a]	Water	—	—	—	60 min
	Clotrimazole	Disintegration[a]	—	—	—	—	20 min
	Estradiol	USP 1	Phosphate buffer	4.75	500	40	10 h

[a]*<701>, Procedure and Criteria for Uncoated or Plain-Coated Tablets.*

Table 9. Methodological Details of Dissolution or Release Performance Tests for Drugs Formulated in Various Vaginal Drug Products, Found in the FDA's Dissolution Methods Database

Dosage Form	Drug	Apparatus	Medium	pH	Volume (mL)	Agitation	Duration
Insert	Dinoprostone (ER)	USP 2	Deionized water	4.5	500	50 rpm	5 h
	Progesterone	USP 2	0.25% SDS in water	6.4	900	50 rpm	30 min
Ring	Estradiol	Incubator shaker	0.9% Saline	6.5	250	130 rpm	45 days
Suppository	Miconazole nitrate	USP 1	0.45% SDS in water	7.4	900	100 rpm	8 h
	Terconazole	USP 1[a]	1% SDS in 0.1 N HCl	4.5	500	100 rpm	40 min
Tablet	Estradiol	USP 1	Phosphate buffer	4.75	500	40 rpm	12 h
	Clotrimazole	USP 2	0.1 N HCl	—	900	50 rpm	45 min

[a]*With a Palmieri type basket.*
ER: Extended release; SDS: sodium dodecyl sulfate; HCL: hydrochloric acid.

exist, the database specifically notes the need to develop an appropriate method for in vitro release testing.

As for oropharyngeal drug products, adapting the volume of the dissolution or release medium is certainly of interest when aiming method standardization as a volume >500 mL might be of little relevance when trying to represent fluid conditions in the vagina. However, before entering too deeply into such a discussion, it should be borne in mind that vaginal dosage forms are also a very heterogeneous group of dosage forms that are used either for local or systemic action and range from dosage forms with very rapid release up to those with sustained release of the active ingredient over a period of weeks or even months. Therefore, as for oropharyngeal drug products, it is not possible at this point to attempt a generally applicable method standardization. One must rather distinguish whether the active ingredient is predominantly delivered into the vaginal lumen, or immediately in the vicinity of the mucosa, whether it is intended for local or systemic action, and whether it is released over minutes, hours, days, weeks, or months. In the end, this distinction will not only determine the volume and composition of the medium, but also a whole range of other analytical parameters. In addition, in the context of considering the standardization of test methods for vaginal drug products, an additional aspect emerges that should definitely be taken into account. Whereas *USP* <4> distinguishes vaginal dosage forms into creams, foams, gels, and inserts (Table 7), both the FDA Dissolution Methods Database and the *Orange Book* refer to dosage forms such as vaginal tablets, rings, ER inserts, and suppositories. Consequently, the question arises as to whether these formulations can be considered subsets of the vaginal drug products listed in *USP* <4>, or otherwise how to deal with these formulations. Especially when it comes to developing an appropriate and standardized performance test, answering this question would be of vast importance. According to *USP* general chapter <1151>, inserts are referred to as solid dosage forms that are inserted into a naturally occurring body cavity other than the mouth or rectum. They can be applied for local or systemic action. Vaginal inserts are described as globular or oviform dosage forms that are intended to dissolve in vaginal secretions. Although the description of vaginal inserts indicates that these are preparations that dissolve in the vaginal fluid, contradictory information follows in the next section, which deals with possible manufacturing processes for inserts (not limited to vaginal inserts). Here, it is stated that the inserts may be molded, pressed from powder or, in the case of extemporaneous formulations, even formulated as capsules, and that they may be formulated so that they melt at body temperature or disintegrate on insertion. These explanations do not necessarily contribute to great clarity. This is why, on the one hand, it would be reasonable to include vaginal tablets and suppositories in the category of vaginal inserts because they are inserted into the vagina and dissolve, melt, or disintegrate there. On the other hand, especially if one considers the composition and the characteristics of the different dosage forms (hydrophilic, lipophilic), they could be considered individual types of dosage forms for which different performance tests will be required. Moreover, it should be noted that ER inserts and vaginal rings do not appear anywhere in the *USP*. Before considering standardization of performance tests for vaginal drug products, thought should be given to standardizing the terminology and clearly distinguishing between the individual dosage forms. In the next step, a suitable method could then be selected depending on the type of dosage form, mode of action (systemic or local), intended drug release time, drug properties, and dose. For hydrophilic formulations containing highly soluble drugs, miniaturized standard methods, as already discussed for oropharyngeal drug products, would certainly be suitable. For lipophilic suppositories, suitable

methods could for instance be established based on EP chapter 2.9.42, *Dissolution Test for Lipophilic Solid Dosage Forms*.

For vaginal rings, there is currently no official method describing a release test using a compendial set-up. The release method for an estradiol ring described in the FDA Dissolution Methods Database is representative of many incubator shaker-based methods reported in the literature (*45*). Such methods are often used to compare different prototypes in the development of vaginal rings. An incubation shaker can be equipped with a variety of vessels so that many rings can be tested in parallel. In addition, the set-up is easy to handle. Both these features are advantageous if the test duration is weeks or even months. In terms of standardization, however, such a method must be viewed rather critically, which is why there is also a need for suitable performance tests here. Because vaginal rings, as already mentioned, are usually intended to release the active ingredient over several weeks or even months, if one wishes to standardize test methods, a completely different question arises at the same time, namely the applicability of accelerated test methods. Based on the results of some studies on the acceleration of drug release without influencing the release mechanism of a vaginal ring (*46*, *47*), the use of such methods as in vitro performance tests seems generally possible, which, however, requires a very precise control of all test conditions as well as an appropriate method validation.

Biorelevance of In Vitro Test Conditions

If one considers the currently described in vitro release methods for vaginal drug products and assesses them with regard to their biorelevance, a similar picture emerges at many points as for oropharyngeal drug products. The site of application, i.e., the vagina, is also a body cavity with a small amount of liquid available and, in contrast to the oral cavity, a much lower fluid exchange. In vitro drug release methods using several hundred milliliters of fluid can therefore hardly reflect the physiological conditions in the vagina, but at best create sink conditions for poorly soluble drugs. As for oropharyngeal drug products, for rapidly dissolving or releasing dosage forms, such as hydrophilic inserts, suppositories, and tablets, smaller media volumes can be used with mini-basket and mini-paddle systems, yet the fluid volume again may still be larger than the fluid volume available in the vagina.

In recent years, especially because it has been shown that microbicides applied topically to the vagina by women can reduce the risk of infection with HIV and other sexually transmitted diseases, there has been a trend towards the development of novel vaginal dosage forms (*48*, *49*). This led with an increasing demand for appropriate in vitro test methods for ensuring a safe and reliable in vivo performance of these novel formulations. Accordingly, there have been several attempts to make release methods more biorelevant, as evidenced in particular by the introduction of various simulated vaginal fluids (*50*). This was a first step toward establishing more biorelevant IVRT methods for these dosage forms. However, the instrumental set-ups used are usually conventional as discussed before and the methods presented so far have generally been used to compare specific formulations in individual experiments. Overall, methods that could be claimed to be biorelevant or even biopredictive and capable of becoming generalizable are still lacking (*51*).

In Vitro-In Vivo Correlation

To date, few efforts have been reported to establish an IVIVC for a vaginal dosage form. Given the lack of physiology-based biorelevant release models for vaginal dosage forms to date, this is not surprising. It should be mentioned, however, that it has recently been possible to retrospectively correlate the mean in vivo release rate of a contraceptive vaginal ring with its in vitro release performance. This was achieved both with a real time release method, in which a medium with a physiologically relevant pH was used, as well as with various accelerated test methods, in which the temperature and/or the medium composition was specifically changed to accelerate drug release (*46*, *47*). Results of the respective studies demonstrate that it is possible to obtain in vivo predictive results for vaginal preparations using appropriate in vitro methods. However, due to fundamentally different release mechanisms and the dependence on the test conditions used, the method developed for the vaginal ring in question cannot simply be transferred to other vaginal dosage forms. Therefore, further substantial work is needed when aiming to predict in vivo performance of vaginal drug products based on in vitro performance testing.

Rectal Route

The rectum represents the last section of the colon and opens into the anal canal. In adults, it has a length of about 10–15 cm and a diameter like that of the sigmoid colon. The anal canal itself has a length of 3–4 cm. The rectum and anal canal have a special sphincter system, which ensures continence and also defecation (*52*). The rectum is normally empty and the anal canal is closed by permanent contraction of the internal sphincter (*53*, *54*). A specific pattern of contraction of the empty rectum

prevents continuous outflow of colonic contents into the rectum, and therefore fecal matter remains in the sigmoid colon until it is ready to be excreted from the body (*54*). When feces pass from there into the rectum, the rectal wall stretches, and the internal anal sphincter relaxes to accommodate the feces. A certain amount of rectal stretching eventually triggers an urge to defecate so that controlled defecation can occur. Even though the rectum can store up to 2 L of stool in the interim, as noted earlier, it is usually empty most of the time, and the open diameter of the rectal lumen is then no more than 1.5–3.5 cm. Histologically, the rectum shows similarities to other sections of the colon. The mucosa has a smooth surface with a total surface area of approximately 200–400 cm^2 (*55*, *56*) and consists of simple squamous enterocytes with straight tubular glands that run through the entire thickness of the mucosa. The rectum is drained by three veins: the inferior, middle, and superior rectal vein. The inferior and middle rectal vein empty into the systemic venous system, thus avoiding a hepatic first-pass effect of rectally administered drugs absorbed via these veins. In contrast, the superior rectal vein opens into the portal venous system. For this reason, complete avoidance of hepatic first-pass metabolism cannot be guaranteed with rectal administration of a drug. In this context, one should also consider that anastomoses between the portal and systemic veins may be present in the wall of the anal canal. Unfortunately, there is very little information on available volumes, secretion rates, composition, and properties of rectal fluid. Secondary literature reports that the adult rectum is "filled" with 1–3 mL of a nearly enzyme-free, viscous fluid with a pH in the neutral to slightly alkaline range (7.2–8) and virtually no buffering capacity (*4*, *57*, *58*). Moreover, there is evidence that age and diet may influence rectal pH. Unfortunately, however, robust data on these statements are not available, and further studies are needed to better understand the rectal environment in different patient groups.

Rectal drug products can be used for both local and systemic administration. Due to its size, the rectal lumen can accommodate relatively large dosage forms. Consequently, high drug doses can be administered rectally. However, although the ability to administer large dosage forms and high doses of a drug provide excellent conditions for controlled-release drug delivery systems, the use of such dosage forms is essentially precluded because drug delivery can be interrupted or terminated at any time by defecation. Therefore, rectal administration is typically used for immediate-release (IR) dosage forms (*59*). In the United States, 145 rectal drug products of which 108 are generic drug products have been approved for clinical use. To date, 83 of these drug products have been discontinued (FDA *Orange Book*).

Performance Tests

USP <4> classifies rectal dosage forms into foams, ointments, suppositories, solutions, and suspensions (Table 10). For performance testing of ointments, reference is made to the methods for determining drug release from semisolid dosage forms described in the existing general chapter <1724>. However, because the test procedures in this general chapter were developed and validated for semisolid formulations for cutaneous application rather than for rectal drug products, appropriate methodological adaptations are required, such as those described in *USP* general chapter <1092>. The same applies to suppositories, for which reference is made to <711> and the use of the basket or the paddle apparatus, the flow-through cell (USP Apparatus 4), and in particular, a modified, dual-chamber flow-through cell also described in chapter 2.9.42. of the EP that prevents analytical interferences caused by oil droplets formed during the melting of lipophilic suppositories. As for vaginal foams and all other solutions for mucosal administration, *USP* general chapter <1004> does not specify performance tests aimed at investigating drug release of rectal foams and solutions, as these are considered preparations in which the active ingredient is dissolved, and the release of the active ingredient is ensured by the nature of the preparation. For drug release testing of suspensions, the basket or the paddle apparatus or a miniaturized basket or paddle system can be used. As already discussed for other mucosal drug products, the use of miniaturized equipment serves to reduce the dissolution or release medium, but not to an extent that would be required to mimic physiological fluid volumes in the rectum.

Table 10. Rectal Drug Products Listed in USP <4> and Current USP Performance Tests According to USP <1004>

Dosage Form, <4>	Performance Test, <1004>
Foams	—
Ointments	<1724>
Suppositories	<711> Apparatus 1, Apparatus 2, Apparatus 4[a]
Solutions	—
Suspensions	<711> Apparatus 2b

[a]*Standard set-up or flow-through cell designed for suppositories.*
[b]*Standard or miniaturized version.*

The *USP* contains a single product-specific monograph (*Indomethacin Suppositories*), that describes a product-specific drug release for a suppository. In that case, the experiment is to be performed in the paddle apparatus in

900 mL of phosphate buffer at pH 7.2 over a test period of 60 min. Three dissolution methods for rectal drug products, i.e., a gel, a suspension, and a suppository can be found in the FDA's Dissolution Methods Database (Table 11). Furthermore, the need to develop an in vitro method for another suppository formulation is expressed.

Methodological Standardization

Although rectal drug products listed in the FDA's Dissolution Methods Database (Table 11) are basically different dosage forms with quite distinct release mechanisms, the paddle or basket apparatus in combination with large liquid volumes (500–900 mL) are to be used for the IVRTs. This is particularly surprising for studying drug release from a gel. While in two cases a phosphate buffer (pH 6.8 or 7.2) has to be used, the release experiment for prochlorperazine suppositories is to be performed in 0.1 N hydrochloric acid, a medium whose composition and pH in no respect correspond to the rectal fluid. It is also interesting to note that instead of the usual basket, a so-called Palmieri basket, named after its developer, representing a suppository basket made of inert plastic with the same dimensions as the standard basket, but in which the meshes have been replaced by 12 linear slits, must be used. By contrast, the indomethacin suppositories monographed in *USP* have to be tested for in the paddle apparatus in 900 mL of phosphate buffer at pH 7.2. The agitation rates in paddle (50 rpm) and basket system (100 rpm) represent standard agitation speeds and the test durations range from 45 and 60 min indicating that all dosage forms represent IR formulations.

As for oropharyngeal and vaginal drug products, a volume of >500 mL is unlikely to be relevant when trying to represent fluid conditions in the rectum. When aiming to standardize test conditions, one should certainly consider an adaptation of the media volume to more physiological volumes. As indicated in <1004>, mini-basket or mini-paddle devices may be suitable to address this issue. Because all rectal dosage forms are rapid-release dosage forms, it should be fairly easy to standardize test durations. As quite reliable pH conditions prevail in the rectum, ranging from neutral to slightly alkaline, a release medium should reflect this if possible. Accordingly, a medium such as 0.1 N hydrochloric acid should not appear in a test method for rectal dosage forms unless there are special reasons for allowing only such a medium. Whereas such a procedure should be appropriate for rectal suspensions, when aiming to standardize IVRT conditions for suppositories one should first refer to <1004>, which distinguishes two types of suppositories: 1) hydrophilic (water soluble), and 2) lipophilic (oil soluble or melting). Whereas drug release (dissolution) of water-soluble suppositories can be studied using the (mini) paddle or basket apparatus or the flow-through cell, as discussed earlier, drug release testing for lipophilic suppositories may need modification of the dissolution procedure to avoid analytical interference from the oil globules. As for lipophilic vaginal suppositories, a suitable method could be established based on *EP* chapter 2.9.42, *Dissolution Test for Lipophilic Solid Dosage Forms*, taking into account the aspects already mentioned (*60*).

Biorelevance of In Vitro Test Conditions

Consideration of the biorelevance of current in vitro release methods for rectal drugs gives a picture similar to that already discussed for a number of other mucosal dosage forms. Like the vagina, the rectum is an application site with a low fluid supply and a very low rate of fluid exchange. Therefore, current IVRT methods that require the application of large volumes of fluid provide, at best, sink conditions for poorly soluble drugs, but they cannot be considered biorelevant. Even with a miniaturized test method, one will hardly be able to match real fluid volumes. Overall, there seems to be quite little activity in the area of in vitro release testing of rectal dosage forms. In contrast to many other mucosal application areas, where there have been at least initial attempts to develop biorelevant media for in vitro testing of various kinds, no simulated or artificial rectal fluid has yet been reported. Therefore, there is a need not only for standardization of test methods, but at the same time also for increasing the biorelevance of IVRTs of rectal dosage forms.

Table 11. Methodological Details of Dissolution or Release Performance Tests for Drugs Formulated in Various Rectal Drug Products, Found in the FDA's Dissolution Methods Database

Dosage Form	Drug	Apparatus	Medium	pH	Volume (mL)	Agitation (rpm)	Duration (min)
Suspension	Mesalamine	USP 2	Phosphate buffer	7.2	900	50	30
Gel	Diazepam	USP 2	0.05 M Phosphate buffer	6.8	500	50	45
Suppository	Prochlorperazine	USP 1[a]	0.1 N HCl	—	900	100	45

[a]*With a Palmieri type basket.*
HCL: Hydrochloric acid.

In Vitro-In Vivo Correlation

To date, no biorelevant IVRT methods have been developed that aim to predict the in vivo performance of rectal drug products and there have been no efforts to correlate data from standard release studies with in vivo data. Accordingly, fundamental work is needed if it is to become possible to establish IVIVC based on results from in vitro performance tests.

Urethral Route

Urethral drug products comprise dosage forms that are inserted into the urethra, typically for local action, but systemic distribution of the administered drugs is also possible.

The urethra is a tubular organ of the urinary and genital apparatus. Due to its close association with the genital organs, which are differentiated by gender, the urethra is also distinct in the genders. It begins at the lower end of the urinary bladder localized in the pelvis and opens at the tip of the penis on the glans in males and in the vaginal vestibule in females. The anatomy and function of the urethra differ significantly in males and females. The male urethra is about 20 cm long and due to its incorporation into the penis has two curvatures as well as three constrictions in its progression. It serves not only to drain urine, but also as a canal for prostatic secretions and semen. The female urethra, on the other hand, is straight and only 3–5 cm long, and its function is limited to the discharge of urine from the bladder (*61*). The average open diameter (~6–8 mm) and the wall structure of some sections of the male and female urethra are similar. The urethral wall consists of three layers: Like all urinary drainage pathways, it has a special lining called the urothelium or transitional epithelium. When the urethra is empty, this lining is raised into longitudinal folds (*61*). Beneath the epithelium are elastic connective tissue and a blood vessel plexus. This is followed further out by smooth muscle and, on the very outside, again by connective tissue, which anchors the urethra in the surrounding tissue. In both men and women, the ducts of various glands open into the lumen of the urethra. Unfortunately, there is little information in the literature about the amount and the composition of fluid present and the pH conditions in the urethra. Overall, however, it can be assumed that the intraluminal pH conditions are dominated by the pH value of the urine (normal range: pH 4.5–7.8) or, in men in the event of ejaculation, also by the pH value of the seminal fluid (normal range: pH 7.2–8.0) (*62*).

To date, a medicated dissolvable urethral suppository, which according to the USP nomenclature would be referred to as an insert, is the only intraurethral drug product approved by the FDA. This suppository contains alprostadil and is inserted into the urethra immediately after urination. The drug is intended to show a fast action by local diffusion into the body tissues to initiate arteriolar vasodilation and penile erection.

Certainly, the application of drugs in the urethra is a very special field of application for which probably only a limited number of dosage forms can be expected in the future. Nonetheless, it should be noted that new therapeutic options have recently emerged for this field of application as well, such as the use of paclitaxel-coated balloon catheters for the treatment of urethral strictures (*63*). For these reasons, it is important to have suitable in vitro performance tests available for the quality assurance of such formulations. To date, eight urethral drug products can be found in the *Orange Book*, five of these are solutions for which a performance test in the sense of a drug release test is generally not required, and three of these are urethral suppositories.

Performance Tests

According to <4>, there is currently only one category of urethral drug products, namely, urethral inserts and no performance tests are described. Like the USP, the FDA's Dissolution Methods Database does not contain a monograph for a urethral drug product. Likewise, no alternative performance test has been described in the literature so far. At this point, it should be noted that there are currently only three suppository formulations on the market, which are alprostadil formulations of various potencies that are inserted into the male urethra in the form of a pellet or rod, referred to as a suppository, immediately after urination and before sexual intercourse. The aim of this procedure is to achieve rapid release and absorption of the active ingredient in order to achieve an erection. For this dosage form, it would first be necessary to develop a performance test, whereby the question arises as to whether this should then also be suitable for new dosage forms for urethral application or whether a universal test method can be developed for urethral drug products in general.

Methodological Standardization

Currently, no official performance tests for urethral drug products are available and general recommendations for their development do not exist. Therefore, it is reasonable to assume that in the case of the development of a new product, if required, a product-specific performance test will be developed. If in the future, several new drug products will be developed, it is likely that, as has happened in the past for other mucosal dosage forms,

different individual methods will be published rather than a standardized method being developed. The latter, however, should be the goal, as all urethral drug products are applied to a site characterized by a narrow lumen, usually direct contact with the tissue and with a small volume of fluid present. Because there are as yet no methods that would have to be considered in the further course of the decision, it would be desirable to develop a biorelevant test method from the very beginning, which would allow an estimation of the in vivo performance, but which would also be robust and simple enough to be used in quality control.

Biorelevance of In Vitro Test Methods

As already discussed, neither standard release methods nor biorelevant in vitro test methods for urethral drug products currently exist. In general, no considerations for the development of biorelevant test methods for these dosage forms can be found in the current scientific literature. As the urethra is normally flushed several times a day during urination, it seems unlikely at first glance that sustained release formulations would be considered for this site of application, because urination could cause washout released drug or the entire drug product and the amount of drug reaching the target site could not be controlled. For IR formulations, one could consider developing an in vitro model comprising a release medium that addresses the average urine pH for both male and female applications. A distinction would have to be made, in general, between dosage forms for systemic and for local action, especially when it comes to establishing sink conditions. Taking into account the apparatus described in *USP* chapters <711> and <724>, the development of a standardized method based on an official *USP* apparatus, such as the flow-through cell, is quite conceivable. Depending on the dosage form under investigation, such a method could probably also be further adapted to suit particular physiological conditions, such as for instance a certain disease state that presents with alterations of urethral fluid pH and/or composition, by varying the release medium and other test parameters if the aim is to increase in vivo predictivity. Since, as already described, there is currently only one commercially available drug product and there are yet little further research approaches towards new dosage forms for this site of application, it would be speculative to discuss further methodological details at this point.

In Vitro-In Vivo Correlation

As there are currently no release methods for urethral dosage forms, the question of an IVIVC does not (yet) arise.

GENERAL DISCUSSION AND CONCLUSION

Mucosal drug products represent a very heterogeneous group of dosage forms that are applied to various sites of the body. The formulations can differ significantly in their formulation design and release properties, whereas the characteristics of the application site, i.e., the various mucosae and other conditions at the site of application, can also differ greatly. Accordingly, it quickly becomes apparent that there can be no universal recommendation for suitable performance tests for all of these dosage forms, but that—as has been done in the previous sections—one must take a closer look at essential characteristics of the dosage form and the application site, but also to take into account whether a local or a systemic effect is to be achieved, to define suitable test conditions. A review of the currently available compendial test methods for mucosal drug products indicates that these considerations were probably not present in the first place during method selection and development. Thus, for many of the dosage forms in question, one finds either no methods at all, or in *USP* general chapter <1004>, reference to standard methods available in *USP* chapters <711>, <724>, <1724>, or <1771> is made, in most cases without addressing the fact that many of the test conditions listed there are unlikely to reflect conditions at the application site and thus may not provide the best basis for developing a meaningful performance test. A closer look at official test methods for individual drug products also reveals a frequent use of standard test methods whose test design often cannot be reconciled with the mode and site of application. This is not to question the suitability of these methods for the quality control of the respective drug products, but it does indicate that it is time to reconsider how meaningful performance tests could be developed and standardized. Results of the gap analysis indicate that, especially in view of evidence that the number of mucosal dosage forms is likely to increase in the future and that more application fields will be identified, it is necessary to update several of the existing methods and to introduce new performance tests for the various subtypes of mucosal drug products.

The need to develop new performance tests that are biorelevant and predictive where possible has also been recognized elsewhere. For example, under the Generic Drug User Fee Amendment (GDUFA), the FDA has funded various research programs to establish equivalence standards for complex pharmaceutical drug products, including several mucosal drug products. Among the stated research objectives here were the development of novel IVRTs that more closely match physiological in vivo conditions, the development of physicochemical

characterization methods to evaluate and compare critical quality attributes of various mucosal products and to determine key physicochemical properties that affect drug release and bioavailability, as well as further development of in silico modeling to study the effects of formulation properties on PK and/or pharmacodynamics (PD).

Some possibilities for the development of biorelevant and predictive performance tests have already been discussed in the individual subsections of this article. However, this *Stimuli* article is not intended to provide specific guidance for each individual subcategory of mucosal drug products, but rather to initiate a discussion among experts aimed at developing appropriate methods, balancing biorelevance, predictivity, robustness, and standardization as best as possible. A whole range of critical aspects and challenges relevant to method development including the choice of the apparatus, the volume, composition, physicochemical properties and temperature of the test medium, agitation or flow rate, that would also need to be considered here have already been discussed in a previous *Stimuli* article of this series (*5*). Although many general aspects apply to all dosage forms, it should be emphasized once again for the mucosal drug products that the dosage form is not permanently surrounded by liquid at any application site, that the available liquid volumes are generally small, but can differ significantly again at the application sites and that, in addition, the condition of the mucous membranes also varies. In addition, as already mentioned, a distinction must be made between dosage forms for local and systemic action.

Biorelevant media have already been proposed for almost all mucosal application sites (*64*, *65*, *50*). These differ considerably in composition and physicochemical properties, as is particularly evident from the example of the simulated vaginal fluids (*50*). A first important step in the direction of meaningful performance tests is certainly to first address the standardization of media for the individual application sites. The desired goal should be a medium that reflects the parameters relevant for drug release at the application site as optimally as possible, can be produced easily and reproducibly and, in combination with other test parameters, results in a robust and discriminatory method that is meaningful without being too complex. The next step would be to consider whether and when it would be appropriate to modify official test methods for certain dosage forms in such a way that they permit the use of smaller media volumes. Due to the diversity of mucosal dosage forms, one would have to consider at the same time whether special hydrodynamic conditions are required and whether and how sink conditions could be guaranteed. In this context, a distinction must certainly be made as to whether the dosage form is to adhere to the mucosa over the entire application time or whether it can or should move freely in the body cavity concerned and whether a unidirectional or multidirectional release is to be achieved. Diffusion methods appear to be particularly useful for dosage forms that are applied to the mucosa to deliver an active ingredient into the systemic circulation. Here, the question of suitable diffusion membranes arises. If the aim is to establish a robust test method, it would seem sensible to work with artificial diffusion membranes, which, however, are not necessarily suitable for mucoadhesive preparations because they probably cannot reproduce the interaction of the preparation and the mucosa that occurs in vivo. For the latter formulations, one would accordingly have to think about biomimetic membranes, cell models, or even natural membranes, although with increasing complexity, standardization of methods will become increasingly difficult. Dialysis-based release methods, in which the dosage form together with a small volume of biorelevant fluid is placed into the lumen of a dialysis tube that is agitated in a larger volume of a suitable acceptor fluid, might represent an interesting approach for the development of performance tests of dosage forms that are applied into body cavities where they can be wetted by or immersed in small amounts of fluid (*23–25*, *66*, *67*).

Because an ideal IVRT method should correlate the changes in the critical quality attributes of the drug product that have directly related to release performance, as has already been suggested or practiced for various types of semisolid drug products, including ophthalmic ointments (68), typical physicochemical properties of the pharmaceutical product in question that have the potential to affect product performance should be correlated with release behavior as part of method development and validation in order to develop meaningful, discriminatory, and robust test methods.

This article was written to raise awareness of challenges in standardizing drug release test methods for mucosal drug products. The points discussed here are intended to provide a starting point for future activities in the area of performance test development and for readers to provide food for thought for a fundamental discussion on this topic. It is our expressed wish that this *Stimuli* article will encourage a collaborative effort to reconsider, and if necessary, revise current methods, as well as to fill currently existing methodological gaps with standardized

and, in terms of in vivo performance, predictive test methods where possible. The EP-NAPPT Subcommittee will therefore greatly appreciate the involvement of as many stakeholders as possible in the activities of the EP-NAPPT by providing comments and suggestions based on their experience, so that the review and revision process now initiated can be jointly pursued further and future activities can move in the right direction.

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