Development of a veterinary orodispersible film with a focus on spectrophotometric quantification of gabapentin

Master's thesis by

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Abstract

To this day, there are no veterinary gabapentin dosage forms available on the market in Finland. Therefore, off-label treatment with human-marketed gabapentin, or compounded dosage forms thereof, are employed in the treatment of epilepsy and pain in cats and dogs. This practice is suboptimal, as there are significant risks of preparation errors and under- or overdosing from manually dividing capsules and tablets. A veterinary formulation, which could be safely and rapidly manufactured at the point-of-care, is needed. However, a hurdle in the development of such small-dose gabapentin dosage forms is the quantification of the gabapentin molecule. Ultraviolet-visible (UV-Vis) spectrophotometric quantification possesses suitable properties for implementation at small production sites, but quantifying gabapentin with the said technique has proven to be challenging as the small molecule is lacking chromophores.

This study aimed to thoroughly assess UV-Vis spectrophotometric gabapentin quantification methods with the intent of finding a reliable method applicable to a veterinary formulation. As a proof-of-concept, an orodispersible film formulation of gabapentin was developed and characterized. A selection of different quantification methods was assessed; one method, based on derivatization of gabapentin with ascorbic acid, stood out as the most precise and robust method. The method exhibited excellent linearity ($R^2 = 0.9998$) in a wide and useful concentration range (0.5–40 µg/ml) at a detection wavelength of 376 nm. The method was successfully applied to the developed formulation for reliable determination of the drug content. The quality of the orodispersible film formulation was assessed according to pharmacopoeial tests and additional analyses. The polymeric films were easy to prepare by solvent casting, and they possessed good mechanical strength and thickness uniformity, neutral surface pH, rapid drug release, and satisfactory disintegration time.

This study proved that pet-friendly gabapentin dosage forms can be easily manufactured and analyzed. The findings can be implemented in practice, for example, in pharmacies, veterinary clinics, and animal hospitals. The findings are a step towards the much-needed goal of improved, safer, and more personalized gabapentin treatment of cats and dogs.

Keywords: gabapentin, veterinary medicine, UV-Vis spectrophotometry, orodispersible films, solvent casting, personalized medicine, compounding, drug delivery

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List of abbreviations

AA – Ascorbic acid

ACN – Acetonitrile

API – Active pharmaceutical ingredient

ATR – Attenuated total reflectance

CC – Copper(II) chloride; cupric chloride

CHA – Chloranilic acid

CMC – Sodium carboxymethylcellulose

DCM – Dichloromethane

DL – Drug-loaded

DMF – N,N-dimethylformamide

DMSO – Dimethyl sulfoxide

DNP – 2,4-dinitrophenol

DSC – Differential scanning calorimetry

EMA – European Medicines Agency

FDA – U.S. Food and Drug Administration

FIMEA – Finnish Medicines Agency

FTIR – Fourier transform infrared (spectroscopy)

GBP – Gabapentin

GLY – Glycerol; glycerin

HPC – Hydroxypropyl cellulose

HPMC – Hydroxypropyl methylcellulose

ICH – The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use

IUPAC – International Union of Pure and Applied Chemistry

LP – Liver powder

NIN – Ninhydrin

NIR – Near-infrared (spectroscopy)

ODF – Orodispersible film

ODT – Orodispersible tablet

PBQ – *p*-Benzoquinone; para-benzoquinone; 1,4-benzoquinone

PEG – Polyethylene glycol

PEO – Polyethylene oxide

Ph. Eur. – European Pharmacopoeia

PVA – Polyvinyl alcohol

PVP – Polyvinylpyrrolidone

RSS – Residual sum of squares

SSE – Semi-solid extrusion

TEC – Triethyl citrate

UL – Unloaded

UV-Vis – Ultraviolet-visible

VAN – Vanillin

VICH – The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products

XRPD – X-ray powder diffraction

1. Introduction

Gabapentin, a derivative of the neurotransmitter γ-aminobutyric acid, is an anticonvulsant used for seizure prevention in epilepsy patients (Taylor, 2002). It is also commonly utilized in the treatment of neuropathic pain, a type of pain resulting from damage to the nervous system (Brannagan, 2009). It is not completely understood how gabapentin exerts its pharmacological effect; the main mechanism of action is likely based on various inhibitory actions on voltage-gated calcium channels, which in turn lead to decreased neurotransmitter release (Kukkar et al., 2013).

Epilepsy and neuropathic pain can affect animals too. Since there are no market-approved veterinary gabapentin dosage forms, many pets are currently treated off-label with human-marketed gabapentin (e.g. Neurontin®, Gabrion®) or extemporaneously manufactured dosage forms thereof. Cats and dogs require significantly smaller doses than those available in the marketed products. The compounding practice, however, is often unsatisfactory due to compliance issues and risks of preparation errors (Davidson, 2017). To achieve the best possible compliance, bioavailability, and therapeutic effect in small pets, a specifically developed veterinary gabapentin dosage form is needed. There is a growing group of potential patients since there are many pets in Finland, and the number of pet-owning households have been growing (Official Statistics of Finland, 2020). In 2016, almost a third of all households had one or several pets, with dogs and cats being by far the most common. Thus, there is clinical potential for a small-dose veterinary gabapentin formulation that is easy and rapid to both manufacture and analyze.

There are, however, challenges in the development of gabapentin dosage forms; these are directly related to reliable identification and quantification of the gabapentin molecule. According to Kostić et al. (2014), ultraviolet-visible (UV-Vis) spectrophotometry is the most utilized quantification technique for zwitterionic epilepsy drugs, a group to which gabapentin belongs together with pregabalin and vigabatrin. UV-Vis spectrophotometry is based on measuring how much a sample absorbs and transmits light in the ultraviolet and visible wavelength ranges (approx. 180–400 and 400–800 nm, respectively). UV-Vis spectrophotometry possesses many advantages over other quantification techniques; low cost, good performance, and simplicity of procedure make it the preferred technique for quantifying gabapentin (Almasri et al., 2019). Besides UV-Vis spectrophotometry, other techniques have been used. These include high-performance liquid chromatography, fluorimetry, liquid chromatography-mass spectrometry, gas chromatography-mass

spectrometry, capillary electrophoresis, thin-layer chromatography, chemiluminometry, potentiometry, and voltammetry (Abdulrahman and Basavaiah, 2012; Kostić et al., 2014). Compared to UV-Vis spectrophotometry, many of these methods are expensive and labor-intensive.

Whereas many molecules can be directly analyzed without further derivatization, the gabapentin molecule does not have significant absorbance in the UV-Vis wavelength range. Because of this, numerous methods have been developed for the determination of gabapentin in bulk, dosage forms, and human or environmental samples. Many methods involve derivatization of the molecule in order to obtain a measurable reaction product. The challenge of quantifying gabapentin has been addressed in the literature, and several theoretical comparisons of methods have been published (see Abdulrahman and Basavaiah, 2011a, 2011b and 2012; Gouda and Malah, 2013; Kostić et al., 2014). Only one practical comparison and assessment of different UV-Vis quantification methods has been published, namely by Fonseca et al. (2017). In the study, three direct methods (no derivatization) and three derivatization methods were assessed together with one fluorometric method. To the best of the author's knowledge, more extensive comparative studies of UV-Vis methods have not been published. Furthermore, many of the proposed quantification methods have been developed for gabapentin in human dosage forms, and their application on small-dose, veterinary formulations has not been investigated.

In order to achieve satisfactory gabapentin treatment of cats and dogs, this study proposes a new, improved formulation: a flavored orodispersible film with adjustable dosage strength, suitable for cats and small dogs. Easy adjustment of the dose would allow for personalized medicine to veterinary patients. To the best of the author's knowledge, this type of veterinary gabapentin formulation has not been explored earlier. As explained above, there is also a need for a thorough assessment of quantification methods to apply to the said dosage form.

2. Literature review

2.1 Gabapentin in veterinary medicine

2.1.1 Prevalence of epilepsy and neuropathic pain in veterinary patients

Seizure disorders, including epilepsy, are not uncommon among pets. Most prevalence studies have been conducted on small patient populations, and exact prevalence numbers are unknown. A recent study by O'Neill et al. (2020), conducted in the United Kingdom, revealed a prevalence of 0.16% in cats over a one-year period. In dogs, seizure disorders appear to be more common; Kearsley-Fleet et al. (2013) gathered data on dogs in the United Kingdom over a two-year period and stated a prevalence of 0.62%.

Pain is difficult to diagnose in animals, and especially neuropathic pain poses diagnostic challenges (Mathews, 2008). Animals express pain differently than humans, and it is challenging to distinguish between pain subtypes. However, it is well known that animals do suffer from neuropathic pain through the same pathophysiological mechanisms as humans. In a prevalence study by Muir et al. (2004), where data were collected specifically on pain patients, 8% of canine and 7% of feline pain patients were classified as having neuropathic pain.

2.1.2 Current practice of gabapentin treatment

Gabapentin has become a part of established clinical practice when treating neuropathic pain or preventing seizures in cats and dogs (Mathews et al., 2014). The use of gabapentin in the treatment of other types of pain (e.g. degenerative joint disease, peri- and postoperative pain, cancer pain) is also common. Gabapentin is given either as monotherapy or as an adjunct therapy to other analgesics or anticonvulsants. Recommended doses for dogs start at 10 mg/kg two to three times daily, and for cats, 5 mg/kg twice daily. Many case studies confirm the effectiveness of these doses. For example, data from a study conducted on greyhound dogs by KuKanich & Cohen (2011) suggested that 10–20 mg/kg three times daily maintained plasma levels equal to therapeutic plasma levels in humans. Three long-term case studies on cats performed by Lorenz et al. (2012) showed good pain management with 6.5 mg/kg administered twice daily. For the treatment of epilepsy, the initial doses are in the same range as in pain treatment (Shell, 2015). Generally, when using gabapentin, dose titration is often necessary to find an effective dose while reducing the risk of side effects

(Mathews et al., 2014). Gradual discontinuation of the medication is also recommended. These regimens mean that a significant number of veterinary patients need to be treated with very small (and often varying) doses of gabapentin, as a cat or a small dog can weigh only a few kilograms. For instance, a cat weighing 3 kg may need single doses of only 15 mg gabapentin.

The Finnish Medicines Agency (FIMEA) database of market-approved medicines in Finland reveals that gabapentin is currently only available as human medicinal products. The marketed dosage forms are tablets and capsules containing 300, 400, 600, or 800 mg gabapentin. According to The Finnish Medical Society Duodecim's medicine database, there is one extemporaneously manufactured (i.e. compounded) gabapentin oral solution for animals. A compounded medicine is made to order in a specific compounding pharmacy. The aforementioned compounded gabapentin solution does not contain any taste-masking agents, and the administration is inconvenient as the pet owner has to measure the correct dose, which inevitably bears the risk of dosing errors. Furthermore, the solution has a shelf-life of only nine months, which means that most pharmacies cannot stock bottles. Thus, veterinary patients may have to wait several days if the gabapentin solution is made to order and shipped across the country. Veterinarians often prescribe human-marketed gabapentin for off-label use, which means that the pet owners themselves are responsible for splitting tables or dividing capsules into appropriate doses. Dividing dosage forms involves a significant amount of manual labor, and there is a risk of under- or overdosing as it is difficult to obtain uniform doses (McDevitt et al., 1998). Breaking the protective coating of tablets or capsules may also alter the stability of the drug (Marriott and Nation, 2002).

Compounding of medicines is common practice in veterinary medicine because the number of approved veterinary medicines are relatively small, and therapeutic gaps must be filled by modifying human medicines. Davidson (2017) has addressed some common issues with compounding. As it is carried out manually in pharmacies, it is labor-intensive and bears risks. Preparation errors are relatively common; at their worst, they can lead to a lack of effect or lethal overdoses. For example, the Missouri Board of Pharmacy annually tests the potency of compounded drugs from Missouri pharmacies. In their annual reports from 2006 to 2020, a wide range of potencies has been documented. The acceptable potency range of a product is usually ±10% from the expected content, but the Board has found potencies between 0% (years 2006 and 2009) and as much as 450.4% (the year 2007) (Missouri Board of Pharmacy, 2006–2021). Every year, the percentage of unsatisfactory products (i.e. with a

potency deviation greater than 10%, or unsatisfactory sterility or endotoxin content) has been fluctuating between 11.1% (the year 2012) and up to 36.4% (the year 2019). As Davidson (2017) further remarks, other notable compounding risks besides varying doses are contaminations (e.g. microbial or from other drugs), physical and chemical instability of the finished product, and a lack of bioavailability in the target animal.

2.2 Development of veterinary dosage forms

There are many aspects to consider when developing veterinary formulations. Animals are not small humans, and therefore human dosage forms cannot always be scaled down according to the weight of the animal (Ahmed and Kasraian, 2002). Due to differences in pharmacokinetics, the required doses can vary significantly between species. Some excipients used in human medicinal products are toxic to animals, and the dosage form or route of administration may not be suitable for an animal (Davidson, 2017). The anatomical aspects must be taken into consideration; for instance, capsules and tablets can get stuck in a horizontal esophagus. Solid dosage forms are also easily spat out by animals. Hence, oral solutions and chewable tablets are common on the veterinary drug market.

A dosage form is chosen based on the target animal and the physicochemical and pharmacokinetic properties of the active pharmaceutical ingredient (API). With some exceptions, many of the same excipients are used in veterinary and human medicines. The toxicology of excipients and residual solvents in the target animal must be assessed, as well as the compatibility of excipients with the API. Excipients that are known to be toxic to animals have been listed by, for example, Davidson (2019). The International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Medicinal Products (VICH), to which the European Medicines Agency (EMA) belongs, has issued a guideline (2011) on residual solvents in veterinary medicines. Herein, toxicological classifications of solvents and recommended residual solvent levels can be found.

As many drugs possess a bitter taste or smell, palatability is of great importance in oral dosage forms. Cats and dogs especially prefer meat-based flavors such as beef, bacon, or liver (Bramwell and Williams, 2009). However, as Ahmed and Kasraian (2002) point out, the stability of a dosage form can be affected by the addition of flavoring agents. The potential effects on, for instance, dissolution, disintegration, and stability must be assessed.

The currently available gabapentin dosage forms are not optimal for administration to cats and small dogs. The compounded oral solution allows for easier dose adjustment, but a liquid drug is inconvenient to administer as it must be introduced with a syringe to the mouth. The lack of taste-masking agents may further decrease the patient compliance. Within human medicine, oral films and orally disintegrating dosage forms have been gaining popularity as they offer many advantages over conventional oral dosage forms. In this study, gabapentin was formulated into a mouth-dissolving film, as the availability of a palatable film for animals would solve many of the administration issues. This specific type of orodispersible film formulation will be reviewed in the following chapter.

2.3 Orodispersible films

2.3.1 Definition and formulation aspects

Orodispersible films (ODFs) are thin polymeric sheets that dissolve rapidly upon placement on the tongue, thus releasing the contained drug instantly (Hoffmann et al., 2011). In different contexts, ODF may also be referred to as oral (thin) film, buccal film, oral thin strip, oral wafer, fast dissolving film, or soluble film. ODF is the term recognized by the EMA and the European Pharmacopoeia (Ph. Eur.) and will also be used in this thesis.

Administration of an ODF does not require water, as the film will disintegrate in the saliva of the mucosal cavity. ODFs typically consist of film-forming polymers, which make up the bulk of the film, and plasticizers, which improve the mechanical properties such as flexibility and toughness. Flavoring or sweetening agents are often needed to mask the taste of APIs and excipients. The addition of saliva stimulating agents, surfactants, fillers, or colors is sometimes also necessary (Joshua et al., 2016). The polymer (or polymer mixture) in an ODF should be water-soluble as the film must disintegrate in saliva. Polymers of both natural and synthetic origin can be used. Some commonly used natural polymers are starch, gelatin, pullulan, sodium alginate, and pectin (Bala et al., 2013). Synthetic polymers can be, for instance, hydroxypropyl cellulose (HPC), hydroxypropyl methylcellulose (HPMC), polyethylene oxide (PEO), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), or sodium carboxymethylcellulose (CMC). A wide range of different molecular weights can be utilized. The polymer(s) should be non-toxic and tasteless and possess sufficient shelf-life. They should not slow down the film disintegration, and it is important that they do not enable microbial growth in the mucosa (a problem that can lead to infections).

Although naturally derived polymers are biocompatible and often available from renewable resources, they possess disadvantages such as impurities and high batch-to-batch variability (Germershaus et al., 2015). Fully synthetic polymers are generally better in terms of homogeneity. In this study, different types of synthetic polymers were investigated in the development of an ODF formulation.

As mentioned by Hoffmann et al. (2011), it is worth noting that the drug-loading capacity of ODFs is limited; the formulation is best for carrying potent, low-dose drugs. A too high load can cause drug crystallization or weakening of the film's mechanical properties. Dry ODFs typically contain 1–25% API, 40–50% polymer, 0–20% plasticizers, and 0–40% additives (colors, flavorings, fillers, etc.) (Arya et al., 2010).

2.3.2 Manufacture

ODFs can be manufactured through casting (solvent or semi-solid), hot-melt extrusion, solid dispersion extrusion, rolling, or various patented methods (Bala et al., 2013). Printing techniques, such as thermal inkjet printing, semi-solid extrusion (SSE), or flexography, have also been utilized (Buanz et al., 2015). Solvent casting is generally preferred since it is a simple process that does not involve heat or pressure. The absence of heating makes it suitable for thermolabile APIs and excipients. The preparation of solvent casting solutions is simple and involves few steps. Thus, solvent casting was also employed as the manufacturing method in this study.

In solvent casting, the polymer and additives are dissolved or dispersed in water or a mixture of water and organic solvents (Hoffmann et al., 2011). The API is added, and the solution is thoroughly mixed, after which it is cast and allowed to dry. The dried films are cut to pieces; the drug amount per dosage unit can be adjusted by changing the thickness of the film or the size of the cut film.

2.3.3 Previous studies

An extensive literature search was conducted, and no publications on ODF formulations of gabapentin were revealed. Soliman et al. (2020) have formulated orodispersible tablets (ODTs) containing gabapentin-saccharin co-crystals for enhancement of gabapentin bioavailability; the formulation is intended for pediatric and adult human patients. There is

a U.S.-based compounding pharmacy that offers veterinary gabapentin dosage forms such as oral pastes, chewable tablets, and melting tablets, but not oral films (Wedgewood Pharmacy, 2021).

ODFs have been explored to some extent within veterinary medicine, and there is one commercially available oral film technology (IntelGenx, 2021). A veterinary ODF formulation of prednisolone has successfully been manufactured through SSE 3D printing (Sjöholm et al., 2020). Huynh et al. (2016) have explored ODFs for drug administration to laboratory animals in preclinical studies and concluded that the administration of ODFs requires less manipulation (e.g. gavage), which in turn is likely to cause less trauma and stress in the animals as compared to administration of conventional oral dosage forms.

Gabapentin has been formulated into polymeric transdermal films for drug delivery through the skin. Sayare et al. (2019) have prepared chitosan films by solvent casting, while Singh et al. (2021) have utilized a solvent evaporation method to produce films consisting of HPMC, PVP, and PVA.

Pregabalin, a structural analog of gabapentin with very similar physicochemical properties, has been incorporated into a mucoadhesive film for transmucosal delivery (Nnamdi and Emmanuel, 2017). This type of film is intended to adhere to the mucosa for a prolonged time, allowing the drug to diffuse into the systemic circulation. The tested formulations were prepared by solvent casting and consisted of varying ratios of HPMC, PVP, and the ethyl acrylate-methyl methacrylate copolymer Eudragit RL 100.

2.3.4 Considerations for a veterinary orodispersible gabapentin film

Gabapentin is most often perorally administered as immediate-release formulations. The drug is rapidly absorbed from the gastrointestinal tract and the relative fraction of absorbed dose is high in both cats and dogs (Adrian et al., 2018; KuKanich and Cohen, 2011). An ODF formulation can therefore be considered suitable given the pharmacokinetics of gabapentin. The limited drug-loading capacity of ODFs should not pose a problem, as the target doses are low (mainly below 100 mg for cats and small dogs) and film strips with adequate flexibility can be rolled or folded into compact dosage forms.

ODFs are often preferred over ODTs; they are flexible and less fragile and thus easier to handle (Dixit and Puthli, 2009). The preparation by means of solvent casting is simple and does not require expensive machinery.

Regarding the excipients, the commonly used film-forming polymers are not known to be toxic to cats and dogs. The most common plasticizers in ODFs, such as glycerol, polyethylene glycol (PEG), triethyl citrate (TEC), and sorbitol, are neither regarded as harmful in the small quantities present in the dosage form. Glycerol, for instance, is widely used as a humectant in moist pet foods (Beynen, 2019a). According to a report issued by the EMA Committee for Medicinal Products for Veterinary Use (CVMP) (1995), PEGs of various molecular weights were well tolerated by dogs when incorporated into the diet in a year-long study. TEC has been studied in both cats and dogs and should be well tolerated in small amounts (World Health Organization Internationally Peer Reviewed Chemical Safety Information database, 1980). Sorbitol is used as a preservative in pet foods and is regarded as safe in the light of current research (Beynen, 2019b).

In the previously described guideline on residual solvents in veterinary dosage forms by VICH, it can be seen that the most common organic solvents used in ODFs (e.g. acetone, acetic acid, and ethanol) are classified as Class 3 solvents. This means that they are of low toxic potential and should not pose a significant risk to the target animal. Their permitted daily exposure is at least 50 mg, but the literature does not reveal specifications on the maximum permitted daily exposure for animals. As organic solvents are often volatile, most of them will evaporate from the ODFs during drying. The safety of the final dosage form should be confirmed by measuring the amount of residual solvent with an appropriate analytical method.

2.4 Quantitative analysis of gabapentin

2.4.1 Properties of the gabapentin molecule

Gabapentin (Figure 1) is an achiral amino acid. Being a zwitterion, it has pKa values of 3.7 for the carboxyl group and 9.4 for the amine group (Zambon et al., 2008). According to the PubChem database (National Center for Biotechnology Information, 2021), gabapentin is freely soluble in water and in alkaline and acidic solutions. In its monograph in Ph. Eur. 10th edition, gabapentin is classified as sparingly soluble in water.

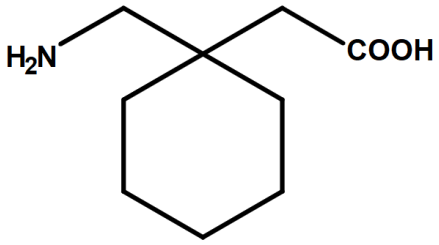


Figure 1. Molecular structure of gabapentin, 1-(aminomethyl)cyclohexaneacetic acid (National Center for Biotechnology Information, 2021).

As can be observed from its molecular structure, gabapentin is a small compound (molecular weight = 171.24 g/mol). The small size of the molecule and the absence of chromophores (i.e. color-yielding parts) are the causes of the weak native UV-Vis absorbance (Kostić et al., 2014).

Gabapentin is known to exhibit polymorphism. In its zwitterionic state, it can exist as a hydrate (form I) or as three different anhydrous forms (forms II, III, and IV; also called alpha, beta, and gamma) (Delaney et al., 2014). Form II (α-gabapentin) is the commercially used drug substance (Zong et al., 2011). Gabapentin undergoes solid-state degradation through intramolecular cyclization, forming a lactam ring. The presence of moisture can decrease the lactam formation (Zong et al., 2011), and the choice of excipients can also significantly affect the lactamization rate (Cutrignelli et al., 2007).

2.4.2 UV-Vis spectrophotometric methods

A central theme in the present study was to investigate gabapentin quantification methods. Thus, literature was extensively searched for UV-Vis spectrophotometric quantification methods developed for, or tested on, gabapentin in bulk or dosage forms.

Quantification methods can roughly be divided into non-derivatization and derivatization-based methods; non-derivatization methods measure the native absorbance of the molecule in various solvents, while derivatization methods are based on coupling gabapentin with detection reagents. The derivatization methods can be further categorized in the manner of Abdulrahman and Basavaiah (2011a, 2011b, and 2012), that is, according to what end product is being measured. When derivatizing gabapentin, the measured reaction products can be condensation products, charge-transfer complexes, or individual ions.

The UV-Vis methods are listed in Table 1 together with the central details of each method: the derivatization reagent, the solvent used for dissolving gabapentin, the detection wavelength (λ_{max}), and the linear range as given by the authors. The linear range is defined as the concentration range where the Beer-Lambert law is obeyed, i.e. where the absorbance is directly proportional to the concentration of the sample. In some cases, the same reagent has been utilized by several authors in different ways; each of these publications is listed separately.

Table 1. Summary of UV-Vis spectrophotometric methods for quantifying gabapentin in bulk or in dosage forms. λ_{max} is the detection wavelength, and the linear range is equal to the concentration range where the Beer-Lambert law is obeyed as given by the authors.

Reference	Reagent	λ_{max} (nm)	Linear range (µg/ml)	Gabapentin solvent
Abdellatef and Khalil (2003)	**A.** Vanillin	376	80–360	Water
	B. Ninhydrin	569	40–280	Water
	C. *p*-benzoquinone	369	80–320	Water
Abdulrahman and Basavaiah (2011a)	Sodium 1,2-naphthoquinone-4-sulfonate	495	7.5–75	Water
Abdulrahman and Basavaiah (2011b)	**A.** 2,4,6-trinitrophenol (picric acid)	415	1.25–15	Acetonitrile
	B. 2,4-dinitrophenol	420	2–18	Acetonitrile
Abdulrahman and Basavaiah (2012)	Sodium hypochlorite	590	0.2–5	Water
Adam et al. (2016)	Ascorbic acid	390, 531	12–60	Water
Adegbolagun et al. (2018)	Chromotropic acid	470	1–6	Water
Adegoke et al. (2018)	Para-dimethylamino-benzaldehyde	430	1–6	Water/HCl/$NaNO_2$
Almasri et al. (2019)	Salicylaldehyde	403	6–100	Water
Al-Zehouri et al. (2001)	Acetylacetone and formaldehyde	415	20–140	Water
Anis et al. (2011)	**A.** Cupric chloride	246	40–95	Water
	B. Bromothymol blue	411	100–800	Water
	C. Bromocresol green	411	10–150	Water
Chandra et al. (2012)	N/A; native absorbance measured	265	2–10	Water/ethanol
Dalvi et al. (2011)	β-naphthol	558	10–50	HCl
Effendi et al. (2013)	Acetylacetone and formaldehyde	340	10.8–80	Water
Fonseca et al. (2017)	**A**. N/A; native absorbance measured	192	5.91–142.42	Water
	B. N/A; native absorbance measured	194	72.09–724.46	Water/ethanol
	C. N/A; native absorbance measured	206	83.25–811.6	HCl
	D. *p*-benzoquinone	360	24.72–241.49	Water
	E. Vanillin	392	64.25–712.08	Water
	F. Sodium hypochlorite	588	4.96–73.72	Water
Galande et al. (2010)	Ninhydrin	405	50–300	Water
Gouda and Malah (2013)	**A.** Quinalizarin	571	0.4–8	Methanol
	B. Alizarin red S	528	0.4–8	Methanol
Gujral et al. (2009)	N/A; native absorbance measured	210	0.25–3.5	Water
Kazemipour et al. (2013)	Vanillin	402	10–90	Water
Mohammed and Elbashir (2015)	4-chloro-7-nitrobenzo-2-oxa-1,3-diazole	476	10–60	Water/methanol

Mohammed and Mohamed (2015)	Vanillin	396	0.1–10	HCl/methanol
Nagaraja et al. (2011)	Ninhydrin and sodium molybdate	570	0.25–4.8	Water
Patel and Patel (2011)	**A.** Bromocresol green	416	10–120	Water
	B. Bromothymol blue	421	40–90	Water
Rassol et al. (2018)	Potassium permanganate	605	2–20	Water
Saleh et al. (2014)	2,5-dihydroxy-benzaldehyde	445	2.57–37.25	Ethanol
Salem (2008)	**A.** Iodine	360	6–30	1,2-dichloroethane
	B. 7,7,8,8-tetracyano-quinodimethane	842	8–24	Acetonitrile
	C. 2,3-dichloro-5,6-dicyano-1,4-benzoquinone	456	12–36	Acetonitrile
	D. 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (chloranilic acid)	535	60–200	Acetonitrile
	E. Tetracyanoethylene	412	40–140	Acetonitrile
	F. 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil)	521	40–120	Acetonitrile
Siddiqui et al. (2010)	**A.** Ninhydrin	568	2–30	Acetonitrile
	B. 2,3,5,6-tetrachloro-1,4-benzoquinone (chloranil)	230	16–70	Acetonitrile
	C. 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (chloranilic acid)	314	6–30	Acetonitrile
	D. 2,3-dichloro-5,6-dicyano-1,4-benzoquinone	304	2–40	Acetonitrile
	E. Tetracyanoethylene	335	6–30	Acetonitrile
	F. 7,7,8,8-tetracyano-quinodimethane	439	4–30	Acetonitrile
Siddiqui et al. (2013)	Ninhydrin	575	10–30	Water
Virupaxappa and Shivaprasad (2011)	**A.** Potassium permanganate	610	17.2–68.8	NaOH
	B. Potassium permanganate	526	17.2–86	NaOH
Winotapun et al. (2012)	Genipin	590	25–85	Water

The presence of both an amino group and a carboxylic group in the gabapentin molecule enables many kinds of derivatization reactions. As can be observed in Table 1, the possible derivatization reagents are many. In several cases, the same reagent has been utilized in different ways; methods may differ in terms of solvents, sample preparation, reaction times, and pH values of potential buffers. Variations in these parameters can shift the detection wavelength. Some detection complexes exhibit multiple absorbance maxima, of which authors have chosen different ones for the publications.

Gabapentin has been measured without derivatization in an aqueous medium by Gujral et al. (2009) and Fonseca et al. (2017). The latter also measured the native absorbance of gabapentin in a 1:1 water/ethanol mixture (v/v) as well as in 0.1 M hydrochloric acid. The method by Chandra et al. (2012) also consisted of measurement in a 1:1 water/ethanol mixture.

Ninhydrin dissolved in organic solvents is widely used for the determination of primary amines or amino acid groups (Abdellatef and Khalil, 2003). Ninhydrin derivatization is also the most commonly encountered gabapentin derivatization method. The reaction is based on oxidative deamination of the primary amino group in gabapentin, which leads to condensation of the reduced ninhydrin and the formation of a colored complex known as Ruhemann's purple. Derivatization with vanillin is another commonly utilized method. Vanillin in the form of a Duquenois reagent is applied to the determination of amino groups; the reaction results in a condensation product due to the aldehyde group in vanillin reacting with the amino group of gabapentin.

One article, namely by Adam et al. (2016), proposes the use of ascorbic acid (vitamin C) for gabapentin derivatization. A condensation product is formed between oxidized ascorbic acid and gabapentin.

Two methods propose the utilization of diazo coupling: either with chromotropic acid (Adegbolagun et al., 2018) or with para-dimethylamino-benzaldehyde (Adegoke et al., 2018). These methods require diazotization, i.e. the conversion of a primary aromatic amine into a diazonium salt ($R–NH_2 \rightarrow R–N{=}N^+$). The diazotized gabapentin is coupled with the reagent.

Almasri et al. (2019) and Saleh et al. (2014) have developed methods based on the formation of Schiff bases, which are imine compounds formed upon condensation of the gabapentin amino group with the carbonyl group in aldehydes. Al-Zehouri et al. (2001) and Effendi et

al. (2013) utilized the Hantzsch reaction, which is also a condensation reaction, but between the gabapentin amino group and reagents acetylacetone and formaldehyde. Hantzsch reactions lead to the formation of dihydropyridines.

The amino group of gabapentin has a lone electron pair on the nitrogen atom. The nucleophilicity of the electron pair is the basis for many derivatization reactions, such as pairing with quinones and quinone derivatives (Abdulrahman and Basavaiah, 2011a). Salem (2008) and Siddiqui et al. (2010) have studied methods based on the formation of electron donor-acceptor complexes with reagents such as iodine, quinone derivatives, and tetracyanoethylene. Gouda and Malah (2013) have developed methods involving quinone derivatives quinalizarin and alizarin red S. Some other reagents that form electron donor-acceptor complexes with gabapentin are picric acid and 2,4-dinitrophenol (Abdulrahman and Basavaiah, 2011b), β-naphthol (Dalvi et al., 2011), bromocresol green and bromothymol blue (Anis et al., 2011; Patel and Patel, 2011), and cupric chloride (Anis et al., 2011). The methods by Mohammed and Elbashir (2015) and Winotapun et al. (2012) are based on nucleophilic substitution reactions.

Two methods are based on measuring products that are not gabapentin derivatives per se, but rather products formed in the presence of gabapentin. The method by Abdulrahman and Basavaiah (2012) begins with treating gabapentin with sodium hypochlorite, which converts the primary amine into a chloro derivative, after which excess hypochlorite is destroyed with the aid of nitrite ions. Finally, a starch and potassium iodide reagent is added, and the gabapentin chloro derivative oxidizes iodide to iodine. A triiodide-starch complex is formed and measured spectrophotometrically. The other method involves potassium permanganate and has been described by Rassol et al. (2018) and Virupaxappa and Shivaprasad (2011). Potassium permanganate is utilized to oxidize gabapentin in an alkaline medium, upon which manganate ions are formed. Absorbance measurements can be carried out at 610 nm for the manganate ions and 526 nm for the unreacted permanganate.

2.5 Validation of quantitative methods

In order to determine how to assess the performance of quantification methods, the validation criteria for analytical methods were investigated. Validation criteria are internationally standardized by The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) in the quality guideline

Q2(R1) (2005). The guideline covers the necessary validation parameters for a wide range of analytical methods and gives guidance on the validation of such analytical procedures that are included in registration applications. The guideline is applied to, for example, identification tests and quantitative tests of the active moiety in drug substance samples or drug products.

There is a variety of validation characteristics to consider when assessing an analytical method; the most important characteristics according to the ICH Q2(R1) are accuracy, precision, specificity, detection limit, quantitation limit, linearity, and range. These should typically be included in method validation; however, exceptions can be justified in some cases. In dissolution and content assays, the typically evaluated characteristics are accuracy, precision, specificity, linearity, and range. Robustness is not listed among the most important characteristics, but the guideline points out that robustness is recommended to consider at an appropriate stage in the method development.

The ICH Q2(R1) glossary defines the aforementioned validation characteristics as follows:

- **Accuracy** ("trueness") is a measure of the closeness between the found value and an accepted reference value.
- **Precision** measures the degree of scatter, i.e. the closeness of values obtained from multiple samplings of the same sample. Precision can be measured as **repeatability** (intra-assay precision, short timespan), **intermediate precision** (within-laboratory variations, i.e. different days, different persons, different equipment), or **reproducibility** (precision between laboratories).
- **Specificity** expresses the ability to specifically measure the expected substance(s).
- **Detection limit** is defined as the lowest amount of substance that is detectable in a sample.
- **Quantitation limit** is defined as the lowest amount of substance that can be accurately quantified in a sample.
- **Linearity** describes the ability to obtain values that are directly proportional to the concentration of the sample. Linearity occurs within a given range.
- **Range** is defined as the concentration interval in which the method has suitable precision, accuracy, and linearity.
- **Robustness** ("reliability") is a measure of the ability to remain unaffected by minor and normal variations in method parameters.

The guideline gives recommendations on how each of these characteristics should be assessed as well as how to express and report them. Within the limited scope of this study, assessing each of the recommended characteristics for all tested spectrophotometric methods is too time-consuming to carry out. The author's decision is that the most relevant characteristics to compare in this context are the linearity, range, and precision of the methods. Furthermore, specificity in the form of an identification test can be observed in practice: a sample containing analyte should give a positive absorbance reading, whereas a blank sample should give negligible to no absorbance readings.

The linearity of analytical methods should be evaluated both visually and statistically after plotting the signal (absorbance) as a function of sample concentration, utilizing a minimum of five concentrations. It is recommended to fit a regression line, for example, by the method of least squares. This will give a calibration curve (standard curve). Data from the line are utilized to evaluate the linearity. The ICH Q2(R1) guideline recommends establishing the regression line's correlation coefficient (R), y-intercept, slope, and residual sum of squares. In a complementary guidance issued by the U.S. Food and Drug Administration (FDA) (2015), it is recommended to also determine the coefficient of determination (R^2).

In this context, it is relevant to define the range with regards to linearity. Precision can be evaluated by applying some of the recommended ICH Q2(R1) methodology: repeatability can be assessed through intra-assay precision (same batch, several measurements) and intermediate precision (between days and between different batches). At least three concentration levels with three replicates of each should be compared.

It is worth noting the remark of the International Union of Pure and Applied Chemistry (IUPAC) (1997) on the use of correlation coefficients (R) in the context of calibration curves and other functional relations. IUPAC does not recommend the usage of R as a measure of calibration curve quality, as it is purely a measure of statistical associations and does not necessarily reflect true correlation throughout a range.

According to IUPAC definitions, in the case of a linear relationship between detected signal and analyte concentration, the slope (dy/dx) of the regression line can be defined as the sensitivity. For instance, Fonseca et al. (2017) have utilized the slope as one of the characteristics for comparing gabapentin quantification methods. The magnitude of the slope is relevant to consider as the slope should preferably be significantly different from

zero; this indicates better sensitivity (i.e. a signal strength proportional to the increase in analyte concentration).

2.6 Quality assessment of orodispersible films

2.6.1 Pharmacopoeial tests

ODFs are still a relatively new dosage form, and there are not yet any detailed pharmacopoeial specifications on their required quality attributes. In Ph. Eur. 10th edition, ODFs are listed under the dosage form category Oromucosal preparations. The pharmacopoeia has defined only two requirements for ODFs: suitable mechanical strength of the films to resist handling, and a dissolution test to demonstrate an appropriate release of the API. The dissolution test can be performed, for example, according to the general chapter Dissolution of solid dosage forms (Ph. Eur. 2.9.3). The chapter describes four different apparatus (basket, paddle, reciprocating cylinder, and flow-through cell). The choice of medium can be distilled water or a buffered solution. For reference, dissolution testing of medicated chewing gums (Ph. Eur. 2.9.25) is recommended to carry out in a phosphate buffer with pH 6.0. However, whereas human saliva typically has a pH value below 7 (Bel'skaya et al., 2017), the pH values of canine and feline saliva are often more alkaline, ranging between 8 and 9 (Iacopetti et al., 2017; Robertson et al., 2003). An acidic buffer may, therefore, not be the most relevant choice for testing veterinary oromucosal dosage forms.

Under the general requirements for all oromucosal preparations, Ph. Eur. requires that the dosage forms comply with the test for uniformity of dosage units: either uniformity of content or uniformity of mass. Under the general chapter Uniformity of dosage units (Ph. Eur. 2.9.40), the choice of uniformity test is made according to the dosage form and the dose and ratio of the API. In the case of gabapentin ODFs, some doses will be less than the threshold of 25 mg, and in these cases, the content uniformity should be determined in lieu of the mass variation.

2.6.2 Other recommended methods

As the current pharmacopoeial specifications were found to be insufficient, the literature was extensively searched for other relevant ODF characterization methods. Several reviews concerning the quality assessment of ODFs can be found. Authors have adapted

characterization methods for other closely related dosage forms (ODTs, medicated chewing gums, mucoadhesive films, etc.), and consensus appears to have formed as to which are the most important characteristics to assess. These will be reviewed in the following sections.

2.6.2.1 Mechanical strength testing

As also pointed out in Ph. Eur., sufficient mechanical strength of ODFs is required. It is, however, not specified what constitutes sufficient mechanical strength and how it should be assessed. In the literature, mechanical strength has been evaluated by performing tensile tests, puncture tests, and folding endurance tests (Dixit and Puthli, 2009; Hoffmann et al., 2011; Preis et al., 2013).

A tensile test is conducted by placing a piece of film between two clamps, which then pull the film in opposite directions until breakage (Preis et al., 2013). Typically, the recorded values are the maximum force required to break the film (tensile strength at break) and the elongation of the film (elongation at break). From the tensile test results, the elastic modulus E (also known as Young's modulus) can be calculated and used as a measure of stiffness. As Preis et al. (2014a) remark, elongation of the films can be disadvantageous, as it might cause uneven batches during industrial cutting. Therefore, a moderate ability to elongate is preferred.

The puncture strength measures the force required to puncture the film with a probe (Preis et al., 2013). The film is fixed to a rig with a cylindrical hole, through which the probe moves down onto the film; the test measures the force needed to displace and break the film. Comparability of the results is obtained by normalizing the results to, for example, the sample area.

Folding endurance is measured by repeatedly folding the films at the same position and recording the number of folds before cracking or breakage of the films (Dixit and Puthli, 2009). The folding endurance of pharmaceutical films has conventionally been tested by manual folding or by bending against a mandrel, because the available industrial folding endurance testers are intended for plastic films, papers, and such. An automated approach to folding endurance testing has recently been suggested by Takeuchi et al. (2020), who demonstrated that a desktop model folding endurance tester could be utilized on ODFs.

2.6.2.2 Thickness uniformity

Variations in the thickness of ODFs are directly related to the accuracy of the dose. Dixit and Puthli (2009) recommend assessing the uniformity of thickness by, for example, accurately measuring the thickness of the films with a micrometer screw gauge at different locations.

2.6.2.3 Dryness and moisture content

The dryness and residual moisture content significantly affect the properties of ODFs (Wasilewska and Winnicka, 2019). It is important to have some residual moisture in an ODF as it yields flexibility; dry films tend to be too brittle. However, too high moisture content is not desirable, as it will yield tacky and sticky films.

The dryness of ODFs can be evaluated with a tack test (Dixit and Puthli, 2009). There are instruments available for this purpose, but stickiness can also be evaluated simply by pressing a piece of paper to the film surface. However, a more precise and comparable method for evaluating dryness is to measure the residual moisture content of the films. This is executed with a suitable assay that determines either the solvent content or the loss on drying.

According to Nair et al. (2013), the ideal moisture content of buccal films is below 5%. In a study by Borges et al. (2017), commercially available orodispersible films (such as breath freshener strips) were analyzed, and moisture content was one of the assessed quality attributes. The studied films exhibited a broad range of moisture content (2.91–9.75%). All films had a moisture content below 10%, with the majority being below 5%. Based on the marketed films, Borges et al. suggest that ODFs should ideally contain 3–6% residual water.

2.6.2.4 Surface pH

It is widely accepted that the surface pH of oromucosal dosage forms should be neutral or close to 7 to avoid mucosal irritation (Bala et al., 2013). Determination of the surface pH has been performed in various ways, but the general principle is to wet a piece of film in a small amount of water and measure the pH of the film surface. One method is to place the ODF on agar gel and measure the surface with a pH paper (Joshua et al., 2016), but utilizing a pH electrode would be more precise. Abdelbary et al. (2014) describe a procedure where

the ODFs were allowed to swell in 1 ml water for 30 minutes before measurement, whereas Sjöholm et al. (2020) performed a similar method but with 30 seconds of swelling time. To obtain the most relevant results, the test setup should simulate the *in vivo* conditions for the dosage form in question. In the case of an ODF, the measurement should be conducted relatively rapidly, as the film will reside in the mouth for a brief time. The liquid volumes should be low to correspond to the amount of saliva normally present in the mucosal cavity.

2.6.2.5 Disintegration

Rapid disintegration is a crucial attribute of ODFs. It is especially beneficial in administration to pets, as fast disintegration will prevent the dosage form from being spit out or getting stuck in the esophagus. Disintegration tests can be conducted with a conventional pharmacopoeial apparatus intended for solid dosage forms, but as it does not mimic the conditions in the oral cavity, alternative methods for disintegration testing have emerged (Wasilewska and Winnicka, 2019). Hoffmann et al. (2011) have discussed these methods in further detail. Many of the proposed methods involve a smaller volume of liquid to better simulate *in vivo* conditions. For example, the Petri dish method consists of placing a frame holding the ODF on a Petri dish and adding a drop of water onto the film; the time until the drop forms a hole is recorded. Another example is the swirling method, which is performed by placing the film into a dish with 25 ml water. The dish is swirled every ten seconds, and the time until the film starts to break is recorded. The main drawback of these disintegration tests is the absence of simulation of the mechanical force exerted by the tongue, a factor that inevitably has an impact on the *in vivo* disintegration of an ODF. One approach to simulating the tongue movement is to place a small weight (for example, a steel ball) on the wetted film and record the time it takes for the weight to fall through when the film breaks (Wasilewska and Winnicka, 2019). A version of this method was employed in the study by Sjöholm et al. (2020). Disintegration tests are generally carried out in distilled water because Ph. Eur. does not recognize simulated saliva as a medium (Hoffmann et al., 2011).

Because of the fast-dissolving nature of ODFs, disintegration and dissolution generally happen simultaneously within a short time (Wasilewska and Winnicka, 2019). In some cases, the guidelines for ODTs may be applied to ODFs, which means that a disintegration test can be used instead of a dissolution test. This is, however, only applicable if the API is

molecularly dispersed in the ODF, in which case the disintegration of the film is the limiting factor for the dissolution of the API.

2.6.2.6 Mucoadhesion

Mucoadhesion expresses the adhesiveness of the film to the mucosal tissue. This characteristic is especially important for mucoadhesive patches, which should reside in the mouth for a prolonged time, but it can also be assessed for ODFs to ensure that they do not float around in the mouth. *In vitro* mucoadhesion is investigated with a texture analysis machine, and the test can be set up in various ways with either artificial or real tissue. Mucoadhesion tests have been performed on, for example, fresh pig buccal mucosa (Puratchikody et al., 2011), chicken pouch tissue (Peh and Wong, 1999), and artificial skin (Sjöholm et al., 2020). In the listed studies, artificial simulated saliva was utilized. Peh and Wong (1999) also assessed the *in vivo* mucoadhesion on human volunteers. Mucoadhesion is evaluated in terms of the force needed to detach the film from the mucosal tissue. The residence time (i.e. how long the film adheres) can also be measured.

2.6.2.7 Organoleptic evaluation

An organoleptic evaluation is recommended to ensure acceptable palatability of ODFs (Dixit and Puthli, 2009). For palatability testing of human medicinal products, taste panels can be employed (Anand et al., 2007). This can also be performed with veterinary medicines by observing animal behavior as a response to different test products, but the drawbacks such as low throughput and animal ethical issues have given way to newer and more accurate biomimetic methods. These include electronic tongues and taste sensors.

2.6.2.8 Solid-state characterization

Solid-state characterization of the raw materials and the films should be included in the quality assessment. Differential scanning calorimetry (DSC) and X-ray powder diffraction (XRPD) can be employed to investigate crystallinity and glass transition temperatures (Woertz and Kleinebudde, 2015a). Near-infrared (NIR) spectroscopy, Raman spectroscopy, or Fourier transform infrared (FTIR) spectroscopy can be used to qualify the API in bulk and in the formulation (Hoffmann et al., 2011).

2.6.2.9 Morphology

In some cases, morphological studies of the ODFs can give valuable information, especially in examining the distribution of poorly soluble or crystallization-prone APIs (Wasilewska and Winnicka, 2019). Texture and morphology are usually assessed with one or several of the following methods: polarized light microscopy, scanning electron microscopy, transmission electron microscopy, X-ray diffraction, and NIR spectroscopy imaging.

2.6.2.10 Stability

In the case of an already existing API in a new veterinary dosage form, the VICH annex on stability testing (1999) advises the implementation of the main quality guideline GL3(R) (2007 revision) on stability testing of new veterinary drug substances. The stability profile should be established with regards to thermal stability and moisture sensitivity. In general, the stability is assessed in long-term studies (twelve months), intermediate studies (six months at slightly higher temperature and humidity), and accelerated studies (six months in significantly amplified conditions). The temperature and humidity ranges, as well as the stability requirements, are specified in the GL3(R) guideline. During the tests, the products should be packaged in the containers intended for the end-use.

The uptake (sorption) of water is a relevant factor affecting the long-term stability of ODFs. As the hydrophilic polymers utilized in the formulations have a tendency of water sorption, it is important to prepare and store ODFs under controlled air humidity. A high water content does not only increase tackiness and complicate the handling of the films, but it may also predispose the films to microbial growth. For instance, Visser et al. (2015) studied the water sorption of drug-loaded ODFs consisting of HPMC and either CMC or HPC. The authors suggest that ODFs should be prepared and stored at a relative humidity below 50%, or that the films are enclosed in protective packaging.

It is important to assess the unique characteristics of ODFs and how they change during stability studies. For example, Puratchikody et al. (2011) performed an accelerated stability study on mucoadhesive patches and chose to assess the residence time (adhesion) of selected patches at specified time points. Other relevant characteristics to evaluate could be, for example, the changes in disintegration, dissolution, and mechanical strength.

2.6.2.11 Properties of the solutions

The properties of the solutions from which ODFs are prepared should preferably be assessed. The solutions are evaluated based on their suitability for the chosen film preparation method; for example, a solvent casting solution is assessed with regards to ease of casting (Visser et al., 2015). Homogeneity and easy removal of air bubbles are important attributes for any solution regardless of the film manufacturing method.

The viscosity of solutions can be assessed, for example, with a viscometer as done by Visser et al. (2015), but for more comprehensive data, rheological analyses are preferable. With a rheometer, the viscosity can be examined under various conditions to determine, for instance, the viscosity in relation to shear stress. Woertz and Kleinebudde (2015a; 2015b) have examined the viscosity of polymer solutions for ODF manufacturing as a function of increasing shear rate.

Particle sedimentation can be of interest, especially if the stability of the solution needs to be assessed. Sedimentation can be estimated rheologically or through manual sampling followed by drug content determination. The latter method was utilized by Woertz and Kleinebudde (2015b) on polymer solutions for ODF manufacturing.

3. Study aims

The ultimate goal of this study is to improve personalized gabapentin treatment of pets by reducing the need for off-label treatment with, and compounding of, human medicines. This would improve drug safety, efficacy, and compliance in veterinary patients. To achieve the goal, this study primarily aims to:

- Investigate different ultraviolet-visible spectrophotometric methods for quantification of gabapentin with the goal of finding one simple, reliable, and inexpensive method applicable to a veterinary formulation.

Furthermore, as a proof-of-concept, the study aims to:

- Develop an orodispersible gabapentin film formulation suitable for administration to cats and small dogs. The preparation method will be manual solvent casting followed by automated film-rolling into compact dosage forms. The practical advantages of the formulation and its manufacturing method are presented in Figure 2.
- Apply the quantification method to the developed formulation and assess the quality of the dosage forms by applying pharmacopoeial tests and other relevant analysis methods.

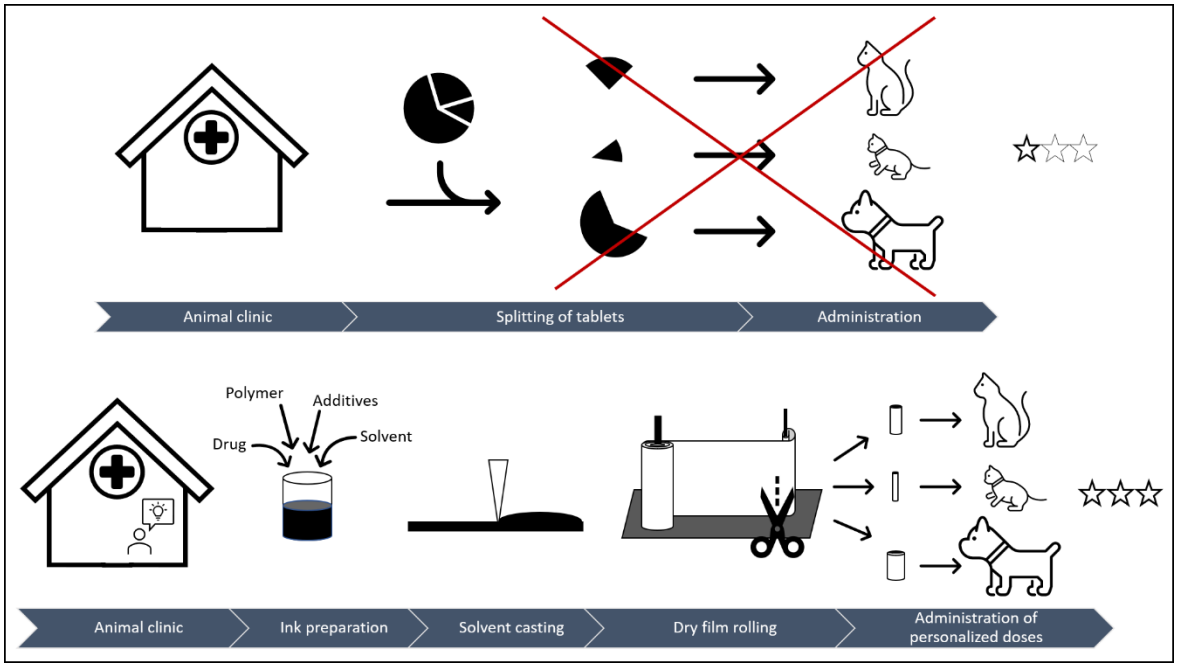


Figure 2. Schematic illustration of the practical advantages of a personalized orodispersible film formulation.

4. Materials and methods

4.1 Materials

4.1.1 Formulation development

Gabapentin (GBP) from Fagron Services B.V. (Uitgeest, Netherlands) was used as received. A selection of synthetic, hydrophilic polymers with film-forming properties was investigated in the formulation development. Hydroxypropyl methylcellulose (HPMC) of three different molecular weights was tested; Methocel E5 Premium LV was kindly provided by Dow Wolff Cellulosics (Bomlitz, Germany), and Benecel E6 PHARM and Benecel K100LV PH PRM were a gift from Ashland (Schaffhausen, Switzerland). Two grades of hydroxypropyl cellulose (HPC), Klucel LF PHARM and Klucel EXF PHARM, were kindly provided by Ashland (Schaffhausen, Switzerland), as well as one sodium carboxymethylcellulose (CMC), Blanose 7MF PH. The polyethylene glycol (PEG) and PVA copolymers Kollicoat Protect and Kollicoat IR were kindly provided by BASF (Ludwigshafen, Germany). Polyethylene oxide (PEO) of two molecular weights (100,000 and 900,000) (Sigma-Aldrich, St. Louis, MO, USA) and two types of polyvinyl alcohol (PVA), Mowiol 4-98 and Mowiol 20-98 (Sigma-Aldrich, Steinheim, Germany), were used. Polyvinylpyrrolidone (PVP) Kollidon VA64 by BASF (Ludwigshafen, Germany) was also tested.

Purified water (Milli-Q®, Merck Millipore, Molsheim, France) and ethanol (Etax 94%, Altia Oyj, Rajamäki, Finland) were used as solvents in the formulations. Polyethylene glycol 400 (PEG 400) from Sigma-Aldrich (Steinheim, Germany) and an 85% aqueous solution of glycerol (GLY) from Fagron (Barsbüttel, Germany) were used as plasticizing agents. Pure liver powder (LP) from CC Moore & Co. (Stalbridge, UK) was added for taste enhancement.

4.1.2 Quantification methods

The derivatization chemicals used for the quantification methods were L(+)-ascorbic acid (Riedel-de Haën, Sigma-Aldrich Laborchemikalien, Seelze, Germany), 2,4-dinitrophenol (Sigma-Aldrich, Steinheim, Germany; product of India), copper(II) chloride (Sigma-Aldrich, Steinheim, Germany; product of UK), chloranilic acid (Sigma-Aldrich, Steinheim, Germany; product of Austria), ninhydrin (Sigma-Aldrich, Steinheim, Germany; product of

India), *p*-benzoquinone (Sigma-Aldrich, Steinheim, Germany), and vanillin (Sigma-Aldrich, Steinheim, Germany).

Besides purified water and ethanol (see 4.1.1), the other utilized solvents were methanol (VWR Chemicals BDH, Fontenay-sous-Bois, France), N,N-dimethylformamide (DMF) (Sigma-Aldrich, Steinheim, Germany; product of France), dimethyl sulfoxide (DMSO) (Sigma-Aldrich, Steinheim, Germany; product of France), 37% hydrochloric acid (HCl) (Fisher Scientific, Loughborough, UK), dichloromethane (DCM) (Sigma-Aldrich, Steinheim, Germany), acetonitrile (ACN) (Sigma-Aldrich, Steinheim, Germany; product of France), and acetaldehyde (Sigma-Aldrich, St. Louis, MO, USA). Additionally, the following chemicals were used for the preparation of buffers: boric acid (Riedel-de Haën, Sigma-Aldrich Laborchemikalien, Seelze, Germany), sodium hydroxide (AnalaR NORMAPUR, VWR Chemicals BDH, Leuven, Belgium), sodium chloride (Sigma-Aldrich, St. Louis, MO, USA), disodium tetraborate decahydrate (Borax) (EMPROVE® ESSENTIAL, Merck, Darmstadt, Germany), disodium hydrogen phosphate (Fluka Analytical, Sigma-Aldrich, Steinheim, Germany), sodium dihydrogen phosphate (Sigma-Aldrich, Steinheim, Germany) and citric acid monohydrate (Sigma-Aldrich, St. Louis, MO, USA).

4.2 Methods

4.2.1 Preparation of solvent casting solutions

The polymers listed under 4.1.1 were investigated in the development of a suitable casting solution. Purified water and ethanol in various ratios were used as the solvent, and either glycerol or PEG 400 was added as a plasticizer. LP was added later in the formulation development after an initial assessment had narrowed down the selection of polymers. The goal was to obtain a smooth, fully dissolved solution runny enough to flow through the casting mold but with enough viscosity for the cast strip to hold its shape. Any air bubbles in the solutions should be few and easy to remove by leaving the solutions to rest for 30–60 minutes before casting.

All solutions were prepared in 100 ml borosilicate flasks by dispersing various amounts of polymer into the solvent under continuous mixing on a magnetic stirrer. GBP, plasticizer, and LP were dissolved in the solvent before the addition of polymer. The flasks were sealed with caps and left to mix slowly on magnetic stirrers for twenty hours. If any undissolved

polymer was present in the solutions, the bottles were placed in a sonicator bath (VGT-1730QT ultrasonic cleaner by GT Sonic, Meizhou, China) for series of 10 minutes. Dissolution of PVA requires heating at approximately 90 °C (Rowe et al., 2009). The PVA solutions were initially mixed at 95 °C hot-plate magnetic stirrers for 10 minutes, after which the bottles were transferred into a 90 °C water bath for six hours, followed by mixing at room temperature for sixteen hours.

4.2.2 Solvent casting

The solutions were cast onto transparency sheets (Folex imaging X-10.0, Paper Spectrum Limited, Leicester, UK) in the form of long strips with a width of 2 cm. Two different in-house made, handheld casting molds were used (Figure 3): one with a fixed-height casting gap of 0.8 mm, and one with adjustable height. The molds were 3D printed with a Zmorph multitool 3D printer (Zmorph, Wroclaw, Poland). The solutions were cast with different heights to investigate suitable thicknesses of the films for the tested formulations. To reduce shaking and to obtain evenly cast films, the casting molds were stabilized parallelly against a long, straight plank, along which they were moved in a constant, smooth motion. The cast films were left to dry under ambient conditions for a minimum of 24 hours. Visual inspection of the test formulations was carried out after 24 hours, while film-rolling and quality assessment of the final formulations were carried out after a minimum of 48 hours of drying unless otherwise described.

Figure 3. Left: the fixed-height casting mold with a gap height of 0.8 mm. Right: the mold with adjustable casting height. The height of the casting gap is adjusted by tightening or loosening the screw.

4.2.3 Film-rolling

The dried film strips were rolled into compact rolls utilizing an in-house made, computer-controlled film-roller (Figure 4). The rolling device consisted of a 28BYJ-48 stepper motor with a ULN2003 driver connected to an Arduino nano board. The Arduino was programmed so that the number of rotations could be programmed by the user; the idea was to adjust the drug dose with the number of rotations incorporated into the film roll. To keep the films tightly rolled, small droplets of 94% ethanol were evenly dispensed onto the film by lightly pressing a microfluidic chip against the rotating film roll. The purpose of the ethanol was to function as a glue. The microfluidic chip (Figure 5) was 3D printed with a Creality Ender 3 printer (Creality, Shenzhen, China). The chip was connected to an ethanol-filled syringe attached to a syringe pump (Pump 33 DDS by Harvard Apparatus, Holliston, MA, USA).

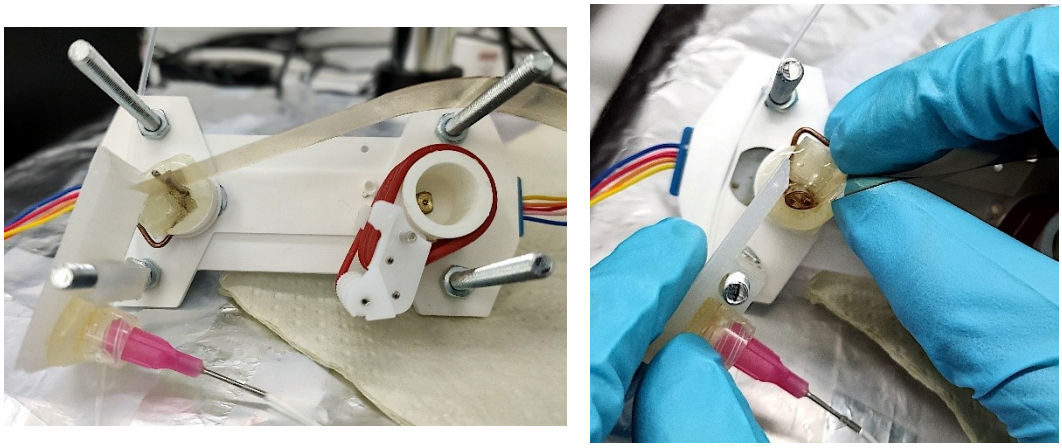


Figure 4. The film-roller setup (left) and demonstration of the film-rolling (right).

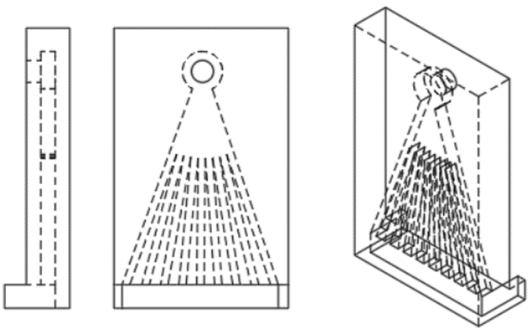


Figure 5. The design of the microfluidic chip for ethanol dispensing.

4.2.4 Spectrophotometric quantification

4.2.4.1 Measurement performance

Absorbance measurements were carried out with a UV-6300PC Double Beam Spectrophotometer (VWR International BVBA, Leuven, Belgium) capable of operating in a wavelength range of 190 to 1100 nm. The spectrophotometer was equipped with matching 10 mm quartz cells (QS High Precision Cell, Hellma Analytics, Müllheim, Germany). Data were gathered and analyzed with the UV-Vis Analyst software v. 5.44.

The system was zero calibrated with blank samples before the wavelength scans and the fixed wavelength measurements. All absorbances were measured against blank samples treated in the same way as the drug-containing samples. For each measurement, 3 ml of sample was pipetted into the cell. The cell was always washed once with the sample before filling and absorbance reading.

4.2.4.2 Choice of methods

A significant part of the study was dedicated to investigating different spectrophotometric quantification methods. From the methods listed in Table 1, a total of twelve were chosen for a practical assessment. The methods were chosen based on certain criteria. Firstly, the methods must be replicable, i.e. described clearly and in enough detail. Secondly, relatively rapid and simple methods were preferred, and methods involving, for example, liquid-liquid extraction (separation) were discarded. The economic aspect was also considered, and thus methods requiring expensive reagents were rejected.

The choice of solvent for gabapentin is highly relevant in terms of the applicability of a method to dissolution studies. When determining the gabapentin concentration in dissolution samples, each sample is treated with the chosen quantification method. Thus, the method must function in a media relevant to dissolution testing (e.g. water or physiological buffer), and therefore, methods utilizing a gabapentin stock solution in water were preferred.

A relevant linearity range is important. When quantifying small-dose formulations, the concentration of a dissolved sample film solution will be low, especially in the first minutes of dissolution sampling. Hence, methods should be linear in low concentrations, and the linearity ranges should be broad enough to cover the working concentrations.

The methods that were included in the assessment are presented in Table 2 together with their assigned abbreviations. The ninhydrin methods were tested in different variations, which are labeled A, B, and C.

Table 2. The chosen quantification methods for the assessment; detection wavelength (λ_{max}) and linear range as reported by the authors.

Method	Reference	Reagent(s)	λ_{max} (nm)	Linear range (µg/ml)	Gabapentin solvent
AQ	Fonseca et al. (2017) Gujral et al. (2009)	No derivatization; measurement of native absorbance in water	192 210	5.91–142.42 0.25–3.5	Water
AQ-ET	Chandra et al. (2012) Fonseca et al. (2017)	No derivatization; measurement of native absorbance in water/ethanol	265 194	2–10 72.09–724.46	Water/ethanol
NIN-MET*	Siddiqui et al. (2013)	Ninhydrin in methanol	575	10–30	Water
NIN-DMF*	Abdellatef and Khalil (2003) Galande et al. (2010)	Ninhydrin in N,N-dimethylformamide	569 405	40–280 50–300	Water
AA	Adam et al. (2016)	Ascorbic acid in dimethyl sulfoxide	390, 531	12–60	Water
VAN7.5	Abdellatef and Khalil (2003) Fonseca et al. (2017) Kazemipour et al. (2013)	Duquenois reagent (vanillin, acetaldehyde, ethanol) + McIlvaine buffer (Na_2HPO_4, citric acid) with pH 7.5	376 392 402	80–360 64.25–712.08 10–90	Water
VAN8.5	Kazemipour et al. (2013)	Duquenois reagent (vanillin, acetaldehyde, ethanol) + McIlvaine buffer (Na_2HPO_4, citric acid) with pH 8.5	402	10–90	Water
VAN-HCl	Mohammed and Mohamed (2015)	Vanillin in 1 M methanolic HCl	396	0.1–10	HCl/methanol
PBQ	Abdellatef and Khalil (2003) Fonseca et al. (2017)	*p*-benzoquinone in ethanol + phosphate buffer with pH 7.5	369 360	80–320 24.72–241.49	Water
CC	Anis et al. (2011)	Cupric chloride in water + borate buffer with pH 7.5	246	40–95	Water
CHA	Salem (2008) Siddiqui et al. (2010)	Chloranilic acid in acetonitrile	535 314	60–200 6–30	Acetonitrile
DNP	Abdulrahman and Basavaiah (2011)	2,4-dinitrophenol in dichloromethane	420	2–18	Acetonitrile

*Ninhydrin derivatization was tested in three variants: **A.** Unequal reaction volumes, dilution after heating; **B.** Equal reaction volumes, dilution after heating; **C.** Dilution to set volume before heating.

4.2.4.3 Assessment of the spectrophotometric methods

The spectrophotometric quantification methods were first tested on gabapentin in bulk. Stock solutions of gabapentin were prepared, and series of dilutions were made to obtain working concentration ranges for the calibration curves. In accordance with the ICH guidance on linearity assessment, a minimum of five concentrations were incorporated into each calibration curve. The methods were also tested in broader concentration ranges which spanned beyond the linear ranges reported by the authors. Each method was tested at least in triplicate on a minimum of two different stock solutions on two separate days to get an estimate of the method's precision. For each method, a sample from the middle of the reported linear concentration range was chosen for the wavelength scan. The absorbance of the sample was scanned through the instrument's whole wavelength range to find the wavelength with maximum absorbance (i.e. the peak), which was then chosen as the fixed wavelength for the absorbance measurements. The most representative data from each method were statistically analyzed with linear least squares regression performed with IBM® SPSS® Statistics v. 25, and the data were plotted with MagicPlot Student v. 2.9.1.

The methods exhibiting the best performance were tested on cast films of various compositions to see whether the methods were applicable to the investigated formulations. This was performed by measuring the drug content of films containing a known theoretical drug amount. The procedure for drug content testing is described under 4.2.5.2.

4.2.4.4 General procedure for sample preparation

The methods were carried out according to the respective authors' descriptions. Stock solutions, reagents, and buffers were prepared in volumetric flasks. Solutions were prepared fresh daily. Solid chemicals were accurately weighed with an analytical scale (AS 220.R2 PLUS by Radwag, Radom, Poland).

All samples were prepared in 10 ml Falcon tubes. All volumes were calculated beforehand and accurately pipetted with manual single-channel pipettes (Rainin Pipet-Lite XLS by Mettler Toledo, Barcelona, Spain). After the addition of all chemicals, the Falcon tubes were mixed with a vortexer. For reactions involving heating, the samples were heated in a temperature-controlled water bath (Julabo SW22 by Julabo GmbH, Seelbach, Germany) and transferred into an ice bath immediately after heating to stop the reaction and speed up the cooling process.

4.2.4.5 Non-derivatization methods

The AQ method, which measures the native absorbance of GBP in purified water, has been described by Gujral et al. (2009) and Fonseca et al. (2017). The AQ-ET method, which has been described by Fonseca et al. (2017) as well as Chandra et al. (2012), was performed in the same way but in a 1:1 (v/v) mixture of water/ethanol. The methods were simply executed by preparing stock solutions of GBP in purified water, or the water/ethanol mixture, from which a range of dilutions was made and measured directly with the spectrophotometer.

4.2.4.6 Ninhydrin derivatization

Apart from the choice of reagent solvent, the main difference between the published ninhydrin (NIN) derivatization methods is whether the samples are diluted with water to 10 ml before or after heating them. The variant where samples were diluted after heating was labeled A.

It was observed that in none of the studies had the reaction volumes been adjusted to equal levels before heating the samples. Since unequal proportions of reagent to reaction volume can potentially affect the accuracy and comparability of the results, a variant B was introduced, where all samples were adjusted to the same (smallest possible) volume before they were heated. The samples were then diluted to 10 ml after heating. In variant C, samples were diluted to 10 ml before heating.

For the NIN-MET method (Siddiqui et al., 2013), a reagent with 2 mg/ml NIN in methanol was prepared, and the flask was covered with aluminum foil to protect it from light. Aliquots of GBP stock solution in water were transferred to Falcon tubes, and 2 ml NIN reagent was added. For variant A, nothing was further added to the samples before heating. For variant B, purified water was added to adjust the volume of all samples to 3 ml. For variant C, purified water was added to a total volume of 10 ml. The samples were heated in the water bath (protected from light) and cooled down on ice, after which variant A and B samples were diluted to 10 ml. After heating, cooling down, and dilution, samples were measured. Different heating conditions have been described in the literature, ranging between 70 °C for 20–80 minutes and 90 °C for 5 minutes. Various conditions were tested: 70 °C for 80 minutes, 70 °C for 20 minutes, 80 °C for 10 minutes, and 90 °C for 5 minutes.

For the NIN-DMF method (Abdellatef and Khalil, 2003; Galande et al., 2010), a reagent with 2 mg/ml NIN in DMF was prepared and protected from light. The method was performed in the same way as the NIN-MET method.

In a study published by Goswami and Jiang (2018), a method corresponding to NIN-MET was utilized to quantify GBP in the aquatic environment. The authors describe the addition of 1 ml of 0.005 M sodium hydroxide to each sample, which is supposed to aid the complex formation between NIN and GBP. The samples were diluted to 10 ml after heating. Although this method is not developed for GBP quantification in bulk or dosage forms, it was decided to also study the effect of sodium hydroxide addition on the NIN-MET variants.

4.2.4.7 Ascorbic acid derivatization

For the ascorbic acid (AA) method described by Adam et al. (2016), a 2 mg/ml AA reagent was prepared by adding 200 mg AA, 1 ml purified water and 20 ml DMSO to a 100 ml volumetric flask. The flask was shaken for five minutes and then completed to the mark with DMSO. The samples were prepared by transferring aliquots of GBP stock solution in water to Falcon tubes and adjusting the volumes to 0.5 ml with purified water. 2 ml AA reagent and 7.5 ml DMSO were added, after which the samples were heated on a boiling water bath for 30 minutes, cooled down, and measured.

4.2.4.8 Vanillin derivatization

Derivatization of GBP with vanillin has been performed on GBP stock solutions in water by Abdellatef and Khalil (2003), Fonseca et al. (2017), and Kazemipour et al. (2013). The methods require a Duquenois reagent of vanillin and a McIlvaine buffer with pH 7.5. Kazemipour et al. (2013) claimed that increasing the buffer pH to 8.5 would optimize the reaction yield., i.e. increase the absorbance intensity. The vanillin methods were therefore tested with a buffer pH of 7.5 (VAN7.5) and a buffer pH of 8.5 (VAN8.5). For comparison, an additional vanillin derivatization method described by Mohammed and Mohamed (2015) was tested, namely, the VAN-HCl method. In the method, both GBP and vanillin solutions were prepared in 1 M methanolic hydrochloric acid.

For methods VAN7.5 and VAN8.5, the Duquenois reagent was prepared by mixing 2 g vanillin with 0.3 ml acetaldehyde and ethanol ad 50 ml. The flask was wrapped in aluminum

foil to protect the reagent from light. The McIlvaine buffer was prepared by mixing 35.5 ml of a 0.2 M aqueous solution of disodium hydrogen phosphate with 64.5 ml of a 0.1 M aqueous solution of citric acid. The pH was measured with an electronic pH meter (edge® meter equipped with an electrode and software v. 1.08, all by Hanna Instruments, Woonsocket, USA). The pH was adjusted to either 7.5 or 8.5 with 0.1 M sodium hydroxide in an aqueous solution. The method was performed by transferring aliquots of the stock solution to Falcon tubes and adding 1 ml of reagent and 1 ml of buffer. The samples were protected from light and left to rest for 30 minutes, after which they were completed to 10 ml with purified water and measured.

For the VAN-HCl method, a reagent was prepared by dissolving 5 g vanillin into 100 ml of 1 M methanolic hydrochloric acid (which was obtained from mixing appropriate amounts of 37% hydrochloric acid and methanol). The GBP stock solution was prepared in methanolic hydrochloric acid as well. The samples were prepared by transferring aliquots of the stock solution into Falcon tubes and adjusting the volume to 1 ml with methanolic hydrochloric acid. After that, 2 ml of vanillin reagent was added and the solutions were set aside for 15 min, after which they were measured.

4.2.4.9 *p*-Benzoquinone derivatization

Derivatization with *p*-benzoquinone (PBQ method) has been described by Abdellatef and Khalil (2003) and also assessed by Fonseca et al. (2017) in their method comparison. The method was carried out on GBP stock solutions in water. A PBQ reagent was prepared by dissolving the appropriate amount of *p*-benzoquinone into ethanol to obtain a concentration of 1 M. Furthermore, a 1 M phosphate buffer was prepared by dissolving 1.4196 g disodium hydrogen phosphate and 1.1998 g sodium dihydrogen phosphate in purified water ad 100 ml and adjusting the pH to 7.5 with sodium hydroxide in an aqueous solution. The method was performed by transferring aliquots of GBP stock solution into Falcon tubes and adding 0.5 ml phosphate buffer and 0.2 ml PBQ reagent. The volumes were completed to 10 ml with purified water, and the samples were heated on a 90 °C water bath for 5 minutes. The samples were measured after cooling down.

4.2.4.10 Cupric chloride derivatization

The cupric chloride/copper(II) chloride (CC) method by Anis et al. (2011) was carried out on GBP stock solutions in water. A 0.1% CC reagent in purified water was prepared (100 mg CC per 100 ml). A borate buffer was obtained by dissolving 2.5 g sodium chloride, 2.85 g disodium tetraborate decahydrate (Borax), and 10.5 g boric acid per 1000 ml purified water. The pH was adjusted to 7.5 with sodium hydroxide in an aqueous solution. Aliquots of GBP stock solution were transferred into Falcon tubes, and 1 ml of borate buffer was added. The samples were mixed, and then 2 ml of CC reagent was added. The volume was made up to 10 ml with purified water, and the samples were measured.

4.2.4.11 Chloranilic acid derivatization

Derivatization with chloranilic acid (CHA) has been described by Salem (2008) as well as Siddiqui et al. (2010). The method has been developed for GBP stock solutions in ACN. The CHA reagent was prepared as 1 mg/ml in ACN. The method was executed by first transferring 1 ml CHA reagent into Falcon tubes and then adding the aliquots of GBP stock solution. The volumes were completed to 10 ml with ACN, and the samples were immediately measured.

4.2.4.12 2,4-dinitrophenol derivatization

Abdulrahman and Basavaiah (2011b) have developed a 2,4-dinitrophenol derivatization method (the DNP method). Like the CHA method, it also requires ACN as the solvent for GBP. For performing the method, a 2 mg/ml DNP reagent was prepared in DCM. Aliquots of GBP stock solution were transferred into Falcon tubes, 1.5 ml DNP reagent was added, and the samples were diluted to 10 ml with ACN. The samples were mixed, covered with aluminum foil, and left to rest for 10 minutes before measuring.

4.2.5 Quality assessment of the dosage forms

As concluded in the literature review, the pharmacopoeial tests must be complemented with additional analysis methods in order to thoroughly characterize orodispersible films (ODFs). A number of relevant methods were selected for the quality assessment in this study; the execution of each one will be described in the following sections. To study the effect of GBP addition as well as LP addition to the formulation, unloaded (UL) and drug-loaded (DL) films were prepared both with and without the addition of LP. The quality analyses were carried out on all four film types.

4.2.5.1 Appearance of the films

The films and the film rolls were visually inspected and photographed with a mobile phone camera. GIMP v. 2.10.22 was utilized for cropping and slightly enhancing the brightness, contrast, and sharpness of the pictures.

4.2.5.2 Drug content

The drug content was determined with the AA derivatization method. The general procedure for content measurement consisted of dissolving the sample films in 50 ml purified water in borosilicate flasks. The sealed flasks were fixed in an orbital shaker (Multi-Shaker PSU 20 by BIOSAN, Riga, Latvia) and shaken at 150 rpm for three hours to ensure that the films were completely dissolved. Samples of 0.5 ml were drawn from each solution and derivatized according to the AA method (see 4.2.4.7). The absorbances of the drug-loaded samples were measured against samples prepared from the corresponding unloaded films. Using unloaded film samples for blank calibration and as reference ensures accurate measurement of the drug's absorbance, as any potential absorbance from the excipients is omitted. The measured absorbance values were inserted into the equation obtained from the AA method calibration curve to obtain the samples' drug concentrations. The drug content of each film was calculated from the sample concentration by taking into account the dilutions made.

The drug content was first determined on film strips of fixed lengths in order to establish the approximate ratio of the drug dose to film length. This was later used to estimate the dose per number of rotations when determining the target doses of the film rolls.

The content uniformity of the final dosage forms, i.e. the film rolls, was determined. The European Pharmacopoeia (Ph. Eur.) specifies under chapter 2.9.6 that in the case of single-dose units, the contents of 10 dosage units should be determined. In the present study with adjustable doses, it was decided to determine the contents of three fixed doses (rotation numbers) and five units of each, making a total of 15 samples. The criteria for uniformity of content from test B (Ph. Eur. 10.0, 2.9.6) were applied, and the acceptance value (AV) was calculated. The content uniformity complies with test B if not more than one individual content is outside 85–115% of the average content, and none is outside 75–125% of the average content. If more than three individual contents are outside 85–115% of the average, or if one or more is outside 75–125% of the average content, the dosage forms fail to comply with the test. If two or three individual contents are outside the 85–115% range but within the limits of 75–125%, another 20 dosage forms must be taken and analyzed. No more than three individual contents of the 10+20 units should be outside 85–115% of the average content, and none can be outside 75–125%. The AV was calculated from the formula $|M - \bar{X}| + ks$, which is described under 2.9.40 (Ph. Eur. 10.0). In the formula, M stands for the reference value and $\bar{X}$ for the mean of the individual contents expressed as a percentage of the target content T. The acceptability constant k = 2.4, and s is the sample standard deviation. The target content T was specified as 100% and therefore, M = $\bar{X}$ and AV = ks. In the case of T = 100%, the maximum allowed acceptance value $L1$ is 15.0.

4.2.5.3 Thickness uniformity

The thickness uniformity of the cast films was determined after 48 hours of drying in ambient conditions. The thickness was measured with a digital caliper (Absolute Digimatic by Mitutoyo, Kawasaki, Japan) from three points 10 cm apart. This was carried out on three separate films of each type. For each film type, the average thickness with standard deviations was calculated.

4.2.5.4 Dissolution

The dissolution profile was determined for DL films with and without LP, as well as for pure GBP. For dissolution testing of ODFs, Ph. Eur. assigns the methods described under 2.9.3 (Dissolution test for solid dosage forms). As each sample will have to be treated with the chosen derivatization method before absorbance measurement, the dissolution setup has

to allow for manual sampling. A suitable setup was modified from Apparatus 3 (Reciprocating cylinder). Further recommendations on dissolution testing are provided under 5.17.1 in Ph. Eur. According to these, dissolution tests should be operated under sink conditions, i.e. in such a manner that the already dissolved substance does not significantly affect the dissolution rate of the remainder. Sink conditions normally occur in volumes at least 3–10 times the saturation volume (solubility). In the case of gabapentin, which according to most sources is classified as freely soluble in water, the minimum volume of the dissolution medium is low. Furthermore, as each sample is diluted during the derivatization, the volume of the dissolution medium must be small in order to obtain detectable absorbances.

The test was carried out in the temperature-controlled water bath equipped with a horizontally reciprocating rack to provide a mixing movement, which was set to 50 rpm. The water bath was kept at a temperature of 37 °C. The test was carried out in purified water as the dissolution medium; 50 ml was chosen as a suitable medium volume. Thus, 50.0 g of purified water was weighed into 100 ml borosilicate flasks, which were sealed with caps to prevent evaporation. The flasks were placed on the reciprocating rack and partially immersed in the water bath. The film rolls were placed in spiral sinkers to prevent them from floating in the flasks. For the dissolution test on the pure substance, weighing boats with GBP were placed inside cylindrical baskets to prevent floating.

Each film type and pure GBP were analyzed in triplicate. Since the sampling requires significant amounts of manual labor, the time points cannot be as frequent as in automated dissolution setups. Sampling was therefore performed at 1, 5, 10, 15, 30, and 60 minutes, and after that, with one-hour intervals until the absorbance values reached a plateau. Samples of 0.5 ml were drawn at the specified timepoints and replaced with the same amount of 37 °C dissolution medium. The drawn samples were derivatized with the AA method. The absorbances of the DL films were adjusted by subtracting the corresponding average UL film absorbance for each timepoint. From the adjusted absorbances, the released drug amount was calculated (taking into account the cumulative amount of drug in the already drawn samples). The average percentages of released drug and the standard deviations were calculated for DL films and pure GBP. The dissolution profiles were plotted as the percentage of released drug as a function of time.

4.2.5.5 Disintegration

The disintegration time of the film rolls was determined with the Sotax DT2 tablet disintegrator (Sotax, Allschwil, Switzerland), which corresponds to apparatus A (basket-rack assembly) described under chapter 2.9.1 in Ph. Eur. 10.0. The test was carried out in 37 °C purified water in 1-liter beakers. Transparent plastic discs were placed in each tube to prevent the film rolls from floating away. The test was carried out on one fixed dose of 4 rotations. Six rolls of each type (UL and DL films with and without LP) were tested. The time until complete disintegration was observed and recorded for each sample, and the machine was operated until all six samples had disintegrated completely.

4.2.5.6 Mechanical strength

A folding test was not needed, as adequate film flexibility was ensured by evaluating how well the films could withstand rolling. All test films were manually rolled during the formulation development to assess which formulations possessed enough flexibility.

Puncture strength was chosen as the sole mechanical strength test in this study. The test was carried out with the texture analyzer TA.XTplus (Stable Micro Systems, Godalming, UK) and the software Exponent, 2013 v. 6.1.4.0 (Stable Micro Systems, Surrey, UK). The texture analyzer was equipped with a 10 kg load cell, a film support rig, and a spherical stainless-steel probe (SMS P/5S) with a diameter of 5 mm (all by Stable Micro Systems, Surrey, UK). The probe was brought down with a speed of 2 mm/s until a trigger force of 0.049 N was achieved, after which the probe continued with a speed of 1 mm/s for 10 mm. The software recorded the maximum force (N) and the probe's distance of travel (mm) at the bursting point. The puncture strength was measured on all film types 24, 48, and 72 hours after casting. At least six replicates were measured, and the average values with standard deviations were calculated for the burst force and the travel distance. The room temperature and relative humidity were monitored during the tests.

4.2.5.7 Moisture content

The moisture content was measured 24, 48, and 72 hours after casting. Each film type was measured in triplicate with samples weighing approximately 0.2 g each. The measurements were performed with a moisture analyzer (Radwag Mac 50/NH by Radwag, Radom, Poland)

which measured the moisture evaporation. The samples were heated up to 120 °C, and the endpoint of the test was an equilibrium where the change in mass was less than 1 mg/min. The weight loss in mass-%, which is equal to the moisture content, was recorded. The average values with standard deviations were calculated for all films. The room temperature and relative humidity were monitored during the tests.

4.2.5.8 Surface pH

The surface pH of the films was measured at room temperature with the electronic pH meter previously described. Small (1x2 cm) pieces of film were placed in glass vials and wetted with 1 ml purified water. After 30 seconds, the pH electrode was brought to the water surface, and the readings were recorded after 1 minute of equilibration. Each film was measured in triplicate and the pH values as well as the temperatures were recorded. The average values with standard deviations were calculated.

4.2.5.9 Differential scanning calorimetry

Differential scanning calorimetry (DSC) is a technique that measures the energy required to increase the temperature of a sample. The heat flow to and from a sample is obtained as a function of temperature. Exothermic events such as crystallization release heat out of a sample, while endothermic events such as evaporation, melting, and glass transition take heat into the sample. These events, such as the dehydration point, melting point, and glass transition temperature, can be observed as peaks in the DSC thermogram. In this study, DSC was utilized to investigate the thermal properties of the prepared films as well as the raw materials and physical mixtures thereof. The analyses were conducted with the Q2000 instrument by TA Instruments (New Castle, DE, USA). Data were analyzed with the TA Universal Analysis software v. 4.5A by TA Instruments. Approximately 3 mg of each sample was weighed, placed in Tzero aluminum pans, and sealed with matching Tzero lids. Nitrogen was used as the purge gas with a flow rate of 50 ml/min. A heating ramp was used, measuring the samples from 40 °C to 220 °C with a heating rate of 10 °C/min. A minimum of two measurements was run for each sample, and if there were any differences observed, a third measurement was performed.

4.2.5.10 Attenuated total reflectance Fourier transform infrared spectroscopy

Attenuated total reflectance (ATR) is a sampling technique for performing Fourier transform infrared (FTIR) spectroscopy. A solid sample is placed on an ATR crystal, which has a high refractive index. Infrared radiation is sent through the crystal and is totally reflected at the surface between the two different optical media. However, a small fraction of the radiation will also extend into the sample, where it is absorbed to various extent based on the composition of the sample. The totally reflected infrared radiation is therefore slightly attenuated since it lacks the absorbed parts. Fourier transform stands for the mathematical process that translates the measured data into a spectrum. ATR-FTIR is a rapid technique that often does not require pretreatment of the samples. Hence, ATR-FTIR spectroscopy was utilized to study the solid states of the prepared films along with the raw materials and physical mixtures thereof. The measurements were carried out with the UATR-2 Spectrum Two by PerkinElmer (Llantrisant, UK). A force of 75 N was applied to all samples on the crystal. The samples were measured over a range of 4000 cm^{-1} to 400 cm^{-1} with 4 accumulations at a resolution of 4 cm^{-1}. A minimum of two measurements was performed on each sample, and if differences were observed in the spectra, a third measurement was run. The spectra were acquired with the PerkinElmer software Spectrum v. 10.03.02 and treated with the program functions baseline correction, normalization, and data tune-up.

4.2.5.11 Rheology

Rheology measurements were conducted in order to investigate the viscosity of the casting solutions under the influence of shearing. The measurements were carried out with the HAAKE™ MARS™ Modular Advanced Rheometer system equipped with a plate rotor of 35 mm in diameter (P35/Ti) and a matching lower plate (TMP35), all by Thermo Fischer Scientific (Karlsruhe, Germany). The measuring gap was set to 0.5 mm and the temperature to 23 °C. Each sample was pre-sheared at a rate of 1 s^{-1} for 30 seconds, followed by 60 seconds of equilibration. After that, a shear rate ramp of 0.01–1000 s^{-1} was applied over a running time of 255 seconds with 5 seconds per data acquisition point. The manufacturer's software HAAKE™ RheoWin Job Manager v. 4.87.0001 was utilized for test setup and monitoring. The obtained flow curves of viscosity vs. shear rate were analyzed with HAAKE™ RheoWin Data Manager v. 4.87.0001. UL and DL solutions with and without LP were measured at least twice, and if differences in the flow curves were observed, a third measurement was performed.

5. Results and discussion

5.1 Formulation

A selection of synthetic film-forming polymers was investigated in the development of a castable, smooth solution that would yield even and flexible orodispersible films (ODFs). The tested polymers were sodium carboxymethylcellulose (CMC), polyethylene oxide (PEO), hydroxypropyl methylcellulose (HPMC), hydroxypropyl cellulose (HPC), polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), and copolymers of polyethylene glycol (PEG) and PVA.

The investigated polymers exhibited highly varying properties in the solution preparation and solvent casting. It was found that in most cases, a 1:1 or 2:1 (v/v) mixture of purified water and ethanol was the best choice of solvent. Using only water tended to give tacky films, whereas a small addition of ethanol shortened the drying time. The amount of air bubbles in the solutions also decreased in the presence of ethanol, due to the diminished surface tension. However, high ratios of ethanol (>50% of the solvent) yielded too dry and brittle films. The polymers that are insoluble in ethanol, i.e. CMC and PEO, were prepared in water only.

Polymers with a higher molecular weight, such as HPMC Benecel K100LV, CMC Blanose, and PEO 900,000, were required in very small amounts (<10%) to obtain a suitable viscosity of the casting solution. However, upon drying of the films, the final mass-% of the polymer was too low, and the resulting films were so thin and brittle that they could not be peeled from the sheet. The same was observed for PVA Mowiol 20-98 in concentrations of 10–13% in the solution and for both grades of HPC (Klucel EXF and LF) in concentrations of 15–17%. A more suitable thickness of the films was consistently obtained from casting solutions containing polymers of somewhat lower molecular weight in concentrations >17%. All test solutions were initially cast with 0.8 mm height, as the casting mold with adjustable height was not obtained until later in the study when the final formulation had already been chosen. Testing various casting heights could have yielded better films, for example, in the case of HPC, which has been successfully cast in previous studies (Takeuchi et al., 2018; Thabet et al., 2018).

The low molecular weight of PEO 100,000 posed a problem as the solutions were too runny. A mixture containing 10% PEO 100,000 and 3% PEO 900,000 in the casting solution was

also tested. The films became grainy, milky, and uneven; PEO was considered not to be a suitable polymer for the purposes of this study.

The PEG:PVA copolymers Kollicoat IR and Kollicoat Protect did not properly dissolve into castable solutions. Heating could potentially have aided the dissolution, but it was not further tested in this study. The PVP polymer Kollidon VA64 was required in concentrations over 50% in the casting solutions but yielded extremely thin and brittle films. However, as the manufacturer also states, Kollidon VA64 is rarely used as the sole film-forming agent and should preferably be mixed with other polymers (BASF, 2021).

PVA Mowiol 4-98 was prepared in water only, as the solutions had to be heated beyond the boiling point of ethanol. Boiling or evaporation of the solvent is not desirable during the preparation process as it will cause batch inconsistencies. Although the recommended procedure is to heat PVA solutions at approximately 90 °C for 5 minutes (Rowe et al., 2009), it was found that the casting solutions had to be heated at a higher temperature for a longer time in order to dissolve all of the polymer. The best results were obtained by initial mixing at 95 °C for 10 minutes, after which the bottles were transferred into a 90 °C water bath for six hours, followed by mixing at room temperature for sixteen hours.

No significant differences between the two tested plasticizers, glycerol (GLY) and polyethylene glycol 400 (PEG 400), were observed in the formulation development phase; these could be used interchangeably. The addition of plasticizer improved the flow of the solutions as well as the flexibility of the films, but a too high content (>2.5% in most cases) was found to cause spreading of the solutions and formation of tacky films. The required amount of plasticizer depends on the intrinsic properties of the polymer. For instance, the PVA formulations required smaller amounts of plasticizer (1.5%) compared to those containing HPMC (2.5%).

Mowiol 4-98 yielded very soft, tough, and flexible films with some elasticity from a solution consisting of 23% polymer, 1% gabapentin (GBP), and 1.5% GLY in purified water. The addition of 1% liver powder (LP) to the solution did not seemingly affect its properties. Although the films possessed good mechanical characteristics, the PVA solutions started drying rapidly in the bottles, turning into a hard gel within two days after preparation. A solution with rapidly changing viscosity would pose quality issues within manufacturing, and thus it was decided not to continue the formulation studies on PVA. Furthermore, it was observed that the PVA films did not disintegrate very well in water and remained intact,

which gave rise to the concern that PVA film rolls would not dissolve or disintegrate rapidly enough to comply with the generally accepted quality standards for ODFs.

The films obtained from HPMC Benecel E6 and Methocel E5 were highly similar. The study continued only on Methocel E5, since it was somewhat easier to dissolve and consistently formed good, smooth casting solutions. Compared to the PVA films, HPMC Methocel E5 formed thinner films with slightly less elasticity. The films, however, were very flexible and could be tightly rolled without cracking or breaking. The findings largely comply with previous studies; HPMC has been widely utilized in ODF preparation due to its excellent film-forming ability, a property that was observed when assessing the various polymers.

In order to keep the dosage forms compact, the thickness of the films or the drug content in the formulation must be increased. This increases the amount of drug per unit of length, thus decreasing the required film length for each roll. These parameters were tested on the HPMC formulations by trying out various casting heights with the adjustable mold. It was found that the casting height could not be increased over 0.8 mm, as the films became hard and rigid. Thus, the films were cast with the fixed-height 0.8 mm mold. Formulations with a GBP content of 1, 3, 5, 6, and 10% were cast, but the drug started crystallizing in the films with a content of 5% or higher (Figure 6).

The compositions of the final formulations are presented in Table 3. Initially, the formulation was prepared with PEG 400 as the plasticizer, but as the production was scaled up for the quality assessment, many of the films started curling up and hardening after casting, making them impossible to roll (Figure 6). A new formulation containing GLY instead of PEG 400 was prepared, and it did not exhibit the same behavior (Figure 7). As both formulations had been cast and dried in the same room in similar conditions, the quality issue was likely related to PEG 400. It is known that liquid PEGs can cause hardening of, for example, gelatin capsule shells through preferential absorption of moisture from the gelatin (Rowe et al., 2009). Although PEG 400 was not suitable for a long, rollable strip, it could potentially be used in thicker and smaller films, for instance, 3D printed ODFs.

Table 3. The composition of the final formulations, percentages given of the wet weight.

Formulation*	HPMC	Gabapentin	Plasticizer	Liver powder	Solvent
UL (PEG)	21%		PEG 400, 2.5%		2:1 (v/v) mixture of purified water and ethanol
UL with LP (PEG)	20.5%		PEG 400, 2.5%	1%	
DL (PEG)	20%	3%	PEG 400, 2.5%		
DL with LP (PEG)	20%	3%	PEG 400, 2.5%	1%	
UL (GLY)	21%		GLY, 2.5%		
UL with LP (GLY)	20.5%		GLY, 2.5%	1%	
DL (GLY)	20%	3%	GLY, 2.5%		
DL with LP (GLY)	20%	3%	GLY, 2.5%	1%	

* UL = unloaded, DL = drug-loaded, LP = liver powder, HPMC = hydroxypropyl methylcellulose Methocel E5, GLY = glycerol 85%, PEG 400 = polyethylene glycol 400.

Figure 6. Upper pictures: gabapentin crystallized in the cast films obtained from 5% drug-loaded solutions. Lower picture: the films with polyethylene glycol 400 as a plasticizer hardened and curled up when drying in ambient conditions.

Figure 7. Films with glycerol as a plasticizer. From left: unloaded film, unloaded film with liver powder, drug-loaded film, and drug-loaded film with liver powder. Right: a drug-loaded film with liver powder.

5.2 Spectrophotometric quantification

A large part of the study was dedicated to assessing gabapentin quantification methods. Each chosen method was assessed with several stock solutions and repeated assays to observe the precision. The methods that exhibited the best performance (i.e. good precision and linearity) were also tested on samples of dissolved film pieces with a known theoretical drug content (film formulations listed in Table 3).

Observations and discussion of the performance are first presented separately for each method along with sample pictures to display the colored reaction products. For comparison between methods, the absorbance spectra displaying the absorbance peaks are gathered in section 5.2.9. The results from the statistical analysis are presented and discussed in section 5.2.10 along with figures presenting the plots and regression lines (calibration curves).

5.2.1 Non-derivatization methods

Measuring the native absorbance of GBP in purified water (the AQ method) and in a 1:1 mixture of purified water and ethanol (the AQ-ET method) were simple and rapid procedures as they did not involve any reaction steps. The maximum absorbance of GBP in water was found to occur below 190 nm, i.e. outside the instrument's limits. The measurements were carried out at 190 nm, where the absorbance was still relatively high. This finding complies with that of Fonseca et al. (2017). However, Gujral et al. (2009) found the absorbance peak to occur at 210 nm; the varying results can potentially be attributed to instrumental differences. Although the U.S. Pharmacopoeia recommends a detection wavelength of 210 nm for analyzing GBP with high-performance liquid chromatography, it is worth noting that the setup with solvents and eluents is different than in direct spectrophotometry and can likely cause a different absorbance maximum.

In the AQ-ET method, it was found that the change of solvent shifted the absorbance peak to higher wavelengths, varying between 199–204 nm. Both groups who studied the method also found this shift in the absorbance maximum: Fonseca et al. (2017) saw a distinct peak at 194 nm, whereas Chandra et al. (2012) found a peak at 265 nm.

As Fonseca et al. (2017) point out, the pH and the polarity of the solvent affect the detection wavelength of GBP. The shift in the absorbance peak between the two non-derivatization methods is attributed to the different protonation states of GBP in the two solvents.

The AQ method was found to have high precision, consistently giving similar absorbance readings between measurements. GBP could not be detected in concentrations below 2 µg/ml, but the method expressed perfect linearity from 5 µg/ml up to the highest tested concentration of 80 µg/ml. Because of the good performance on GBP in bulk, the method was also tested on GBP film formulations (compositions listed in Table 3). However, the low native absorbance of GBP posed problems, and the drug content in the formulations could not be reliably quantified. The presence of excipients was interfering, and the drug's native absorbance could not consistently be detected.

The AQ-ET method appeared to give rise to a more unstable state of the GBP molecule as the absorbance peak varied between measurements. The native absorbance of GBP was measured at 199, 202, and 204 nm, and the absorbance readings were not reliable in concentrations below 20 µg/ml. At 204 nm, the method exhibited linearity in concentrations above 20 µg/ml. As the linear range and the solvent are not applicable to the requirements in this study, the method was not further examined. It did, however, make for an interesting comparison to the AQ method, as some significant differences were observed between the two.

As Kostić et al. (2014) remark, poor sensitivity is an issue with the non-derivatization methods. This was observed in both of the tested methods, as the slopes of the calibration curves were low. The methods were also lacking specificity, as detection of true positives was not reliable in the very low concentration ranges.

5.2.2 Ninhydrin derivatization

The condensation product formed between ninhydrin (NIN) and GBP is a purple complex known as Ruhemann's purple (Abdellatef and Khalil, 2003). As elaborated under 4.2.4.6, NIN derivatization has been conducted in several ways with different solvents, heating conditions, and reaction volumes.

When comparing the different heating conditions, no differences in color intensity were observed between heating the samples at 70 °C for 80 minutes, 70 °C for 20 minutes, 80 °C for 10 minutes, or 90 °C for 5 minutes. This is supported by the observations of Bali and Gaur (2011), who utilized ninhydrin derivatization on pregabalin and studied the effect of different heating times and temperatures. The authors concluded that heating the samples for longer than 20 minutes at 70–75 °C did not produce an improvement in color.

Abdellatef and Khalil (2003) remark that prolonged heating at the higher temperatures weakens the color intensity, so the heating time should be controlled.

The obtained reaction products of GBP and NIN can be observed in Figure 8. With a NIN reagent in methanol (the NIN-MET method), absorbance maxima were consistently found at 402 and 568 nm. With the NIN reagent in N,N-dimethylformamide (DMF), i.e. the NIN-DMF method, the absorbance peaks shifted slightly to 404 and 568 nm. The intensities of the peaks at 402 nm and 404 nm were marginally higher than those at 568 nm. The found absorbance maxima correspond to those reported in the original studies.

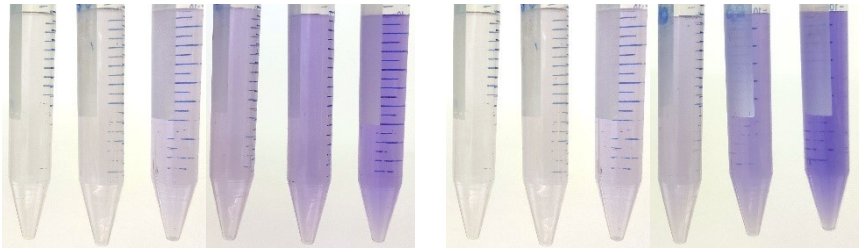


Figure 8. Samples of gabapentin (5, 10, 15, 20, 30, 40 µg/ml) derivatized with ninhydrin in methanol (left) and in N,N-dimethylformamide (right).

As expected, adjusting the reaction volumes to equal (variant B) improved the results, especially for the NIN-MET method. The plots were visibly more linear as compared to variant A with unequal reaction volumes. Diluting the samples to 10 ml before heating (variant C) did not work for either method as the reaction medium became too dilute; no complex formation and therefore no absorbance readings were obtained in the samples. Variant C was also tested in concentrations up to 150 µg/ml to find the threshold GBP concentration where the reaction would start to occur. Complete dilution before heating worked only for the NIN-DMF method in the concentration range 70–150 µg/ml, which is too high to be applicable to the analysis of the dosage form in this study.

The effect of adding 1 ml 0.005 M sodium hydroxide to each NIN-MET sample, as adapted from Goswami and Jiang (2018), was studied but found not to be useful in the context of this study. The addition of sodium hydroxide did not improve the reaction yield but rather diluted the samples, decreased the absorbances, and caused even less linear plots. Goswami and Jiang quantified GBP in the aquatic environment in a notably low concentration range

(0–6 µg/ml) and at a different detection wavelength (281 nm); the findings in this context do not rule out the usefulness of sodium hydroxide in other applications.

It was observed that the NIN-DMF method overall yielded higher color intensity (i.e. higher absorbance values) than NIN-MET. However, both methods expressed highly varying absorbance values between stock solutions and on different days. This finding indicates poor precision and robustness. The NIN-DMF method showed exponential plots in the lower concentration range, but linearity was obtained in concentrations ≥40 µg/ml, which correlates with the findings of Abdellatef and Khalil (2003).

Both methods exhibited some linearity in specified ranges and were therefore tested on GBP quantification in the film formulations. However, no visible complex formation or measurable absorbance was obtained in any of the samples, and thus the NIN methods could not be applied to the quantification of GBP in this study. It is possible that the excipients in the orodispersible film (ODF) formulation caused too much interference and hindered the derivatization reaction. Excipients can be removed from samples, e.g. through centrifugation or filtration, which could potentially aid the detection of GBP in formulation with NIN derivatization. This approach was, however, not investigated as the overall performance of the NIN methods was not satisfactory.

5.2.3 Ascorbic acid derivatization

The ascorbic acid (AA) method is based on the formation of a condensation product between GBP and AA (Adam et al., 2016). An advantage as compared to NIN derivatization is the lack of several pipetting steps; all of the pipetting was executed at once, after which the samples were heated, cooled down, and measured.

The method showed good performance with high precision between assays, and it was found to be both reliable and robust. The quantification range was broad, and samples containing as little as 0.5 and 1 µg/ml GBP were detected and fit the calibration curves. Three useful absorbance maxima were found: 309, 388, and 531 nm. In the original article, peaks at 390 and 531 nm were utilized.

The reaction product of GBP and AA gives a pink color (Figure 9). It was noticed that the complex changed into a more orange hue when moving towards higher sample concentrations. This was also observed in the absorbance peaks; the peak at around 390 nm

shifted to 376 nm along with the increasing concentration. When the whole concentration range was measured at 376 nm, it was found that the said wavelength yielded the best linearity throughout.

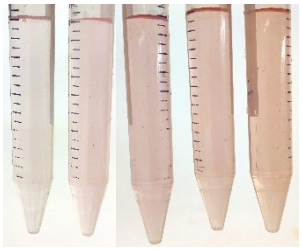


Figure 9. Samples of gabapentin (5, 10, 20, 30, 40 µg/ml) derivatized with ascorbic acid.

The most linear plots were obtained by keeping the total water volume (stock solution + additional water) of the samples at 0.5 ml and measuring the absorbances at 376 nm. The AA method exhibited perfect linearity from 0.5 to 40 µg/ml with a steep calibration curve. The method was tested several times on the selected GBP ODF formulations (Table 3) and the recovered drug content was consistently very similar to the theoretical drug amount.

5.2.4 Vanillin derivatization

GBP derivatization with vanillin is also based on the formation of a condensation product (Abdellatef and Khalil, 2003). The reaction proceeds at room temperature in the presence of Duquenois reagent (vanillin, acetaldehyde, and ethanol) and McIlvaine buffer (Na_2HPO_4 and citric acid). The method does not involve heating of the samples; the reaction is stopped when the samples are diluted to the final volume. An alternative method performed in methanolic hydrochloric acid as solvent was also tested.

All of the vanillin derivatization methods had to be repeated several times to obtain readable absorbance values. Many attempts at calibration curves failed because there was no detectable absorbance in the majority of the samples, indicating that a reaction had not occurred. Similarly to the NIN methods, the vanillin methods showed high variation in the absorbance values between assays, indicating a lack of precision. The GBP-vanillin complex did not yield any visible color.

The methods utilizing the McIlvaine buffer (VAN7.5 and VAN8.5) exhibited an absorbance peak at 393–394 nm, which is in line with the peaks reported in the original studies: 392 nm

(Fonseca et al., 2017) and 402 nm (Kazemipour et al., 2013). Abdellatef and Khalil (2003) carried out their measurements at 376 nm.

Contrary to the findings of Kazemipour et al. (2013), increasing the buffer pH to 8.5 did not increase the absorbance of the GBP-vanillin complex. Instead, the change seemed to yield poorer results with a higher degree of scatter. Given the many required attempts at vanillin derivatization, the highly varying results may unfortunately be characteristic of vanillin methods in general.

Both methods VAN7.5 and VAN8.5 exhibited some linearity in higher concentrations ≥30 µg/ml. Especially VAN7.5 yielded an acceptable calibration curve in a broad concentration range, but the range was too high to be applicable to GBP quantification in the ODF formulation. Furthermore, considering the unreliable performance of the methods, vanillin derivatization was not a preferred choice.

The vanillin method in methanolic hydrochloric acid (VAN-HCl) could not successfully be replicated. Minimal absorbance could be observed around 400 nm, but the intensity was not enough to give a useful absorbance maximum; the sample measurements did not give positive absorbance readings, which indicates a lack of specificity of the method. In the original study, Mohammed and Mohamed (2015) reported an absorbance peak at 396 nm.

5.2.5 *p*-Benzoquinone derivatization

Derivatization with *p*-benzoquinone (the PBQ method) also belongs to the category of condensation reactions (Abdellatef and Khalil, 2003). The reaction between GBP and the PBQ reagent in ethanol occurs in the presence of a phosphate buffer. Like the AA method, all pipetting is performed before heating, which means that the working steps are few. GBP and PBQ formed complexes with an intense reddish-brown color (Figure 10). The color intensity is not attributable to GBP alone, as the zero samples also obtained a strong color. Despite their visual similarity, the absorbances of the samples differed distinctly and gave rise to steep calibration curves. The PBQ method showed good precision with similar absorbance readings between assays. In most cases, the method exhibited good specificity. Somewhat more scatter and decrease in specificity were observed in the lower concentrations (<20 µg/ml), but calibration curves with acceptable linearity were also obtained throughout a broad range.

A distinct absorbance peak was not found, but measurements were carried out at 364 nm where the absorbance was relatively high. This value is close to the detection wavelength of 369 nm utilized in the original study by Abdellatef and Khalil (2003), and to 360 nm, which was used by Fonseca et al. (2017) in their method comparison. Again, instrumental differences may cause these slight variations in detection wavelength.

As the PBQ method was found to be relatively reliable, it was also tested on the GBP ODF formulations. However, the PBQ-derivatized drug-loaded samples gave absorbance readings that were too low and, therefore, did not accurately quantify the GBP content. As with NIN derivatization, it is likely that the excipients interfered with the reaction.

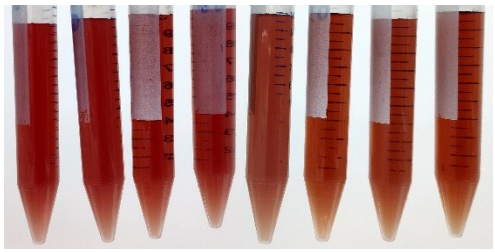


Figure 10. Samples of gabapentin derivatized with *p*-benzoquinone. To the left is a placebo sample, followed by 5, 10, 15, 20, 30, 40, and 50 µg/ml samples.

5.2.6 Cupric chloride derivatization

The cupric chloride (CC) method described by Anis et al. (2011) is based on the formation of binary complexes between GBP and copper(II) ions. The reaction proceeds at room temperature after mixing the GBP sample, borate buffer, and CC reagent; the reaction is stopped when the samples are diluted to the final volume with water. Based on the information given in the original article, it was assumed that the dilutions and measurements were carried out immediately without allowing for reaction time. When the method was initially tested according to this procedure, detectable absorbance was not obtained in the majority of the samples. Therefore, the effect of different reaction times was investigated. After adding the borate buffer and the CC reagent to the stock solution aliquots, the samples were left to rest for 10, 15, 20, or 30 minutes before the final dilution to 10 ml.

Increased absorbance readings were obtained when the samples were given time to react. The absorbance maximum occurred at 244–246 nm, and most measurements were carried

out at 245 nm; the findings are in line with the value of 246 nm reported in the original study. The GBP-CC complex did not obtain a visible color.

The method was difficult to successfully replicate. It had to be repeated several times to obtain absorbance readings in enough samples to be able to analyze the results. The GBP-CC complex was seemingly unstable, as the absorbance values varied highly depending on the reaction time. Furthermore, the values did not seem to stabilize at any point; after 20 and 30 minutes of reaction time, the absorbances in the lower concentrations (<40 µg/ml) evened out, and in concentrations above 50 µg/ml, the absorbance could not be detected at all. The best results were obtained from a 10-minute reaction time, after which a somewhat linear relationship between absorbance and concentration could be observed between 60–120 µg/ml. Due to the unreliable performance and the lack of linearity in relevant concentration ranges, the CC derivatization was not investigated on the ODF formulations.

5.2.7 Chloranilic acid derivatization

Chloranilic acid (CHA) acts as an electron donor to the electron acceptor GBP, leading to the formation of a charge-transfer complex (Salem, 2008). The reaction proceeds instantly at room temperature and does not involve heating. The method was indeed found to be very rapid; the complex formation took place instantly upon combining the reagent with the GBP solution, and a red color developed in the GBP-containing samples (Figure 11). The samples were diluted to 10 ml with acetonitrile (ACN) before absorbance measurements. Absorbance maxima were found at 312 nm and 518 nm, of which the former had a considerably higher intensity. Siddiqui et al. (2010) utilized the same absorbance peak, which in their study was found at 314 nm. Salem (2008) carried out the measurements at 535 nm.

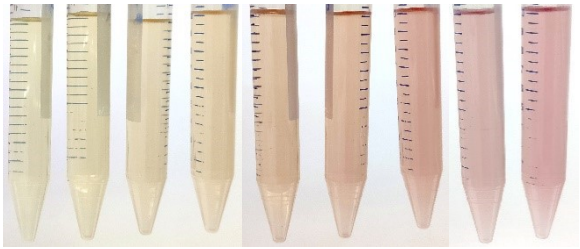


Figure 11. Samples of gabapentin derivatized with chloranilic acid. To the left is a placebo sample, followed by 5, 10, 15, 20, 30, 40, 50, and 60 µg/ml samples.

The precision of the method was relatively good; some variation in the absorbance values between different stock solutions was observed, but the precision was better than in many other tested methods. The limitation of the CHA method is the low solubility of GBP in ACN. It was found that the stock solutions had to be prepared in low concentrations (50–100 µg/ml) to dissolve GBP. If the CHA method is utilized for content assays on GBP dosage forms, the poor solubility has to be taken into account by dissolving the dosage forms in large enough volumes of ACN.

It was briefly assessed whether CHA derivatization could be applied to GBP in an aqueous medium. A GBP stock solution in purified water was utilized, and the samples were diluted to 10 ml with ACN. However, it was found that CHA reacted strongly with water and formed the same intense purple color, which can potentially overlap the absorbance of the GBP-CHA complex. The effect of the CHA-water reaction would have to be investigated further.

The CHA method exhibited good linearity from 1 to 60 µg/ml when carrying out the absorbance measurements at 312 nm. The obtained calibration curves were steep, indicating good sensitivity. Salem (2008) found that the method also was linear in a very broad and high concentration range of 60–200 µg/ml when measuring the absorbance at 535 nm; this information indicates that by combining both absorbance peaks, the CHA method could have a broad and useful linear range. Although the method showed good performance, it was not further investigated since the medium (ACN) is not relevant for dissolution testing on the ODFs in this study.

5.2.8 2,4-dinitrophenol derivatization

Similarly to CHA, the 2,4-dinitrophenol (DNP) derivatization is an electron donor-acceptor reaction (Abdulrahman and Basavaiah, 2011b). The DNP method exhibited characteristics similar to those of the CHA method, namely, rapid reaction and development of an intensely colored complex. The DNP method also suffers from the same limitation regarding the low solubility of GBP in ACN.

The procedure was simple and all pipetting was performed in one step; the samples were diluted to 10 ml with ACN immediately upon mixing the GBP samples with the DNP reagent. After this, the reaction was allowed to proceed at room temperature for 10 minutes before absorbance measurements. The formed ion-pair complex yielded an intense yellow color (Figure 12) with an absorbance peak at 423 nm, which is close to the value of 420 nm

reported in the original study by Abdulrahman and Basavaiah (2011b). The precision and sensitivity of the method were similar to those of the CHA method. The method was found to exhibit good linearity in two separate concentration ranges of 1–10 and 15–70 µg/ml.

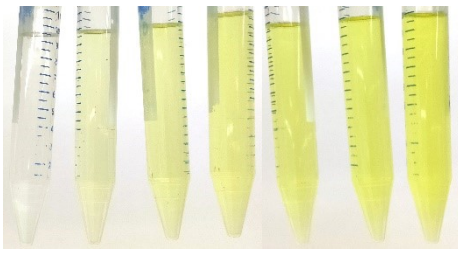


Figure 12. Samples of gabapentin derivatized with 2,4-dinitrophenol. To the left is a placebo sample, followed by 5, 10, 15, 20, 30, and 40 µg/ml samples.

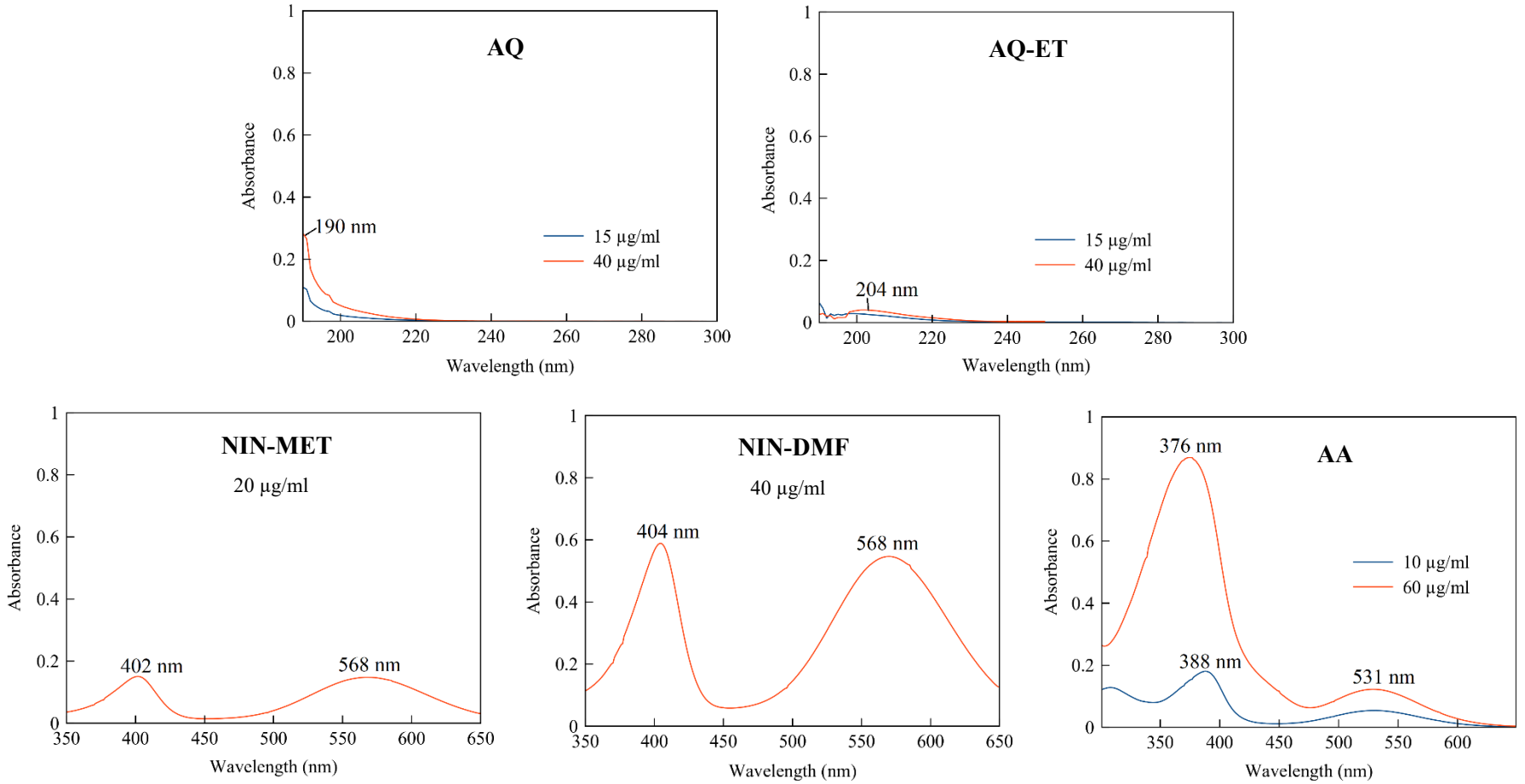
The applicability of the DNP method to GBP in an aqueous medium was briefly investigated. Because of the low solubility of water in dichloromethane, the DNP reagent and the GBP stock solution in water started separating into immiscible layers. This gave rise to the question whether the DNP reagent could have been prepared in another solvent. Gouda and Malah (2013) have discussed the choice of solvent in charge-transfer reactions and remark that the polarity of the solvent has a great impact on this type of derivatization reactions. For example, water would otherwise be a suitable choice of solvent, but DNP is not soluble in water. Interestingly, DNP has also been utilized for the quantification of pregabalin (Sher et al., 2015), where the authors describe the successful application of the method to pregabalin in an aqueous medium. In the study, a DNP reagent in dichloromethane was utilized, but the reagent volumes and total water volumes of the samples were much higher than in this tested method on GBP. An absorbance peak identical to the one found in this study is pictured in the pregabalin study. A thorough investigation of various parameters would have to be executed in order to determine whether DNP derivatization could be applied also to GBP in an aqueous medium.

5.2.9 Absorbance spectra

The absorbance spectra displaying the absorbance peaks of each method are presented in Figures 13a and 13b. For easier comparison of the peaks, the y-axes have been scaled to the same size for all spectra. The absorbance spectra (wavelength scans) were gathered on a

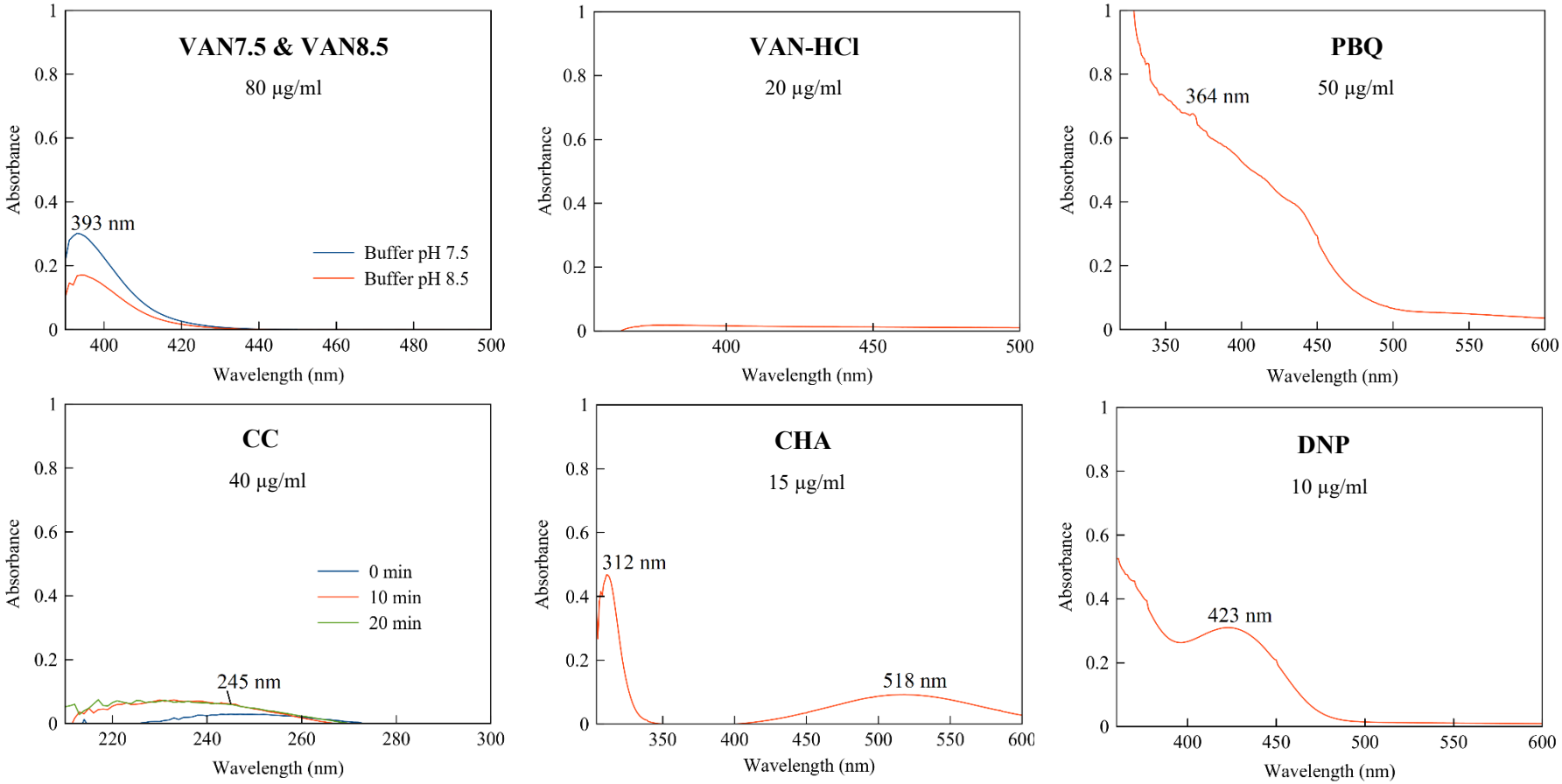
sample approximately from the middle of the linear concentration range reported by the respective authors, which explains the varying choices of concentration. It was, however, not possible to choose a concentration from the middle range in all methods. In the case of the AQ, AQ-ET, and VAN-HCl methods, the wavelength scans were performed on chosen concentrations above the reported linear ranges, as the absorbances of the samples within the reported linear ranges were too low to properly detect the absorbance maxima. In some other methods, the reported linear ranges occurred in very high concentrations, and a lower concentration more relevant for the study purposes was chosen.

The previously discussed shift in the absorbance peak in the AA method can be observed when comparing the wavelength scan performed on samples of 10 µg/ml and on 60 µg/ml. The lower peak clearly shifts from ~390 nm to ~376 nm with the increase of the analyte concentration.



AQ = native absorbance in water; AQ-ET = native absorbance in water/ethanol; NIN-MET = derivatization with ninhydrin in methanol; NIN-DMF = derivatization with ninhydrin in N,N-dimethylformamide; AA = ascorbic acid derivatization.

Figure 13a. Absorbance spectra of gabapentin quantification methods. The sample concentration on which the scan was performed is given, and the wavelength of each absorbance maximum is labeled.



VAN7.5 & VAN8.5 = vanillin derivatization with buffer pH 7.5 or 8.5; VAN-HCl = vanillin derivatization in methanolic HCl; PBQ = *p*-benzoquinone derivatization; CC = cupric chloride derivatization; CHA = chloranilic acid derivatization; DNP = derivatization with 2,4-dinitrophenol.

Figure 13b. Absorbance spectra of gabapentin quantification methods. The sample concentration on which the scan was performed is given, and the wavelength of each absorbance maximum is labeled.

5.2.10 Statistical analysis

For each assay, the absorbance values were plotted as a function of GBP concentration. The linearity of the plots was visually evaluated and statistically analyzed to obtain calibration curves. When plotting the data, a few obvious outliers were discarded. In accordance with the recommendations in the ICH Q2(R1) validation guideline, a minimum of five concentrations were included in each regression analysis and linearity assessment. Some methods consistently exhibited varying and non-proportional absorbances in the lower concentration ranges; these values were included in the plots to illustrate the characteristics of the method. For each method, regression analysis was conducted on chosen concentration ranges where the absorbance was proportionally increasing with the GBP concentration. In some methods, the plot could be split into two separate linear ranges.

In general, a linear calibration curve should have a slope statistically significant from zero to ensure sensitivity of the method and that the absorbance increases proportionally with increasing analyte concentration. To ensure specificity, the (y-)intercept should not be statistically significant from zero – if the analyte concentration in the sample is zero, the absorbance should ideally be negligible. The coefficient of determination (R^2) is often used as a measurement of linearity, but as it was discussed in the literature review, it should not be trusted as the only tool for linearity assessment. A perfectly linear relationship yields an R^2 value of 1; thus, an R^2 close to 1 is considered an attribute of a good quality calibration curve. However, R^2 can return seemingly good values, for instance, if the plot is curve-shaped or the data points are symmetrically scattered around the regression line. Therefore, it is equally important to visually inspect the data points. Investigating the residual sum of squares (RSS) can be useful in evaluating the quality of a calibration curve (Moosavi and Ghassabian, 2018). The RSS comes from the sum of all the squared deviations from the fitted line – in other words, the RSS is a tool for expressing the degree of scatter and how well the values fit the model (the regression line). A small RSS indicates a tight fit of the data points to the model.

The results from the regression analysis are presented in Table 4, in which the most relevant parameters have been included. Comparing the RSS values was found not to give much valuable information in this method assessment; the RSS values were overall very low (all except one were <0.005), and the values did not predict the quality of the calibration curves or usefulness of the methods, and therefore, the information was excluded from the table.

Table 4. The statistical parameters of the tested quantification methods.

Method*	Tested concentration range (µg/ml)	Linear concentration range (µg/ml)	λ_{max} (nm)	Slope	Intercept	R^2
AQ	0.25–80	5–80	190	0.0079	-0.0171	0.9997
AQ-ET	0.5–80	20–80	204	0.0007	0.0052	0.9829
NIN-MET						
A	1–80	10–70	402	0.0035	0.1674	0.9627
B	1–80	5–80	"	0.0069	-0.0053	0.9922
C	5–150	N/A	N/A			
NIN-DMF						
A	1–80	5–30	404	0.0221	-0.0823	0.9945
		30–80	"	0.0348	-0.4116	0.9946
B	1–80	1–20	"	0.0042	-0.0030	0.9778
		30–80	"	0.0120	-0.2459	0.9898
C	5–150	70–130	"	0.0008	-0.0178	0.9907
AA	0.5–80	0.5–40	376	0.0158	-0.0023	0.9998
		40–80	"	0.0118	0.1594	0.9972
VAN7.5	1–140	30–120	393	0.0019	0.0283	0.9949
VAN8.5	1–140	40–140	394	0.0020	-0.0304	0.9729
VAN-HCl	1–20	N/A	N/A			
PBQ	1–180	2.5–60	364	0.0120	0.1134	0.9924
		60–140	"	0.0079	0.3669	0.9933
CC	0.5–140	60–120	244	0.0019	0.0513	0.9904
CHA	1–80	1–60	312	0.0159	0.0132	0.9993
DNP	1–80	1–10	423	0.0400	0.0496	0.9981
		15–70	"	0.0115	0.3275	0.9927

* AQ and AQ-ET = native absorbance of gabapentin in water or water/ethanol
NIN-MET and NIN-DMF = derivatization with ninhydrin in methanol or N,N-dimethylformamide. Variants A, B, and C stand for different reaction volumes (see 4.2.4.6).
AA = ascorbic acid derivatization; VAN7.5 and VAN8.5 = vanillin derivatization with buffer pH 7.5 or 8.5; VAN-HCl = vanillin derivatization in methanolic HCl;
PBQ = *p*-benzoquinone derivatization; CC = cupric chloride derivatization; CHA = chloranilic acid derivatization; DNP = derivatization with 2,4-dinitrophenol.

As can be observed from Table 4, the R^2 values were reasonably high for all methods. In most cases, however, the R^2 did not describe the true linearity very well on its own. This is, in fact, accurate for all statistical parameters if they were to be examined individually; thus, when interpreting the regression analysis and validating quantification methods, several parameters must be assessed. Visual evaluation of the scatter and the goodness of fit is also important. A high slope, which generally indicates good sensitivity, was not always equal to a good quality calibration curve; the lines with a relatively high slope also exhibited a y-intercept significantly different from zero, or an R^2 below the preferred minimum of 0.999.

An interesting observation was that in many methods, the results were affected by the concentration of the stock solutions. This was observed as variations in the linearity and the precision of the absorbance readings. All methods were tested on at least two different gabapentin stock concentrations, and differences were noticed in all methods except for the AA method. In theory, different stock concentrations should not affect the results, as the molar proportions in the reactions remain the same (assuming that the solvent proportions are not changed). This variation can indicate decreased robustness and precision of a method.

The calibration curves corresponding to the regression analysis results are presented in Figures 14a and 14b. All other methods could be performed throughout the whole analyzed concentration range with the same stock solution, but for the AA method, two separate stock solutions had to be utilized. Since the total water volume of each sample was adjusted to 0.5 ml, the samples with higher GBP concentration required the use of a stronger stock solution in order not to exceed the defined water volume. A stock solution of 1 mg/ml was utilized for the concentration range 0.5–40 µg/ml, and respectively, 2 mg/ml for the range 40–80 µg/ml.

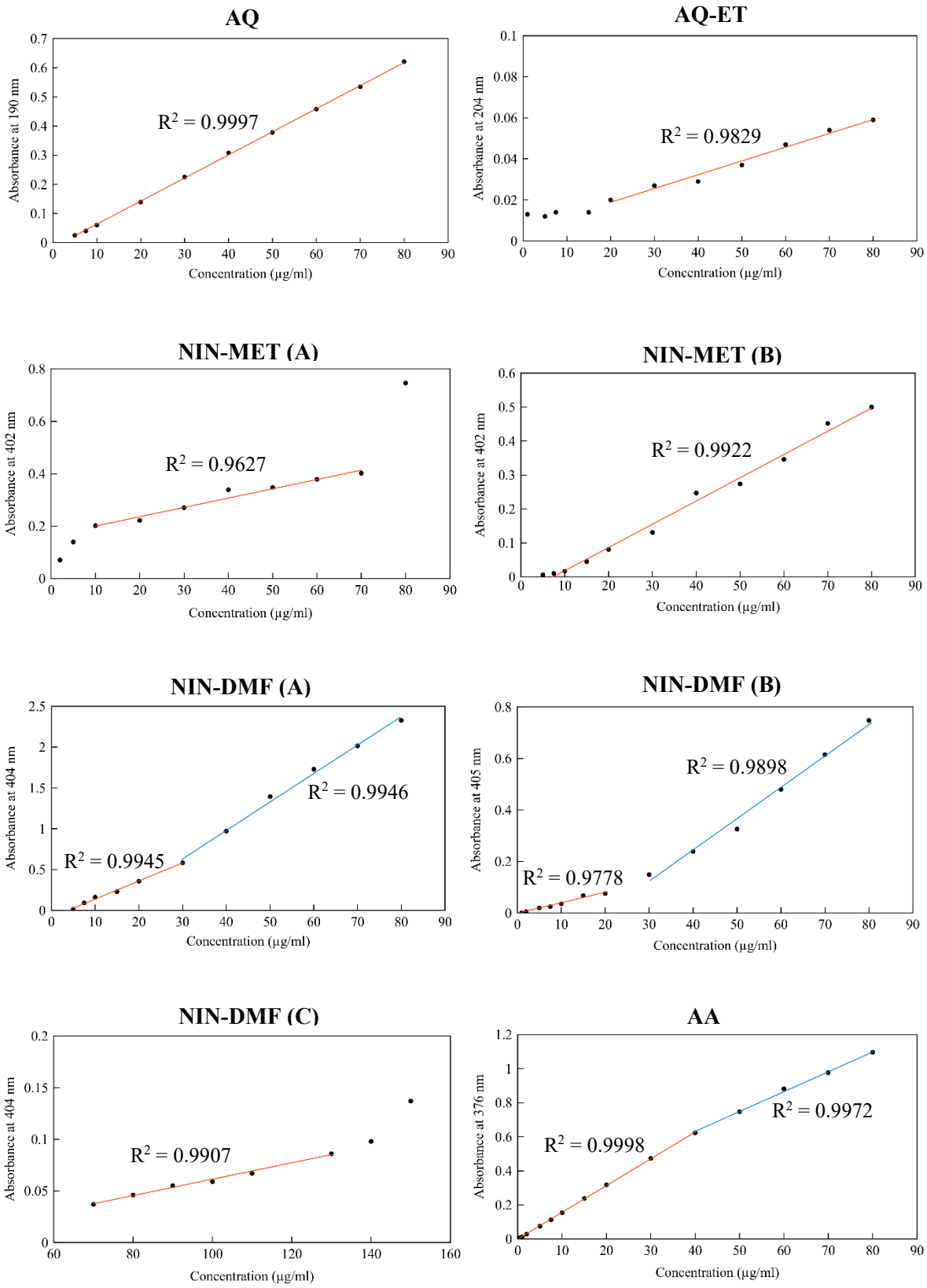


Figure 14a. The absorbance-concentration plots for the assessed methods with calibration curves showing the linear ranges.

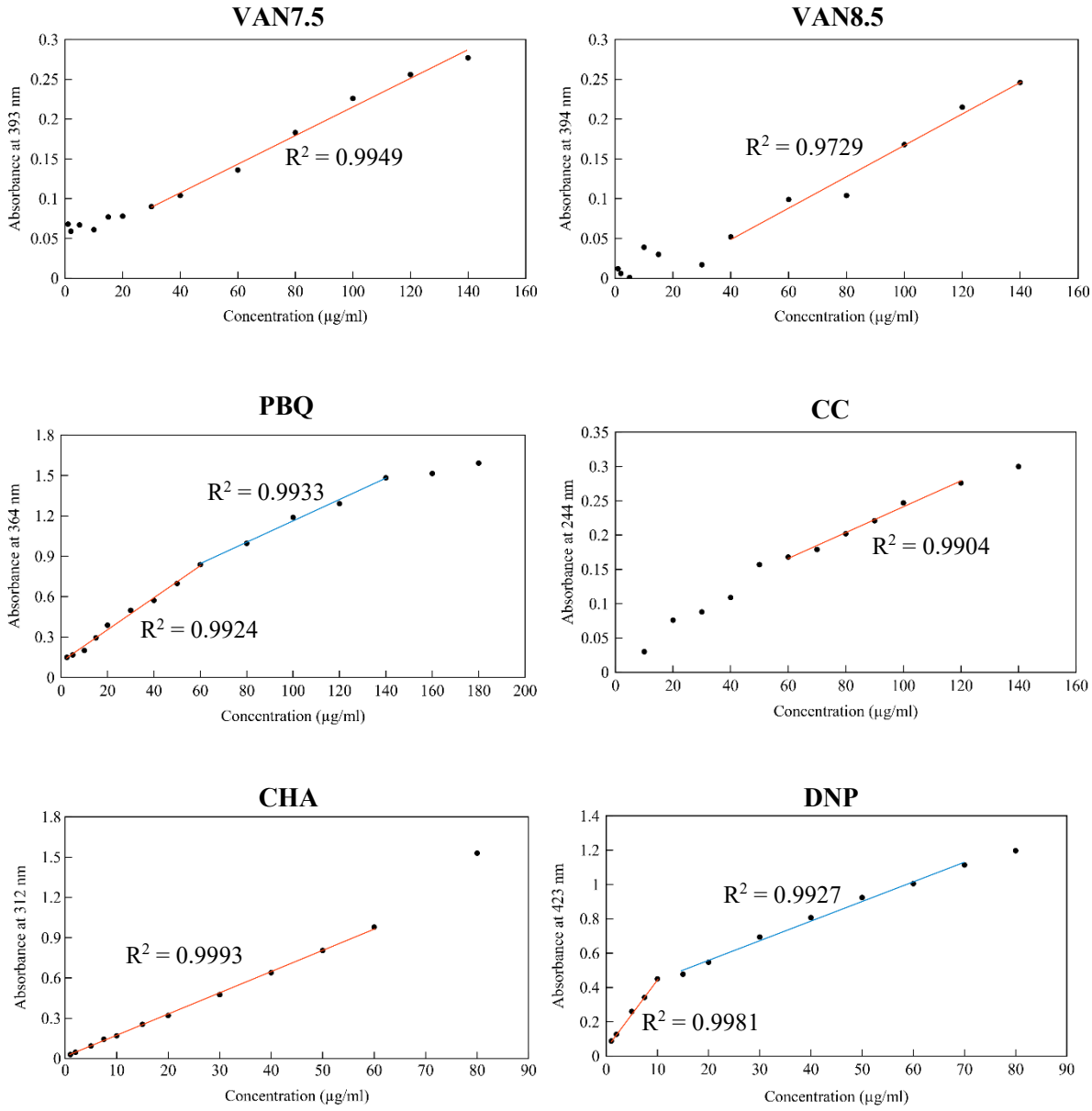


Figure 14b. The absorbance-concentration plots for the assessed methods with calibration curves showing the linear ranges.

The AA method exhibited the best performance throughout each assay. The method also performed well in the statistical analysis, returning the highest R^2 and the intercept closest to zero. Furthermore, excellent linearity was achieved in a relevant concentration range (0.5–40 µg/ml), making the method applicable to dissolution testing of the small-dose formulation in this study. Of the methods which were tested for determining the drug content in ODF samples, the AA method was the only precise and reliable method. Thus, a

quantification method that fulfilled the study aims had been found, and the study could proceed with the proof-of-concept part consisting of dosage form manufacture and analysis.

5.3 Quality assessment of the dosage forms

The HPMC-based formulations listed in Table 3 were cast into approximately 25 cm long film strips onto the A4-sized transparency sheets. UL and DL films with and without the addition of LP were manufactured and compared throughout the quality assessment. The film-rolling was conducted 72 hours after casting to ensure that the films had properly dried. As the majority of the films with PEG 400 curled up (see Figure 6), only the films with GLY were rolled utilizing the film-rolling technique described under 4.2.3. Thus, the analyses intended for the rolled films (dissolution, disintegration, and uniformity of content) were only carried out on the GLY formulation. All other analyses were performed on both formulations to compare the effect of the different plasticizers. The appearance and size of the film rolls are displayed in Figure 15. Because of limited raw material availability, only three fixed doses were produced, namely, film rolls with three, four, and five rotations.

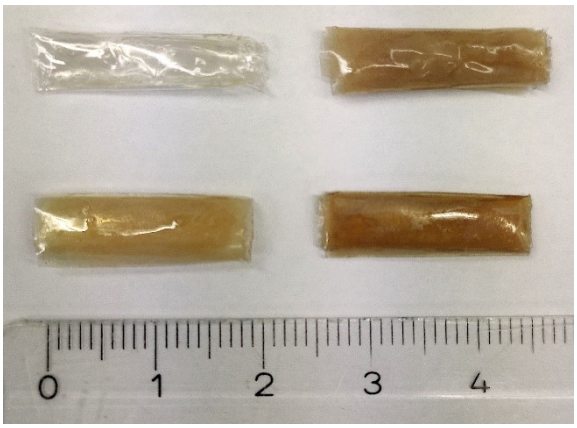


Figure 15. Film rolls with four rotations. The upper row shows an unloaded film (left) and an unloaded film with liver powder (right); on the lower row, a drug-loaded film (left) and a drug-loaded film with liver powder (right).

5.3.1 Drug content

The drug content of the films with GLY was determined with the AA derivatization procedure after dissolving the sample films (or rolls) in purified water. The films were visibly dissolved already after two hours of mixing on the orbital shaker, but the mixing was

continued until three hours to ensure complete dissolution. Samples of 0.5 ml were drawn and derivatized with AA; the returned drug content was obtained from the regression equation y = 0.0158x - 0.0023, where y is the absorbance and x the sample concentration.

In the first content assay, film strips of determined lengths were analyzed in order to determine the drug dose vs. film length. The information was used for determining the fixed-dose rotation numbers. 10 and 20 cm strips of DL films and DL films with LP were dissolved in water, as well as corresponding UL films of the same lengths. Three samples of each DL film solution were drawn, derivatized with AA, and measured spectrophotometrically against an UL film sample treated in the same way. The results are presented below in Table 5. The drug amount in the DL films was found to be proportional between the 10 cm and 20 cm films, although the 20 cm DL film with LP returned a lower drug amount than expected; this can potentially be a consequence of the manually performed casting. The deviation was, however, relatively small. The phenomenon of lower recovered drug content in LP-containing films was not encountered in any other samples in the study.

Table 5. The drug content in pieces of drug-loaded (DL) films with and without liver powder (LP). The average values and standard deviations have been calculated from three samples.

Film	Drug content (mg)
10 cm DL film	25.5 ± 0.3
10 cm DL film with LP	25.8 ± 0.7
20 cm DL film	50.0 ± 0.7
20 cm DL film with LP	45.9 ± 0.7

The second content assay was carried out on film rolls of three, four, and five rotations to determine the uniformity of content according to the specifications in the European Pharmacopoeia (Ph. Eur.). The drug content of the film rolls is presented in Table 6. The content uniformity complies with pharmacopoeial requirements if not more than one individual content deviates more than ± 15% from the average content, and none more than ± 25% from the average content. Furthermore, the acceptance value (AV) should not exceed 15. No individual film roll exceeded the allowed deviations in the percentage of drug content, but the AV exceeded the limit for two batches of DL films with LP. Although the film-rolling process was partly automated, it involved some manual labor, which is always a source of variations. As the thickness uniformity of the films was good (see the following section), the variations in drug amount were most likely caused by the rolling process.

Table 6. The average weight and drug content, the maximum deviation (max. dev.) from the average content, and the acceptance value (AV) of the film rolls. Each analyzed batch contained five film rolls.

Film roll batch	Weight (mg)	Drug content (mg)	Max. dev. (%)	AV
DL film, 3 rotations	120.7 ± 4.0	12.4 ± 0.7	7.5	13.7
DL film with LP, 3 rotations	132.7 ± 14.8	13.7 ± 1.4	-16.6	25.2
DL film, 4 rotations	160.1 ± 6.9	16.6 ± 1.0	7.3	14.3
DL film with LP, 4 rotations	163.6 ± 3.8	16.2 ± 0.8	-8.2	11.2
DL film, 5 rotations	190.8 ± 16.5	18.7 ± 1.0	5.6	12.4
DL film with LP, 5 rotations	180.9 ± 13.3	18.0 ± 1.5	-9.6	19.5

When applying the AA derivatization, it was observed that the UL samples with LP obtained a slight red hue. Although paler than in the corresponding DL samples, it still indicated that AA, to some extent, also reacted with the amino acids in LP. The phenomenon did not pose a problem in the content assays, as the spectrophotometer was blank calibrated with the UL samples before measuring the DL samples, which minimized the interfering absorbance. This observation was also taken into account in the dissolution studies by subtracting the UL absorbance from each DL sample absorbance.

The difference in drug dose between the rotation numbers was small; preparing and analyzing more doses in a wider range would have been preferable, but in this context, the results suffice as a proof-of-concept that small-dose veterinary GBP formulations can be easily prepared and accurately quantified. Refining and perhaps further automating the manufacturing process would likely produce more uniform dosage forms. The polymer-based formulation in this study could easily be modified to suit, for example, semi-solid extrusion (SSE) 3D printing, which was proven to be a satisfactory production method for veterinary ODFs as demonstrated by Sjöholm et al. (2020). An SSE ink would have to be more viscous than a solvent casting solution; the GBP formulation in this study could be modified by incorporating higher amounts of HPMC E5, by testing other types of HPMC, or by changing it into another film-forming polymer.

5.3.2 Thickness uniformity

Variations in thickness of an ODF can lead to non-uniform drug content (Dixit and Puthli, 2009). Thus, it is important to ensure the uniformity of thickness. The thickness was measured from three points on three separate films after 48 hours of drying. As can be seen in Table 7, all film types exhibited good thickness uniformity with only minor deviations. This was also indicated by the behavior of the casting solutions, which flowed well and distributed evenly while still holding their cast shape. The formulation with PEG 400 as a plasticizer yielded thicker films than the one with GLY, which may be attributed to the tendency of PEG to harden, as was discussed previously under 5.1. As expected, the addition of more solid material to the solution increased the bulk of the film. Adding LP or GBP slightly increased the thickness of the films as compared to the films without.

Table 7. The average thicknesses of the dried films. Measurements were taken from three points 10 cm apart on three separate films.

Film	Plasticizer	Thickness (mm)
UL film	PEG 400	0.10 ± 0.01
UL film with LP	PEG 400	0.11 ± 0.01
DL film	PEG 400	0.12 ± 0.01
DL film with LP	PEG 400	0.12 ± 0.01
UL film	GLY	0.08 ± 0.00
UL film with LP	GLY	0.09 ± 0.01
DL film	GLY	0.10 ± 0.01
DL film with LP	GLY	0.10 ± 0.01

5.3.3 Dissolution

The dissolution profiles were determined separately for each film type and for pure GBP. The sampling was performed manually at specified time points, and each sample was derivatized with the AA method to determine the released drug amount. The absorbances of the DL films were adjusted by subtracting the average absorbance of the corresponding UL film for each time point. The average drug release (%) of three samples was calculated with the previously presented regression equation utilized in the content assays. The cumulative drug release was plotted as a function of time (Figure 16).

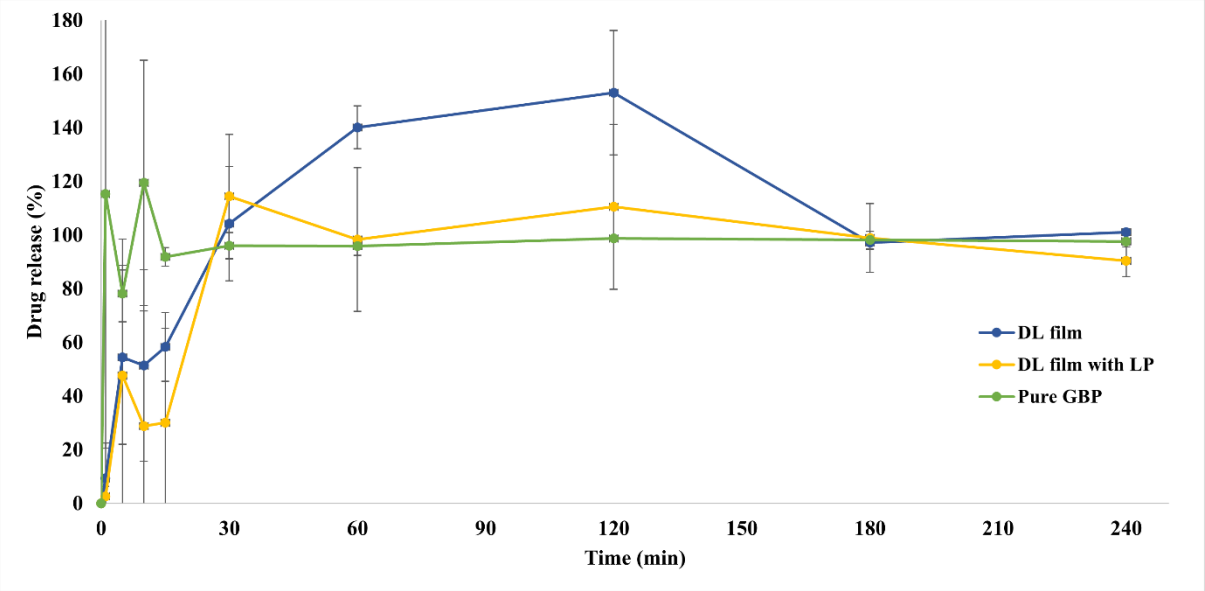


Figure 16. The drug release in water of pure gabapentin (GBP) and drug-loaded (DL) films with and without liver powder (LP). The averages (n = 3) with standard deviations are shown.

As can be observed from Figure 16, the standard deviations are high for most sampling points. The cumulative drug release also exceeds 100% at several points. These results are due to the properties of the film and the dissolution test setup. The rapid disintegration of the film into smaller pieces caused burst release and drug gradients in the media, and the test setup could not provide proper mixing of the media. The reciprocation could not exceed 50 rpm, as an elevated speed would have hindered precise sampling at the same position every time. It was also not possible to incorporate a paddle or other continuous mixing equipment into the vessels due to the setup. Another drawback of the manual sampling method is that each sampling requires time and labor, which means that the sampling time points must not be too close.

Despite the high variations in the drug release values, some conclusions can be drawn from the dissolution test. As expected, pure GBP dissolved very rapidly in water, reaching 100% release in less than 30 minutes. It was also found that the drug release from the films was rapid; 100% drug release was achieved in 30 minutes. As is typical for ODFs, the drug release is closely correlated to the physical disintegration of the films (Wasilewska and Winnicka, 2019). In this study, it was observed that the drug release was almost as rapid as the disintegration times of the films, which are presented in the following section.

5.3.4 Disintegration

Rapid disintegration is an important characteristic of ODFs. The disintegration time of the film rolls with four rotations (n = 6) in purified water was investigated with a tablet disintegrator. The film rolls were continuously observed when the machine was running, and the disintegration time for each unit was recorded; the test ended when all rolls had disintegrated completely.

As the disintegration apparatus A (Ph. Eur. 2.9.1) was utilized in this study, the requirements for solid dosage forms analyzed with this setup were applied in the absence of specifications for ODFs. Ph. Eur. specifies that capsules should disintegrate within 30 minutes, uncoated tablets within 15 minutes, and film-coated tablets within 30 minutes. The average disintegration times of the film rolls are presented in Table 8.

Table 8. The average mass and disintegration time of film rolls with four rotations.

Film roll	Mass (mg)	Disintegration time (min)
UL	113.3 ± 11.3	15:16 ± 03:26
UL with LP	141.5 ± 18.1	16:36 ± 01:51
DL	160.4 ± 22.3	13:47 ± 03:09
DL with LP	151.1 ± 20.1	17:43 ± 02:21

Given the standard deviations and the individual disintegration times of each sample, it appeared that the addition of LP or GBP did not significantly affect the disintegration time of the rolls in this test setup. The maximum disintegration time (in minutes) was 18:11 for UL films, 19:00 for UL films with LP, 17:10 for DL films, and 20:50 for DL films with LP. The maximum disintegration times of the film rolls were longer than the accepted time for uncoated tablets (15 minutes) but well within the accepted time for capsules and film-coated tablets (30 minutes).

Orodispersible tablets (ODTs) are a closely related dosage form to ODFs. According to Ph. Eur., ODTs should disintegrate within 3 minutes. With this as a reference, the obtained disintegration times of 15–20 minutes are too long for a dosage form that should disintegrate rapidly in the mouth. This test setup did, however, not replicate *in vivo* conditions. The medium volume was significantly larger than the saliva volume in the mucosal cavity, and more importantly, the test setup did not involve the mechanical forces exerted by the tongue. Thus, the *in vivo* disintegration time of the film rolls would likely be shorter. Preis et al. (2014b) have discussed these challenges with determining the disintegration time of ODFs.

In their study, the authors suggest a testing method that utilizes the conventional disintegration apparatus but modified to better suit ODFs. In the said setup, the upper end of the ODF is fixed by a clamp and a small weight is attached to the lower end. The films are half-immersed in the medium during the dipping movement, and the end-point is the dropping down of the weight. The advantages of this setup are a clear end-point, and the presence of a force (the weight) similar in magnitude to the licking of a tongue. This serves as one example of how the disintegration time of the film rolls in this study could be determined.

5.3.5 Mechanical strength

Ph. Eur. requires that ODFs should possess sufficient mechanical strength to withstand handling. As it was discussed in the literature review, there are several approaches to mechanical strength testing. In this study, the mechanical strength of the films was assessed with a puncture test. Both the PEG and GLY formulations were analyzed 24, 48, and 72 hours after casting.

The output from the puncture test consists of the maximum force exerted by the probe to break the film and the distance of travel (displacement) of the probe after contact with the film. As explained by Radebaugh et al. (1988), the maximum force can be normalized to the contact area to express the puncture strength (N/mm^2), and the probe displacement can be used to calculate the elongation (%) of the film radius, i.e. how much the film stretches before bursting. The puncture strength is calculated by dividing the maximum force by the cross-sectional area of the film located in the path of the probe. The elongation percentage is obtained from the formula $\frac{\sqrt{R^2+D^2}-R}{R} * 100\%$, where R is the radius of the film located in the rig hole, and D is the distance of travel (displacement) of the probe. The elongation percentage can be regarded as a measure of film flexibility.

The maximum force and the percentage of elongation at break for each film are presented in Figure 17. The maximum force has not been normalized to the sample area because the contact area between a spherical probe and the sample cannot be determined (Preis et al., 2014a). The contact area can only be determined in the case of a flat probe. The average thicknesses of the analyzed films were the same as presented under Thickness uniformity (5.3.2.). The room conditions were monitored every 15 minutes throughout the assays, and the temperature and relative humidity remained stable throughout the test days;

21.9 ± 0.2 °C and 10.4 ± 1.7% relative humidity (RH) when analyzing the PEG films, and 21.7 ± 0.4 °C and 9.1 ± 1.2 % RH when analyzing the GLY films.

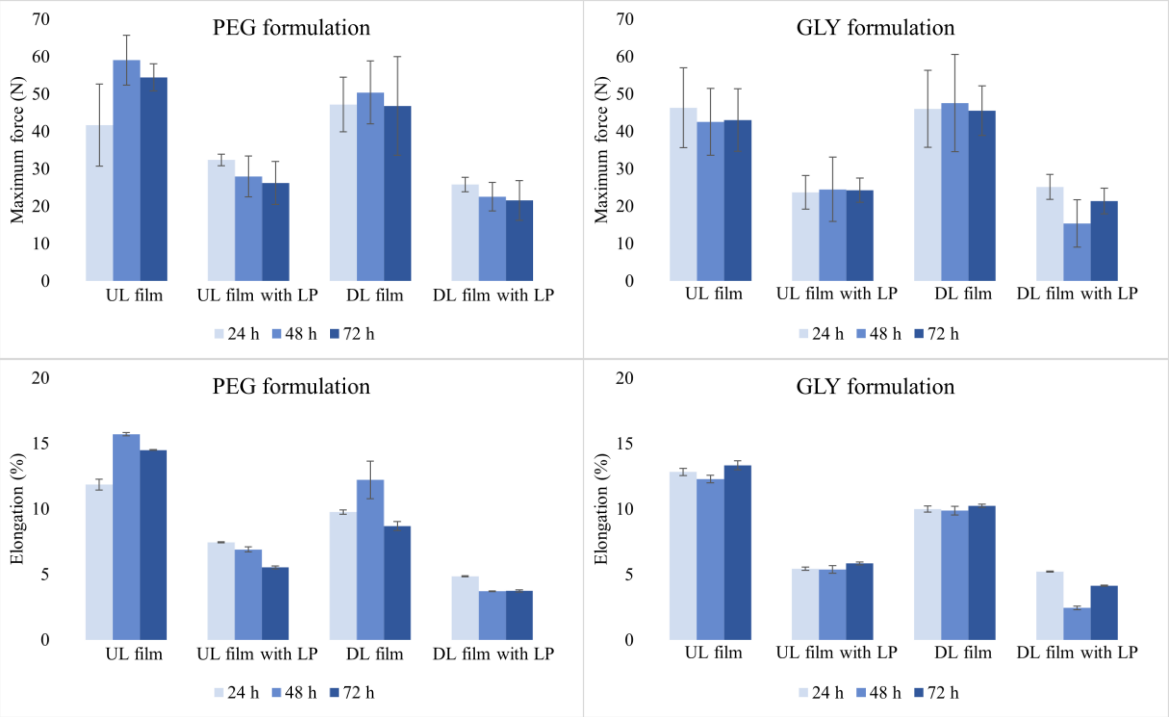


Figure 17. Measured puncture strength of the films containing either PEG 400 or glycerol as a plasticizer on days one, two, and three after casting. The results are presented as the maximum force (N) required to puncture the film (upper row) and the elongation of the film (%) until bursting (lower row).

The mechanical strength did not change significantly between days one, two, and three in either formulation. For unknown reasons, the GLY-containing DL film with LP exhibited decreased strength and elongation on day two but not on day three. The addition of LP reduced the maximum strength and elongation of the films; this phenomenon has been observed in previous studies and can be attributed to the presence of undissolved particles in the film (Sjöholm et al., 2020). The impact of GBP was not as significant on the burst strength, but the addition of the drug reduced the film flexibility slightly.

The numerous adjustable parameters in a puncture test pose some challenges in the comparability of results between studies. The exposed sample area, the probe's traveling speed and distance, and the force threshold vary between setups. As Preis et al. (2014a) demonstrate, the choice of probe significantly affects the results. A large, flat probe yields higher force values because film penetration is easier with a small and pointy probe.

In their study, Preis et al. assessed the puncture strength of commercially available oral films and compared them with prepared test films of various polymers. A flat, cylindrical probe larger than the spherical one in this study was utilized. The obtained maximum puncture forces of the commercial films ranged between 1.39 ± 0.13 N and 7.18 ± 1.59 N, and the elongation percentages between 1.03 ± 0.21% and 6.54 ± 0.89%. The recommended minimum value of puncture strength was defined as 0.06 N/mm^2, which in their setup corresponded to a maximum force of 1.09 N. The films in the present study exhibited values well above this, even though a smaller and pointier probe was utilized. Thus, the overall mechanical strength of the films can be considered sufficient. It is, however, advisable to perform additional tests with the texture analyzer to further gain understanding on the mechanical properties: the mucoadhesion of the films could be assessed with a suitable setup to ensure proper adherence of the ODFs to the oral mucosa, and a tensile test with pulling clamps could also be conducted as an additional evaluation of the mechanical strength of the films.

5.3.6 Moisture content

Like the mechanical strength, the moisture content of the PEG and GLY formulations was measured on days one, two, and three. The ambient conditions were the same as during the puncture tests. The moisture was measured in triplicate on film samples weighing 0.2 ± 0.02 g. The average moisture content of the films is presented in Figure 18. Given the standard deviations, the moisture content did not change significantly between days in either formulation. This result correlates with the stability of the mechanical strength between days. The films with PEG as a plasticizer were slightly harder than the films with GLY; this can be observed as a somewhat lower average moisture content in the PEG-containing films. The majority of the analyzed films comply with the recommendations on moisture content in ODFs. In the literature, the recommendations are <5% according to Nair et al. (2013), and more specifically, 3–6% as defined by Borges et al. (2017). Although the films dried relatively rapidly (within 24 hours), it could be explored whether the drying process could be accelerated without compromising the quality of the films. The moisture analyzer measured the total residual moisture content, but it would also be desirable to specifically determine the residual ethanol content of the films to ensure that the levels are low.

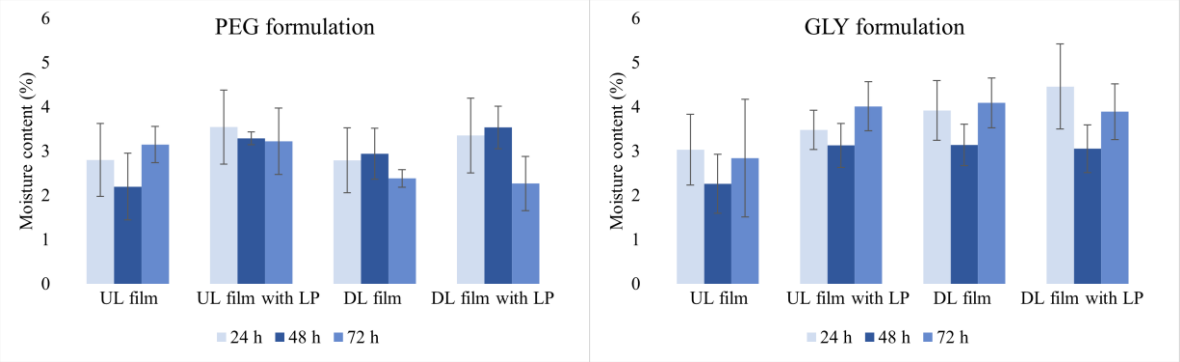


Figure 18. The moisture content (% of total mass) in films with PEG 400 or glycerol (GLY) as a plasticizer. The moisture content was measured one, two, and three days after casting.

5.3.7 Surface pH

Administering an ODF with a too acidic or basic pH value can cause irritation and damage to the oral mucosa (Nair et al., 2013). Ideally, the surface pH of oromucosal dosage forms should be close to 7. The surface pH of the films was measured after wetting the films with 1 ml water and bringing a pH electrode to the surface after 30 seconds. The pH values were recorded after 1 minute of equilibration. The measurements were carried out at room temperature (T = 19.5 ± 0.2 °C). The average pH values (n = 3) are shown in Table 9. It was observed that the UL films yielded alkaline pH values, which were brought close to neutral with the addition of LP or GBP. The pH values of the DL films with and without LP were close to physiological values. Thus, the administration of these studied formulations should not cause mucosal irritation.

Table 9. Surface pH of the films with either PEG 400 or glycerol as a plasticizer.

	UL film	UL film with LP	DL film	DL film with LP
PEG formulation				
Surface pH	8.5 ± 0.1	6.8 ± 0.1	6.9 ± 0.1	6.7 ± 0.2
GLY formulation				
Surface pH	8.7 ± 0.1	7.0 ± 0.1	7.3 ± 0.1	6.8 ± 0.0

5.3.8 Differential scanning calorimetry

To investigate the thermal properties of the raw materials and the films, samples of 3.4 ± 0.3 mg were analyzed with differential scanning calorimetry (DSC) in a temperature range of 40–220 °C at a heating rate of 10 °C/min. The DSC curves are presented in Figure 19. The 3% DL formulations exhibited identical curves regardless of plasticizer; only the GLY formulation is presented in the figure. The crystallized films obtained from 5% DL solutions were also analyzed for comparison. The evaporation of water around 100 °C was present but only marginally noticeable in most samples. The onset of the melting point at 162 °C was evident for pure GBP and the physical mixtures containing GBP, namely, physical mixture 1 (HPMC, GBP, LP) and physical mixture 2 (HPMC, GBP). The melting point complies with literature values (O'Neil, 2013). Physical mixture 3 (HPMC, LP) only exhibited slight water evaporation. The melting point of GBP could not be observed in the 3% DL films, which suggests that the drug is in an amorphous state. Two small endothermic peaks could be observed in the crystallized 5% DL films: one at 70 °C and one around 160–166 °C. The degradation product gabapentin lactam should display a distinct melting point around 87–91 °C (Braga et al., 2008; Cutrignelli et al., 2007), so the former endothermic peak is most likely not attributed to gabapentin lactam. The peaks could possibly display phase transitions of the β polymorph (form III) around 70 °C (Reece and Levendis, 2008) and for the α polymorph (form II) around 160 °C (Hsu and Lin, 2009).

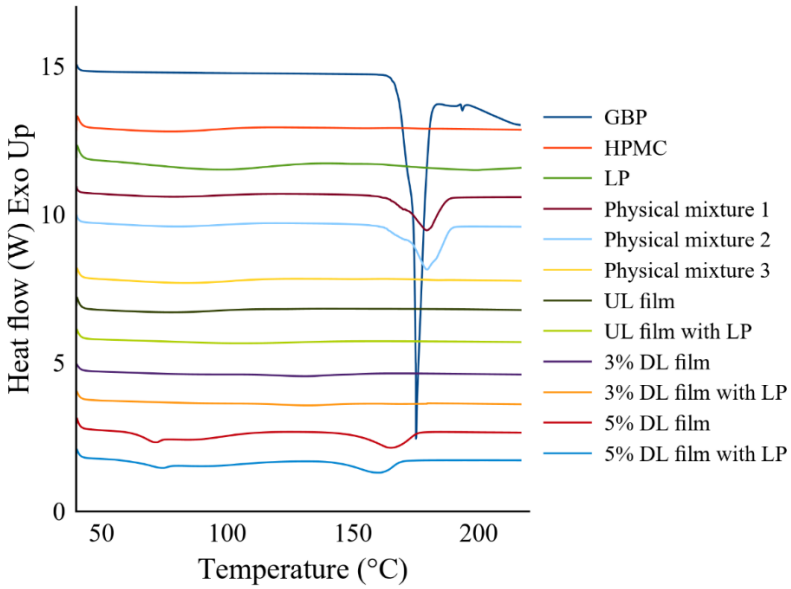


Figure 19. DSC curves of the pure substances, physical mixtures, and unloaded (UL) and drug-loaded (DL) films with and without liver powder (LP). Physical mixture 1 contains hydroxypropyl methylcellulose (HPMC), gabapentin (GBP), and LP. Physical mixture 2 contains HPMC and GBP. Physical mixture 3 contains HPMC and LP.

5.3.9 Attenuated total reflectance Fourier transform infrared spectroscopy

Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was utilized to study the solid-state characteristics of the raw materials and the films. The FTIR spectra are presented in Figure 20. As with the DSC results, the FTIR spectra of the GLY and PEG formulations displayed similar peaks; thus, only the GLY formulation is presented.

In the solid state, zwitterionic GBP can form four different polymorphs, of which form II is the commercially used drug substance (Zong et al., 2011). Peaks in the typical NH stretching region (3500–3300 cm^{-1}) would indicate the presence of unionized amine groups, whereas the absence of these peaks confirms the existence of GBP in the zwitterionic state (Lin et al., 2010). The stretching vibrations of the ionized groups ($NH_3^{+)}$ are typically noticeable in the 3200–2800 cm^{-1} range (Ranjous and Hsian, 2013); in Figure 20, they can be seen as a doublet at 2920 and 2857 cm^{-1} in the pure GBP sample. The aforementioned findings are also in line with those of Rimawi et al. (2019) and Siddiqui et al. (2010). The asymmetric stretching vibration of COO^- typically occurs in the 1650–1600 cm^{-1} range (Sinha et al., 2013). Siddiqui et al. (2010) observed the carbonyl stretch at 1615 cm^{-1}, which corresponds to the observed peak at 1611 cm^{-1} in the pure GBP sample in Figure 20. The found peaks in the pure GBP sample are in line with the peaks characteristic for polymorph II as reported by Lin et al. (2010) and Ranjous and Hsian (2013). The most distinct of these peaks are labeled in the spectrum for pure GBP.

In the HPMC spectrum, a broad band characteristic of C–H stretching can be observed at 2800–2900 cm^{-1} (Ding et al., 2015). It was noticeable in every sample containing HPMC. The band hides the characteristic NH^{3+} doublet peak in physical mixtures 1 and 2 and in the 5% DL film. The peaks could still be observed in the 3% DL films and in the 5% DL film with LP. The presence of GBP was evident in physical mixtures 1 and 2 because the spectra displayed the same peaks in the range 1611–708 cm^{-1} as were labeled in the pure GBP spectrum. The presence of LP in the physical mixtures or the films could not be observed in the spectra.

Ranjous and Hsian (2013) have found the characteristic peaks of the degradation product, gabapentin lactam, to occur around 3202, 2928, and 1699 cm-1. These peaks were not visible in the obtained spectra of the DL films, which indicates that GBP had likely not degraded into gabapentin lactam during the manufacturing process.

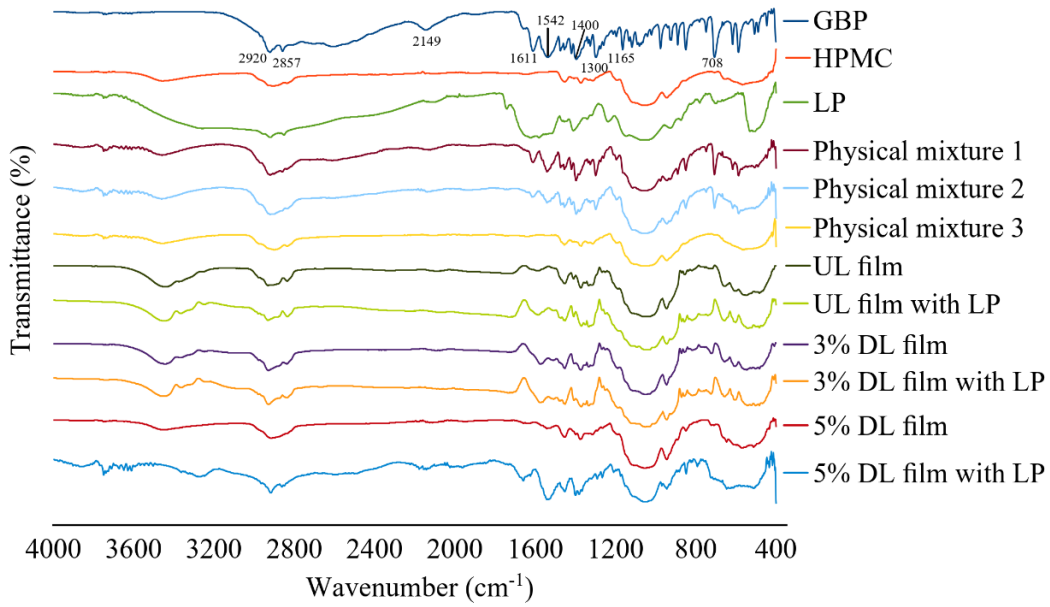


Figure 20. FTIR spectra of the pure substances, physical mixtures, and unloaded (UL) and drug-loaded (DL) films with and without liver powder (LP). Physical mixture 1 contains hydroxypropyl methylcellulose (HPMC), gabapentin (GBP), and LP. Physical mixture 2 contains HPMC and GBP. Physical mixture 3 contains HPMC and LP.

5.3.10 Rheology

The rheological behavior of all solutions was examined by measuring the change in viscosity as a function of increasing shear rate. An attempt was made to analyze the thixotropic behavior as well, but the solutions started drying during the longer periods of constant, slow shear rate; useful results could therefore not be obtained. The small sample volume combined with the rapid evaporation of ethanol caused drying of the sample from the edges inwards. Successful analysis of the thixotropic behavior would require a larger sample volume and additional equipment to cover the plate area and protect the samples from the air during the test.

The viscosity curves are pictured in Figure 21. As can be seen in the curves, the solutions exhibited non-Newtonian fluid behavior. The solutions with LP initially exhibited shear-thickening before the shear rate was increased to 1 s^{-1}, after which they started to exhibit shear-thinning. The solutions without LP showed shear-thinning properties throughout the shear rate range. Overall, the addition of 1% LP to a solution decreased the viscosity, but the addition of 3% GBP did not significantly affect the rheological properties. The GLY formulations, in general, had lower viscosity than the PEG solutions. The rheological

properties are directly related to the castability of the solutions and affect, for example, the optimal casting speed. The observed shear-thinning behavior of the solutions confirms their castability.

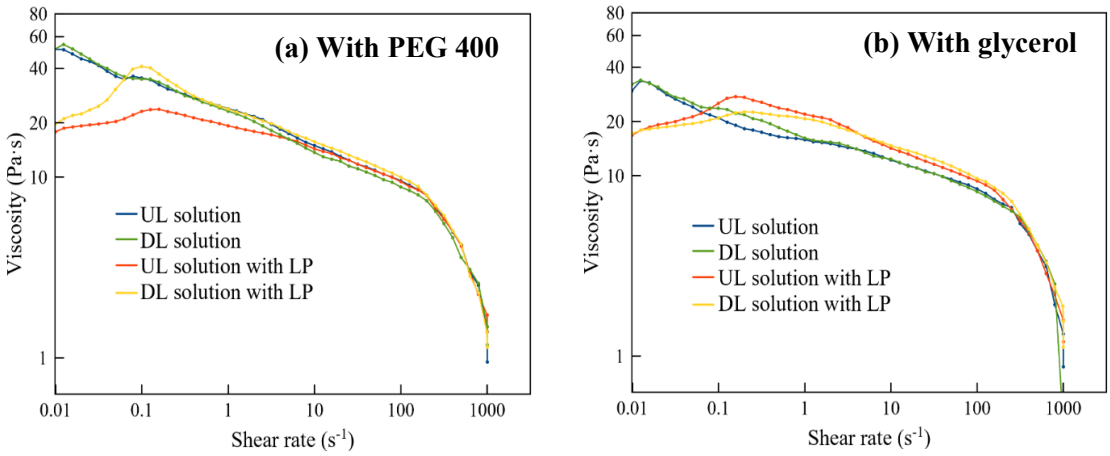


Figure 21. Viscosity vs. shear rate curves of the formulations in base-10 logarithmic scale: (a) shows the formulation with PEG 400 as a plasticizer, and (b) the formulation with glycerol as a plasticizer.

6. Conclusions

This thesis aimed to investigate ultraviolet-visible (UV-Vis) spectrophotometric quantification methods for application to the manufacture of a veterinary gabapentin (GBP) dosage form. There are known challenges with quantifying GBP, and the question of interest in this study was whether an easy, rapid, and reliable quantification method could be applied to small-dose veterinary formulations. To the best of the author's knowledge, GBP quantification methods have previously not been assessed for the purpose of veterinary medicine. There is a dire need for veterinary GBP dosage forms, but the quantification challenges have been a hurdle in their development.

A selection of quantification methods was assessed, and the methods exhibited highly varying performance. A solvent-cast orodispersible film (ODF) formulation of GBP was developed alongside with the UV-Vis method assessment, and the quantification methods were tested on GBP in bulk and on the developed formulation. One method based on derivatizing GBP with ascorbic acid (AA) exhibited excellent performance and was successfully applied to the quantification of GBP in the developed formulation. As a proof-of-concept, the AA derivatization method was applied for determining the content uniformity and the *in vitro* dissolution profile of the developed dosage forms. Furthermore, the quality of the ODFs was assessed through various analysis methods.

The findings proved that GBP can be reliably quantified with the AA derivatization method, both in bulk and in formulation. The developed formulation exhibited good mechanical strength, rapid drug release, neutral surface pH, and easily adjustable doses to personalize the treatment for each patient's needs. These findings are important as there are no commercially available veterinary dosage forms of GBP, and there is a great unmet need within GBP treatment of small pets. Cats and dogs are currently treated with human dosage forms, which have to be manually divided into smaller doses. Oral films for veterinary patients is yet an underexplored field, even though ODFs have great clinical potential. ODFs are convenient dosage forms for administration to animals, as they disintegrate rapidly in the mouth, and flavoring agents are easy to incorporate into the formulation to improve compliance. The polymer utilized in this study, hydroxypropyl methylcellulose (HPMC), showed excellent film-forming properties and yielded flexible, durable films with the incorporation of glycerol into the formulation. HPMC could be utilized for solvent casting of other drugs as well, expanding the field of veterinary ODFs. The addition of liver powder as a taste-masking agent did not have a negative impact on the film quality; in fact, it

improved, for example, the surface pH of the films. Liver powder addition slightly decreased the mechanical strength of the films, but the strength was still very well sufficient to withstand handling.

Since there are roughly 50 UV-Vis quantification methods for GBP described in the literature, one topic of interest for further studies would be to investigate the methods omitted in this study. A more thorough validation of the methods would be recommendable; for example, assessing the accuracy according to ICH guidelines would be highly relevant. The AA method could be further tested on various formulations with different polymers. Successful application of the method to various formulations would enable the utilization of more effective manufacturing methods, such as 3D printing. The quality assessment conducted in this study was by no means exhaustive, and several analyses should be performed to more thoroughly characterize the ODFs. For example, studies on the stability and shelf-life of both the casting solution and the dried ODFs should be carried out, as well as determination of optimal packaging and storage conditions. The palatability of the ODFs should be assessed, for instance, with an electronic tongue or through *in vivo* palatability studies. However, there is a dire need for pharmacopoeial specifications on the quality attributes of ODFs. ODFs are a relatively new but very promising dosage form, and the current ODF monograph in the European Pharmacopoeia is insufficient. Establishing proper pharmacopoeial quality requirements would aid in bringing more ODFs to the market.

The findings in this thesis carry many practical benefits, as they demonstrate that pet-friendly GBP dosage forms can be easily manufactured and analyzed. The UV-Vis quantification method with AA derivatization is simple and can fairly easily be implemented in pharmacies, veterinary clinics, animal hospitals, and such. The suggested ODF formulation of GBP serves as an example of a dosage form that is simple to prepare and enables personalization of the dose. Implementing these findings in practice could diminish the need for the extensive manual labor which is compounding of GBP dosage forms and splitting of tablets and capsules. Instead, safe and effective veterinary medicines could be rapidly manufactured at the point-of-care.

7. Summary in Swedish – Svensk sammanfattning

Utveckling av en munsönderfallande film för djur med fokus på spektrofotometrisk kvantifiering av gabapentin

7.1 Inledning

Gabapentin är en antiepileptisk medicin som även används för behandling av olika smärttillstånd, främst neuropatisk smärta. Epilepsi och och neuropatisk smärta förekommer förhållandevis ofta hos husdjur, varvid gabapentin är ett vanligt behandlingsalternativ. För närvarande finns det dock inga djurläkemedel med gabapentin på marknaden, och husdjur behandlas med gabapentinpreparat avsedda för människor. Katter och små hundar kräver betydligt mindre doser än människor, vilket leder till att starka gabapentinkapslar måste delas för hand eller ex tempore-framställas i mindre doser. Dessa förfaringssätt kräver betydligt mycket manuellt arbete och medför risker för såväl över- som underdosering, kontamineringar och stabilitetsproblem samt det faktum att djur tenderar att spotta ut de illasmakande medicinerna. Antalet husdjur i de finländska hushållena ökar kontinuerligt och det finns ett växande behov för gabapentinpreparat specifikt anpassade för katter och mindre hundar.

Inom läkemedelsutveckling och -framställning är kvantifieringen av den aktiva substansen en av de mest centrala analysmetoderna. I litteraturen har gabapentin kvantifierats medelst flertalet olika tekniker såsom till exempel kromatografi, elektrofores och olika spektroskopiska metoder. Spektrofotometri som mäter absorptionen av ultraviolett och synligt ljus (UV/Vis-spektrofotometri) föredras framom de andra eftersom tekniken är snabb, enkel och relativt ekonomisk. Problemet är att gabapentinmolekylen inte har några kromoforer och därmed besitter mycket låg absorbans i det ultravioletta och synliga våglängdsspektret; molekylen måste derivatiseras med någon reagens för att kunna mätas spektrofotometriskt. Flera forskare har påpekat problemet med gabapentinkvantifiering, och i litteraturen finns flera tiotals förslag på derivatiseringsmetoder för UV/Vis-spektrofotometri. Däremot har endast en praktisk jämförelse av olika metoder genomförts, och den omfattar blott ett fåtal metoder. Därutöver är alla hittills publicerade metoder ämnade för kvantifiering av gabapentin som ren substans eller i människoläkemedel, och

det har inte undersökts huruvida metoderna går att tillämpa på små gabapentindoser i djurläkemedelsformat.

7.2 Målsättning

Målsättningen med studien var att testa och utvärdera spektrofotometriska kvantifieringsmetoder för gabapentin med avsikten att finna en pålitlig metod att tillämpa på veterinärmedicinska gabapentinpreparat. I studien ingick även att utveckla och analysera ett gabapentinpreparat som lämpar sig för katter och små hundar.

7.3 Material och metoder

En omfattande litteratursökning genomfördes och ett urval UV/Vis-spektrofotometriska metoder sållades fram för vidare studier. Gabapentin kvantifierades utan derivatisering i vatten och i vatten/etanolblandning, och av de olika derivatiseringsmetoderna testades ninhydrin, askorbinsyra, vanillin, *p*-bensokinon, kopparklorid, kloranilsyra samt 2,4-dinitrofenol. Metoderna genomfördes på ren gabapentin samt på testfilmer av den utvecklade formuleringen, och metoderna utvärderades på basis av precision, robusthet, linearitet och genomförbarhet.

Valet av dosform föll på munsönderfallande filmer, vilka är tunna polymerfilmer som sönderfaller snabbt i munnen vid kontakt med saliven. Fördelarna med munsönderfallande filmer är många: enkel framställning utan upphettning, möjlighet att justera och personalisera läkemedelsdosen, samt underlättad administrering då filmen inte kan spottas ut eller fastna i halsen på djur. Filmerna framställdes genom en typ av manuell formgjutning som skapade långa filmremsor. Remsorna rullades ihop till kompakta dosformer i en automatiserad filmrullare, med vilken läkemedelsdosen kan justeras genom att ändra antalet rotationsvarv. I formuleringsutvecklingen utvärderades ett flertal syntetiska, vattenlösliga polymerer. Som lösningsmedel användes renat vatten och 94 % etanol i varierande proportioner. Leverpulver användes som smaksättning, och polyetylenglykol (PEG) 400 eller 85 % glycerol tillsattes som mjukgörare för att förbättra filmernas flexibilitet.

7.4 Resultat

Kvantifieringsmetoderna uppvisade mycket varierande egenskaper. Gabapentin gick visserligen att mäta utan derivatisering, men med låg sensitivitet. Metoden gick inte att applicera på filmformuleringen; de uppmätta absorbansvärdena motsvarade inte de teoretiska gabapentinmängderna i provet. Ninhydrin, *p*-bensokinon, kloranilsyra och 2,4-dinitrofenol gav starkt färgade reaktionsprodukter och uppvisade linearitet i viss mån, men metoderna gick inte att applicera på formuleringen. Ninhydrin och *p*-bensokinon klarade inte av att skapa komplex med gabapentin i filmformuleringen. Trots goda statistiska egenskaper kunde kloranilsyra och 2,4-dinitrofenol inte användas på formuleringen, eftersom metoderna utfördes i acetonitril som lösningsmedel, vilket inte kan användas som medium i studier av läkemedelsfrisättning. Vanillin och kopparklorid gav upphov till instabila komplex och stora variationer mellan varje analys, dvs. dålig precision och robusthet. Den enda metoden som genomgående gav utmärkta resultat var derivatiseringen med askorbinsyra. Metoden uppvisade god precision och robusthet samt hög linearitet (R^2 = 0.9998) i ett brett koncentrationsspann (0.5–40 µg/ml gabapentin) vid absorbansmätning på våglängden 376 nm. De uppmätta absorbanserna motsvarade i hög grad de teoretiska gabapentinmängderna i de analyserade testfilmerna. Askorbinsyrametoden kunde således användas för att bestämma läkemedelsmängden i dosformerna samt läkemedelsfrisättningen ur dem.

I formuleringsutvecklingen uppvisade hydroxipropylmetylcellulosa (HPMC) de bästa egenskaperna. Den slutgiltiga filmformuleringen bestod av 20 % HPMC som filmbildare, 3 % gabapentin, 2.5 % glycerol som mjukgörare och 1 % leverpulver som smaksättning, upplöst i en 2:1 (v/v) blandning av renat vatten och etanol. PEG 400 testades som ersättare för glycerol, men det orsakade att merparten av filmerna hårdnade och rullade ihop under torkningen. Därmed utfördes filmrullandet enbart på glycerolformuleringen. Som konceptvalidering framställdes dosformer (filmrullar) av tre olika styrkor och analyserades med relevanta farmaceutiska kvalitetsanalyser. Filmerna uppvisade god mekanisk styrka och flexibilitet som inte nämnvärt ändrade mellan dag ett, två och tre efter formgjutning. Eftersom enstaka gabapentindoser för hundar börjar från 10 mg/kg, och för katter från 5 mg/kg, var det önskvärt att producera filmrullar med gabapentindoser från ca 10 mg och uppåt för att kunna medicinera även de minsta hundarna och katterna. Den uppmätta genomsnittliga läkemedelsdosen (utan leverpulver/med leverpulver) för tre rotationer var 12.4 ± 0.7 mg/13.7 ± 1.4 mg, för fyra rotationer 16.6 ± 1.0 mg/16.2 ± 0.8 mg, och för fem

rotationer 18.7 ± 1.0 mg/18.0 ± 1.5 mg. Variationen var dock relativt hög och ledde till att vissa satser inte uppfyllde farmakopékraven på enhetlighet. Detta berodde på filmrullningsmetoden, som inte var särskilt exakt. Vid analys av bestämda längders filmer uppnåddes nämligen utmärkt korrelation mellan filmlängd och gabapentindos. Överlag var det lätt att uppnå relevanta terapeutiska doser för katter och hundar med den utvecklade formuleringen. Läkemedelsfrisättningen från filmrullarna visade sig vara snabb; i genomsnitt 100 % läkemedelsfrisättning uppnåddes efter 30 minuter. Sönderfallstiden i vatten mättes med en tablettsönderfallsmaskin och så gott som alla filmrullar disintegrerade på mindre än 20 minuter. Sönderfallstiden *in vivo* är troligtvis betydligt kortare i och med att tungans rörelser påskyndar sönderfallet. Vid mätning av filmytornas pH-värde vid kontakt med vatten observerades att filmerna innehållande läkemedel hade ett neutralt pH-värde nära 7, vilket innebär att filmerna lämpar sig för sönderfall i munnen och troligtvis inte orsakar slemhinneirritation vid administrering.

7.5 Slutsatser

Sammanfattningsvis uppfylldes studiens målsättning om att finna en fungerande, enkel och ekonomisk kvantifieringsmetod för gabapentin i veterinärdosformer. Därutöver påvisades att en djurvänlig, munsönderfallande gabapentinfilm går att producera enkelt och relativt snabbt med goda resultat. Det finns flera frågor för vidare studier: bland annat vore det idealt att utveckla en mer automatiserad produktionsmetod – munsönderfallande filmer kunde till exempel 3D-printas. Flera kvantifieringsmetoder kunde prövas, eftersom endast en del valdes ut för denna studie. Mer omfattande kvalitetsanalyser bör även utföras, såsom stabilitetsstudier på såväl polymerlösningarna som de färdiga filmerna. Dessutom bör det utvärderas hur väl djur accepterar smaken av filmerna.

Resultaten från denna studie är ett steg på vägen mot förbättrad, säkrare och personaliserad gabapentinbehandling av djur. De relativt enkla och ekonomiska framställnings- och analysmetoderna går att implementera i praktiken på apotek, djursjukhus och veterinärkliniker och skulle kunna minska på behovet av ex tempore-framställning och delning av tabletter och kapslar.

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