Journal of Drug Delivery Science and Technology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Research paper

Investigation of hydroxypropyl-β-cyclodextrin inclusion complexation of two poorly soluble model drugs and their taste-sensation - Effect of electrolytes, freeze-drying and incorporation into oral film formulations

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ARTICLE INFO

Keywords:
Solubilization
Indomethacin
Furosemide
Oromucosal preparations
Electronic tongue

ABSTRACT

The goal for any formulation design of poorly soluble drugs is to increase the solubility. However, increased solubility is a challenge when the drug is administered to the oral cavity as rapidly dispersing or mucoadhesive buccal films. Most drugs are bitter and increased solubility may correlate with perceived worsening of the taste profile. The aim of the present work was to investigate the dual effect of inclusion complex formation, namely solubilization of two lipophilic model drugs (indomethacin and furosemide) in the hydrophobic cavity of hydroxypropyl-β-cyclodextrin with the aim of increasing the solubility in different electrolyte solutions, and at the same time hinder the taste sensation of the solubilized drug. Taste perception investigations were performed using an electronic tongue on simple solutions, inclusion complexes and on multi-component formulations such as orodispersible films and buccal films. The electrolyte media was found to have an effect on solubilization, association constant and complexation efficiency of both model drugs. Buffers containing phosphate ions were generally better than other electrolyte media with respect to the solubility parameters, and freeze-drying had a favorable effect on all the desirable properties. This work demonstrated that freeze-dried drug-hydropxypropyl-β-cyclodextrin complexes in solution, or added to orodispersible or buccal film, exhibit different taste sensation as compared to the plain drug in solution or reference films without complexes, indicating a successful taste-masking.

1. Introduction

Poor organoleptic properties are a great concern for oral drug delivery, especially when the dosage form is intended to dissolve rapidly in the mouth cavity as an orally disintegrating film (ODF) [1,2]. Although dissolution properties and solubilization of poorly soluble drugs are important parameters in terms of sufficient bioavailability, the dissolved fraction of the drug will have the opportunity to interact with taste buds and produce a taste sensation. Many drug substances have an unpleasant taste, and compliance can be hampered by the unpleasant taste of a drug product [3].

The most accepted method to evaluate taste is by human taste panels, but they are expensive and subjected to ethical considerations. Therefore, *in vitro* analytical methods, such as electronic tongue assays have become increasingly popular [4,5]. Electronic tongues are sensor array systems capable to detect single substances or complex mixtures by means of particular sensor membranes and electrochemical techniques. From an analytical point of view, these systems are based on a different composition of sensors with variable properties and characteristics of partial or cross-selectivity, which can detect a range of substances of different tastes and intensities [4]. It is important to remember that the sensor values (mV) should not be interpreted as measures of real taste intensity, even though in the case of the Insent e-tongue the various sensors may be associated to particular taste qualities, such as bitter and salty [6,7].

To counteract the bitter taste of a drug substance, taste-masking technologies are often employed [3]. Taste-masking efficiency is related to a reduction or inhibition of the interaction between drug and the oral taste receptors of the taste buds [8,9]. Hindered contact between the drug in aqueous solution and the taste receptors in the oral cavity is of key importance for taste-masking. One such way to prevent the interaction between the drug substance and taste-receptors is

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https://doi.org/10.1016/j.jddst.2020.102245
Received 19 August 2020; Received in revised form 16 November 2020; Accepted 21 November 2020
Available online 26 November 2020

molecular complexation of the drug by cyclodextrins [10]. Cyclodextrins are also known to form water-soluble complexes with lipophilic drugs by inclusion in the hydrophobic cavity [11]. Hence, a dual effect of the inclusion complex formation might be hypothesized, which would be attractive for oromucosal preparations. It will increase the solubility of the poorly soluble drug and produce a strong complexation to assure sufficient taste-masking. Oral film formulations are prominent examples of dosage forms that would benefit from such approach.

Since the loading capacity of films are limited, the complexation efficiency and the drug to cyclodextrin molar ratio, needed to achieve the maximum complexation, are of high importance. In the current study, two poorly water-soluble model drugs, indomethacin (IMC) and furosemide (FM), BCS class II and IV, respectively, were subjected to solubility studies and taste assessment studies with and without complexation with hydroxypropyl-β-cyclodextrin (HPβCD). Both drugs have been described in literature to have an unpleasant taste [12]; Kawano et al., 2010). The objective of the present study was to investigate the dual effect of inclusion complex formation, namely solubilization of the lipophilic drug in the hydrophobic cavity of the HPβCD, thereby increasing the solubility in different electrolyte solutions, and at the same time preventing the taste sensation of the solubilized drug by formation of the inclusion complex. Taste perception investigations were performed using electronic tongue on simple solutions, inclusion complexes and on multi-component formulations. Two types of oral film formulations were evaluated, rapidly disintegrating ODFs and recently developed mucoadhesive buccal films [13].

2. Material and methods

2.1. Materials

All salts for buffer preparations were from Sigma Aldrich (St. Louis, MO, USA). HPβCD (Cavasol® W7 HP Pharma) was purchased from Wacker Chemie (Munich, Germany), IMC was from Sigma-Aldrich (St. Louis, MO, USA) and FM was from Fagron (Copenhagen, Denmark). Water was purified with a Milli-Q integrated water purification system for ultrapure water (Merck Millipore, Darmstadt, Germany), and is referred to as Milli-Q water, or in some cases, in-lab distilled water obtained by reverse osmosis was used, and is referred to as demineralized water. Lycoat® RS720 was kindly gifted from Roquette Pharma (Lestrem, France) and glycerol was purchased from Apoteksproduksjon AS (Oslo, Norway). Quinine hydrochloride was purchased from Caesar & Loretz (Hilden, Germany). Potassium chloride (KCl) was acquired from Grüssing (Filsum, Germany). Tartaric acid was purchased from Sigma–Aldrich Laborchemikalien (Schnelldorf, Germany). The saturated silver chloride (AgCl) inner solution for sensors and reference electrodes in the electronic tongue, consisting of 3.33 M KCl in saturated AgCl solution, was provided by Insent (Intelligent Sensor Technology, Kanagawa, Japan). All chemicals used were of analytical grade.

2.2. Preparation of buffer solutions

Eight different aqueous solutions with varying grades of electrolytes and buffer capacity were used throughout the studies. 0.15 M phosphate buffered saline (PBS) with pH 7.4 was prepared from tablets acquired from Sigma-Aldrich (St. Louis, MO, USA) and Milli-Q water. 0.1 M phosphate buffer (pH 7.4) and 0.1 M sodium citrate buffer (pH 7.4) were prepared according to Ph.Eur. (4.1.3. Buffer Solutions). 0.01 M phosphate buffer (pH 7.4) was prepared by diluting from the 0.1 M phosphate buffer. 0.15 M (isotonic) NaCl, 0.1 M NaCl and 0.1 M NaBr were prepared by dissolving suitable amount of the salts in Milli-Q water. Saliva substitute was prepared according to the Documenta Geigy Scientific Tables [14] of natural saliva contents, and was prepared as a solution of 0.21 g/L of $NaHCO_3$, 0.43 g/L NaCl, 0.75 g/L KCl, 0.22 g/L $CaCl_2{\cdot}2H_2O$, 0.91 g/L $NaH_2PO_4{\cdot}H_2O$.

2.3. Osmolality and pH

Osmolality was determined through measurement of freezing point depression using a Semi-micro Osmometer K-7400 from Knauer (Berlin, Germany). pH meter (pH 562 MultiCal®, WTW, Weilheim, Germany), was used to measure the pH value of the samples at room temperature. All samples were measured in triplicate.

2.4. Solubility studies and interaction with HPβCD

Phase solubility studies were conducted according to the shake-flask method by Higuchi and Connors [15]. Briefly, solutions of various concentrations of HPβCD dissolved in the respective solvents were added to glass vials with an excess of either FM or IMC present. 0–75 mM HPβCD was used for FM phase solubility, and 0–10 mM HPβCD was used for IMC phase solubility. The flasks were then shaken at 220 rpm in 25 °C for 72 h (Environmental Shaker-Incubator ES-20, BioSan, Latvia), until equilibrium occurred and saturated inclusion-complexes were obtained. The solutions were then filtered with 0.45 μm syringe filter (25 mm, polyethersulfone membrane, VWR Europe, Darmstadt, Germany) and diluted with ethanol (EtOH) to a suitable concentration. Filtrates were quantified on UV-VIS (Spectro UV-2550 spectrophotometer, Thomas Scientific, Swedesboro, NJ, USA) at wavelength 276 and 254 nm for FM and IMC, respectively. All concentrations were prepared and tested in triplicate.

The association between drug and CD is explained by the following equation

$$mD + nCD \overset{K}{\rightleftarrows} D_m : CD_n \tag{1}$$

when m drug molecules (D) associate with n cyclodextrin molecules (CD) a drug:cyclodextrin complex with the association coefficient (K) is formed. The results from the phase solubility experiments were plotted with concentration of drug in sample against the known CD concentrations and the resulting regression curve was used to calculate the association constant. The slope is the linear part of the plotted curve and S_0 is the intrinsic solubility of the respective drug, which is often extrapolated as the intercept from the linear equation in case of very poorly soluble drugs [16]. In cases where one drug molecule associates with one CD molecule, the association constant ($K_{1:1}$) is described by the following equation

$$K_{1:1} = \frac{[D:CD]}{[D]\ x\ [CD]} = \frac{Slope}{S_0(1 - Slope)} \tag{2}$$

The complexation efficiency (CE) of the different systems was calculated from the slope of the phase solubility profiles

$$CE = \frac{[D:CD]}{[CD]} = S_0\ x\ K_{1:1} = \frac{Slope}{(1 - Slope)} \tag{3}$$

The drug-cyclodextrin molar ratio was calculated from the CE

$$D:CD\ molar\ ratio = 1 : \frac{(CE + 1)}{CE} \tag{4}$$

Phase solubility studies were conducted for IMC:HPβCD in Milli-Q water, saliva substitute, phosphate buffer (0.01 M, 0.1 M), PBS (0.15 M), citrate buffer (0.1 M), NaCl (0.1 M and 0.15 M) and NaBr (0.1 M). For FM:HPβCD the solubility studies were limited to Milli-Q water, saliva substitute, phosphate buffer (0.1 M) and PBS (0.15 M). The following descriptors were collected for all drug:HPβCD systems: concentration of the electrolyte solution (M), osmolality of the electrolyte solution (mOsmol/kg water), pH of solutions at the end of the phase solubility study for all HPβCD concentrations (reflecting the pH change from 0 to max. concentration HPβCD), regression coefficient of the plotted isotherm, S_0, drug concentration at max. concentration HPβCD, $K_{1:1}$, CE and D:CD molar ratio.

To evaluate the impact of freeze-drying on CE and $K_{1:1}$, a phase

solubility study was also conducted as follows; the phase solubility was performed in the same manner in PBS as described above, until after 72 h shaking, when instead of dilution and analyzing, an additional freeze-drying step of the samples was added. Known amounts of the respective stirred solutions (3 mL) were freeze-dried, protected from light in a freeze-dryer (Christ Alpha 2–4 LSC plus, Osterode, Germany), for a minimum of 24 h at 72 °C and a vacuum of 0.019 mbar, and then followed by a final drying for up to 4 h at 76 °C and a vacuum at 0.010 mbar, and stored in airtight containers. For the analyzes, freeze-dried samples were rehydrated with PBS in the same volume (3 mL), before filtering and diluting with EtOH and finally quantifying on UV-VIS as described earlier.

2.5. Preparation of freeze-dried HPβCD complexes in specific molar ratios for further studies

Freeze-dried complexes were prepared for FM in a 1:1 M ratio with HPβCD and for IMC in a 1:1, 1:2 and 1:3 M ratio, also with HPβCD. Briefly explained, calculated molar ratios of drug and HPβCD were added to Milli-Q water or PBS buffer and complexes allowed to form while stirring for 24 h. The solutions where then filtered (0.45 μm) and aliquots frozen with liquid nitrogen before freeze-drying as described previously. The resulting lyophilized powders were stored in airtight containers protected from light until use. Freeze-dried drug-cyclodextrin complexes were used for film preparation as well as in direct assessments of taste-masking properties.

2.6. Electronic taste sensation assay

A commercially available system for assessing taste sensation was used; TS-5000Z (Insent, Atsugi-Chi, Japan), that was equipped with 5 lipid membrane sensors corresponding to human taste attributes, astringency (SB2AE1), saltiness (SB2CT0) and three different bitterness sensors (SB2AC0, SB2AN0, SB2CO0). Experiments were performed according to Woertz et al. who published a complete method description for qualification of the electronic taste system [7,17]. 100 mL liquid was needed per sample and all samples were run five times with the first two runs considered as preconditioning the membranes and the results discarded. The results were displayed as a change of the membrane potential in mV calculated in relation to the respective reference solution correlating to the specific sensor [7]. Quinine hydrochloride (QiHCl) in demineralized water (0.5 mM) was used as external standard as this concentration is in the linear range of all sensors [7]. Two different washing solutions were prepared, for negatively and positively charged sensors respectively. 100 mM hydrochloric acid (HCl) for the negatively charged sensors and 100 mM KCl and 10 mM potassium hydroxide (KOH) for the positively charged sensors with EtOH used as co-solvent. 30 mM KCl and 0.3 mM tartaric acid in demineralized water were used as conditioning and reference solutions.

Drug solutions of both IMC and FM were prepared in demineralized water at logarithmic concentration for calibration curve, in order to establish the relationship and possible linearity between drug and sensor responses. EtOH was used as co-solvent as EtOH does not interfere with the sensor response signal. The concentration ranges were 0.001 M – 0.1 M for IMC and 0.001 M–1 M for FM.

Sensor output for taste, called relative value (R) was calculated in relation to the sensor response that was determined from the reference solution as according to the following equation:

$$R = V_s - V_r \quad (4a)$$

where R is the final sensor output of the sample, V_s is the sample solution and V_r is the output from the reference solution.

Based on the last three results of each sample, the mean and standard deviations were calculated. Sensors were dipped into the sample beakers and samples were then measured for a period of 120 s, before a washing cycle was performed, and the next sample was measured. The sample measurement was randomized, and a sensor check was performed before each sample set was run.

2.7. Oral films

2.7.1. Film compositions

ODF formulations based on Lycoat®, with IMC, glycerol and HPβCD were prepared with compositions outlined in Table 1. The amount of freeze-dried complex added per film was calculated so that drug content was the same in all film formulations (0.1% w/w); hence, the amount of HPβCD varied according to the molar ratio 1:1, 1:2 and 1:3. The theoretical drug content was estimated to be approximately 400 μg indomethacin per 2 × 2 cm dry single-unit dose. The Lycoat®-based formulations were named LC1-LC5.

Buccal film formulations and corresponding complexes based on Soluplus® were prepared according to earlier studies [13] and composition is presented in Table 1. Based on the reported studies, the drug content in these formulations were made so that the furosemide content per 2 × 2 cm dry single-unit dose was approximately 500 μg. The Soluplus®-based formulations were named SP1-SP4.

2.7.2. Preparation of films

Films were prepared using the solvent casting method. Lycoat®-based films were prepared as follows: polymer and glycerol were mixed together, and Milli-Q water added. Appropriate amounts of freeze-dried drug-HPβCD complexes were added, and the solution stirred until homogeneous in appearance, making sure no air bubbles or foam was formed. Films were then cast on a levelled glass plate of a film caster equipment (Coatmaster 510, Erichsen GmbH & Co. KG, Hemer, Germany) with cellophane (Panduro AS, Gressvik, Norway) as release liner. Soluplus®-based films were prepared by adding appropriate amounts of Soluplus in Milli-Q water and allowing a homogeneous micellar solution to form. FM was solubilized directly in the micelles and last, glycerol was added, and the solution was cast as above. The casting height was set as standard height of 550 μm on all Lycoat®-films and 1000 μm for Soluplus®-based buccal films. Films were allowed to dry in ambient conditions for a minimum of 24 h before cutting into rectangular single-unit dose pieces, which were defined as 2 × 2 cm.

2.7.3. Film characterisations

Single-unit doses were characterized as follows: Film thickness was measured using a micrometer screw (Mikrometer Cocraft, Clas Ohlson, Sweden) with a resolution of 0.01 mm. The mass was measured using a Sartorius Research R160P balance (Richmond Scientific, England). Disintegration time was defined using a petri-dish method [13,18])

Table 1
Film formulations. All composition expressed as the wet formulation before drying.

ODF formulations with indomethacin as drug					
Component	LC1	LC2	LC3	LC4	LC5[a]
Lycoat® RS720 (% w/w)	17	17	17	17	17
IMC:HPβCD (molar ratio)[b]	1:1	1:1	1:3	1:3	1:2
Glycerol (% w/w)	2.0	6.0	2.0	6.0	4.0
Buccal film formulation with furosemide as drug					
Component	**SP1**	**SP2**	**SP3**	**SP4**	
Soluplus® (% w/w)	25	16	16	16	
FM:HPβCD (molar ratio)[b]	1:0	1:0	1:1	1:1	
HPMC (% w/w)	–	0.5	0.5	0.5	
Glycerol (% w/w)	3.5	3.5	3.5	3.5	
Preparation method of complexes	–	–	Freeze dried	Physical mixture	

[a] n = 3.
[b] Drug content was the same in all wet film formulations (0.1% w/w) but amount of cyclodextrin varied according to the given molar ratio.

where a single-unit dose was covered with PBS in a petri-dish under constant shaking (3 mL and 200 rpm, respectively) and the time in seconds for complete film disintegration was recorded.

2.8. Statistical analysis

2.8.1. Software and methods

The values are represented as mean ± standard deviations. All standard statistical analysis was performed using the software program GraphPad Prism 8® (Graphpad Software San Diego, CA, USA) with the statistical significance set to $p \leq 0.05$. The effects were evaluated univariate and multivariate. The Unscrambler® software version 9.8 (Camo ASA, Trondheim, Norway) was used for multivariate analysis. Principal component analysis (PCA) was applied to identify trends and extract latent variables.

2.8.2. Multivariate investigation of electrolyte solutions of drug:HPβCD complexes

PCA was performed on the phase solubility descriptors from IMC:HPβCD and FM:HPβCD in different electrolyte media. In order for the inclusion complexation to fulfil the dual action (solubilizing drug and hindrance of interaction with the taste buds), the most desired descriptors were defined as $K_{1:1}$ and CE, which should both be at the highest possible level. Therefore, positive correlations to these descriptors identified the electrolyte media that had the most desirable properties. These were identified by using a bi-plot displaying the superimposed scores (different electrolytes) and loadings (solubility descriptors) on the first two principal components (PC1 and PC2). In case of covariate variables, only one of the variables were included in the PCA. Prior to analysis, all variables were centred and normalized through division by their standard deviation (1/SD).

2.8.3. Univariate evaluation of electronic tongue sensor responses

The electronic tongue sensor responses were first evaluated in a univariate manner to establish the concentration dependency of sensor responses, and further on to investigate if there was a change in sensor signals when HPβCD was added to the drug, complexed or as a physical mixture and in different molar ratios. The two bitter sensors SB2AC0 and SB2AN0, where used as bitterness reduction was of main interest.

2.8.4. Multivariate evaluation of results from the electronic tongue

PCA was also used for evaluation of the sensor output for the experiments with the electronic tongue. Each PCA was based on the output from all tested sensors (SB2AE1, SB2CT0, SB2AC0, SB2AN0 and SB2CO0) and the samples. Three PCAs were investigated: I) drug:HPβCD complexes in different electrolyte solutions, and drug:HPβCD complexes in film formulations based on II) Lycoat or III) Soluplus®. For evaluation of results from the electronic tongue, the PCA score plot is typically used to map the samples along the first two principal components, and look at the location relative to each other [6,7]. The closer the formulation is located to the reference film and the larger the distances to the pure drug (unpleasant taste), the better taste masking may be obtained.

3. Results and discussions

3.1. Solubility studies

Formation of inclusion complexes with cyclodextrins can increase the solubility of poorly soluble drugs, and the complexation can potentially have a favorable effect on taste masking of bitter taste sensation [3,19,20]. Normally, increasing solubility correlates to increased free drug available in the oral cavity free to interact with taste receptors, but by forming an inclusion complex with cyclodextrin, the bitter drug entrapped in the cyclodextrin cavity cannot act at the taste receptors in the taste buds of the oral cavity [20].

IMC and FM are both lipophilic drugs (log*P* 3.10 and 2.29, respectively) and poorly water-soluble weak acids with pKa values of 4.5 for IMC and 3.8 for FM [21,22]. The pKa values suggests pH-dependent solubility.

Table 2 shows an overview of results from a series of phase solubility studies of IMC with HPβCD in various test media. The results for FM can be found in Table SI in the Supplementary information. The linear slope of the phase solubility profiles for both drugs, IMC and FM, in all electrolyte media, indicated that all the drug cyclodextrin interactions were of the A_L type and form 1:1 drug-cyclodextrin complexes [11]. In a dilute system this is expected to be the dominating structure, but cyclodextrins are known to not exclusively form 1:1 inclusion complexes, but also non-inclusion complexes [23]. Cyclodextrin and drug interactions, namely inclusion complex formation, are described by the association constant (e.g. $K_{1:1}$), which is affected by pH, temperature, presence of electrolytes and properties of the guest molecule, e.g. drug substance, and many other factors [16]. The solubilization is usually correlated to cyclodextrin concentration, with rising concentration resulting in the formation of drug-cyclodextrin aggregates. Aggregate formation are a complex matter when it comes to assessing taste-masking and solubility, since they technically contribute to increased drug solubilization, but on the other hand, aggregate formation has been shown to correlate negatively to taste-masking, since not all of the drug is in an inclusion complex [3]. The taste-masking capacity has also been shown to correlate with the association constant ($K_{1:1}$), indicating complexation is necessary for taste-masking efficiency [10,24]. If non-inclusion complexes are a predominant species in a system, this can hinder the effect of taste masking, hence, complexation efficiency is an equally important parameter to define [25]. The phase solubility theory of Higuchi and Connors does not differentiate between different kinds of complexes (inclusion or non-inclusion), but merely measures solubility in aqueous media [15]. This may cause problems when interpreting the association constant values ($K_{1:1}$), as it relies heavily on the relationship between S_0 and the slope. This is especially a problem when working with very poorly soluble compounds, as S_0 is often extrapolated from the intercept and probably does not reflect the true value, thus making $K_{1:1}$ unreliable. A more accurate way of describing the drug-cyclodextrin relationship is to calculate the complexation efficiency (CE), which does not rely on an accurate measurement of S_0 [16,25]. CE is calculated from the slope of the phase-solubility diagrams (Eq. (3)) and is thus more reliable when comparing various solvent media. The drug-cyclodextrin molar ratio is calculated from the CE and is an important parameter when it comes to formulation technology, as this ratio indicates how much cyclodextrin needs to be added to ensure that all of the drug is dissolved and will therefore serve as a useful tool in calculating formulation bulk [26].

One main finding is that HPβCD is not as efficient in solubilizing FM as IMC (Table 2 versus Table SI, Supplementary information). This can be seen from the low association constant ($K_{1:1}$) obtained for FM compared to IMC, indicating a low affinity for FM towards the cyclodextrin cavity, also the CE for FM did not research the same high levels as for IMC with the same electrolyte solution. Another main finding is that CE and $K_{1:1}$ for both drugs were enhanced by freeze-drying of the complexes in solution as compared to conventional shake flask studies at room temperature over 72 h.

Increased solubility of drug in the medium (S_0) correlated with increased solubility of drug at max CD concentration ($R^2 > 0.98$; each drug separately). Increased solubility of the drug showed a general tendency to increase CE, but not in a direct linear relationship. Since molar ratio was derived from CE, these descriptors are not independent. Neither osmotic strength (osmolality) or pH of the solution seemed to correlate directly to increased CE and thus, reduce CD in the estimated molar ratio. Looking closer at pH, saliva substitute, phosphate buffer, PBS and citrate buffer are all solutions with pH in the range of 6.9–7.3, well above pKa of IMC at 4.5, but they showed CE ranging from 0.161 up to 1.453 for IMC:HPβCD. The composition of the electrolytes might be more important; Looking at the phosphate buffer at different molarities,

Table 2
Solubility studies of indomethacin (IMC) with hydroxypropyl-β-cyclodextrin (HPβCD) in various electrolytes (n = 3, mean ± SD). Gray area emphasizes differences in complexes prepared by two different methods employing otherwise identical conditions.

Drug	Compl. method[a]	Electrolytes			HPβCD	Drug: HPβCD inclusion complex (D:CD)						
		Type of solutions	conc. M	Osmolality mOsmol/kg water	conc. range mM	Δ pH low - high [CD].	R^2	S_0 μg/ml	[D] at max [CD] (approx. μg/ml)	$K_{1:1}$ (M^{-1})	CE	Molar ratio D:CD
IMC	PS	Saliva substitute	0.01	49 ± 4	0–10	6.82–6.89	0.946	272	780	181	0.161	1:7
IMC	PS	phosphate buffer	0.01	25 ± 1	0–10	7.21–6.90	0.959	452	1054	128	0.175	1:7
IMC	PS	phosphate buffer	0.10	239 ± 3	0–10	7.30–7.23	0.994	1381	3100	363	1.453	1:2
IMC	PS	isotonic PBS (pH 7.4)	0.15	283 ± 3	0–10	6.90–6.56	0.996	789	1500	132	0.281	1:5
IMC	PS-FD	isotonic PBS (pH 7.4)	0.15	283 ± 3	0–10	6.90–6.56	0.995	921	2232	207	0.559	1:3
IMC	PS	citrate buffer	0.10	219 ± 6	0–10	6.95–6.50	0.974	260	1100	356	0.356	1:4
IMC	PS	NaCl	0.15	268 ± 3	0–10	5.28–4.27	0.965	10[b]	50	414	0.011	1:92
IMC	PS	NaCl	0.10	185 ± 2	0–10	5.10–4.18	0.990	1[b]	27	722	0.007	1:151
IMC	PS	NaBr	0.10	192 ± 1	0–10	5.45–4.62	0.999	2[b]	23	321	0.005	1:204
IMC	PS	Milli-Q water	–	–	0–10	5.41–4.75	0.991	11[b]	43	255	0.009	1:116

[a] PS: phase solubility, PS-FD: phase solubility followed by freeze-drying.
[b] Extrapolated from intercept (isotherm).

increased osmotic strength seemed to have a positive correlation with increased CE. However, evaluating the results in a multivariate manner is better suited to identify the desired properties of the electrolytes that will provide high CE, but at the same time a high association constant $K_{1:1}$.

In order to fulfill the dual role of the drug-cyclodextrin inclusion complex in an oromucosal formulation, i.e. stable complexes (high $K_{1:1}$) and high complexation efficiency (high CE, i.e. low CD in D:CD molar ratio), the different electrolyte solutions can be used to tailor the system. To understand the large data sets and find correlations, a principal component analysis (PCA) was performed separately for each drug. Fig. 1 show the bi-plot of the IMC (for a similar plot for FM, see Fig. SI, Supplementary information).

The first two principal components explained over 95% of the variation. To reduce the risk of over-fitting the model, for descriptors that were identified as covariate only one of the descriptors were included. (e.g. S_0 and concentration of drug at max cyclodextrin concentration, and CE and molar ratio). The bi-plot of the PCA of the selected descriptors from the phase solubility data (S_0, pH at max CD concentration, $K_{1:1}$ and CE) from IMC:HPβCD systems show that the samples (type of electrolyte solution) were ranked from high to low correlation with each of the descriptors in the coordinate system of PC1 and PC2 (Fig. 1); gray arrows in the plot indicate the span from highest and lowest correlation with $K_{1:1}$ and CE. High $K_{1:1}$ indicates strong associations between drug and cyclodextrin, thus efficiently hindering drug molecules from reaching the taste receptors (i.e. taste-masking effect) and a high CE, meaning efficient solubilization of drug into cyclodextrin. Drug ionization has a key role in the values of $K_{1:1}$ and CE. The lipophilic unionized form of the drug is likely to have greater affinity for the hydrophobic cyclodextrin cavity and results in a higher $K_{1:1}$ value [27]. NaCl seems to have a favorable effect on increasing $K_{1:1,}$ but the solubilization and CE remain very poor and higher osmotic strength of the NaCl solution does not have a favorable effect. Phosphate buffer 0.1 M has the best overall qualities with the highest CE and a $K_{1:1}$ in the mid-upper range. The isotonic PBS 0.15 M showed an inverse correlation to $K_{1:1}$ and mid-level CE, but by freeze drying the IMC: HPβCD both of these values can be improved and thus the sample PBS-FD can be seen migrating to increasing correlations with both CE and $K_{1:1}$ in the PCA (Fig. 1). Solubilization is an effect of pH and buffers as the solvent media. Weak acidic drugs and are in their ionized form in physiological pH meaning the aqueous solubility is at its highest here also. More free drug in solute form will also increase the rate at which free drug molecules can form complexes with the cyclodextrin, provided the affinity is strong enough for the complexation to happen.

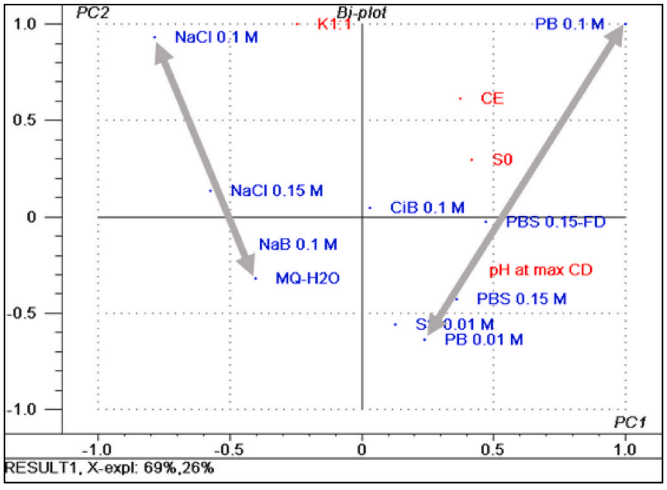

Fig. 1. Bi-plot, where the scores and loadings are superimposed, of a principal component analysis of phase solubility descriptors from the investigations on indomethacin and hydroxypropyl-β-cyclodextrin in various media (PB: phosphate buffer, PBS: phosphate buffered saline, FD: freeze-dried complexes, CiB: citrate buffer, SS: saliva substitute, MQ-H2O: Milli-Q water, and the numbers specify molarity, K1:1: association constant, CE: complexation efficiency, S0: solubility of indomethacin or furosemide, pH at max cyclodextrin concentration in respective media). For a similar plot for furosemide, see Fig. SI in Supplementary information.

There is a major difference in the affinity towards HPβCD exhibited by IMC and FM, respectively (Table 2), as illustrated by the association constant ($K_{1:1}$). Less investigations were performed with FM (results in Supplementary information) since the solubilizing effects of association with HPβCD was found to be less favorable than for IMC. IMC has a higher affinity for the lipophilic HPβCD cavity resulting in overall higher $K_{1:1}$ values whereas FM has very low affinity, and even though the solubility of FM is relatively high in the buffers with free drug molecules available, the $K_{1:1}$ values are very low and so is the CE. Comparing to IMC, some degree of explanation might be given by the fact that IMC is more lipophilic, log*P* 3.10 versus 2.30 for FM, and is thus more likely to favor the lipophilic HPβCD cavity in a hydrophilic environment. Nevertheless, in all tested media, A_L type phase solubility profiles were

found, indicating a 1:1 complex formation and linear relationship between HPβCD concentration and drug solubilization.

The role of buffered aqueous solutions is noteworthy, as the same solubilities could not be obtained by simply adding electrolytes at the same osmotic pressure. It was also shown in IMC solutions that if a non-buffered solvent media was used, the solubilized drug would eventually lower the pH of the solvent and thus hinder further solubilization, resulting in an type curve instead of the usually obtained linear curves (data not shown). This phenomena has also been described in literature [28]. The atypical decrease in IMC concentration with an increase in cyclodextrin concentration is due to the effect of the dissociation constant in decreasing pH. By using a buffered solvent media, this problem can be avoided and linear solubilization achieved as well as a favorable effect on the CE. For FM, especially the buffered electrolyte solutions exhibited poor complexation, which might be due to that the conditions for FM solubilization were more favorable outside the HPβCD cavity than inside the cavity.

On the other hand, ionization increases the intrinsic solubility and if the increase in S_0 is greater than the decrease of $K_{1:1}$, CE will improve. Salts in the solute media can also have a profound effect on the CE values. Sometimes the salts can interact directly with the complex and a drug-cyclodextrin-salt complex is formed [26], but likewise salts can have an effect through the solubility enhancement pathway (increase in S_0) or in case of buffers, by modifying the pH and thus controlling the degree of ionization of the drug (descriptor "pH at max CD" in the PCA, Fig. 1). There were some notable differences in the increase in CE by addition of electrolytes for both FM and IMC. Phosphate buffer 0.1 M and PBS showed the highest increase, and even though PBS was higher in osmotic strength (0.15 M) phosphate buffer was still more efficient. This might be because PBS is made saline with large amounts of NaCl, whereas phosphate buffer contains much more phosphate ions. The phosphate anion is a very kosmotropic agent and this might partly explain why it is so efficient in increasing complexation [29]. The mechanism has many other aspects, but it has been shown that strongly hydrated solutes stabilize complexes. Kosmotropic ions in the interfacial water layer results in minimizing the solvent accessible surface area [30]. This is an interesting paradox of systems with seemingly unfavorable environment for solubilization resulting in a favorable complex formation and thus increasing drug concentration in solute form, which also increases the CE. The effect of NaCl was studied by preparing pure NaCl solutions of different concentrations (0.1 and 0.15 M), where the $K_{1:1}$ increased and CE remained almost the same as water. NaBr (0.1 M) was investigated as an example of chaotropic salt, i.e. ions that disrupt the hydrogen bonds and increase the solubility of poorly soluble macromolecules [29]. Br^- resulted in poorer $K_{1:1}$ and CE as compared to the same concentration of NaCl.

Although FM does not show favorable complexation in buffers where solubility is high, a number of methods can be employed to increase the complexation, for example freeze drying [31]. Freeze drying is a known method to increase the solubility of poorly water-soluble compounds. Freeze drying can have a positive effect on solubility through increasing the surface area of particles, and also, has a favorable effect on complexation and stabilizing the complexes [32–34]. For both FM and IMC, freeze drying of the complexes resulted in increased values of CE and $K_{1:1,}$ indicating that the complexation became more stable but also less cyclodextrin is needed to solubilize the drug as CE and molar ratio are directly correlated.

In order to achieve the most desired properties of high $K_{1:1}$ and CE at the same time, IMC:HPβCD should be prepared in 0.1 M PB whereas the FM:HPβCD should be prepared with PBS and freeze drying. From an in use perspective, the results obtained for the simulated saliva should be taken into account. For both IMC:HPβCD and FM:HPβCD complexes, the results in simulated saliva is negatively correlated to the desired properties. This might be interpreted as a reduced taste masking efficiency and solubilizing efficiency if the complexes remain in the saliva in the oral cavity over a prolonged period of time.

3.2. Evaluation of the in vitro taste perception

3.2.1. Taste-assessment of drug: HPβCD complexes

Masking of an unpleasant taste perception of a drug by the use of cyclodextrins is based on the hindrance of the drug molecule to get in touch with the taste receptors [20]. Earlier studies have showed that cyclodextrins are effective as taste maskers through complex formation [10], and that an electronic tongue combined with multivariate evaluation of the results is a good method to evaluate the effect [6,17]. However, it is known that not all drugs are easily investigated using the electronic tongue. In some cases, there is no direct response to different drug concentrations or the response is not linear [6,35].

In the current study, taste-masking capacity of freeze-dried complexes of HPβCD and either IMC or FM was evaluated using the electronic tongue. The evaluation was mainly based on the two bitter sensors (SB2AC0 and SB2AN0). Both IMC and FM could be detected using the e-tongue. The taste response to logarithmic concentrations of IMC and FM was near-linear showing a concentration dependent response from 0.001 to 0.1 or 1 M, respectively, on both sensors (Figure SII, Supplementary information). Higher concentrations of both IMC and FM resulted in values closer to the standard, indicating an increased bitterness detected by the e-tongue. Both sensors were able to detect changes in concentrations and could thus be used further in assessing taste masking of the bitterness in the pure compounds and complexes.

Fig. 2 demonstrates that all samples with drug in complexed form have a significantly more negative response than the corresponding pure drug in solution without HPβCD ($p < 0.05$), indicating a taste-masking effect or reduction of the bitter taste. The response was not influenced by varying drug to cyclodextrin ratios, indicating that the effect of bitterness reduction, or taste-masking capacity is not increased by excess HPβCD once an optimal complexation has been achieved.

3.2.2. Taste assessment of inclusion complexes in various electrolytes

The score plot from the principal component analysis (PCA) of the responses from all included sensors (SB2AE1, SB2CT0, SB2AC0, SB2AN0 and SB2CO0) maps out behavior of the two drugs with and without HPβCD in selected electrolytes (See Fig. 3). The first principal component (PC1) explains 88% and PC2 explains 10%, thus, the horizontal distance between the samples are the most important.

The multivariate analysis of the sensory output data on different electrolytes with drugs dissolved in water, PBS and phosphate buffer and either with or without HPβCD complexation to the two drugs show that the electronic tongue can distinguish between the different samples. The clustering of the samples is according to solvent; however, the PCA indicate that in this sample set, solvent type is the main factor governing clustering of samples, i.e. taste-differences. Complexation with HPβCD or even which drug was employed was not significant.

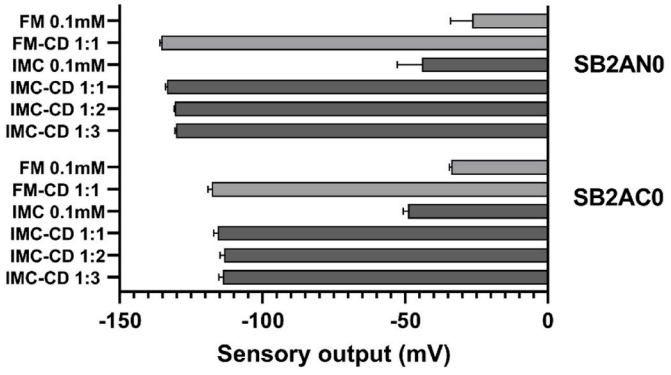

Fig. 2. Univariate data evaluation of two bitter sensor responses (SB2AN0, SB2AC0) of FM (light gray) and IMC (dark gray) pure drug dissolved in Milli-Q water compared to same amount of drug but complexed with hydroxypropyl-β-cyclodextrin (freeze-dried complex). Values represent mean ± SD.

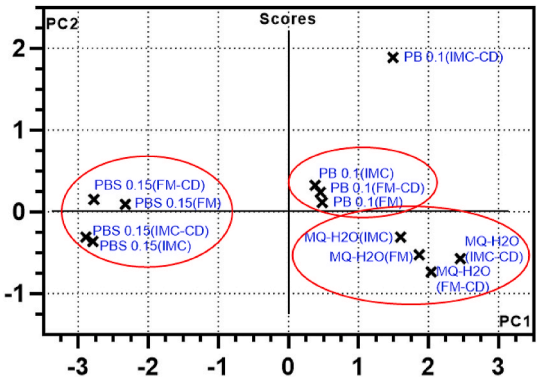

Fig. 3. Score plot mapping the results from PCA of the output from the electronic tongue on five sensors (SB2AE1, SB2CT0, SB2AC0, SB2AN0 and SB2CO0) for two drugs in various solutions without and with HPβCD complexes (IMC = indomethacin, FM = furosemide, CD indicates that drug is complexed with hydroxypropyl-β-cyclodextrin, solvent media: PB = phosphate buffer, PBS = phosphate buffered saline, MQ-H20 = Milli Q-water, the numbers specify molarity of the electrolyte solution).

3.2.3. *Taste assessments of oral film formulations*

The Lycoat®-based ODFs with IMC and HPβCD in three different molar ratios were found to disintegrate at approximately 35 s (34.7 ± 3.2 s) mostly dependent on their dry film thickness. Glycerol content or molar ratio D:CD were not found to be factors influencing the disintegration time. The taste perception of these formulations were evaluated with the e-tongue and compared to free drug and two different reference films (either no drug or no cyclodextrin included) (Fig. 4A). In total, 97% of the variation was explained on two PCs, where PC1 contained 70%. The free drug ("IMC-free" in Fig. 4A) was located in the lower right quadrant with information explained on both PC1 and PC2. The reference samples, film samples one with no IMC and one with no CD, were found close together in the upper right quadrant, indicating different taste than the free IMC. The value on PC 2 was rather low. The various film formulations containing inclusion complexes in different molar ratios were located close to the center, on the opposite side of the free drug mostly explained by PC1. This suggests different taste sensation for the film formulations compared to the free drug solutions and to the films without the combination of both drug and CD. The scattering of the data points of the ODF formulations in Fig. 4A is not straightforward to interpret. The amount of drug was kept constant in the different formulations, so the molar ratio expresses an excess of HPβCD to IMC. It seems that the 1:1 complex provide the taste sensation that is most different from that of the free drug along PC1. Based on this, one may not conclude that increasing the cyclodextrin to drug ratio has any positive effect when it comes to taste sensation. This correlates well to the univariate taste-assessment data from the cyclodextrin complexes (Fig SII, Supplementary information), where the taste-masking efficiency was not significantly improved by increased drug to cyclodextrin ratios. Overall, it may be suggested that the latent variable explaining PC1 would be attributed to the taste assessment of the film former Lycoat®, whereas the latent variable explaining PC2 would be taste assessment of IMC.

B) Principal Component Analysis on taste-assessment of Soluplus films. SP1 and SP2 are reference films containing furosemide but with no cyclodextrin, SP3-FD contains freeze-dried FM:HPβCD inclusion complexes and SP4-PM is equivalent to SP3-FD except the FM:HPβCD is added as a physical mixture, FM-free is furosemide in equivalent amount to film content dissolved in water.

Buccal films are formulations that will remain in the oral cavity for a prolonged period, providing more time for dissolving the drug and reaching the taste receptors. Fig. 4B displays the results from the PCA of the taste investigations of Soluplus®-based buccal films The films studied here were found to have a disintegration time of approximately

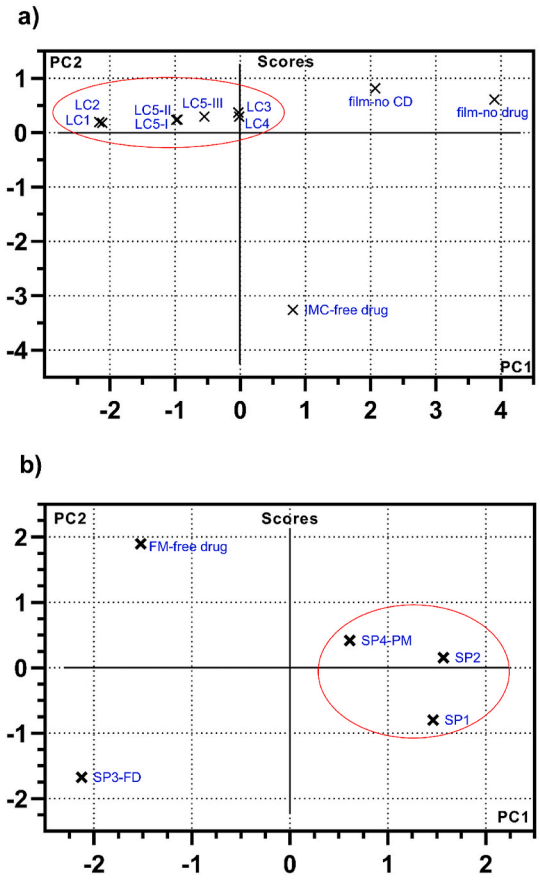

Fig. 4. A) Principal Component Analysis on taste-assessment of Lycoat®-films with freeze-dried IMC-HPβCD inclusion complexes in different molar ratios. (film-no CD = control, Lycoat® film loaded with IMC without addition of cyclodextrin, film-no drug = control, Lycoat® film with cyclodextrin but no IMC).

210 and 280 s, respectively, for the film with Soluplus® as single polymer (SP1) and a mixed formulation of Soluplus® and HPMC (SP2) [13]. Some of the same trends were observed as for the ODFs: film with HPβCD in a freeze-dried complex (SP3-FD) was distinctly different in taste-profile from the rest of the films, even the one containing a physical mixture of FM and HPβCD in equivalent amounts (SP4-PM). Most notably SP3-FD showed the least correlation to free FM, indicating that the freeze dried complexed FM is reduced in unwanted flavor profile and bitter sensation masked in the film. Also, in this test-set PC1 seemed to be explained by the taste assessment of main polymer Soluplus® whereas the taste assessment of the drug FM was explained by PC2, with the freeze-dried FM:HPβCD complexes having an inverse assessment as compared to the free drug.

In the current study, it seemed like the taste assessment was more influenced by the electrolyte solution for drug:cyclodextrin complexes (Fig. 3) or the polymers in the film formulations (Fig. 4). However, since in the electronic tongue we are comparing a simple solution of the drug with a more complex formulation containing several components, we cannot be sure that an unpleasant taste of the drug has been masked by HPβCD complexation; it could also be altered by the electrolyte solution, the excipients or the formulation [5]. Human taste perception can be different if a chemical substance is presented in a mixture than when presented alone [36,37]. Furthermore, molecular interactions between different formulation components could introduce misinterpretation of the results [5]. Also, the interaction with the sensor membrane in the electronic tongue can be influenced by other components, e.g. electrolytes, surfactants or other excipients. A weakness in the current study is that we do not have the taste assessment of the electrolyte solution

without any drug or cyclodextrin complexes present, and we therefore cannot eliminate this effect of the final response (Fig. 3), also, for the evaluations of the film formulations the taste assessment of each component alone would have been helpful (Fig. 4).

The sample preparation, meaning dissolution/dispersion and time until measurement, is a critical factor for the correct interpretation of the taste results [5]. For a dissolved drug molecule to reach the taste receptor, the drug must be released from the formulation first. It has been suggested as rule of thumb that solid oral dosage forms with dissolution within 30 s, e.g. ODFs, would benefit from taste-masking since the drug otherwise will be released while the formulation is in the oral cavity giving the drug molecules the possibility to interact with the taste receptors [5]. In the current set-up, all samples were tested after complete dissolution/dispersion because of the time it took to measure one sample using the electronic tongue including the washing procedures. Therefore, the results do not capture any time difference between the dissolved free drug and the dissolved film formulations. Since ODF formulations are expected to disperse within 30 s and then be swallowed before the drug has had the chance to dissolve out of the complex and interact with taste receptors, this set-up would under-estimate the difference between the formulations and the free drug in a patient setting. Buccal films on the other hand will remain in the oral cavity for a prolonged period providing more time for dissolving the drug and reaching the taste receptors, and hence, masking of unpleasant taste is more critical. For both tested film types (orodispersible and buccal films) a different taste profile was found compared to reference films and free drug in solution, this suggests that the unpleasant taste of the drug might be successfully masked in the formulated films, but as mentioned above it could also mean that the taste perception is different because of the additional excipients and not necessarily less unpleasant.

Taste evaluation is a complex matter and the results from the current study should be interpreted with the limitations discussed above. More studies are needed, in particular human taste studies. Nevertheless, these results may provide some support in the formulation optimization of different types of oromucosal film formulations using cyclodextrin inclusion complexes.

4. Conclusions

Solubility of IMC and FM were found to depend on the electrolyte composition of the aqueous media. Osmolality and osmotic strength were not directly correlated to solubilization and CE with HPβCD. Buffer capacity was favorable for solubilization when controlling drug ionization by the right pH, and increased solubilization was correlated to CE. Buffers that contained a lot of phosphate ions were more efficient in solubilizing both model drugs alone as well as in HPβCD complexes, and by freeze-drying the complexes both could be increased when compared to the same complex that was not freeze dried. Freeze-dried HPβCD complexes of both IMC and FM were also found to reduce the bitter response in the taste-assessment using the electronic tongue as compared to free drug in solution, which indicates that the unpleasant taste may be improved by complexing the drug substance by HPβCD. Freeze-dried complexes were also added to formulated ODFs and buccal films, and in both systems taste-perception differences as compared to free drug in solution and reference films, were an indication that the taste perception of the drug was changed.

Author contributions

Julia F. Alopaeus – Conceptualization; Data curation; Formal analysis; Design of Figures and Tables; Writing original draft, **Anja Göbel** –Methodology, Writing - review & editing, **Jörg Breitkreutz** – Supervision, Writing - review & editing, **Sverre-Arne Sande** - – Supervision, Writing - review & editing, **Ingunn Tho –** Conceptualization, Supervision, Project Administration, Writing - review & editing.

Declaration of competing interest

The authors state that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Acknowledgements

BASF and Roquette Pharma are acknowledged for donating the polymers Soluplus® and Lycoat®, respectively. The Norwegian Pharmaceutical Society is kindly thanked for funding the research visit to University of Düsseldorf.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2020.102245.

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