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Research Article

Potential Application of Ionic Liquid 1,4-Diazabicyclo-Octane for Enhanced Buccal Permeability of Diphenhydramine Hydrochloride in Oral Dissolving Polymeric Film

Hayley Harrod[1], Sarthak M Shah[1], Safa Ahmed[1], Rawas-Qalaji Mutasem[2], Mohsin Kazi[3], Babak Minofar[4], Samir A Kouzi[5]* and Mohammad N Uddin[1]*

[1]*Department of Pharmaceutical Sciences, College of Pharmacy, Mercer University, Atlanta, GA, USA*

[2]*College of Pharmacy, University of Sharjah, Sharjah, UAE*

[3]*Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, KSA*

[4]*Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic*

[5]*School of Pharmacy, Wingate University, Wingate, NC, USA*

***Corresponding Author:** Samir A Kouzi, School of Pharmacy, Wingate University, Wingate, NC, USA and Mohammad N Uddin, Department of Pharmaceutical Sciences, College of Pharmacy, Mercer University, Atlanta, GA, USA.

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Abstract

To improve patient compliance and experience, oral dissolving film (ODF) is a favorable drug delivery method. Advantages of using ODFs include bypassing the first pass effect, no degradation in the gastrointestinal tract, and quick onset of action. Films were prepared by using the casting method. Physical characterizations of the films were performed. Drug release studies and cytotoxic studies using dendritic cells were also conducted. An *ex vivo* permeability study was performed using the ionic liquid DABCO as a permeability enhancer and diphenhydramine hydrochloride as a model drug. In addition, differential scanning calorimetry (DSC) and X-ray diffraction (XRD) analyses were conducted. The results showed that ionic liquid DABCO-containing films disintegrate in < 5 minutes, have a neutral surface pH, and are noncytotoxic. Stability tests showed that there was no significant difference in appearance, physiochemical properties, and weight variation. Differential scanning calorimetry (DSC) analysis revealed films are in amorphous form. X-ray diffraction (XRD) analysis confirmed that the drug was not crystalline. Results suggest that films with the ionic liquid are capable of enhancing not only the permeability of the drug diphenhydramine hydrochloride but also the release of the drug from the film.

Keywords: *Ionic Liquid; 1,4-Diazabicyclo-Octane (DABCO); Oral Dissolving Film (ODF); Permeability Enhancer; Buccal Films; Permeability*

Introduction

There is a growing demand for novel dosage forms that can address swallowing-related challenges in the pediatric and geriatric populations. To overcome these challenges and fulfill the needs of the market, intraoral film has emerged as a new dosage form. This quick disintegrating film can be provided in various packages convenient for use, especially for children and elders. Thus far, various

bioadhesive mucosal dosage forms have been formulated, including adhesive tablets, gels, ointments, patches, and, more recently, the use of polymeric films for buccal delivery, known as mouth disintegrating films [1]. Film dosage forms are also being tested in different other routes. Shouvik., *et al*. investigated the anti-inflammatory property of vildagliptin using a topically applied plasticized ocular film formulation that was prepared by a solvent cast and evaporation method using triethanolamine (TEA), dimethyl sulfoxide (DMSO), and polyethylene glycol 400 (PEG 400) as the plasticizer in the HPMC hydrogel matrix base [2]. Mohapatro., *et al.* also evaluated the transcorneal permeation of diclofenac potassium (DCP) as a function of temperature from a hydroxypropyl methylcellulose (HPMC) matrix film containing triethanolamine as a plasticizer and benzalkonium chloride (BKC) as a preservative [3]. The most common film dosage forms are for oral administration. Oral dissolving films are thin polymeric films designed to deliver the active pharmaceutical agent directly to the bloodstream via the mucosa. These films are usually applied in the oral cavity on the buccal or sublingual area.

Both buccal and sublingual are ports of the mucous. The mucous membrane that lines the structures within the oral cavity limits is known as the oral mucosa. Histologically, the major layer of oral mucosa is the oral epithelium, whose thickness and degree of keratinization depend on the location and functional requirements [4]. The entire surface of the oral mucosa is covered by squamous stratified epithelium. The area is used as the site of drug administration because it is a highly organized, avascular, and semipermeable tissue. When the film dosage form is administered in the buccal or sublingual area, the film quickly dissolves in the saliva without the need for water, releasing the drug immediately to be absorbed from the oral cavity, and offering a rapid onset of action [6,8]. According to the European Pharmacopoeia, oromucosal films are solid, semisolid, or liquid preparations intended for administration to the oral cavity and/or the throat to obtain a local or systemic effect [9]. Oral dissolving film is a highly useful and well-needed dosage form for pediatric, geriatric, psychiatric, and dysphagic patients due to its ease of administration. It also minimizes the risk of patient choking or breathing issues, thus ensuring patient safety. Oral films can also serve bedridden and noncooperative patients, as they pose minimal risk during their administration, even if they are to be spit out.

Oral films should be designed to be highly water soluble with good mechanical and mucoadhesive properties while being compatible with the loaded drug. Hydrophilic polymers such as hydroxypropyl methylcellulose (HPMC), methylcellulose, polyethylene glycol (PEG), pullulan, polyvinylpyrrolidone (PVP), gelatin, maltodextrin, and Kollidon form adhesive hydrogels when wetted and have been successfully used in the preparation of fast dissolving films [7,21,23]. Although the higher surface-to-mass ratio enables faster film dissolution, the film's size and thickness are largely dependent on the drug potency and the therapeutic dose required to be loaded into the film, which may negatively impact the film's desired properties.

The most common routes for film administration are sublingual and buccal routes. The sublingual route is commonly used for the treatment of acute disorders due to high drug permeability across the mucosa and quick onset of action, while the buccal route is preferred for the treatment of chronic conditions where a relatively short extended drug release is desired [12].

The oral cavity for drug buccal and/or sublingual administration has several advantages. The oral mucosa offers excellent accessibility, blood supply, permeability, and continuous cell turnover and repair system compared with other mucosa. Therefore, the buccal and sublingual areas of the oral mucosa are potential routes for drugs for enhanced permeability and quick onset of action [13]. Oromucosal film preparations can also be applied to a specific site within the oral cavity, such as the gums (gingival preparations) or the throat (oropharyngeal preparations), to induce local effects or to be absorbed primarily through the oral mucosa for systemic effects [9]. These routes also avoid the hepatic first pass effect, thus providing higher drug bioavailability and offering safer and more effective alternatives to other conventional routes for drug administration [11,24].

Along with the mucoadhesive polymer, another important excipient for oral dissolving films (ODF) is permeability enhancers, as they increase the absorption of the drug through the membrane [16]. Permeability enhancers modulate membrane permeability and enhance the passage of drugs through the membrane [16]. In this report, we evaluated the ionic liquid (IL) 1,4-Diazabicyclo-octane (DABCO) as a permeability enhancer using diphenhydramine HCl (g) as a model drug. DABCO is a caged tertiary diamine commonly used in chemical synthesis. It has a closed ring structure (Figure 1).

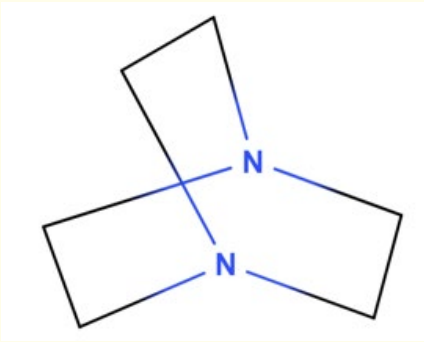

Figure 1: *Chemical structure of 1,4-diazabicyclo [2.2.2] octane (DABCO).*

Previously, it was found that DABCO can enhance the permeability of membranes in transdermal drug delivery. Monti., *et al*. showed that DABCO enhanced the transdermal delivery of diltiazem, a nondihydropyridine calcium channel blocker that treats hypertension. The results confirmed that the nature of the ionic liquid DABCO enhanced the degree of transdermal permeation [19]. Since DABCO enhances the transdermal delivery of drugs by enhancing the permeability of the membrane via the transdermal route, we investigated the capability of IL to serve as a permeability enhancer of drugs via the buccal route. To test the permeability of DABCO, we used diphenhydramine as a model drug. Diphenhydramine is an antihistamine drug that is commonly used to relieve symptoms of allergy, hay fever, and the common cold. It is also used to prevent and treat nausea, vomiting, and dizziness caused by motion sickness. Adverse effects of diphenhydramine include drowsiness, dizziness, constipation, stomach upset, blurred vision, and dry mouth/nose/throat [18]. Current dosage forms of diphenhydramine are tablets, capsules and liquid dosage forms. However, the film dosage form of the drug will be more patient compliant and easier and quicker to use without the need for water [19].

Orally administered diphenhydramine has a low bioavailability issue. Several clinical studies have reported that the bioavailability of the drug is approximately 50% among children and adults [20]. The tablet form of the drug showed a significant first-pass effect among men [21]. Although diphenhydramine HCl is a BCS class I compound with high aqueous solubility and permeability, the bioavailability is low because diphenhydramine undergoes rapid and extensive first-pass metabolism [22]. Additionally, the drug is highly protein-bound (approximately 80%). The protein-bound drug tends to stay longer in the blood instead of the site of action [23]. Therefore, the film dosage form will eliminate the issue of bioavailability of the drug. Based on these findings, we hypothesize that using an ionic liquid as a permeability enhancer may increase the absorption of the drug from the film in the sublingual route of administration. The objective of this study was to evaluate the permeation-enhancing effect of DABCO from the ODF following buccal administration. The film will be applied in the buccal area of the mouth cavity, from which the drug will be absorbed via the highly permeable membrane of the area. This dosage form will also provide the opportunity to reduce the dose, which will reduce the adverse effects exerted by the drug. Another advantage of ODFs is that they avoid the gastrointestinal (GI) route, eliminating the first-pass effect; therefore, they have a quick onset of action.

Materials and Methods

Materials

Kollidon F90, Kollidon VA64, Kollidon K90 and Kollidon K64 were purchased from BASF (Texas, USA). 1,4-Dioxane was purchased from Alfa Aesar (Massachusetts, USA). Ethyl alcohol (EtOH, 190 proof) was purchased from Spectrum Chemical MFG Corp (New Jersey, USA). PEG 200 was purchased from VWR (Pennsylvania, USA). Diphenhydramine hydrochloride, hydroxypropyl-methylcellulose (HPMC), hydroxypropyl-cellulose (HPC) and isopropyl-alcohol (IPA) were purchased from Sigma–Aldrich (Missouri, USA). Side-by-side diffusion cells were purchased from PermeGear (Pennsylvania, USA). Artificial saliva was obtained from a local Walmart store (Georgia, USA).

Methods

Preparation of orally dissolving films (ODF)

Six single-layered films were prepared by the solvent casting method in petri dishes [14]. The films contained both Kollidon F90 and Kollidon VA64 as hydrophilic polymeric carriers, PEG 2000 as a plasticizer, HPMC grade K4M to enhance the flexibility of the film and diphenhydramine hydrochloride as a model drug. All components were accurately weighed (Table 1) and dissolved under heat (40°C) in ethyl alcohol by stirring to obtain a homogenous viscose liquid. Formulations with ionic liquid DABCO were prepared using different w/v % of 1% (F1), 2% (F2), 3% (F3), 4% (F4), 5% (F5) and 6% (F6). The control film does not contain any ionic liquid. The formulation mixtures were then sonicated for 15 minutes using a bath sonicator (Branson 3510, Hampton, NH, USA) to eliminate the bubbles, and 8 mL of the formulation was measured and placed into a petri dish. The petri dish was then rotated for 20 minutes using a rotator (Barnstead 2309) to evenly spread the formulation on the plate. All the films were then placed in an oven for 30 minutes at 50°C to dry. After drying, films were placed at room temperature for 30 minutes to cool, and then they were removed and cut into 4 cm^2 pieces. In each formulation, the same amount of HPMC was added. The formulation composition of the batches is shown in table 1.

Excipients and drug	Control	F1	F2	F3	F4	F5	F6
K90 (g)	2.8	2.8	2.8	2.8	2.8	2.8	2.8
K64 (g)	0.6	0.6	0.6	0.6	0.6	0.6	0.6
PEG2000 (g)	1	1	1	1	1	1	1
Ethanol (mL)	18	18	18	18	18	18	18
Water (mL)	82	82	82	82	82	82	82
HPMC K4M (g)	1	1	1	1	1	1	1
DABCO (w/v%)	None	1	2	3	4	5	6
Diphenhydramine HCl (g)	0.48	0.48	0.48	0.48	0.48	0.48	0.48

Table 1*: ODF formulation of control and ionic liquid and composition. K90 and K64 = Kollidon F90 and VA64, PEG 2000 = Polyethylene-Glycol 2000, EtOH = Ethyl Alcohol, HPMC K4M = Hydroxypropyl-Methylcellulose K4M Grade.*

Appearance of ODF

Film appearance was categorized as opaque, semitransparent, or transparent. Observations were made based on the appearance of three films.

Fold endurance

Three film strips were cut into 2 cm x 2 cm pieces. Three trials were conducted. Data are represented as the average ± standard deviation. Each formulation was folded repeatedly until the film strip broke.

Average film thickness

The thickness of the prepared films was measured using a Mitutoyo electronic digital caliper (Japan). The thickness of each film was measured at 8 different points, and the average thickness was calculated [5]. The final average thickness was recorded in mm.

Weight variation

Five batches of ODF were cut into 2 cm x 2 cm pieces and weighed. The average weight of the films from the five batches and standard deviation were calculated. The final average weight was recorded in grams.

Disintegration test of ODF formulation

Each ODF formulation film strip was placed in a petri dish (141 cm^2). Then, 3 ml of PBS solution was added to each formulation. The petri dish was then placed on a shaker to allow the film strip to completely disintegrate. The time required for the film to completely dissolve in the solution was recorded from 3 parallel experiments.

Tensile strength and percent elongation of the ODF formulation

Tensile strength and elongation were tested with a MARK-10 force gauge (Copiague, NY). Three strips, 2 cm x 2 cm, were used from each formulation. The average tensile strength in N/m^2 was measured. The standard deviation was calculated from the obtained data.

Surface pH of the ODF formulation

Each ODF formulation was cut into 4 cm^2 pieces and placed in 2 mL of artificial saliva, which is a combination of water and viscosity enhancer carboxymethylcellulose (CMC). The surface pH was noted by placing the pH electrode directly on top of the ODF. The pH of the artificial saliva was measured separately by placing the electrode only in artificial saliva in a small beaker.

Stability test

Stability testing was performed as per ICH guidelines under several conditions. Three film strips from each formulation were wrapped with butter paper and then with aluminum foil to be stored in the stability chamber. Films were stored under two storage conditions: 25°C/60% relative humidity (RH) (Zone II) and at 40°C/75% R.H. (zone IVb) [15,16]. Stored film strips were sampled at varying intervals (0, 1 week, 1 month, 3 months, 6 months and 12 months) for further testing for overall appearance, physiochemical properties, and weight variation.

Differential scanning calorimetry (DSC)

Pure DABCO and the film prepared with DABCO (F2) were characterized using a differential scanning calorimeter (DSC-60, Shimadzu, Kyoto, Japan). Samples (3 - 5 mg) were placed in aluminum pans and hermetically sealed by a crimp sealer. The sealed pans were then heated from 10 to 250°C at a heating rate of 100°C/min under a nitrogen gas flow rate of 50 mL/min.

X-ray diffraction (XRD) analysis

Powder x-ray diffraction of the samples was evaluated by Ultima IV diffractometer (Rigaku) over 3 - 60° 2θ range at a scan speed of 1.00 deg./min. The tube anode was Cu with Ka = 0.1540562 nm monochromatized with a graphite crystal. The pattern was collected at 40 kV of tube voltage and 40 mA of tube current in step scan mode (step size 0.02°, counting time 1 second per step).

In vitro release study

An *in vitro* release study was conducted using an IncuShaker, a Crystal Technology dissolution apparatus. The dissolution and release of the film containing diphenhydramine hydrochloride as a model drug was conducted for 30 minutes. Samples were withdrawn at 1, 3, 5,

10, 15, 20, 25 and 30 minutes. The artificial saliva solution (25 mL) was used as a dissolution medium. Collected samples were quantified using a U.V. spectrophotometer (NanoDrop 2000c, Thermo Fisher) at a wavelength of 210 nm [17]. The calibration curve was linear with $R^2 = 0.997$ over a range of 50 µg/mL to 1000 µg/mL.

Cytotoxicity study

This study was conducted using a modified version of the MTT assay (2,5-diphenyl-2H-tetrazolium bromide). Antigen-presenting dendritic cells (DC) 2.4 (ATCC) were placed on a 6-well plate with a seeding of 0.3×10^6 cells/well [25]. D.C. Cells were exposed to each ODF formulation film strip, which was dissolved in 2 mL of Dulbecco's Modified Eagle Medium (DMEM) and added to each well. After the exposure period, 50 µL of MTT reagent was added (5 mg/ml in PBS), and the 6-well plate was incubated for 4 hours in an incubator at 37°C and 5% CO_2. Then, the medium was removed, and the cells were washed with sterile PBS. After washing, 50 µL of DMSO was added to each well to dissolve the formazan. The 6-well plate was shaken for 20 minutes at room temperature. The concentration of dissolved formazan crystals was spectrophotometrically quantified using a microplate reader at a wavelength of 570 nm (BioTek® Synergy H1, Winooski, VT). The percentage viability was determined relative to the viability of cells that were treated with DMEM only.

Permeability study

The *ex vivo* permeability of diphenhydramine hydrochloride from buccal films was carried out through porcine buccal mucosa using side-by-side diffusion cells (5 mm clear, 20 cm^2 with 7 ml volume, 37 degrees). The porcine buccal mucosa was sliced to a thickness of 0.5 mm per membrane. The thickness of each buccal mucosa was carefully measured at all sides using a thickness gauge (Mitutoyo). The donor compartment was filled with 3 mL of PBS. One milliliter sample was withdrawn at different time points from the receptor chamber, and 1 mL of PBS was added back to maintain a constant volume. In addition, the amount of drug remaining within the buccal mucosa was determined by cutting out the site of permeation into three pieces and stirring them in PBS for 4 hours using a plate stirrer. The extracted drug in PBS was quantified by a UV spectrophotometer as described above.

Statistical analysis

The recorded results were expressed as the mean ± standard deviation (SD). GraphPad Prism 8 statistical software (San Diego, CA) was used to analyze the results. One-way ANOVA followed by post hoc Tukey's multiple-comparisons tests were used to analyze cell culture and stability data. Student's t test was used to analyze cell culture data at a significance level of p value < 0.05.

Results

Oral dissolving film dosage forms are designed to release the drug quickly and permit the drug to pass through the membrane easily. Therefore, characterizing the film dosage forms in terms of solubility, disintegration time, permeability, *in vitro* release and cytotoxicity is important for making an efficient film formulation.

Formulated orally dissolving films (ODF)

The ionic liquid DABCO was added as a permeability enhancer. DABCO was added in different ratios in different formulations. Diphenhydramine hydrochloride was added as a model drug that can pass through the membrane. Equal amounts of 0.48g of diphenhydramine hydrochloride were added to each formulation.

Film appearance

Film appearance was categorized as not transparent, semitransparent, or transparent. Observations were made based on the appearance of three films. Microscopic images were taken for each formulation to observe their appearance. The images are shown in figure 2. The appearance of the film formulations in this study shows that the formulations were smooth, transparent, bubble-free and stable.

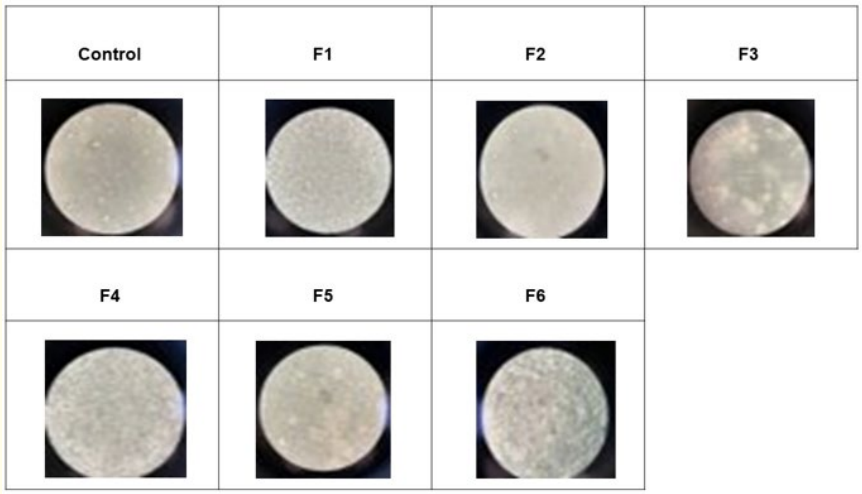

Figure 2: *Microscope images of control and six formulations of DABCO at 10x magnification.*

Fold endurance

The fold endurance shows that formulations F1, F2 and F4 have the highest endurance value. All the F1, F2, F3 and F4 formulations have endurance higher than the control film. On the other hand, the endurance values for F5 and F6 are almost similar to that of the control. All these fold endurance values are shown in figure 3.

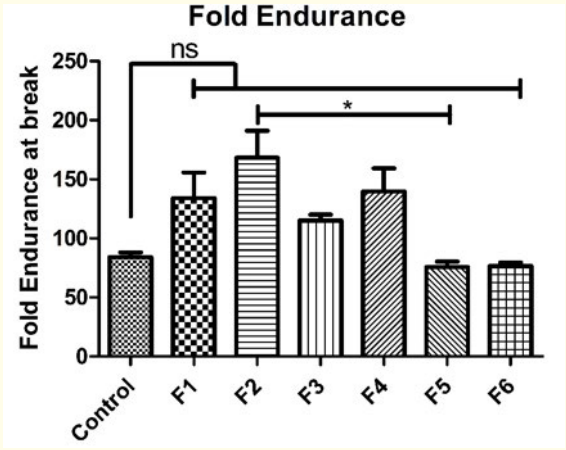

Figure 3: *Fold endurance values of the control, F1, F2, F3, F4, F5 and F6 film formulations. One-way ANOVA followed by Dunn's multiple comparison test was conducted.*

Average film thickness

The thickness of the prepared films was measured using a Mitutoyo electronic digital caliper (Japan). The thickness of each film was measured at 8 different points, and the average thickness was calculated. The average thickness of all the films was found to be 0.08 mm or 80 micrometers, which is in the acceptable range of very thin films. The film thickness of different formulations is shown in figure 4.

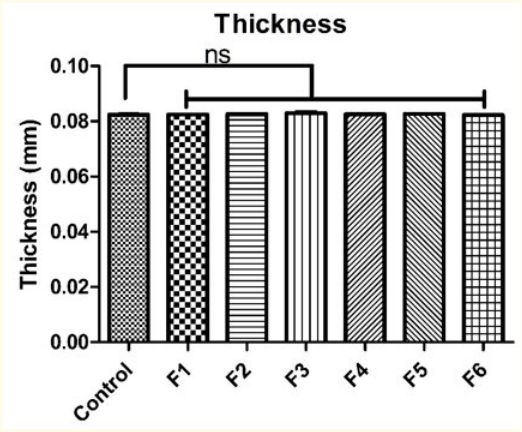

Figure 4: *Thickness of control, F1, F2, F3, F4, F5 and F6 film formulations.*

Weight variation

Five batches were cut into 2 cm x 2 cm pieces and weighed. All six formulations and the control films have similar weights, ranging from 25 to 30 mg. The weight variations of the film formulations are shown in figure 5.

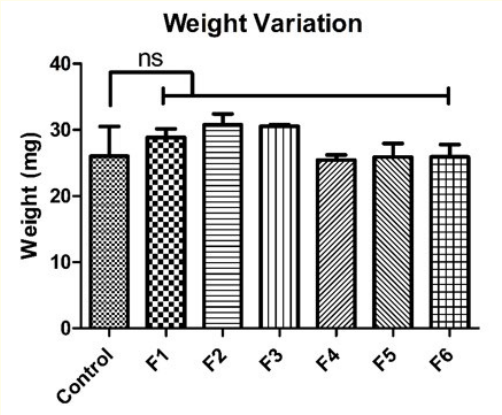

Figure 5: *Weight of control, F1, F2, F3, F4, F5 and F6 film formulations. One-way ANOVA followed by Dunn's multiple comparison test was conducted.ns.*

Disintegration test

The disintegration time of oral dissolvable films is one of the important parameters. All disintegration times were less than 5 minutes. The F2 formulation showed the highest disintegration time of 4 minutes, and the F6 formulation had the lowest disintegration time of 3 minutes. The disintegration times of all the formulations are shown in figure 6.

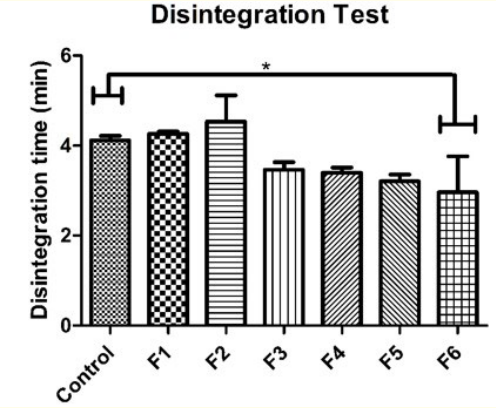

Figure 6: *Disintegration time of control, F1, F2, F3, F4, F5 and F6 film formulations. One-way ANOVA followed by Dunn's multiple comparison test was conducted.*

Tensile strength and percent elongation

Tensile strength and elongation were tested with a MARK-10 force gauge (Copiague, NY). Three strips, 2 cm x 2 cm, were used from each formulation. The results showed significant differences in formulations F3 and F4 versus the control. Elongation of the films was also conducted during the tensile strength measurement. One-way ANOVA followed by Dunnett's multiple comparison test was conducted in tensile strength and elongation measurement calculations. The tensile strength and elongation of the films of different formulations are shown in figure 7a and 7b.

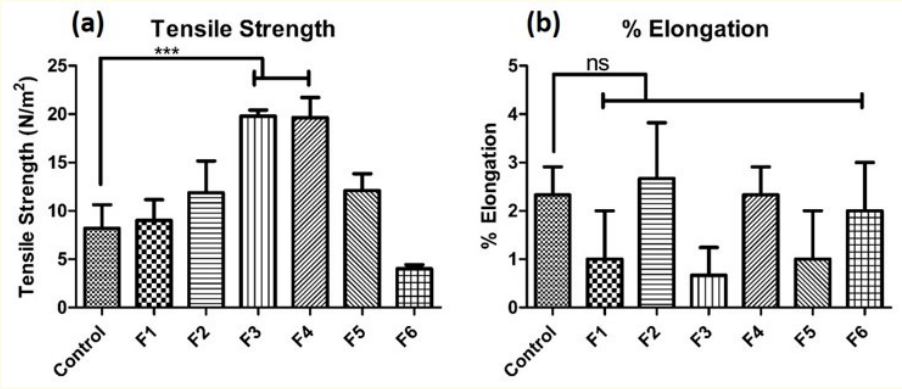

Figure 7: *Tensile strength value (a) and Percent elongation (b) of the control, F1, F2, F3, F4, F5 and F6 film formulations.*

Surface pH

Each ODF formulation was cut into 4 cm^2 pieces and placed in 2 mL of artificial salvia. The surface pH was noted by placing the electrode directly on top of the ODF. The surface pH of all the formulations was consistent and in the range of 6 to 6.5, which is close to neutral pH. The surface pH of all the film formulations is shown in figure 8.

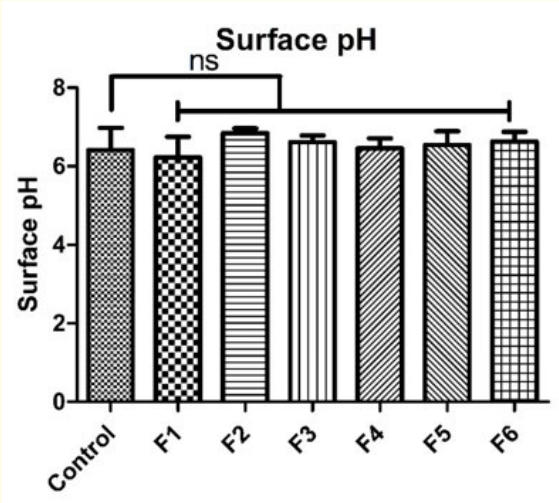

Figure 8: *Surface pH value of the control, F1, F2, F3, F4, F5 and F6 film formulations.*

Stability test

The stability study was conducted according to the International Council on Harmonization guidelines. The stability of ODFs was maintained under controlled conditions (25°C temperature/60% relative humidity and 40°C temperature/75% relative humidity) for 12 months [18,20]. The results showed that the films were stable for six months in this condition.

Differential scanning calorimetry (DSC) analysis

The DSC thermograms of pure DABCO and the representative film formulation F2 are shown in figure 9. A sharp endothermic peak of pure DABCO was observed at 82.20°C, while the F2 film formulation showed complete disappearance of the DABCO peak in the same temperature range.

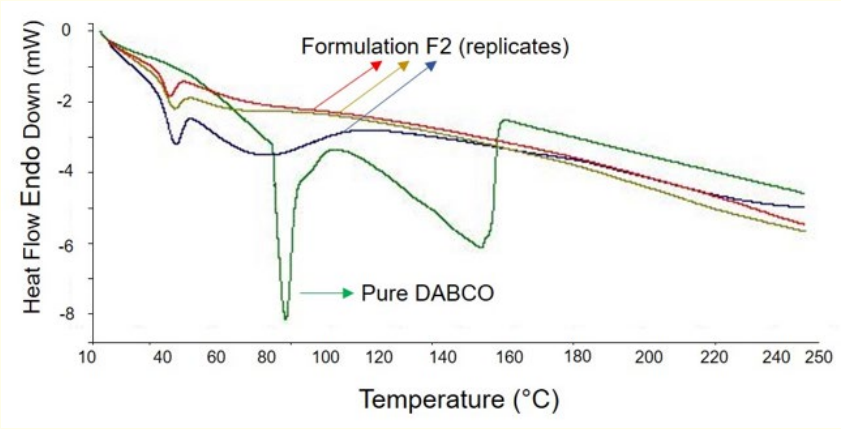

Figure 9: *DSC chromatograms of pure DABCO and the film formulation (F2) of DABCO.*

X-ray diffraction (XRD) analysis

XRD analysis is a distinctive method to determine the crystallinity of a drug compound with proper interpretation before and after loading in the formulation. It allows the identification of the drug crystalline changes. Figure 10 shows the X-ray diffraction (XRD) pattern of pure DABCO powder and F2 formulation loaded with diphenhydramine hydrochloride. Pure DABCO manifested the distinct peaks at 2θ: 17.22º, 19.1º, 19.48º, 19.68º, 23.5º, 25.34º, 30.48º, 32.66º, 33.58º, 34.08º, 35.58º, 38.04º, 39.6º, 43.28º, 45.08º, 46.1º, 51.3º, 54.18º, 59.44º indicating the highly crystalline form of DABCO, whereas the drug loaded formulation F2 showed broad two peaks with very low intensity.

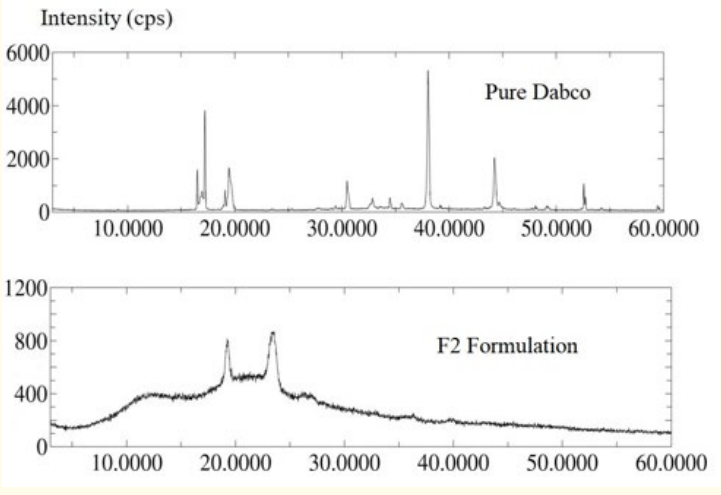

Figure 10: *XRD of (A) pure DABCO powder, (B) F2 formulation loaded with diphenhydramine hydrochloride*

In vitro release study

The results of the *in vitro* release study are shown in figure 11. The study shows that all the formulations had higher release in comparison with the control. More than 50% of the drug was released within two minutes for all of the DABCO-containing formulations (F1 to F6), which indicates that the ionic liquid induced faster release of the drug. Formulations F2 and F4 show quicker release of drug compared with other formulations. It has been found from the data that more than 80% of the drug was released in less than 5 minutes for both formulations.

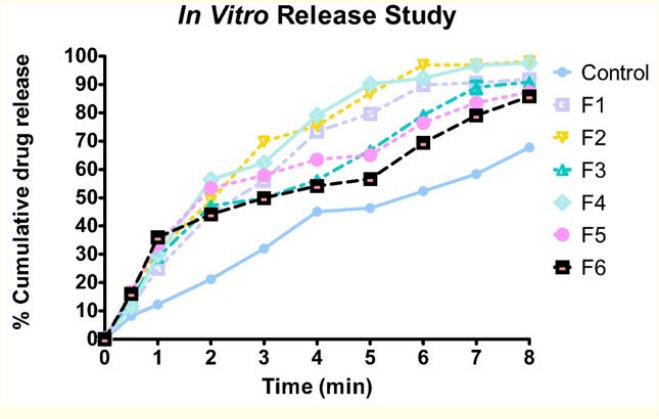

Figure 11: *In vitro release study of control, F1, F2, F3, F4, F5 and F6 film formulations. In vitro release study for the control and IL formulations with error bars (SD).*

Cytotoxicity study

The results of the cytotoxicity study are shown in figure 12. The results of the cytotoxicity study showed that none of the ionic liquid DABCO-containing films were cytotoxic. The results were analyzed by one-way ANOVA followed by Dunnett's multiple comparison test.

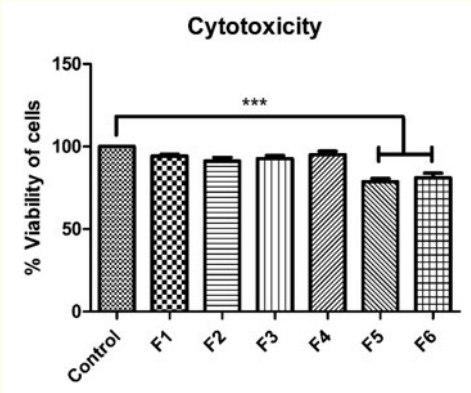

Figure 12: *Cytotoxicity study of control, F1, F2, F3, F4, F5 and F6 film formulations.*

Permeability study

The results of the permeability study of the different formulations are shown in figure 13. The study shows high permeability in comparison with the control, which does not contain any ionic liquid. Formulations F5 and F6 have higher permeabilities than F1 and F3. More than 80% of the drug was released within 8 minutes for both formulations. This is because the F5 and F6 formulations have higher contents of the ionic liquid. Interestingly, the F2 formulation also has higher permeability than F1 and F3.

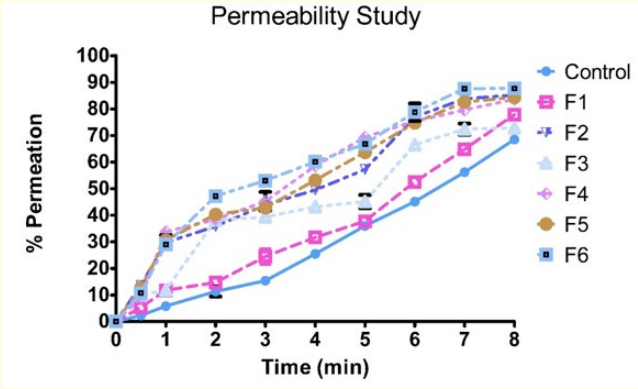

Figure 13: *Permeability study of the control, F1, F2, F3, F4, F5 and F6 film formulations. An ex vivo permeability study using porcine buccal mucosa for the control and IL formulations with error bars (SD).*

Discussion

Film dosage forms can address the swallowing problem of most common oral dosage forms, including tablets and capsules. The buccal area in the mouth cavity is the most common site of administration for film dosage forms. Along with other excipients, a safe ionic liquid

can be used in film dosage forms in appropriate amounts to enhance the permeability of the drug in buccal administration. Enhanced permeability in the buccal area quickens the drug onset time and increases bioavailability. Thus, physical characterizations such as solubility, thickness, tensile strength, elongation, and toxicity are important studies along with permeability to prepare an effective and quickly soluble film dosage form.

Fold endurance studies are important for film stability, packaging, storage, and handling by patients. The fold endurance study shows that the films were able to display endurance when folded multiple times. Each formulation was folded repeatedly until the film strip broke.

The thickness of the film determines the solubility time, drug release, and permeability. If the thickness of the film is high, it will take a longer time to dissolve, which will slow the release of the drug and reduce the permeability. On the other hand, if the thickness is low, the film will dissolve in a shorter time, which will increase the drug release and enhance the permeability. The ideal thickness of buccal thin films should be between 50 and 1000 µm. The average thicknesses of all the films in this project were found to be 0.08 mm or 80 micrometers, which is in the range of very thin films. This average thickness indicates that the thickness of the formulations is very close to that of thin films. There was no significant difference in terms of the thickness of the drug-containing film strips versus the control film. Additionally, the consistency of thickness would have similar disintegration times and permeability.

The weight of the films is important, as films with different weights can have different amounts of content and solubility times. The average weight determines the consistency of the dose in each administration. The average weight of the films from the five batches and standard deviation were calculated. In this study, all six formulations and the control had similar weight ranges from 25 to 30 mg.

The disintegration time of the oral dissolving film is also another important parameter. This indicates the time needed for the drug to be absorbed in the buccal and sublingual areas. A disintegration time of less than 5 minutes is ideal for oral dissolving film dosage forms. In this case, the disintegration time ranges from 4.5 to 5 minutes, showing the ideal character of an oral dissolving film.

The tensile strength is the maximum tensile force applied until the thin-film specimen breaks. It is obtained by dividing the applied force by the cross-sectional area of the film and multiplying by a hundred [34]:

Percent of Tensile Strength = {(Load at Failure)/(Film Thickness × Film Width)} * 100.

When a pulling force is applied to the film, the tensile strength increases. This tensile strength continues until the integrity of the film form deteriorates. The percentage of elongation can be determined by measuring the final size of the film before its integrity deteriorates. This rate increases as the amount of plasticizer is enhanced. The elongation percentages of the ODF formulations are calculated by the same formula [34]. The tensile strength and percent elongation show that the films are sufficiently stable. A significant difference was seen in the F3 and F4 formulations versus the control. However, there was no significant difference between the control and the other formulations.

Determining the pH of ODFs is important in terms of their solubility/dispersion in the oral cavity, taste properties, and rapid release of the drugs [35]. Ideally, ODFs should not irritate the buccal mucosa; therefore, the assessment of their pH after dissolution is critical to ensure their convenient use by patients. Therefore, the pH should be close to 7 in film dosage forms. Determining the pH of the film is also important, as the pH can change the physical character of the membrane. Such a change can lower the permeability and absorption of the drug. Additionally, the pH should not be different from the saliva pH. The pH of the films in this study was very close to the saliva pH, which indicates that this film will not exert undesirable effects on the membrane.

The stability study of the ODF is very important, as these formulations are not robust (unlike tablets or capsules). With a change in the temperature or humidity, the thin film can be distorted or dissolved or stick to the container or other films. The stability study in the project shows that the films are stable for up to 6 months. The stability study was conducted according to the International Council on Harmonization guidelines, where ODFs were kept under controlled conditions of 25°C temperature/60% relative humidity and 40°C temperature/75% relative humidity for 6 months [18,20]. After this time period, physical characterizations such as weight uniformity, morphological properties, film thickness, tensile properties, and dissolution tests were conducted, and similar results were obtained.

Differential scanning calorimetry (DSC) is widely used for studies of heat capacities, enthalpies of phase transitions (fusion, mesomorphic, vaporization), and chemical reactions. Previously, the enthalpies of formation of these ionic liquids in the liquid state were determined using the DSC results according to Hess's Law, which can dictate the stability of the ionic liquid [36]. Thus, the DSC study can provide information on the stability of the ODF and whether the ionic liquid is in amorphous or crystalline form in the ODF. The results show that DABCO forms crystals in its pure form. However, when it is in film dosage form, it is in amorphous form. The amorphous form is more suitable for drug solubility and permeability. The disappearance of the peak in the F2 film at DABCO's melting temperature is probably related to partial amorphization of the film formulation. On the other hand, the formation of crystals causes a reduction in solubility and permeability. The results show that the ionic liquid DABCO forms crystals in its pure form; however, when it is in the film dosage form, it is in the amorphous form.

X-ray crystallography study was conducted to determine whether the ionic liquid in the film is in crystalline forms or not. The XRD pattern of F2 formulation showed broad peaks at about 19.26º, and 23.48º, indicating the minimum crystalline nature of the drug loaded formulation. The characteristic peaks of F2 formulation indicated that the drug was not in crystalline nature in the formulation and that the amorphous state would contribute to the higher drug up taking capacity. Furthermore, the major peak of drug loaded formulation had a reduced intensity as compared to pure DABCO. This may be attributed to the incorporation of DABCO in the formulation, leading to a change in the crystallinity of the drug loaded formulation.

A release study was conducted to mimic the mouth condition of buccal administration. Standard USP dissolution apparatus is used to mimic the gastrointestinal tract using simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) in large amounts. However, in this study, we used artificial saliva in a small amount of 25 mL. The efficiency of film dosage forms depends on the release time of the drug from the film. The release of drug from F2 formulations shows the highest and quickest release within 8 minutes. Therefore, the use of DABCO enhances the release of the drug from the film. The use of DABCO not only enhances the permeability of the buccal membrane but also helps enhance the release of the drug.

In general, one of the major problems with ionic liquids is that they can be toxic. Previous studies have correlated the toxicity of ionic liquids with increasing alkyl chain length of the cationic constituent, proposing that the alkyl chains insert through the polar head-group region of the membrane bilayer and consequently induce membrane damage and cell death [10,22]. The toxicity of ionic liquids may vary and depend on the concentration and structural features. However, it can be mitigated by rational design and the right selection of ionic liquid [39]. In this study, the ionic liquid DABCO, triethylenediamine, is a caged tertiary diamine that does not have any alkyl chains, indicating that it is a nontoxic molecule. This was consistent with the cytotoxicity study, which showed that DABCO is nontoxic. The ionic liquid was used at different concentrations of 1%, 2%, 3%, 4%, 5%, and 6% to observe whether higher percentages can cause cytotoxicity. None of the formulations were cytotoxic ($p > 0.5$). In general, DABCO is nontoxic when used as a permeability enhancer, which may be due to its ring structure.

Ionic liquids have the ability to disrupt the cell membrane and enhance permeability through the membrane. There are several hypotheses on how ionic liquids enhance permeability. In general, all ionic liquids with permeation enhancement are hydrophilic or hydrophobic. Hydrophilic ionic liquids open up the tight junction of the epithelial cell layers; thus, they promote paracellular transport.

On the other hand, hydrophobic ionic liquids improve partitioning into the epithelial membrane by providing channels, thus promoting transcellular transport of the drug in the lipid regions [40]. In this study, we used the ionic liquid DABCO (triethylenediamine), which does not contain any alkyl chains and therefore has minimal cytotoxicity. The results showed high permeability of the drug in the presence of the ionic liquid DABCO in comparison with the control, which does not contain any ionic liquid.

Conclusion

In this study, different ODF formulations were prepared with different percentages of the ionic liquid DABCO as a permeability enhancer. Permeability enhancers are one of the major components used for the preparation of an efficient oral dissolving film dosage form. All of the prepared formulations showed similar physical characteristics, such as appearance, thickness, weight variation, and surface pH. Additionally, the cytotoxicity study revealed that all of the prepared formulations are not toxic even though up to 6% of the ionic liquid has been used. However, in the tensile strength, percent elongation, *in vitro* release, and permeability studies, the F2 formulation showed higher values than the other formulations. The results of this study suggest that film dosage forms with the ionic liquid DABCO are capable of enhancing not only the permeability of the drug but also the release of the drug from the film. The higher values of the F2 formulation in the *in vitro* release and permeability studies make this particular formulation a candidate for further investigations, including *in vivo* studies. More research aimed at investigating the benefits of ionic liquids is certainly needed to further optimize drug delivery formulations.

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Informed Consent Statement

Not applicable.

Bibliography

1. M Malke., *et al*. "Formulation and evaluation of oxcarbazepine fast dissolve tablets". *Indian Journal of Pharmaceutical Sciences* 69.2 (2007): 211-214.

2. N Souvik., *et al*. "Vildagliptin plasticized hydrogel film in the control of ocular inflammation after topical application: study of hydration and erosion behavior". *Zeitschrift für Physikalische Chemie* 236.2 (2021): 275-290.

3. M Rajaram., *et al*. "Transcorneal permeation of diclofenac as a function of temperature from film formulation in presence of triethanolamine and benzalkonium chloride". *Colloids and Surfaces B: Biointerfaces* 123.1 (2014): 170-180.

4. S Groeger., *et al*. "Oral mucosal epithelial cells". *Frontiers in Immunology* 10 (2019): 208.

5. Alipour Shohreh., *et al*. "Development and *in vitro* evaluation of fast-dissolving oral films of ondansetron hydrochloride". *Trends in Pharmaceutical Sciences* 1.1 (2015): 25-30.

6. Almeida Nilsa Maria Galvão., *et al*. "A novel dosage form for buccal administration of bupropion". *Brazilian Journal of Pharmaceutical Sciences* 51.1 (2015): 91-100.

7. Arun Arya., *et al*. "Fast dissolving oral films: an innovative drug delivery system and dosage form". *International Journal of ChemTech Research* 2.1 (2015): 576-583.

8. Chougule Pankaj C., *et al*. "Design and evaluation of formulated mouth dissolving film of domperidone and study the effect of concentration of polymers on drug release". *Asian Journal of Pharmaceutics* 11.4 (2017): 846-853.

9. European Directorate for the Quality of Medicines & HealthCare. 1 European Pharmacopoeia - 8th edition. Strasbourg: EDQM (2013).

10. Evans Kervin O. "Room-temperature ionic liquid cations act as short-chain surfactants and disintegrate a phospholipid bilayer". *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 274.1-3 (2006): 11-17.

11. Gandhi Rajesh B and Joseph R Robinson. "Oral cavity as a site for bioadhesive drug delivery". *Advanced Drug Delivery Reviews* 13 (1994): 43-74.

12. Hao Jinsong and Paul WS Heng. "Buccal delivery systems". *Drug Development and Industrial Pharmacy* 29.8 (2003): 821-832.

13. Hearnden Vanessa., *et al*. "New developments and opportunities in oral mucosal drug delivery for local and systemic disease". *Advanced Drug Delivery Reviews* 64.1 (2012): 16-28.

14. Hoffmann Eva Maria., *et al*. "Advances in orodispersible films for drug delivery". *Expert Opinion on Drug Delivery* 8.3 (2011): 299-316.

15. "ICH Requirements for Pharmaceutical". Q1 Scientific (2017).

16. Kaza Rajesh., *et al*. "Formulation and characterization of gliclazide oral dissolving films". *Drug Delivery Letters* 4.3 (2017): 185-192.

17. Kirkpatrick Douglas., *et al*. "UHPLC assay and impurity methods for diphenhydramine and phenylephrine hydrochloride oral solution". *Separation Science Plus* 3.1-2 (2020): 4-11.

18. Mahboob Muhammad Bilal Hassan., *et al*. "Oral films: a comprehensive review". *International Current Pharmaceutical Journal* 5.12 (2016): 111-117.

19. Monti Daniela., *et al*. "Ionic liquids as potential enhancers for transdermal drug delivery". *International Journal of Pharmaceutics* 516.1-2 (2017): 45-51.

20. Murthy AV., *et al*. "Formulation and evaluation of tramadol hydrochloride oral thin films". *International Journal of Pharmaceutical Sciences and Research* 13.5 (2018): 1692-1698.

21. Pathare Yogyata S., *et al*. "Polymers used for fast disintegrating oral films: a review". *International Journal of Pharmaceutical Sciences Review and Research* 21.1 (2013): 169-178.

22. Schaffran Tanja., *et al*. "Toxicity of N,N,N-Trialkylammoniododecaborates as new anions of ionic liquids in cellular, liposomal and enzymatic test systems". *Green Chemistry* 11.9 (2009): 1458.

23. Teodorescu Mirela and Maria Bercea. "Poly(Vinylpyrrolidone) - A versatile polymer for biomedical and beyond medical applications". *Polymer - Plastics Technology and Engineering* 54.9 (2015): 923-943.

24. Uddin Mohammad Nasir., *et al*. "Overview and future potential of fast dissolving buccal films as drug delivery system for vaccines". *Journal of Pharmacy and Pharmaceutical Sciences: A Publication of the Canadian Society for Pharmaceutical Sciences, Societe Canadienne Des Sciences Pharmaceutiques* 22.1 (2019): 388-406.

25. "Useful Numbers for Cell Culture". Thermo Fisher Scientific (2021).

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