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Original Article

FORMULATION DEVELOPMENT OF MOUTH DISSOLVING PRINTED FILM OF KETOROLAC AND *IN VITRO* EVALUATION

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ABSTRACT

Objective: The present research work was carried out to prepare Ketorolac printed Oral Thin Films using a pneumatic pressure printer. In this research, we attempted to prepare a non-contact printing system by using pneumatic pressure-based printer that incorporates printing of active pharmaceutical ingredients onto a medical-grade Orodispersible film for developing personalized medication.

Methods: In the present work Ketorolac Trometamol was used as a model drug. Placebo substrate was developed by using cellulosic polymers like HPMC, MCC, Neusilin, and starch to impart paper-like properties that are desirable for printing. It was evaluated for various physicochemical properties like disintegration time, mechanical strength, folding endurance, surface properties, etc. Polymers and plasticizers were evaluated for the development of drug loaded Printing ink. The drug-printed films were characterized for physicochemical properties and *in vitro* drug dissolution.

Results: Various film-forming polymers were evaluated for the development of printing substrates. The F3 substrate had desired mechanical properties i.e. the thickness of 0.157±0.003, the tensile strength of 0.331±0.016, disintegration within 60 seconds, and this substrate also maintained its integrity after the printing of the drug ink. The HPMC-based ink (I4) with polyethylene glycol for modulating flow properties of ink in the concentration of 1.40%w/v was selected among various ink formulations. The drug release from the printed films was 98±1.94% in 1 h.

Conclusion: Through this new drug printing technology the limitation of low drug dose loading associated with ink-jet and flexographic printing can be solved by increasing the drug loading ranges from micrograms to milligrams by a single pass of the print head.

Keywords: Ketorolac printed OTF, 2D printing, HPMC ink, Pneumatic-based printer

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INTRODUCTION

Printing of medicine on an oral thin film (OTF) is an emerging technology that is not yet fully established. It has multiple advantages compared to other conventional manufacturing processes of OTF. Firstly, printing active pharmaceutical ingredients (APIs) is potentially an enabling technology to produce personalized medicine [1]. Personalization is crucial, for drugs that require careful dose adjustments, such as low therapeutic index drugs, and potent drugs. The OTF disintegrates within seconds when placed on the tongue intentional swallowing is not essential for effective treatment [2]. Therefore, OTF is an ideal oral dosage form for drug delivery in children and the elderly. Solvent casting is the most common method for the preparation of oral thin films. During solvent casting, the API is stressed by the solvent used, the high shear mixing process, and subsequent drying. Unstable APIs can be affected by mixing and drying. Achieving a uniform distribution of APIs throughout the film can be difficult, especially when using potent drugs at low doses and characteristics of coating mass i.e., Viscosity or density is affected by the properties and quantity of APIs processed. Therefore, the formulation of coated mass often needs to be adjusted for each new active substance and each new dose and waste containing API is also generated by this method. So in this perspective, manufacturing Orodispersible films by printing APIs onto placebo substrates can overcome these constraints, increasing the production yield and quality.

(Gaisford, 2011) Evaluate the use of thermal ink-jetting the printing drugs onto oral films. Hewlett-Packard printer was modified the drug solution was replaced by ink. They used potato starch-based film for the deposition of Salbutamol solution. The printer used in this work was operated most successfully when the viscosity of the feed solution was between 1.1 and 1.5 $mm^2\ s^{-1}$, corresponding to glycerine concentrations of 10–20%v/v [4]. The dose deposition was achieved with a single pass of the print head on multiple passes of the printhead the dose deposition was always lower because of shearing forces eroding the existing dose during paper handling. (Georgios K. Eleftheriadis 1 ID, 2018) Applied ink-jetting for the printing of diclofenac sodium, a nonsteroidal anti-inflammatory drug, commonly used to treat pain and inflammation onto an edible sugar sheet [5]. They found that drug solution can be deposited up to 9 passes of the print head. (Yasmin Thabet, 2018) Applied the piezoelectric inkjet printing technique for the printing of enalapril maleate ink during continuous OTF production. Macrogol, methanol, and water-based inks were printed on OTF. No enalapril maleate crystallization was found in water-based ink [6]. The same approach was also applied to print enalapril maleate ink on hydrochlorothiazide (HCT) containing OTFs to prepare a fixed-dose combination. (Jana Pardeikea, 2011) Printed the nanosuspensions of Folic acid. Printing of the folic acid nanosuspensions was performed using an inkjet-based micro-dosing dispenser head at a frequency of 200Hz and voltage of 100V and impulse width of 25µs. Despite the potential advantages, there are still technical limitations for printing medicine that have not yet been overcome most critically, inkjet printing of APIs has been restricted to low viscosity fluids. Inkjet printing of viscous liquids can be attained by using heated piezoelectric-based inkjet systems but the heat-sensitive drugs cannot be printed through a heated print head because this results in degradation of API [7]. Alternatively, contact printing techniques such as flexographic printing, which are common in industrial roll-to-roll printing, have been used. However, contact with the board can lead to mutual contamination and damage to the board [3, 8]. Moreover, such printing techniques can lead to excessive waste of ink, and their implementation on an industrial scale involves a high cost of capital. Therefore, to avoid the challenges associated with the above-mentioned printing techniques in this current study we focus on the use of extrusion-based printing. Although several 2D printers were available on the market these all utilize a drug ink of water-like consistency and the main printing principle is based on inkjet printing or flexographic printing. So, in this research work, we used a 3D pneumatic-pressure extrusion-based printer for 2D printing on

a pharmaceutical-grade substrate. The majority of the published studies on printed medicine were conducted using non-edible substrates such as polytetrafluoroethylene (PTFE) sheets, glass plates, and various paper substrates [9]. While a few studies have used placebo Orodispersible films. Therefore, in the current study, we developed a unique printing platform using placebo Orodispersible films and extrusion-based printing.

MATERIALS AND METHODS

Material

The excipients used for the preparation of placebo substrate and ink were Hydroxypropyl methylcellulose (E5, LV) from Plaquemine Methocel, US; MCC from Ankit pulps and Boards India; Neusilin from Fuji chemical industries Japan; Starch was from Universal starch-chem allied ltd India; Carbopol was from Lubrizol advanced material USA. The propylene glycol, glycerol, butylated hydroxyanisole (BHA), and Butylated Hydroxytoluene (BHT) were from Finar Limited Ahmedabad India. The ketorolac Trometamol has obtained from Zim laboratories in Kalmeshwar India. The Carmosine supra dye was from Rohan A JJT group company, Mumbai, India. The water used for the preparation of substrate and ink was ultrapure.

Development of placebo substrate for printing

Three different substrates have been investigated in this work. These were HPMC containing Neusilin substrate, HPMC containing MCC substrate, and starch-based substrate. All the substrates were prepared by the solvent casting method. Table 1 gives the composition of each formulation. The weighed amount of film-forming polymers HPMC/Starch (87% w/w) were first dissolved in 300 g water and stirred on a mechanical stirrer at 2500 rpm for 20 min to get a homogenous mixture. The Neusilin/microcrystalline cellulose (2% w/w) was added to the solution and stirred at 1000 rpm for 10 min. Propylene glycol, glycerine, (4% w/w), and other excipients were added to this mixture and stirred with the help of a mechanical stirrer at a speed of 1500 rpm for 15 min. Placebo films were cast on the polyester sheet using an automated film-making machine (TB 300, China) equipped with an adjustable coating roller. The wet film thickness was adjusted to 700 µm and the speed was 0.3 m/min. The width of the coating was 310 mm. The obtained films were dried in an oven with two heating zones (40 °C and 45 °C) before being rolled up into a jumbo roll. The jumbo roll was then cut into daughter rolls with a width of 3 cm (the final OTF width) and a length of up to 100 m. the resulting film sheets were used as a substrate for printing.

Table 1: Formulation composition of various oral soluble substrates

S. No.	Ingredients	Role	% composition		
			F1	F2	F3
1	HPMC	Film-forming polymer	87.00		87.00
2	Starch	Film-forming polymer	-	87.00	
3	Neusilin	Pore-forming agent	2.00	-	-
4	Microcrystalline cellulose	Pore-forming agent	-	-	2.00
5	Propylene glycol	Plasticizer	4.00	4.00	4.00
6	Glycerine	Plasticizer	4.00	4.00	4.00
7	Sucralose	Sweetener	1.5	1.5	1.5
8	Mentha oil	Flavoring agent	1.0	1.0	1.0
9	Purified water	Solvent	q. s	q. s	q. s

Placebo substrate characterization

Digital microscopy

The front and back sides of printed films were examined through a digital microscope to perceive the presence of micro-holes on the placebo substrate and drug printed films. The Digital microscope used was Olympus BX 51 polarization microscope (Olympus Corp., Tokyo, Japan), with both transmission and reflection modes. MP3 software was used to improve the depth of focus. The magnifications varied from 5 to 100%.

Thickness

The thickness of each film was measured by using digital venire calliper (Mitutoyo, Japan) at five different points (at the centre and four corners) of the film and the average was calculated. This is critical to establish uniformity in the thickness of the film this is directly associated with the accuracy of the dose in the film. The test was performed in triplicate for each sample and the results are presented as mean value±standard deviation (SD).

Folding endurance

The folding endurance was measured manually for the prepared films. A strip of 33x23 mm was cut evenly and repetitively folded at an equivalent place till it broke [10]. The number of times the film might be folded at an equivalent place without breaking gave the precise value of folding endurance. The test was performed in triplicate for each sample and the results are presented as mean value±standard deviation (SD).

Tensile strength

Tensile strength was determined using a TA. XT plus Texture Analyser (Linux Machines incorporation Mumbai, India). It is the maximum stress applied to a point at which film breaks [11] and is measured by dividing the applied load at the break by the cross-sectional area which is given by

$$\text{Tensile strength} = \frac{\text{load at breakage}}{\text{strip thickness}} \times \text{strip width}$$

The test was performed in triplicate for each sample and the results are presented as mean value±standard deviation (SD).

Percentage elongation

% elongation was calculated by dividing the extension at the point of rupture by the initial length of the specimen.

$$\%\text{Elongation} = \frac{\text{increase in length}}{\text{original length}} \times 100$$

Development of ink for pneumatic-based microextrusion printing

A solvent providing the highest saturation solubility of the API, was used to develop drug-loaded inks with appropriate rheological properties for efficient printing of the ink on the substrate. To achieve desired rheological properties of ink, various polymers were used like Hydroxypropyl cellulose (HPC), Hydroxypropyl methylcellulose (HPMC), Starch, and Carbopol. Table 2 gives the composition of each formulation. All the ink formulations were prepared by dissolving polymers into distilled water. Propylene glycol, BHA, BHT, and coloring agents were added to this mixture and stirred with the help of a mechanical stirrer at a speed of 300 rpm for 20 min. The weighed quantity of ketorolac was added to the above solution and stirred at 350 rpm for 30 to form a homogenous dispersion. Propylene glycol was selected because it acts both as an excipient for enhancing viscosity as well as it acts a humectant. Thus slowing crystallisation of the solute and helping to make sure that cartridge orifice doesn't become blocked. BHT (Butylated hydroxytoluene) and BHA (Butylated hydroxyanisole) were added to control the degradation of KT.

Table 2: Formulation composition of ink

S. No.	Ingredients	Role	% composition			
			I_1	I_2	I_3	I_4
1	Ketorolac Tromethamine	API	10.00	10.00	10.00	10.00
2	HPC	Viscosity modifier	24.10	-	-	-
3	Starch	Viscosity modifier	-	24.10	-	-
4	Carbopol	Viscosity modifier	-	-	24.10	-
5	HPMC	Viscosity modifier	-	-	-	24.10
6	Propylene glycol	Plasticizer	1.40	1.40	1.40	1.40
7	BHA	Antioxidant	0.69	0.69	0.69	0.69
8	BHT	Antioxidant	0.34	0.34	0.34	0.34
9	Vitamin E acetate	Antioxidant	0.415	0.415	0.415	0.415
10	Sunset yellow	color	0.05	0.05	0.05	0.05
11	Purified water	solvent	63	63	63	63

Ink characterization

Viscosity

The viscosity of each formulation was measured using a cone plate rheometer (MCR 300, Anton Paar GmbH, and Graz, Austria). After the sample temperature was equilibrated at 20 °C, a fixed shear rate of 60 s^{-1} was applied for 1 min, during which 10 data points were recorded [12]. The formulation viscosity was calculated as the average of these 10 values. Newtonian and shear thickening properties were therefore not considered here.

Density

The density of each ink formulation was measured by using a digital oscillating U-tube density meter (Abbemat1 RXA 170, Anton Paar GmbH, and Graz, Austria). All measurements were performed in triplicate at 20 °C. The test was performed in triplicate for each sample and the results are presented as mean value±standard deviation (SD) [12].

Spreadability test

The sample was placed between the two glass slides and 50 g weight was placed on the glass slide for 5 min to compress the sample to a uniform thickness. The time in seconds required to separate the two slides was taken as a measure of spreadability. The test was performed in triplicate for each sample and the results are presented as mean value±standard deviation (SD) [12].

$$S = m \times \frac{l}{t}$$

In which S is the spreadability of the ink, m is the weight (g) tied to the upper plate, l is the length (cm) of the glass plates, and t is the time taken (s) for the plates to slide the entire length.

Printing of ketorolac loaded ink

For printing of ketorolac loaded ink the "Cellink Inkredible+Bioprinter" was used. The INCREDIBLE 3D Bioprinter is a pneumatic-pressure based microextrusion bioprinter with dual printheads and a UV LED curing system. The ink-filled cartridge was attached to the printhead and calibration was done. After calibration, the design was printed by converting three-dimensional CAD models into coordinates. The printing process works through the extrusion of ink or hydrogel. Once the printing of the design was completed the printed films were dried in a hot air oven at 50 °C for 10 min.

Characterization of printed films

Determination of moisture content

The prepared films were weighed and kept in a vacuum desiccator containing anhydrous silica at room temperature. The films were weighed repeatedly until a constant weight was achieved. Percent moisture content was determined using the formula

$$\%\ \text{Moisture content} = \frac{(\text{initial weight of strip} - \text{final weight of strip})}{\text{initial weight of strip}} \times 100$$

Results are presented as mean value±standard deviation (SD), n=10.

Tensile strength

Tensile strength was determined using a TA. XT plus Texture Analyser (Linux Machines incorporation Mumbai, India). It is the maximum stress applied to a point at which film breaks and is measured by dividing the applied load at the break by the cross-sectional area which is given by

$$\text{Tensile strength} = \frac{\text{load at breakage}}{\text{strip thickness}} \times \text{strip width}$$

Results are presented as mean value±standard deviation (SD), n=10.

***In vitro* disintegration test**

Disintegration time is the time when an oral film starts breaking when brought in contact with water or saliva. For a fast-dissolving film, the time of disintegration should be in the range of 5-30s [13]. The apparatus consists of two baths which were filled with purified water up to 1100 ml. In a beaker take 90 ml purified water and insert this beaker into the bath. The heater was ON to achieve set temperature (Beaker and Bath) then with the help of two binder clips suspend the film deep in water. The time when the clip falls down at the bottom of the beaker was recorded as disintegration time. Results are presented as mean value±standard deviation (SD), n=10.

Assay

The assay of ketorolac printed film was carried out by HPLC. The Princeton SPHER-100 column was used for analysis. The mobile phase was prepared by mixing 550 ml of methanol, 440 ml of water, and 10 ml of glacial acetic acid. The mixture was then filtered through a 0.45 µm pore size nylon membrane which was degassed by sonication. A mixture of methanol and water in a 1:1 ratio was used as a diluent.

Standard solution

About 50 mg of ketorolac Trometamol was transferred in a 100 ml volumetric flask and 70 ml of diluent was added. Then the solution was sonicated to dissolve solid particles. From the resulting solution, 5.0 ml was pipette out in 100 ml of the volumetric flask and diluted to 100 ml with diluent. The obtained solution had a concentration of 25µg/ml.

Sample solution

In a 200 ml volumetric flask 10 printed films (equivalent to 100 mg of Ketorolac) were transferred to it 150 ml of diluent was transferred and sonicated for 5 min and volume was made up to 200 ml by diluent. Then the sample was stirred for 30 min at 350 rpm using a magnetic stirrer. The obtained solution was then centrifuged at 2000 rpm for 5 min. From the resulting solution, 5.0 ml solution was pipette out in a 100 ml flask and diluted with diluent then filtered through a 0.45 µm pore size nylon membrane. The obtained solution had a concentration of 25µg/ml. The prepared solutions were injected into Colum as per the following sequence:

Table 3: Sequence of injections

Sequence of Injections	No. of Injections
Blank Solution	01
Standard Solution	05
Sample Solution	02

Uniformity of dosage unit

Content uniformity was carried out by the HPLC method. The chromatographic conditions Mobile Phase, Diluent, and Standard Solution were prepared in the same manner as per the assay procedure.

Sample solution

In a 100 ml volumetric flask one printed film was transferred and 70 ml of diluent was added. The resulting solution was sonicated for 30 min with intermediate shaking and volume was made with diluent. Then the solution was centrifuged at 2000 rpm for 5 min. the 5.0 ml of the resulting solution was pipette out in a 20 ml volumetric flask and diluent was added to make up the volume. Then the solution was filtered through a 0.45 µm pore size nylon membrane. The prepared solutions were injected into Colum as per the following sequence:

Table 4: Sequence of injections

Sequence of injections	No. of injections
Blank Solution	01
Standard Solution	05
Sample Solution	01

Dissolution

The *in vitro* drug release study of the printed film was carried out using a USP Type 1 (Basket) dissolution test apparatus. 600 ml of distilled water was used and maintained at 37±5 °C while the basket was set at 100 rpm. Dissolution was carried out for 45 min by the HPLC method. One dosage unit was placed in each of the dissolution vessels containing the dissolution medium and the apparatus was run as per the above-mentioned conditions. 10 ml aliquot was withdrawn after a stipulated time point from each vessel and a distance not less than 1 cm from the vessel wall by using sampling cannula was maintained. The solution was filtered through a 0.45 µm membrane.

Standard solution

Accurately weighed about 40 mg of ketorolac Trometamol working standard was transferred in 100 ml volumetric flask and 70.0 ml of mobile phase was added. Then the resulting solution was sonicated for 5 min to dissolve the particles and volume was made with diluent. In a 50 ml volumetric flask, 2.0 ml of the above solution was pipette out and volume was made with dissolution medium. The mobile phase was the same as used for the assay. Chromatographic conditions were the same as the ones used for the assay. The prepared solutions were added in Colum as per the following sequence:

Table 5: Sequence of injections

Sequence of injections	No. of injections
Blank Solution	01
Standard Solution	05
Sample Solution	01

The *in vitro* dissolution study was performed in sextuplicate and the results are presented as mean value±SD.

RESULTS AND DISCUSSION

Selection of printing substrate

The various printing technologies such as thermal ink-jet, piezoelectric and flexographic printing have been well established for active pharmaceutical ingredient (API) printing. The restrain problems with these printing technologies [14] are the development of a porous high ink absorption capacity edible substrate without affecting the integrity and mechanical properties of the substrate upon the printing of a high volume of ink [15] and secondly loading a high dose of the drug. the ideal substrate should remain intact after deposition of the high volume of the ink followed by a possible drying step This means that extensive disintegrations and/or dissolution of the substrate in contact with the ink should not take place [16] The substrate in this research was modified by adding one or more ingredients such as microcrystalline cellulose in the range of 5 to 10% that make the substrate act as adsorbents and imparts required roughness to the surface of the substrate which can hold the ink and absorbed the ink solvent resulting in prevention of cavity formation on the substrate after the printing of drug ink. Gaisford *et al.* printed the salbutamol on potato-starch-based film through a thermal ink-jet printer. When the deposition was achieved with multiple passes under the print head, the measured dose was always lower, and outside the±5% limit, of the theoretical dose. It is posited that this is a result of shearing forces eroding the existing dose during paper handling [4]. Georgios K. *et al.* used a sugar sheet as a printing substrate for inkjet printing of diclofenac sodium similarly Natalja Geninaa *et al.* used an icing sheet and polyethylene terephthalate sheets for printing Loperamide. In many studies, the researchers used either an edible icing sheet or non-edible polyethylene terephthalate sheets for medicine printing not much work is done on the development of an edible printing substrate. In this study, we develop an edible substrate using cellulosic derivative polymers. The developed printing substrate has the desired morphological, mechanical, and absorption properties. The Cavity formation or dissolution of the substrate after printing is a major challenge that needs to be addressed during the development of the substrate [17]. In this research work, this problem was assessed by visual and Digital microscopic observation after drug printing on a substrate. As presented in fig. 1. The prepared trial ink was printed on F1, F2, and F3 substrates. When starch-based substrate (F2) was appraised through the digital microscope (Olympus BX 51 polarization microscope) microscopic pores were presented throughout the substrate. After printing of trial ink on the same substrate, a hole in the substrate formed which represent the dissolution or disintegration of the substrate. The Neusilin in the F1 substrate was added to enhance the adsorption property of the substrate so that upon printing cavity formation or dissolution of the substrate can be avoided. After printing when the printed strips were inspected through the digital microscope cavities on the substrate were detected. Since F1 and F3 exhibited the cavity formation or disintegration of the substrate hence both substrates were excluded. When printing was executed on MCC containing substrate (F3) and printed strips were observed through a digital microscope no cavity formation was detected.

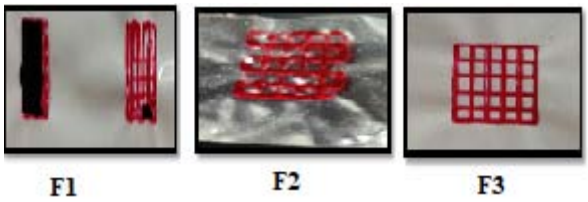

Fig. 1: Visual of cavity formation on the substrate

Mechanical properties

The film's mechanical properties can be changed by changing the film-forming polymer. In this work, we altered the mechanical property of the substrate by using different types of polymers like HPMC and starch. Tensile strength (TS) and elongation at break (%E) are generally reported responses to define the mechanical properties of thin films. The tensile strength of the HPMC-based substrate was 0.423±0.01 which was the highest. This specified that the films were resilient and flexible. This may be because the HPMC provided a more flexible structure compared to all other polymers due to a large number of side chains, which resulted in molecules sliding easily.

Table 6: Physical properties of prepared substrates

Formulation code	Thickness (mm) (mean±SD, n = 6)	Folding endurance (mean±SD, n = 6)	Tensile strength (N/m²) (mean±SD, n = 6)	% Elongation (mean±SD, n = 6)
F1	0.125±0.004	64±3	0.303±0.010	17.51±7.90
F2	0.139±0.0028	77±1	0.423±0.01	64.64±3.129
F3	0.157±0.0039	106±4	0.331±0.016	31.31±3.66

Selection of printing ink

Rheology deals with the study of deformation and flow of fluid materials under controlled shear conditions. During the dispensing process, the ink undergoes various deformations and changes due to the applied pressure. Rheological properties provide a means to measure such changes (such as viscosity) since stability, ease of flow, and self-supportability can directly correlate with observed behavior. Newtonian flow behavior, where viscosity is independent of shear rate. This ink type is typical of low-viscosity, prone to surface wetting, lacks self-supportability, and therefore not suitable for printing [18]. The remaining three are categorized as non-Newtonian materials, in which viscosity depends on the shear rate. The shear-thinning behavior is characterized by a decrease of viscosity as the shear rate increases and is considered ideal for printing, because this decrease in viscosity facilitates flow through nozzles, and rapidly increases upon deposition to enable shape retention [19]. On the other hand, the shear thickening behavior is characterized by an increase in viscosity as the shear rate increases. Printing is required to be carried out under high pressure for viscous inks. The Newtonian and non-Newtonian flow behavior of inks was characterized by determining the viscosity of inks at a different shear rate through cone and plate rheometer. The Hydroxypropyl cellulose (HPC) based ink represents Newtonian flow property but the resulting viscosity of the ink was very low and was not suitable for printing by the pneumatic based printer. The Carbopol-based ink printing was not uniform due to lots of air entrapment in the ink which result in variability in printing. The viscosity value was given in table 8. The starch-based ink represents Non-Newtonian shear thickening behavior (fig. 2). The HPMC-based ink represented Non-Newtonian shear thinning behavior (fig. 3). Which is desirable for printing. The viscosity results were given in table 9. The quality of a particular print depends on the ink density and the density of ink depends upon its viscosity. The high-density ink produced a dense print and the low-density ink produced a very light print. The density of HPMC-based ink was 1.19±0.01. At this density, the printed designs had color uniformity. Spreadability of ink is important for sustaining the integrity of printed design and also for evenly spreading ink on the substrate for proper adhesion of printed design on the substrate. The Spreadability of HPMC-based ink was 1.82±0.03 g. cm/s. With this Spreadability the printed designs were well-consolidated (table 7).

In inkjet and flexographic usually, low viscosity inks are used because viscous inks are difficult to jet from cartridges. This makes these technologies limited to smaller molecule and less viscous fluids printing only which result in low deposition of drug dose on the substrate. Janßen *et al.* printed rasagiline solutions and tadalafil suspensions by flexographic printing. The viscosity of rasagiline solution was 45 mPa. s and the viscosity of tadalafil suspension was relatively high about 70-80 mPa. s. During each printing process 0.28±0.03, mg API was transferred. Therefore, flexographic printing technology is highly suitable for manufacturing ODFs in small doses and individualized doses. Up to four printing cycles were performed [3]. Planchette *et al.* utilized both piezoelectric-and solenoid valve-based inkjet technologies, in which a sodium picosulfate solution, polymeric nanosuspensions, and PEG solutions are used to print onto (1) Rapid film, (2) hydrophilic porous, (3) hydrophobic non-porous films. The viscosity range of ink formulation was from 1 to 8 mPa. s [1]. Similarly, Georgios K. Eleftheriadis *et al.* and Asma B. M. Buanz *et al.* used inkjet printing technology for printing Diclofenac and Salbutamol orally disintegrating films. The viscosity of diclofenac ink was from 5 to 28.67 mPa. s and the viscosity of salbutamol ink ranges from 1 to 8.9 mPa. s [4, 5]. In this research work through the pneumatic-based printer, we were able to print a high viscosity ink on orally disintegrating films. The viscosity of ink ranges from 10000 to 50000 mPa. s. The clogging of the nozzle due to printing of this much high viscosity ink was not found. The printing process and system were stable during the printing process. Due to the higher amount of ink loading as compared to other printing technologies the desired dose of a drug or a higher dose can be deposited on oral thin films by a single print.

Table 7: Density and Spreadability values of different inks

S. No.	Formulation code	Density (g/cm³) (mean±SD, n = 3)	Spreadability (g. cm/s) (mean±SD, n = 3)	Adherence of printed design to substrate
1	I_1	1.01±0.03	3.00±0.05	**X**
2	I_2	3.01±0.06	2.66±0.06	**X**
3	I_3	7.03±0.07	1.09±0.01	**X**
4	I_4	1.19±0.01	1.82±0.03	✓

Table 8: Viscosity values of I_1 and I_2 ink

Formulation code	Shear rate Y (1/s) (mean±SD, n = 10)	Shear stress τ (Pa) (mean±SD, n = 10)	Viscosity η (Cps) (mean±SD, n = 10)	Flow behavior
I_1	60	120.91±0.64	1526.667±2.08	Newtonian
I_2	60	2287.54±0.57	139171±230.65	Non-Uniform

Table 9: Shear thinning and shear thickening behavior study of I_3 and I_4 ink

S. No.	Formulation code	Shear rate Y (1/s)	Shear stress τ (Pa)	Viscosity η (Cps)	Flow behavior
1	I_3	10	2575.89	42944.38	Non-Newtonian
		20	2251.58	45206.99	Shear thickening
		30	1914.48	48246.74	
		40	1548.48	52546.08	
		50	1138.84	59019.43	
		60	661.34	72159.84	
2	I_4	10	161.09	72305.09	Non-Newtonian
		20	346.3	59931.91	Shear-thinning
		30	479.01	54562.71	
		40	596.89	49517.58	
		50	700.7	45877.27	
		60	804.97	42966.49	

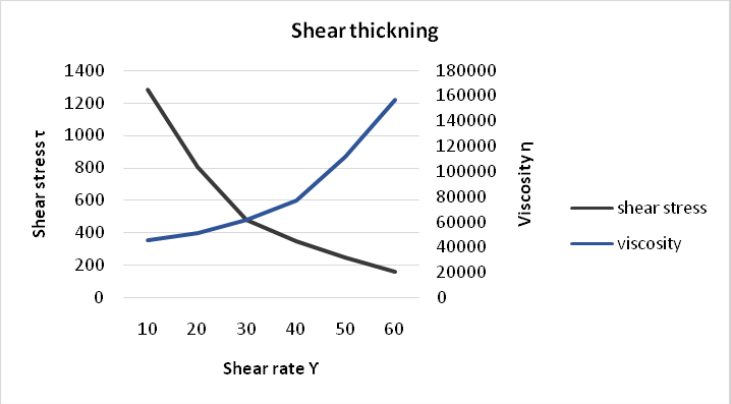

Fig. 2: Viscosity flow curve (I_3)

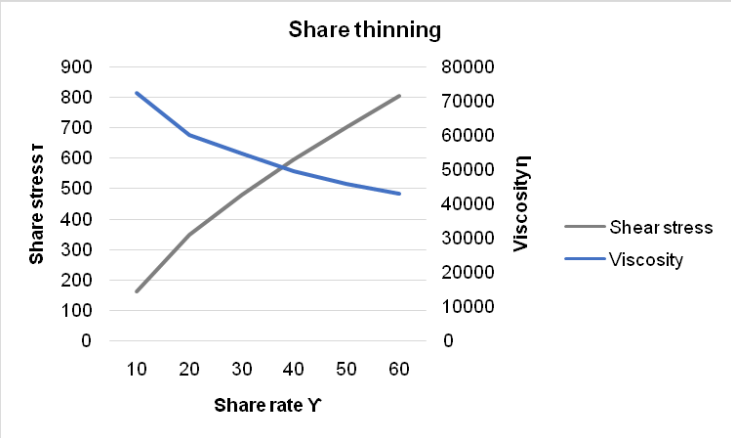

Fig. 3: Viscosity flow curve (I_4)

Printing process characterization

The printer used in this work was based on the Micro extrusion principle. The printer comprised of a syringe extruder system, a compressor, and a computer with 3D designing and slicing software. The semisolid ink of synthetic polymer is filled in a metal or plastic syringe and mounted in the extruder attached to a pressure-controlled air pump with the help of the pressure, the material inside the syringe is extruded through small nozzles of different shapes [20]. The printer head is moving horizontally in different directions to deposit the first layer because we prepared the G-code of a design through the slicer software so that it can print a single layer of design. The printer stage is not allowed to move down to allow a new layer to be created on top of the previous one.

The commercially available TIJ printers are only capable to deposit very small doses (approximately a maximum of 35 µg/print cycle). So, this technology is presently only appropriate for the printing of highly potent drugs [14]. This provides a challenge when attempting to formulate narrow therapeutic index drugs that typically require dosing within the milligram range, such as aminoglycosides, cyclosporine, carbamazepine, digoxin, digitoxin, flecainide, lithium, phenytoin, phenobarbital, rifampicin, theophylline, and warfarin. Researchers have attempted to increase drug deposition by several methods, for example by using multiple printing cycles of drug-loaded ink on a substrate [7] and increasing feed concentrations of the printing ink [21]. However, challenges surrounding the non-linearity of drug deposition and crystallization of active pharmaceutical ingredients were found. To extend the applications of drug printing on oral thin films, it is clear that a novel method to increase the amount of drug deposition is required. In this research, we demonstrated a printing technology that is capable of loading a high amount of drug on the substrate by a single print. Multiple times printing is not required. The amount of drug-loaded ranges from micrograms to milligrams.

To qualify printing as stable and accurate, the extrude ejection should be consistent and satellite-free. To achieve this, inks with suitable properties must be used and several process parameters optimized. Appropriate ink properties, which are precise to the printing system and nozzle, have been identified in an initial study allowing us to consequently develop and select the inks used in this work. Typically, the Cellink Inkredible+Bioprinter can print inks whose dynamic viscosity is between 0.001 to 250 Pa. S. A major challenge for printing stability is to prevent nozzle blocking. Practically, this was attained by selecting inks of low volatility to avoid API precipitation. As for the process parameters, with the Inkredible+Bioprinter the calibration, pressure, and nozzle size were tuned for the inks printed. The symmetric drop shape and the absence of satellite droplets were additional indicators of a good and stable printing process. The optimized printing parameters of the Inkredible+Bioprinter were given in the table.

Characterization of drug printed film

To verify that the pneumatic-based printing method allows the production of final dosage forms free of defects, such as holes or solid protuberances. The final dosage forms of typically a few $cm^2$of each substrate comprising, 10 mg Ketorolac Trometamol were prepared and characterized. The weighed amount of ink was printed over the targeted area of 120x80 mm of the substrate in the form of an array of 96 drops. This film was dried in a hot air oven and cut into 8 films of dimension 33x23 containing 12 drops per film. Twelve drops/film were equivalent to a 10 mg dose of the drug. These printed films were then subjected to evaluation of mechanical properties, assay, content uniformity, and dissolution studies.

Table 10: Optimized printing parameters

S. No.	Parameters	Optimized printing parameters
1	Printer	Cellink Inkredible+Bioprinter
2	Operating printhead	Printhead 1
3	Operating pressure*	37±1.8 k. Pa
4	Printing tip size	22 gauge
5	Nozzle diameter of the tip	838 µm
6	Printing Design	30*30 mesh
7	Drying of printed strips	Hot air oven
8	Drying temperature	60 °C
9	Drying time	10 mints.
10	printed film contains	10 mg ketorolac

*Optimized pressure for required drug loading mean±SD, n=10

Physicochemical properties

The thickness of printed films differs as compared to the thickness of non-printed films due to moisture loss during the drying of printed films. It was detected that the initial thickness of the non-printed film was 0.158 mm and the thickness after printing and drying of the film was 0.148±0.63 mm. Tensile strength and percent elongation of the film is important to resist the mechanical movements that occur during the packing, storage, and shipping of the films.

Moisture content, pH, and disintegration time

Adequate moisture content is required to maintain desirable flexibility and folding endurance. Moisture content may also affect the degradation and microbial growth of the drug product. The Moisture content of printed films was 12.39%±0.03 (mean±SD, n=6). The disintegration time of printed films was found to be 32±0.063 seconds (mean±SD, n=6). The acidic or basic pH of the film causes irritation of the oral mucosa hence should be close to salivary pH. The ph of printed films was found to be 6.62±0.07 (mean±SD, n=6).

Table 11: Physical properties of printed films

S. No.	Evaluation parameter	Result (mean±SD, n=10.)
1	Thickness	0.148±0.63 mm
2	Folding endurance	102±4
3	Tensile strength	0.353±0.09 N/m^2
4	% elongation	29%±0.03 %
5	Moisture Content	12.39%±0.03%
6	pH	6.62±0.07
7	Disintegration time	32±0.085s

Assay

Drug content was evaluated and it varied within the range of 96.9 to 108.1%. The drug Content was found to be102.5%±0.73 (mean±SD, n=12). As per USP requirements, drug content was found to be within the limits i.e. between 90 to 110%

Uniformity of drug content

Ten film strips of 33X23 mm were cut and drug content was estimated using the HPLC method. It was found that the drug was uniformly dispersed throughout the film. The results are given in table 12.

Table 12: Content uniformity of printed films

S. No.	Weight(mg)	Area/Abs	% content (mean±SD, n=10)
1	193.79	1315308	100.5
2	191.04	1320211	100.9
3	195.35	139226	106.4
4	193.64	1324503	101.2
5	184.30	1268012	96.9
6	188.45	1386533	105.9
7	192.07	1361759	104.0
8	191.61	1311683	100.2
9	191.67	1321689	101.0
10	190.80	1415089	108.1
			102.51±3.45

Table 13: *In vitro* dissolution of ketorolac printed films

Area/Abs					% Drug release (mean±SD, n=6)			
Vessel	**15 min**	**30 min**	**45 min**	**60 min**	**15 min**	**30 min**	**45 min**	**60 min**
1	570367	845154	827924	820539	66	98	98	99
2	595200	844480	840641	831111	69	98	100	100
3	297266	606454	799371	827316	34	70	94	99
4	573351	827045	816942	811185	66	96	97	98
5	518761	816249	816942	796976	60	95	97	96
6	576595	838211	827885	790467	66	98	98	95
					60±13.15	93±11.09	97±1.96	98±1.94

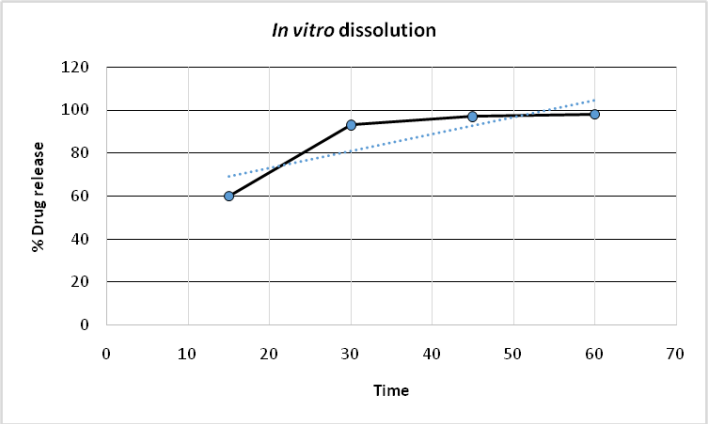

Fig. 4: Graph of % drug release from ketorolac printed oral thin films

In vitro dissolution studies

In vitro drug release studies were carried out in USP basket type dissolution apparatus using 900 ml purified water. Ketorolac printed film formulation released more amount of drug i.e., 98% within 60 min. See table 6 for % drug release with respect to time. More amount of drug release indicates the rapid onset of action.

CONCLUSION

In conclusion, we were able to demonstrate that Pneumatic-Based Microextrusion printing technology enables the printing of APIs on ODFs. Therefore, through this technology, the processing of high-potent low-dose, and heat-sensitive APIs have been possible. This technology is feasible for highly flexible small-scale printing, i.e., for personalized medicine. The ketorolac-loaded ink was prepared using HPMC as a thickening agent and Propylene glycol as a plasticizer to get a desired flow property of ink from the printer cartridge and nozzle. This ink was printed on HPMC containing MCC placebo substrate through Cellink Inkredible+Bioprinter. The ink viscosity was modified for 2D printing on the edible substrate through this Bioprinter. The critical quality attributes (CQAs) like mechanical strength, disintegration time, and *in vitro* drug release are analogous to those of ODFs manufactured by the common solvent casting method.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

The author(s) declare(s) that there is no conflict of interest

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